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HIGH PELLETING TEMPERATURES REDUCE BROILER PERFORMANCE

D. CRESWELL[1] and M. BEDFORD[2]

Summary

Six separate studies are reviewed in which a relationship between pelleting temperatures and broiler performance was measured. The studies included both wheat and corn-based diets, but were predominantly with wheat diets. The studies covered pelleting temperatures from 65 to 105 C. In all cases a relationship was established, such that the higher pelleting temperatures were associated with poorer broiler performance, in terms of weight gain and feed:gain, and in one study, mortality. Specifically it appears that pelleting temperatures over 85 C should be avoided. The reasons for this relationship are not clear, but presumably are due to losses of some heat labile nutrients, which may include vitamins, and binding of lysine and perhaps formation of indigestible complexes of starch with protein.

I. INTRODUCTION

Broiler feeds in Australia are virtually all produced in crumble or pellet form. This form of feeding is known to maximise broiler performance, both growth rate and feed conversion. During the conditioning of the mash feed, steam is introduced under pressure, which subjects the mash to high temperatures, prior to entering the die where the pellet is formed. The mash temperature achieved is measured close to the exit point of the conditioner, and this is generally referred to as the pelleting temperature. Most pellet presses have a visible gauge to record the temperature at this point. The conditioner temperatures which Australian feed mills employ are generally in the range of 85-95 C. Some feedmillers will say they operate in the range of 85-90 C, while others will say they are in the range of 90-95 C. These temperatures are generally higher than used in other markets, such as USA and Asia.

Pelleting temperatures are known to affect degrees of gelatinisation, feed throughput, and possibly pellet quality. However it is not generally known that pelleting temperatures may affect broiler performance. This paper reviews six research studies which have examined the relationship between pelleting temperature and broiler performance.

II. RESEARCH

a) Raastad and Skrede (2003)

A mash compound grower feed, based on corn, wheat, oats and soybean meal was subjected to steam conditioning and pelleting at three different temperatures (69, 78 and 86 C), measured as the core temperature at the outlet of the conditioner. Metabolisable energy (ME) content of diets was measured in 2-3 weeks old chickens. The pelleted diets were fed to chickens from 1-35 days. Results are shown in Table 1.

Pellet quality determined as durability and hardness was improved by increasing pelleting temperature. Conversely, the ME content of the diets declined significantly with increasing

[1] Creswell Nutrition, 12 Beaconsfield Road, Mosman, NSW 2088, Australia;
[2] Zymetrics, Golden Valley, MN, USA.

temperature, the ME content of feed pelleted at 86 C being approx. 3% lower than for feed pelleted at 69 C. Body weight at 21 days of age was significantly lower for birds fed diets pelleted at 86 C compared with 69 or 78 C. Feed conversion showed no difference between pelleting at 69 or 78 C, but there was poorer feed conversion when the pelleting temperature was increased from 78 to 86 C, especially in the 1-21 day period. Utilisation of ME during the 0-21 and 0-35 day periods was significantly lower with a temperature of 86 C than by pelleting at 69 or 78 C.

It was concluded that the temperature during conditioning and pelleting of the feed influenced technical quality of the pellets as well as the nutritive quality for young broiler chickens. High temperature improved the physical quality of the pellets, but caused lower levels of ME and poorer growth performance.

Table 1. Effect of pelleting temperatures on energy utilisation and broiler performance, 1-35 days

Pelleting temperature (C)	69	78	86
ME (Mj/kg)	13.280[a]	13.138[a]	12.866[b]
Liveweight (g)			
21 days	356[a]	361[a]	340[b]
35 days	1737	1740	1700
Feed:gain (g/g)			
0-21 days	1.52[a]	1.53[a]	1.64[b]
21-35 days	1.78[a]	1.79[a]	1.85[b]
0-35 days	1.73[a]	1.74[a]	1.81[b]

[ab] Means in the same row with no common superscript differ significantly (P<0.05)

b) Bedford (Zymetrics Quantum Report QR-3-04)

A trial in corn-based diets tested three enzymes at pelleting temperatures of either 85 or 93 C. The main effects for temperature are shown in Table 2. The higher temperature significantly depressed weight gains and feed:gain.

In this research, two additional 21d trials at 85 and 93 C pelleting temperatures were completed which showed the same significant weight gain and feed:gain effects as in Table 2.

Table 2. Effect of pelleting temperatures on broiler performance, 8-42 days[1]

Pelleting temperature (C)	Feed intake (g)	Weight gain[1] (g)	Feed:gain[1] (g/g)	Feed:gain[2] (g/g)	Mortality[3] (%)
85	3909	2062	1.900	1.887	1.91
93	3868	1987	1.952	1.927	2.86

[1]The weight gain and feed:gain effects were significant at P<0.001
[2] Feed:gain, corrected for mortality
[3] P=0.14

c) Bedford *et al.* (2003)

A trial in wheat-based diets tested several enzymes at pelleting temperatures of 65, 75 and 85 C respectively. None of the parameters measured exhibited an enzyme by temperature interaction so the main effects of temperature are tabulated. Weight gain and feed:gain deteriorated significantly with increasing temperature (<0.0001) and mortality increased (Table 3). This particular trial suggested that the negative effects of higher temperature pelleting were evident once temperatures exceeded 65 C.

There was a significant linear, negative effect of temperature on both gain and feed:gain, with 75 grams of gain and 4 points in feed:gain being lost for each 10 C increment in die temperature.

Table 3. Effect of pelleting temperature on broiler performance, 0-42 days

Pelleting temperature (C)	Weight gain (g)	Feed intake (g)	Feed:gain (g/g)	Mortality (%)
65	2518	4449	1.736	2.74
75	2461	4428	1.779	3.80
85	2383	4295	1.842	6.24

d) Finnfeeds International Ltd. 1998 (TR 1300:UK 98.29)

Wheat-soybean meal diets were steam-pelleted after conditioning at four different temperatures for 30 seconds with saturated steam at 1.5 bar. Processing temperatures of 90 to 95 C depressed 42 day broiler performance independently of enzyme addition, which may be due to damage to heat-labile nutrients (Table 4).

Table 4. Effects of pelleting temperatures on broiler performance, 0-42 days

Pelleting temperature (C)	Weight gain (g)	Feed:gain[1] (g/g)
70	2250	1.743
80	2276	1.730
90	2215	1.784
95	2230	1.762

[1]FCR corrected to 2.3 kg body weight, 3 points per 100 grams

e) Finnfeeds International Ltd. 1996 (TR1300:UK 96.24)

Wheat-soybean meal-fishmeal diets were extruded with conditioning at five different temperatures for 60 seconds residence time. Processing temperatures of 95 and 105 C tended to depress 21 day broiler performance (Table 5). Extract viscosity of the feed was also increased by pelleting temperature, due presumably to xylan solubilisation and starch gelatinisation.

Table 5. Effects of pelleting temperatures on broiler performance, 0-21 days

Pelleting temperature (C)	Weight gain (g)	Feed:gain (g/g)	Feed extract viscosity (cPs)
65	639	1.53	6.5
75	648	1.52	7.0
85	652	1.52	8.5
95	643	1.54	9.3
105	628	1.58	15.2

f) Finnfeeds International Ltd. 1997 (TR 1300:97.26)

Wheat-soybean meal diets were pelleted after conditioning at six different temperatures for 55 seconds at 18.5% moisture in a twin screw extruder. Processing temperatures of 90 and 95 C tended to depress 21 day broiler performance (Table 6). In this particular trial it seems that the relationship between pelleting temperature and performance was quadratic, with optimum performance being achieved at approximately 80-85 C with performance deteriorating at temperatures above or below this range.

Table 6. Effects of pelleting temperatures on broiler performance, 0-21 days

Pelleting temperature (C)	Weight gain (g)	Feed:gain (g/g)
70	649	1.497
75	665	1.443
80	661	1.417
85	677	1.404
90	650	1.455
95	630	1.509

III. DISCUSSION AND CONCLUSIONS

There are a number of research studies which suggest that high pelleting temperatures depress broiler performance. The research includes both corn and wheat-based diets. Whilst the depression may be quadratic in some trials and linear in others, there is a clear point to argue that excessive conditioning temperatures will impede growth performance. Specifically, it appears that pelleting temperatures over 85 C should be avoided.

The reasons for this relationship are not clear, but presumably are due to losses of some heat labile nutrients, which may include vitamins, the binding of lysine and perhaps formation of indigestible starch and starch-protein complexes. For example, it has been observed that technological treatment may cause the formation of starch that is not susceptible to enzymatic hydrolysis (resistant starch). It has been shown that there is considerable variation in the rate and extent of starch digestion in the chicken, and that this variation affects energy utilization (Aar *et al.*, 2003; Svihus, 2003). However, there is a lack of knowledge on the exact causes for these variations in starch digestibility.

In the case of diets based on viscous grains (wheat and barley), increased viscosity due to greater fibre disruption may occur at higher pelleting temperatures. This impedes both nutrient absorption and the resorption of endogenous excretion products in the small intestine, thereby increasing the amount of substrate available for bacterial growth in the hind gut. A moderate growth of endogenous bacteria may prevent invasion and growth of a pathogenic microflora. An excessive microbial growth, however, may disturb the gut environment and cause problems such as sticky droppings or necrotic enteritis. Interestingly, work by Fernandez, *et al.,* (2000) demonstrated a positive relationship between viscosity and susceptibility to Campylobacter colonisation. Since higher processing temperatures are known to be linked to higher intestinal viscosity (see Table 5), it is possible that in an attempt to sterilise the feed we could unwittingly be putting the bird at higher risk of infection from other sources.

Normal pelleting temperatures are generally not thought sufficient to solubilise dietary fibres or to form resistant starch. However, conditions within the feedmill conditioner, which involve combinations of temperature, moisture, pressure and time may be such as to cause damage to nutrient availability.

For corn (and sorghum) -based diets there may be loss of lysine and arginine due to maillard complexing with sugars and loss of available energy due to a kind of retrogradation of starch into resistant complexes following high temperature pelleting.

One reason given for the use of high pelleting temperatures is to control *Salmonella* in feed. It is suggested that *Salmonella* control only really requires 80 C for 30 seconds, according to a study by Dalgety conducted in the UK. A pelleting temperature of 80 C plus organic acids plus enzymes should allow for good *Salmonella* control.

Holding pelleting temperature to around 80 C should maximize broiler performance, and also allow for easier use of microbial enzymes.

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FEED PROCESSING – IMPACTS ON NUTRITIVE VALUE AND HYGIENIC STATUS IN BROILER FEEDS

M. PEISKER[1]

Summary

Most of the technological processes in compound feed manufacturing impact on nutritive value and hygienic status of the resulting feed and sometimes act synergistically, (e.g. expansion plus pelleting on salmonella decontamination). The nutritive effect is mainly exerted on feed digestibility and efficiency of energy utilisation. The return on energy invested in feed processing (electrical and steam energy), is positive for the majority of processes when balancing against the gain in available feed energy. In terms of dietary protein (amino acids) and feed additives like vitamins, enzymes and probiotics, special attention must be paid when applying thermal processes. Only a feed/purpose specific selection of treatment processes and fine-tuned process parameter setting brings optimal results in a scenario with sometimes conflicting objectives: maximum improvement of nutritive value combined with optimal decontamination of pathogenic germs. Whereas an improved nutritional status of the processed feed is durable, precautionary measures must be taken to maintain its hygienic status.

I. INTRODUCTION

Domestication and breeding of animals has improved animal performance in an unprecedented manner. The requirements in terms of nutrients have increased accordingly. Feedstuffs as offered by nature cannot meet these requirements, even if they are offered in a variety, without further processing. The coverage of dietary energy needs poses a particular challenge in feed formulation and subsequent manufacture. In the process of making dietary nutrients available for metabolic purposes the digestion process is a major influencing factor.

Digestive capacities of animals has remained largely unaffected despite centuries of breeding and selection (Wenk, 1982). Therefore, the preparation of feedstuffs before ingestion to improve digestibility became a major focus in nutrition research. On the other hand, the production units for livestock have become larger driven by economy of scale. This has heightened the importance of hygienic regimes including feed hygiene to prevent disease outbreaks and securing hygiene and feed ingredients of animal origin. This paper highlights some key processing steps in compound feed processing and their impact on nutritive value and hygienic status. Table 1 gives an overview of the major technological processes for making poultry feed. It can be stated that short and long conditioning have very little effect on nutritive value. Grinding and crumbling have no hygienic effect. Conditioning with subsequent pelleting has some and expansion plus pelleting has pronounced effects on both nutritive value and hygienic status. Toasting is reserved for the treatment of single ingredients like soybeans, peas, beans, canola and others. It is used to eliminate or reduce the content of anti-nutritional factors in these ingredients, before they can be used in compound feed. From that angle this process is very significant in terms of improving the nutritional value of these ingredients, which otherwise could be used only with limitations. The toasting process is not primarily applied for microbial decontamination, however, the processing conditions would allow for this.

[1] ADM Specialty Ingredients (Europe) B.V. Stationsstraat 76, 1541 LJ Koog a/d Zaan, The Netherlands

Table 1. Overview of technological process in poultry feed manufacturing

Process	Equipment	Nutritive Effect	Hygienic Effect
Grinding	Hammer / Roller mill	*	-
Short Term Cond.	Mixer/Conditioner	-	-
Long Term Cond.	Conditioner	*	***
Pelleting	Ring die/ Flat die press	*	*
Crumbling	Roller crumbler	*	-
Mash Coditioning	Batch/continuous cond.	-	***
Expansion	HTST- expander	**	***
Toasting[1)]	Hydrothermal toaster	(***)	***

1) Applied only for single ingredients

II. GRINDING

Poultry have a short digestive tract and therefore the digestibility must be high; but is capable of grinding entire grain in the gizzard. Nir and Hillel (1994) found a correlation between particle size and weight of gizzard and duodenum in 21-old broilers (Table 2). Also the gizzard pH-value is significantly lower when the feed particles are coarser. Feeding partly entire grains thus is seen as a possibility to prevent intestinal infections in poultry. Kamphues *et al*. (2005) made similar observations in weaning piglets when testing the effect of potassium diformate on *Salmonella* in faeces. The number of animals excreting *Salmonella* and the duration of excretion was significantly lower, when the feed was coarse ground (control diet: 32% > 1.0 mm; 26% < 0.4 mm; test diet: 58% > 1.0 mm; 10% < 0.4 mm). The authors concluded that *Salmonella* shedding is related to feed structure. In broiler feeding, pelleted feed is the preferred choice whereas in layers its mash feed. Finely ground mash increases the time for feed intake and reduces feather picking (Walser, 1997). Particle size structure should be uniform to prevent nutrient imbalances by selective feed intake. An optimal mash structure can be achieved with a combination of expansion and crumbling process. It also must be mentioned that the level of fineness of mash impacts on subsequent processes like pelleting (throughput, pellet quality). Grinding of feed ingredients is a pre-requisite of mixing different ingredients and achieving a low coefficient and variation % of nutrients in the feed mash.

Table 2. Influence of particle size on intake, growth and gizzard weight (Nir and Hillel, 1994)

Particle size (mm)	0.6	1.1	2.3
Weight at 21 d (g)	491[a]	568[b]	540[b]
Feed Intake 7-21 d (g)	591	662	645
FCR (7 - 21 d)	1.66	1.56	1.61
Gizzard wt. d-7 (% of LW)	3.95	4.5	4.87
Gizzard wt. d-21 (% of LW)	2.22	2.8	3.13

[a,b)] Means in the same row followed by different letters differ significantly (P<0.05)

III. CONDITIONING AND PELLETING

Before pelleting, mash feed needs a certain degree of steam conditioning. Figure 1 shows different options for conditioning prior to pelleting. All processes require the use of a short-term conditioner for steam and water addition. Pelleting agglomerates smaller feed particles with the help of mechanical pressure, moisture and heat to larger particles. This tends to improve animal performance due to less feed wastage, no selective feeding, and

improved palatability and starch gelatinization. Numerous trials have shown better daily gain and feed conversion broiler feeds.

Average daily gain and feed conversion ratio is improved by 5-8% and 3-5% respectively, when feeding a pelleted diet with zero fines versus a mash diet. Pelleting has an effect on the hygienic status of the feed; however, a short-term conditioner alone, or in connection with a pelleting press, will not be sufficient for decontamination of pathogenic microorganisms. Already Friedrich (1979), Hacking *et al.* (1978) and Pietzsch (1985) have stated that pelleting is not sufficient for safe decontamination as relatively large numbers of

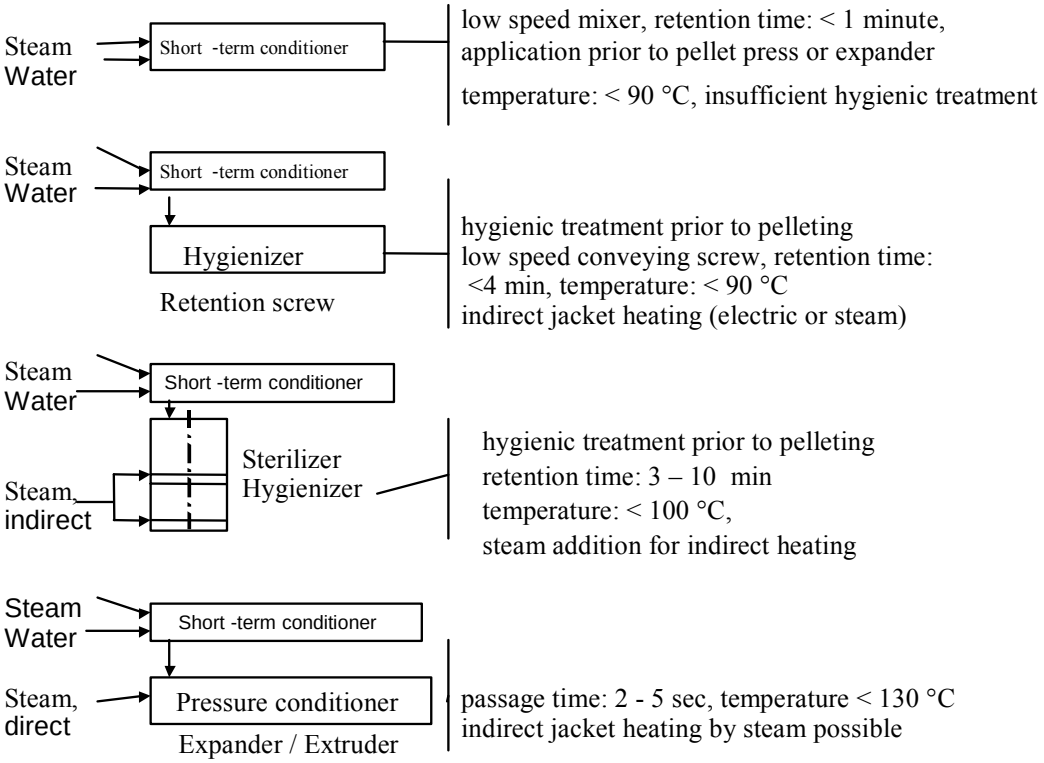

Figure 1. Thermal treatment prior to pelleting

micro-organisms remain in the finished feed. Sufficient decontamination with pelleting as the final processing step is achieved, when using a hygienizer with horizontal retention screw or vertical shaft (similar to long term conditioner or ripener), assuring minimum retention times of three minutes. It is suggested to increase retention time at the technically highest possible moisture level; however, limits apply for the proper functioning of the pellet press (< 16% moisture).

IV. EXPANSION

Expansion of mash feed is an established thermal processing technology that is widely used for broiler diets to improve pellet quality, include higher liquid and fat levels and increase operational productivity by increasing pelleting line capacity, and to enhance the flexibility of ingredients usage and animal performance (Wilson *et al*., 1998). This technology simultaneously leads to achieve a hygienic status, described below as "commercial sterility".

Table 3. Influence of different treatments of broiler diets on performance

	Mash	Pelleted	Exp. + Pell	Exp.+ Crumbled	
Diet effect					Jackson and Bollengier (1994)
Feed Intake (g)	5105[a]	5050[a]	5112[a]	5028[a]	
Body Weight (g)	2546[a]	2595[a]	2677[a]	25931[a]	
FCR	2.02[a]	1.95[ab]	1.91[b]	1.94[ab]	
Trial 1					Smith *et al*. (1995)
Body weight (g- 41 d)		2,068[a]	2,118[b]		
FCR		1.74	1.73		
Trial 2					
Body weight (g- 41d)		2,202[a]	2,237[b]		
FCR		1.82[a]	1.80[b]		
No enzyme added					Nissinen *et al*. (1993)
Body weight (g- 28d)		1.155	1.124		
FCR		1.50[a]	1.53[a]		
Enzyme added					
Body weight (g- 28d)		1.158	1.177		
FCR		1.47[ab]	1.39[b]		

[a,b)] Means in the same row followed by different letters differ significantly (P<0.05)

Table 3 shows results from broiler trials comparing different treatments of broiler feed. Most studies document an improvement in body weight gain and feed conversion ratio; however, depending on the selection of ingredients, enzyme supplementation to expanded diets proves to be obligatory to achieve this. Gauer (2002) reported no differences in FCR, when energy level in a corn-soy broiler diet (ME 3200 kcal) was decreased by 3.1% (Fig. 2).

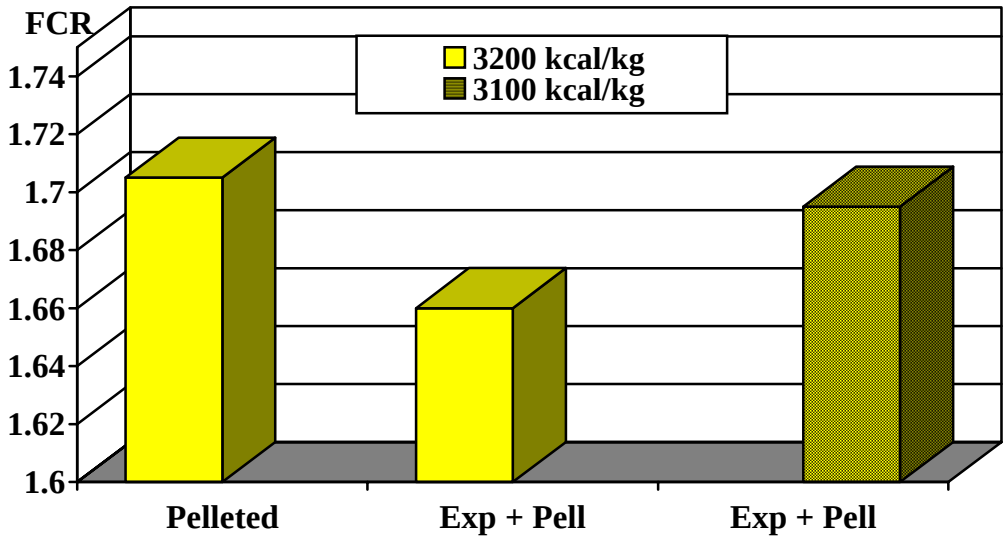

Figure 2. FCR in pelleted and expanded/pelleted corn-soy diets with and without ME reduction (Gauer, 2002)

Separate treatment of single ingredients can prove beneficial for some but not all ingredients (Table 4).

Table 4. Influence of expansion on ME in peas and corn and feed conversion ratios (FCR) in broiler diets containing full fat soy (FFS)

(AME, MJ/kg DM)	control	+ enzyme	expanded	exp + enz	
Peas	13.8[a]	14.0[a]	14.4[b]	14.45[b]	(Liebert, 2002)
Corn[1)]	13.85[a]		13.85[a]		
FCR					
FFS (% in diet)	pelleted		exp + pell		(Gauer, 2002)
0	1.68		1.55		
15	1.66		1.57		
30	1.62		1.53		

[1)] No differences occurred between 80°C and 100°C conditioning before expansion

[a,b)] Means in the same row followed by different letters differ significantly (P<0.05)

Table 5 shows a significant effect of expanding on the digestibility of the dietary fat fraction, leading to an increase in metabolizable energy. The expansion of the corn alone did not improve digestibility or energy value of the diet. This supports the results in table 4.

Table 5. Nutrient digestibility (%) and energy content of differently processed broiler feeds (Armstrong,1993)

	Organic Matter	Crude Protein	Crude Fat	Starch	NDF	ADF	Cellulose	ME (MJ/kg)
A	68.6[a]	78.5[a]	70.6[a]	97.9[a]	8.1[a]	19.8[a]	8.2[a]	11.7
	± 3.4	± 1.9	± 4.5	± 0.7	± 2.1	± 4.5	± 5.2	(100)
B	70.2[a]	77.2[a]	82.9[b]	98.9[b]	13.8[b]	22.3[a]	16.1[b]	12.2
	± 1.2	± 0.8	± 6.1	± 0.1	± 3.5	± 6.0	± 4.0	(104.3)
C	67.7[a]	76.9[a]	67.7[a]	98.1[ab]	13.8[b]	20.0[a]	12.0[a]	11.5
	± 2.3	± 0.8	± 10.0	± 0.7	± 4.6	± 3.2	± 3.6	(98.3)

A = pelleted; B = expanded + pelleted; C = only maize expanded (diet contained 40% maize)

[a,b)] Means in the same row followed by different letters differ significantly (P<0.05)

The results show that expansion is not acting on all ingredients in the same manner. In corn-soy diets an increase in dietary energy improves feed conversion ratio. In wheat or barley based diets, the addition of enzymes is recommended to reduce the gut viscosity that is inevitably increased by expansion of these ingredients. Only then a positive effect on body weight gain and feed efficiency is achieved.

V. ENERGY RETURN

Table 6 shows ranges of expenditure for steam and electrical energy in major feed manufacturing processes. The values are dependent on selected feed ingredients; their moisture content and the technical design and wear and tear impact on equipment. The expenditure of technical energy is balanced against the gain in feed energy (ME) in Table 7.

The increase in metabolisable energy for pelleting and expanding and pelleting has been measured in several broiler trials. In pelleting, energy expenditure (57 kWh/t) and gain in ME (56 kWh/t) are about the same. Thus for pelleting the technical energy expenditure is

recovered by the increase in metabolisable energy of 1 – 1.5%. In expanding and expanding plus pelleting a net gain in feed energy verus. technical energy is observed. About 73 kWh/t are expended in return for 122 kWh/t extra feed energy. As trials have shown, the ME increase in corn-soy-based broiler diets can exceed 4% (Table 5).

Table 6. Ranges of energy expenditure in feed processing (kWh/t)

	Low	High
Grinding		
Coarse	3	5
Fine	5	10
Mixing	2	5
Conditioning + Pelleting*	**37**	**57**
Steam** (2-3%)	15	22
Electrical	15	20
Conditioning + Expanding*	**47**	**68**
Steam** (4-5%)	30	38
Electrical	10	15
Condit. + Expand. + Pelleting*	**52**	**73**
Steam** (4-5%)	30	38
Electrical	15	20

* Includes grinding and mixing ** 1 kg steam = 2.755 MJ = 0.77 kWh

These calculations consider only the nutritional improvement by enhancing the digestibility at equal feed intakes. Better economic feed conversion ratios by reduced feed losses (dust, fines, uneaten feed) will further improve the value of these technological processes. A total energy balance should include energy for transportation and storage processes of feed. High-density feeds resulting from pelleting after expansion are advantageous in terms of transport and storage space and feed intake in broilers.

Table 7. Ranges of technical energy input verus nutritional energy increase in different feed processing methods

	Technical Energy Expenditure (kWh/t)		Dietary ME increase (%)		(kWh/t)	
	Low	High	Low	High	Low	High
Pelleting	37	57	1	1.5	37	56
Expanding	47	68	2	3	75	112
Expanding + Pelleting	52	73	2	3	75	112

1 MJ = 0.278 kWh

VI. HYGENIC FEED PREPARATION

a) Decontamination

Sterility cannot be achieved in feedstuffs; however, a so-called "commercial sterility" (Asquith, 2002) is possible. This means that pathogenic microorganisms have been eliminated (coliform bacteria, S*almonella*, moulds, etc.). A higher, but still incomplete level of sterility can be reached at temperatures of 130°C, pressures of 3 bar and treatment times of 20 minutes; as practiced when sterilizing meat-meal (autoclaving). This technology is

reserved for special applications because valuable components, such as amino acids and vitamins, are damaged at these temperature and pressure conditions.

Appropriate processing technology depends on proper judgement of how these organisms live, grow and die. *Salmonella*, for example, have the highest growth rate at a temperature of 35-38°C. But, they can also grow at temperatures between 5-50°C if the ambient conditions are optimal. This depends mainly on the moisture content. *Salmonella* can only reproduce at an a_w-value (available water) of >0.92, which is not found in normal feedstuffs with ~13% moisture as it requires a moisture level of >25%. The a_w-value is the part of the water in feedstuffs, which is not bound to other substances but is completely available to microorganisms. This value varies between 0 (anhydrous substance) and 1 (pure water). It indicates the equilibrium moisture, adjusted between the sample and the relative air humidity. *Salmonella* and coliform bacteria need an a_w value of >0.92 for growth, while moulds need >0.8. The a_w value of cereals with 17% moisture is ~0.8. To avoid spoilage, the cereals needs drying to <16% moisture. The temperature range allowing growth of *Salmonella*, other bacteria and moulds is in the range of 5-55°C. When heating the product to more than 60°C, microorganisms stop growing and die. They do not die abruptly, but according to a logarithmic function.

Apart from a_w values and temperatures, the mortality rate also depends on the pH value. *Salmonella* and other bacteria have good growth conditions in the pH range of 7.0 to 8.5, moulds from 5.0 to 7.0. The reduction of pH value by adding organic acids can be used to decontaminate feeds. For complete decontamination, the addition of about 2-4% of organic acids is required. The costs involved are much higher when compared with thermal treatment; also, special attention is needed for the selection of acid-resistant equipment. In dry feed at ambient temperatures, *Salmonella* cannot reproduce, but they do survive by downscaling their energy metabolism. When the a_w-value increases, the energy metabolism rekindles. Activated, moist *salmonella* can be eliminated much easier than non-activated *Salmonella*. For this reason, hydrothermal treatment should always be coupled with an increase in moisture. *Salmonella* are not uniformly distributed in the feed, but are found in spots throughout the mixture. Therefore, it is difficult to "locate" them and to account for them in samples. For this reason coliform bacteria counts are used as a measure for the decontamination effect of different treatments since they exhibit the same heat resistance as *Salmonella* and are ubiquitously and uniformly distributed in feedstuffs. Some organisms such as spores of aerobic bacteria and anaerobic sporoform bacteria (*Clostridium perfringens*) cannot be eliminated readily by thermal processes. The same applies for toxins formed by moulds. Figure 3 displays the correlation between temperature and time for *Salmonella* decontamination at normal product moisture in meat meals (Beumer, 1992). Heidenreich (2002) showed that moisture content of about 14% in expanding at 105 °C is needed for a significant reduction in total germ counts (Figure 4). Israelsen *et al*. (1996) (Figure 5) and König (1994) aimed at similar conclusions. In expander processing prior to pelleting the expander peak temperature must clearly exceed 100°C to result in total elimination of *Salmonella*. In expanders and extruders, next to temperature and moisture, the sudden pressure drop at the outlet is a key element in killing bacterial cells, causing the living cells to burst (Peisker, 1991). Without pelleting, the expander temperature must reach 110°C for total *Salmonella* elimination.

b) Prevention of recontamination

Recontamination is a true hazard in feed production. A multitude of steps must be taken to secure the hygienic status achieved by feed processing. The addition of organic acids, such as propionic or formic acid (~ 0.5%) is generally accepted to protect a clean feed

mixture after hygienic treatment against recontamination. As a consequence, the design of the cooling area for pellets, the finished product sector, the bulk transport to the farm and the storage of the feed in the farm silos must be taken into account for maintaining hygienic status of finished compound feeds. Figure 6 shows a typical "recontamination pattern" from the feed plant to the farm silo when no chemical stabilisers such as organic acids are added. Moulds were completely eliminated and total germ count considerably reduced after thermal treatment. The weak spot at the feed plant level are the ducts from the pellet press to the cooler and the cooler itself. Such equipment in particular should be addressed in HACCP-programs. Also unloading pits, dust filters, elevator legs, bins and trucks must be checked on regular basis. In the farm silo the feed maybe completely re-contaminated due to feed residues and insufficient cleaning before recharge. Fully integrated poultry companies can address this and establish a successful *Salmonella* control system, comprising raw materials, feed processing (broiler, parent stock and layer feed), transport systems and farms. For example, the German poultry integrator "Wiesenhof" has adopted a policy enabling them to offer guaranteed *Salmonella* free hatched chicken, broiler meat, table and breeding eggs (Gill, 2002). However, in the commercial feed industry sector similar policies can be agreed upon contractually and several "tracking and tracing" systems have been developed by private or governmental entities and are quickly gaining importance in the industry.

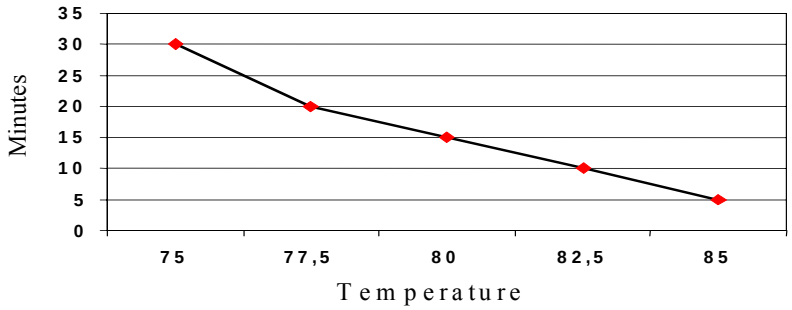

Figure 3. Decontamination of *Salmonella* as a function of time and temperature (Beumer, 1992)

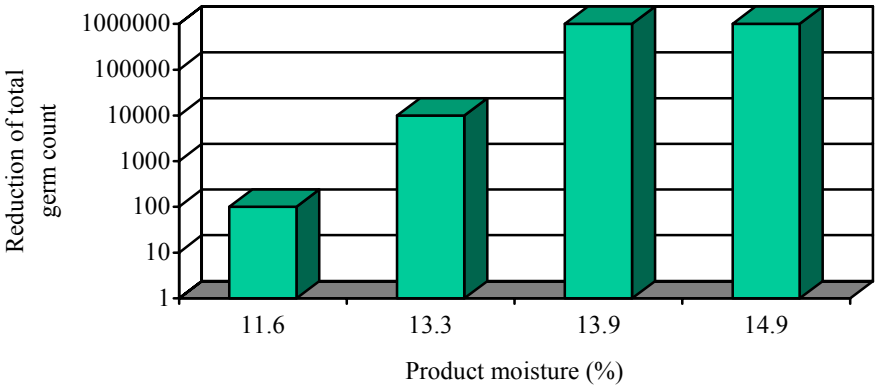

Figure 4. Reduction of total germ count in broiler feed with expander (105°C) at different moisture levels (Heidenreich, 2002)

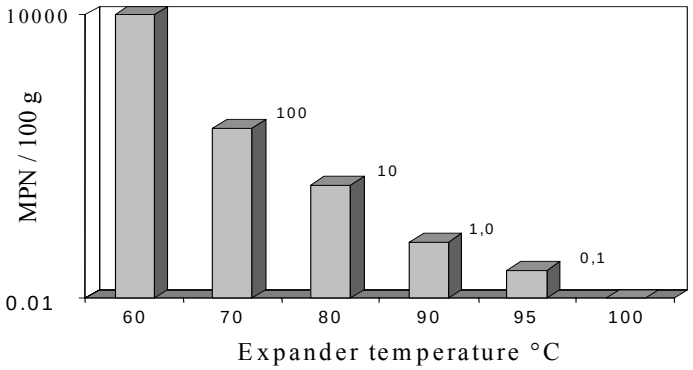

Figure 5. Decontamination of *salmonella* with expander as function of expander temperature (Israelsen *et al.*, 1996)

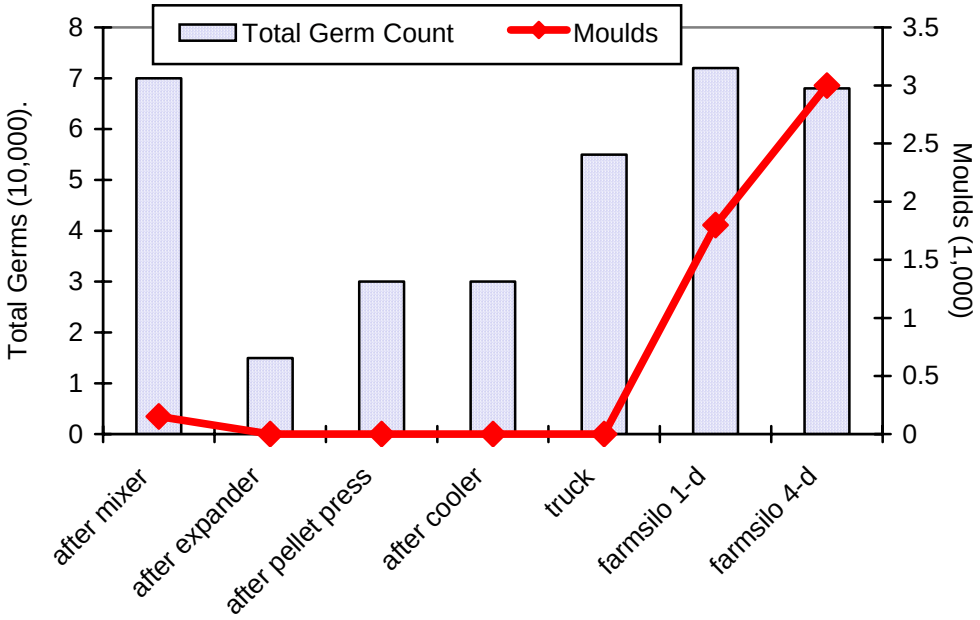

Figure 6. Recontamination pattern (total germ count and moulds) from feed factory to farm silo without chemical stabilizer

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TRENDS AND NEW TECHNOLOGIES FOR FEED MILLING

I. BUICK[1]

Summary

The development of feed production technology has made a subtle shift during the past ten years. In general, development of primary machinery has slowed with changes principally in scale rather than method. This paper will describe that the most important developments are those on the fringe of these core processes, but no less important. They can be characterised under the heading of smart manufacture, obtaining the best outcomes through optimising technologies new to feed milling. Much of the developing milling technology is used widely in other industries. The major aspects of new trend in feed processing will be stated in the order they appear in most post grinding feed mills.

I. FEED PROCESSES

a) Weighing and blending

The fundamentals of weighing have not changed significantly for years but smarter computer systems and cheaper load cell technology involve greater use of weighers in plant to control flows, monitor process and weighing rather than metering liquids. Demands for greater traceability and higher efficiency with increasingly smaller work forces are the driving force.

b) Grinding

As with presses, grinders have generally reached the end of their development both in terms of size as well as operating speeds and efficiency. Smart developments in this area are those, which aim to achieve the best particle size for each raw material. Pre grinding mills, however have largely been superseded with post grinding mills on the grounds of high capital cost. I have undertaken mill design projects where the difference between pre and post grinding plants can be as much as AUD 2.5 million. The past ten years have seen the development of slow speed grinders and rollers in post grinding, especially in the poultry feed sector, accompanied by pre-screening, but in terms of optimal particle size, there is no substitute to size reduction of separate raw materials (Goodband *et al*., 2002; and Ricker, 2004).

c) Conditioning

With *Salmonella* control being the principle driving force, many different conditioners have been developed during the past seven years. In the late 90's Camden and Chorleywood Research Centre in the UK found that single short-term conditioners with sufficient meal temperature from steam injection, achieved satisfactory microbiological kill rates, typically at 72 ^{o}C for 15 sec (Gaze, 2000). Much debate has continued on this subject, with many large supermarkets creating their own criteria for feed heat treatment. This has led to a plethora of new conditioning machines (see Figure 1 as example), all generally increasing the time of conditioning to many minutes. This however carries potential problems of nutritional damage to raw materials and additives (Garland, 2005)

Scandinavia in particular have taken a much more measured view. The minimum conditioning temperature has been set at 81 ^{o}C, and short term conditioners can achieve the necessary kill rates at this level when used with presses and or expanders (Langstrand, 2003)

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Common conditioning temperatures used in Australian feed mills around 90 ^{o}C with normal press conditioners exceeds the minimum requirement. I believe future trends will lead to shorter conditioning times coupled with acid treatments, as well as moves to optimise raw material particle sizes for healthy digestion.

The behaviour of the *Salmonella* bacterium (adapted from Wagner, 1998; Miles, 1999):

- *Salmonella* multiplies between 5 and 60^{O}C
- Optimum growth occurs between 25 and 40^{O}C
- Total destruction occurs at 130^{O}C for one second
- Total destruction occurs at 72^{O}C for 15 seconds
- Total destruction occurs at 65^{O}C for 30 minutes

It is clear that the *Salmonella* bacterium kill rates at increasing temperatures are exponential and elements of overkill have resulted in many unnecessary capital alterations to feed mills.

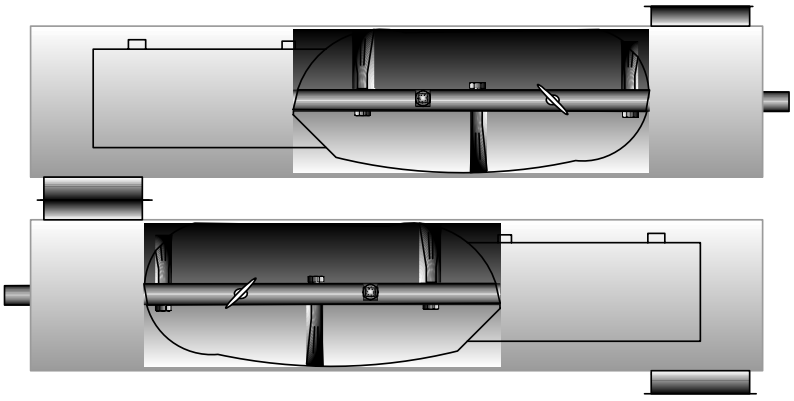

Figure 1. Short term conditioners doubled up

Smart conditioning involves working with liquids to achieve lower energy inputs, both in conditioning and downstream at the presses, expanders and extruders. Recent developments in emulsifier technology indicate the possibilities for improved gelatinisation and homogeneity in providing optimum digestibility of feed. For example, feeds, which contain high levels of added fat in the mix, will generally suffer low durability, and limited gelatinisation due to the difficulty of adding high steam levels. Emulsifier technology allows the fat to properly penetrate the feed as well as absorbing heat more completely from steam. The end result can be increased pellet moisture, better physical quality and reduced wastage from breakage without an effect on feed conversion. Table 1 shows the result from a feeding trial in Belgium. Feed containing 1% extra water and emulsifier using a patented process had the same FCR as the lower moisture feed without emulsifier. The subsequent microbiological tests also showed lower counts with the emulsifier, even with higher moisture content, normally a trigger for faster mould development. Conclusions:

1. The additional moisture combined with emulsifier had no effect on FCR.
2. The feed with emulsifier had a lower anerobic germ count.
3. The feed with emulsifier had a lower mould count after 3 months.
4. Both feeds were still considered safe after 3 months, but the feed with emulsifier had not developed mould in spite of having an extra 1% moisture and being over 13%.
5. The feed with emulsifier, included as an emulsion with oil retained 1% extra moisture after manufacture.

Notes:

1. Both feeds were manufactured from the same batch mix of dry ingredients.
2. Both feeds were manufactured using the same pelleting plant within a four hour period.
3. No compensation was made for the reduction in dry matter content of the feed with emulsifier.

Table 1. Results table for broiler feeding trial to evaluate the effect of castor oil ethoxilate emulsifier and additional water on FCR and other factors (Samuelsson, 2004)

	Control + 2% added water	Emulsifier + 2% added water
Water contents in feed after production	12.5	13.5
Body weight (g) 42 d	2321	2312
Daily food intake (g/bird/d)	90.8	90.5
Feed conversion ratio	1.76	1.76
Adjusted FCR	1.63	1.64
	Microbial Analysis of feed	
Aerobic germ KVE/g	4700	2000
Moulds KVE/g	850	<150

Heavily fat coated product can cause a barrier to effective digestion. Moving fat to the inside of the pellet without reducing physical quality is possible. Practical experience in integrated Broiler feed production indicates this is a successful strategy, but the precise nutritional effect has yet to be fully identified. In addition, acids used to further control *Salmonella* and other bio risks can be added in the mix and achieve much better distribution and penetration.

d) Presses

Pelleting presses have reached the practical limits of size for die handling and cooling capacity, and attention has to be given to effective batch sizes. It is no use having a 30 tonne per hour press making 5 tonne batches of feed, as the machine will never reach its optimum operating level. The start up and shut down phase of a feed batch is the most risky from a bio security aspect. Ideally, feed from each end of the batch should be recirculated or rejected, and this would be too high a penalty if the press is over sized. Better use of existing presses can be made with advances in liquid conditioning techniques, also presenting advantages in feed performance on farm, which is best when feed is fully homogenous.

e) Mixing

Dry mixing is acceptable up to a point, but different particle sizes mean good dispersion only at point of mixing. Subsequent handling and storage even for short periods often results in separation. The solution is to extend liquid ingredients and liquid combinations to allow significantly increased dispersion.

II. CONTINUING TRENDS IN FEED MILLING

Continuing trends in Feed Milling can be broadly grouped under the following headings

1. Liquid technology
2. Developments in feed hygiene
3. Computer technology
4. Mill security
5. Staff training and innovation

a) Liquid Technology - dispersion

A long standing problem in milling is the failure of mixers to properly disperse liquids. It is now possible to inject these liquids directly into the powder using rotating dispersers such as the one illustrated below

Figure 2. Liquid Disperser for use in mixers

The liquids are injected into the centre of the disperser and the rotating beaters mix the liquid with the dry meal. The result is a clean dispersion, without quantities left on the mixer sides and scroll.

This is a rapidly developing technology used in all areas of feed production, particularly in both poultry pellets and meal. There are few restrictions in the types and number of liquids, which can be added. Combined with emulsifier, the bulking of liquids acts as an effective pre-mix. Micro additions are dispersed in a significantly better away. In one application, no fewer than 10 different liquids are being added together in poultry mash, leading to more even egg production particularly in yolk colour where liquid colour is one ingredient. The following two diagrams show how this technology is used.

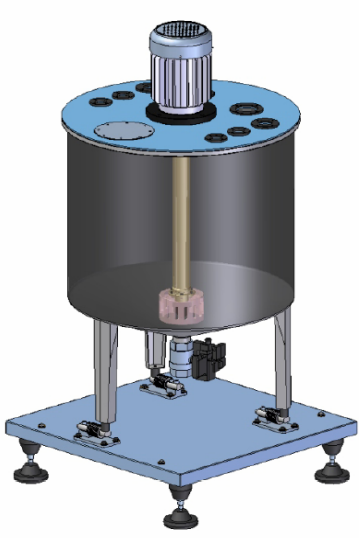

Figure 3. A weighing and mixing tank

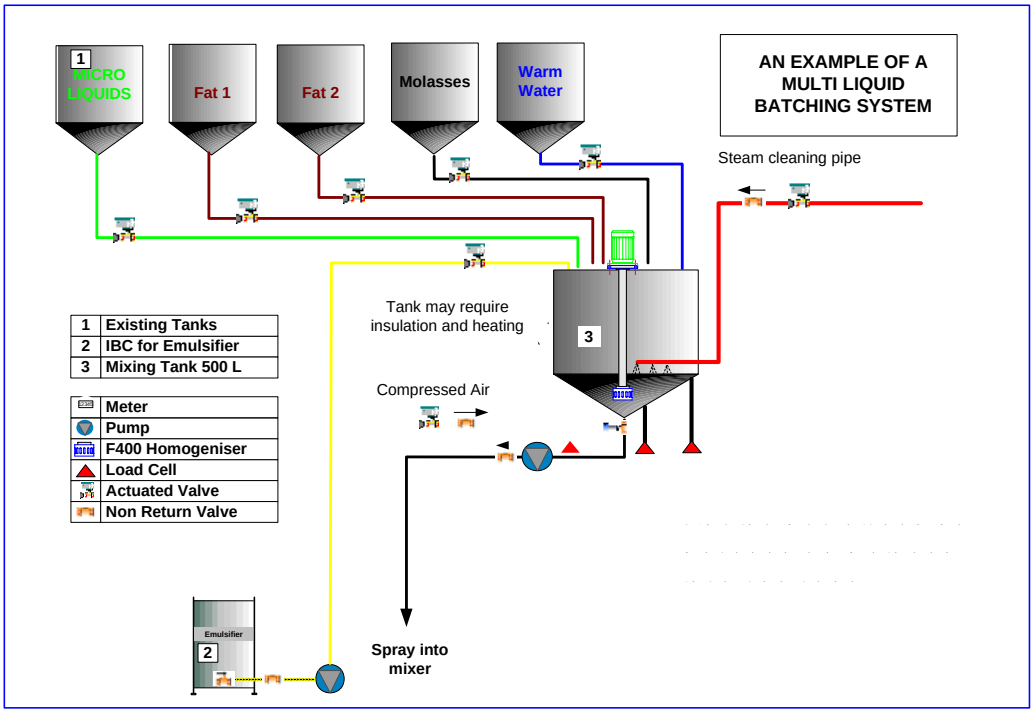

Figure 4. Example of Liquid Batching System

This is a technology, which has been developing for the past 25 years, but which primarily existed to spray fat onto finished pellets. Enzyme damage during the pelleting process, led to an expansion of external liquids applied.

With the development of emulsifier technology and other types of coating liquids, two very significant developments are in progress.

Vitamin spraying on the finished product has been carried out for a number of years in one Danish feed mill. They have indicated to me that they can save up to 25% of their vitamin requirements in A, E and D (Duer, 2000). This particular application demands a total premix to be created for each feed type. Current experimental work is ongoing to develop individually sprayed vitamins, so that computers can adjust levels to suit product types. This eliminates the need for many premixes and holds the prospect of considerable savings in vitamin addition.

Medicated feed is the biggest headache for any mill manager, as new legislation and codes of practice force ever more stringent separation from 'clean feed'. At the time of writing, a new, patented hard coating process for feed is undergoing production trials in two Italian Feed Mills. This enables powdered medicines to be attached to the feed and subsequently coated with a very palatable hard shell, which prevents the powder being abraded from the pellet. There are three patented coatings, derived from human medicine use. The coatings vary from a cold coating for general dry feed use, through a warm coating for feed in humid climates, to a highly water resistant hot coating for fish feed. This can resist water for up to 24 hours, but still be easily digested by the fish. The substances are all food grade ingredients, and therefore have none of the disadvantages existing with some additives. The other major advantage is that the coating can be coloured, enabling colour coding of different drugged feed and showing very clearly if even one coloured pellet is present in clean feed. The process involves environmentally controlled drum coaters specially adapted to achieve the necessary stages in the application. This process can be placed in an isolated position at the very end of the production line, eliminating the risk and cost factors currently associated with drug cross contamination. The testing programme is

expected to take up to one year, but further details are likely to be available at Victam Asia in March.

Figure 5. Examples of coated feed – colour coded

b) Developments in feed hygiene

Mill cleanliness has undertaken a revolution, particularly in Europe. Feed assurance schemes are leapfrogging each other in severity and cross border trading inevitably means keeping up with the best. European supermarkets are already having an effect far from Europe with quality demands. New mills are being developed with food grade wash down facilities both internal and external. In order to achieve clean/dirty separation for disease control between raw materials and processed feed, new buildings require physical separation between areas. This requirement is likely to extend to existing mill buildings, especially where non ruminant feeds are being produced. To prevent re-infection, coolers are increasingly being supplied with filtered air.

In existing mills, cleanliness levels are being increased, particularly in relation to internal parts of machines. As I indicated earlier, there is a very fine line in terms of temperature between killing and growing *Salmonella*. Coolers especially have such temperature zones, which maintain ideal growing conditions and have to be properly maintained.

Another neglected area of cleanliness is the interior of vehicles. New robot steam cleaners have been developed which can totally sterilise vehicle boxes and tanks.

c) Computer advances

Computers continue to grow in capacity, and free up previously over sized software issues. Mills are increasingly using more computer capacity in the following areas.

1. Information gathering for traceability and HACCP compliance
2. Linking intake raw material analysis to formulations
3. Staff training via the Internet
4. Information exchange via the Internet
5. Multi mill comparisons e.g. Kansas State or in house multi mill arrangements

d) Mill security

This is a widely neglected issue particularly in rural areas where nobody thinks anything can go wrong. Bio terrorism is becoming an increasing threat. Many mills have

adopted a full lock out system with access control not only at reception, but on every mill door. Access to bin tops is a difficult one to secure, but potentially the highest risk area. Drop down escape ladders are a good solution and external bin lids should be locked. Lorries should also be in a locked compound, with adequate lighting to deter intruders. CCTV is now a realistic option with low cost cameras and recorders, which can be switched on via movement sensors.

e) Staff training and innovation

The best teams are achieving high levels of business success and optimum quality. A summary of the important areas where progress is being made, are listed below;

1. Raw material selection to get the best overall outcome for the business
2. Optimising ingredient pack sizes
3. Introducing automated weighing
4. Quality circles
5. Performance guarantees on new machines or technology
6. Investment decisions
7. Mill maintenance

The one big cloud on the horizon is a general lack of training and development at every level of staffing. There are very few effective management programmes in feed milling around the world.

The trend in business generally, is for managers who only manage people but have no technical know how. This only works if strong technical back up is also provided. Feed Milling is depopulating everywhere and these skills are severely lacking.

In the recent World Economic Forum survey, Finland was judged to be the most competitive country in the world for the second year running (Lopez-Claros, 2005). This would seem at first to be a strange decision. This country has a very high level of training and employee involvement. Companies are not afraid to employ technical experts, who get the best out of their factories. Recently I undertook consultancy in a Finnish feed mill, and spent some days with production engineers whose sole responsibility was to optimise the process.

This is a common concept around the world in other industries such as general engineering, but normally only seen in the biggest feed companies and then normally combined with other tasks. If mills are not able to justify the cost themselves, there is a real need for either increasing co-operation between companies who retain their independence but pool technical resources or to use independent consultants for short periods.

Mill staff who feel that they are at the bottom of the 'mushroom management' process do not perform properly. Well trained and involved staff at every level results in high performance both in output as well as quality. The areas of production nutrition and business management can achieve more than the sum of the individual parts by using joined up thinking, taking advantage of the technological advances described earlier, particularly in the area of liquids management, and most importantly involving all their staff in issues, before they become critical problems.

III. CONCLUSION

The most exciting developments in feed manufacturing technology, stem from the fast moving innovations in liquids. These developments are happening on many fronts and satisfy many criteria, in food safety, feed quality and performance, economic manufacture, ecologically friendly power savings and lower capital requirement. Feed Milling has the

opportunity to develop further, but this can only be achieved with a combination of both smart production methods like these and smart staff.

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OPTIMISING BROILER PERFORMANCE – THE ROLE OF PHYSICAL FEED QUALITY

M. KENNY[1] and E. FLEMMING[1]

Summary

To maximise broiler performance physical feed form must be of optimum quality. Feed form will have a significant effect on feeding behaviour, in terms of frequency of meals, meal size, duration and time spent feeding and, consequently, will influence growth performance. Improved feed production processing conditions will improve pellet quality; however, excess thermal conditioning may result in reduced broiler performance.

I. INTRODUCTION

Successful broiler development is dependant on optimal feed intake throughout the growing period. Optimal feed intake is dependant on a number of factors such as environmental temperature, diet nutrient density and physical feed quality is considered to have a very significant impact on broiler growth. A review on the effect of pellet quality on broiler performance shows wide variations of impact on growth and feed efficiency; growth can be improved by as much as 39% and feed efficiency by as much as 12% when comparing pellets to mash as shown in Table 1 (McCracken, 2002).

Table 1. Increases (%) in feed intake, bodyweight gain and gain:feed ratio with pelleted feed relative to mash (McCracken, 2002).

Reference	Period (days)	Feed Intake	Weight Gain	Gain:feed ratio
Choi *et al.* (1986)	1 - 28	5	5	0
Zatari and Sell (1990)	1 – 49	9	13	4
	1 - 21	6	16	10
Douglas *et al.* (1990)	1 - 21	5	13	8
	1 - 21	12	19	6
	1 - 21	2	8	6
Petterson *et al.* (1991)	1 - 20	20	27	6
	1 - 20	25	29	3
Deaton (1992)	21 - 42	3	4	2
Kratzer *et al.* (1994)	1 - 24	12	18	6
	1 - 22	20	26	5
Hamilton and Proudfoot (1995)	1 - 42	3	8	5
McCracken *et al*. (1994)	7 - 28	22	27	4
Munt *et al.* (1995)	21 - 42	3	7	4
Nir *et al*. (1995)	1 - 49	1	3	2
Plavnik *et al*. (1997)	28 - 49	15	20	4
	28 - 49	9	13	4
Preston (1997)	7 - 28	26	39	12

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More recent research work (Figure 1) has shown the impact of introduction of increased levels of fines to birds resulting in higher levels of growth depression and growth retardation than previously recorded. From the literature it would seem the broilers response to pellets has been increasing over time emphasising the need for further data.

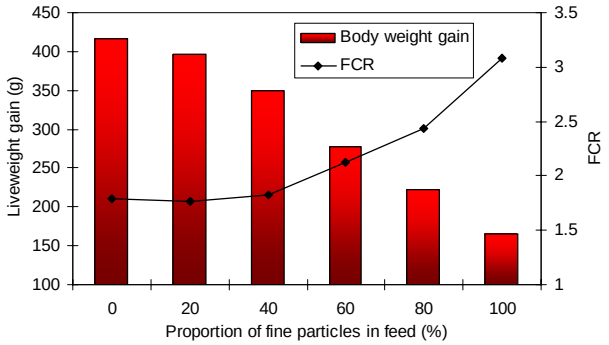


Figure 1. The influence of fine particles in the feed on broiler performance (Quentin *et al*., 2004)

II. TRIAL DATA

The different levels of fines fed to broilers to 31 days of age was investigated. The control was a starter crumb and grower pellet, treatment 1 was 100% fines, treatment 2 was an intermediate level of fines created by mixing equal weights of the control and treatment 1 feeds, treatment 3 was created by alternating the fines/pellet treatment throughout the growing period.

The results showed treatment 1 (100% fines) reduced liveweight by 20% and FCR by 4.9%; which was slightly worse than the previous trial. Treatment 2, (intermediate fines) reduced liveweight by 7% and FCR by 3.7% and treatment 3, (alternating regime) reduced liveweight by 5% and FCR was increased by 1.8%. It was unclear why the alternating regime improved FCR compared to the control but one reason may be that growth was depressed up to the last phase (21 days) lowering maintenance requirements of the birds, feeding pellets for the last phase (22 to 31 days) resulted in compensatory growth to 31 days. The result was a lower liveweight for age with a lower maintenance requirement to 21 days which may explain the improved FCR. The results of this trial concur with other trials conducted by Aviagen examining the effect of poor feed physical quality of broiler performance.

The trials confirm that the higher the level of fines fed the lower the performance will be, regardless of the manner in which the fines are presented.

Table 2. The effect of physical feed form on broiler liveweight gain and feed conversion ratio (FCR) at 10, 21 and 31 days of age (Aviagen data 2005).

Treatment	Liveweight:			FCR:		
	10d	21d	31d	10d	21d	31d
1. Control	297	975	1972	1.39	1.53	1.63
2. Meal	264	797	1579	1.54	1.67	1.71
3. Mix	287	916	1835	1.42	1.60	1.69
4. Alternating	284	812	1872	1.42	1.65	1.60
s.d	5.32	9.80	17.65	0.0203	0.0241	0.0182
P Value	0.016	0.000	0.000	0.003	0.011	0.008

III. THE EFFECT OF FEED FORM ON ACTIVITY

It is suggested that as pellet quality increases either the bird expends less energy for consumption or the bioavailability of nutrients and/or energy increases. Previous reports indicate that pelleted feeds modulate bird energy expenditure. Jensen *et al.* (1962) observed that birds provided pellets as compared to mash, visited the feeder less frequently and spent less time at the feeder while consuming similar amounts of feed. Behaviour observations by Teeter *et al.* (2005) showed that as the proportion of pellets in the feeder increased, birds were observed to eatless frequently and rest more frequently compared to birds fed mash.

Recent Aviagen data shows differences in feeding behaviour between birds fed either pelleted or crumbed products. Comparing an average of individual bird intake data from 14 to 32 days shows that birds fed crumb had lower cumulative feed intake and lower bodyweights compared to pellets. The amount of feed consumed per meal and duration of each meal increased when birds were fed crumb (Figure 2 and 3); hence the total amount of time spent eating was much higher in crumb fed birds than pellet fed. This agrees with earlier work where duration of each meal increased when feeding mash compared to pellet (Jensen *et al*., 1962).

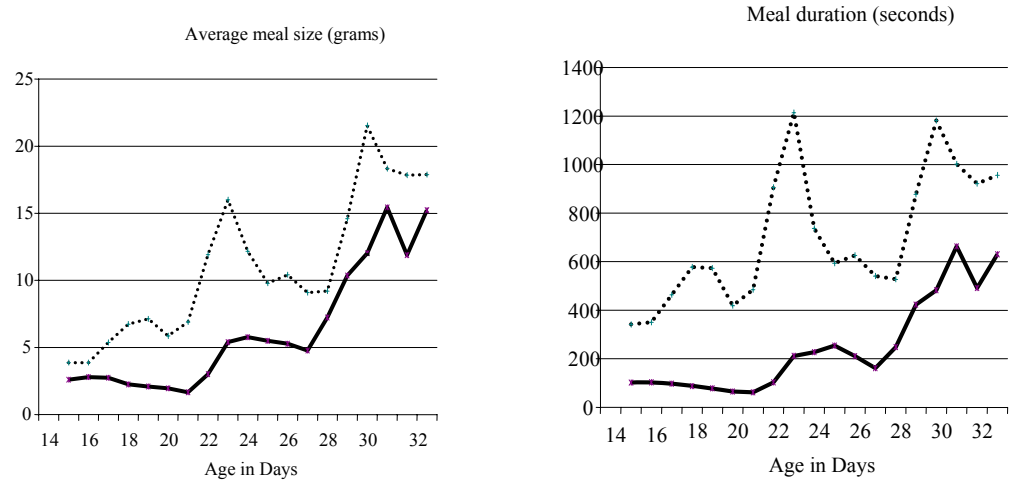


Figure 2 and 3. The effect of feed form (Pellet …; Crumble solid) on meal size and duration.

Birds fed pellets fed more frequently than those fed crumb which contrasts with work by Teeter *et al*. (2005) who showed that as the proportion of pellets increased relative to mash fed birds the number of meals reduced.

The total time spent eating was higher on crumb fed birds which will increase energy expenditure relative to pellet fed birds (Figure 4). Our data would suggest that birds fed pellets tend to 'snack' or eat smaller quantities but more frequently than birds fed crumbs which seem to be 'diners' preferring larger and longer meals less frequently.

These observations emphasise the importance of providing adequate feeding space especially when birds are fed crumb type products.

It is apparent that feed physical form will have a dramatic impact on bird behaviour and biological performance. There is an economic consequence of improving feed form, based on Aviagen trial data and UK economic conditions a 10% improvement in level of pellets will result in a return of 1.8 Euro cents per bird. This calculation is based on response data from Aviagen trials and may not always reflect field conditions however it does show that there is significant

scope for improvement financial performance if feed form is improved. This agrees with previous work by Skinner-Noble *et al*. (2005) who calculated that energy sparing attributable to pelleting versus mash was as high as 187 kcal ME_n/kg feed consumed and suggested that pellet quality could change the energy value of a diet by about 60% of what is considered routinely possible by nutrition. It is imperative that means of improving feed physical form are investigated.

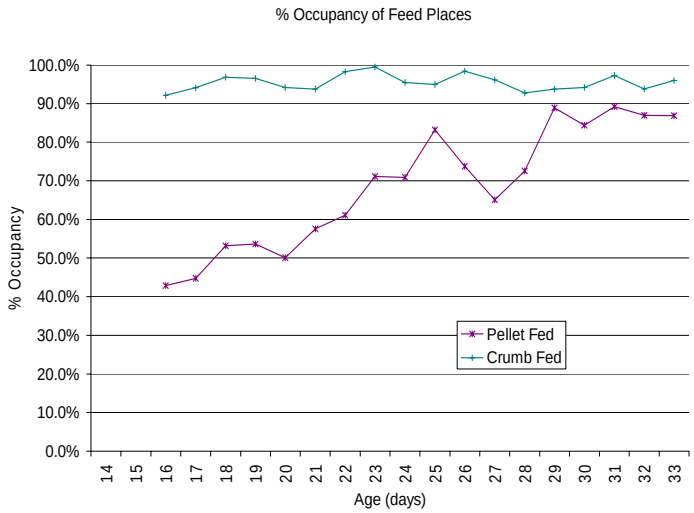
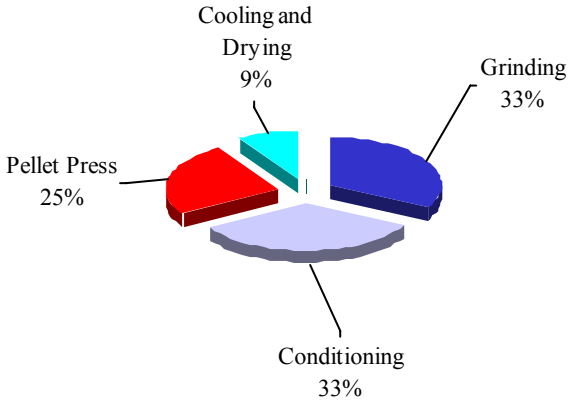


Figure 4. The effect of feed form on percentage occupancy of feeding station.

IV. FACTORS AFFECTING PELLET QUALITY

Pellet quality may be improved by manipulation of diet formulation; use of raw materials with good binding ability such as wheat, barley, rape and use of pellet binders will have an influence (Borregaard, 2001).

Feed manufacturing practises will also have an effect on pellet durability (Figure 5).



(Adapted from Behnke, 1994)

Figure 5. Factors affecting pellet quality – excluding raw materials.

Grinding and conditioning are considered to have the greatest effect on pellet quality. Grinding decreases the particle size of ingredients resulting in a greater surface area to volume ratio allowing greater penetration of conditioning heat and moisture to the core of a particle (Anand, 1970).

Thermal conditioning activates the natural adhesives in ingredients which are critical to the formation of intra particle bonds necessary for the formation of strong durable pellets (Woods, 1987; Briggs *et al.,* 1999). Activation of these natural adhesives is both heat and moisture dependant for instance if water is limiting the amount of gelatinised starch is restricted (Tester and Sommerville, 2000). Hence adequate processing conditions will have a positive impact on pellet quality.

Recent data shows that there maybe negative effects of increased processing temperature on the nutrient digestibility of feeds and on broiler growth (Raastad and Skrede, 2003). Cowieson *et al*., 2005 showed that increasing conditioning temperature from 80 to 90 degrees centigrade reduced bodyweight gain of broilers by 7%, diet viscosity was also increased significantly suggesting an increased release of non-starch polysaccharides. The conclusion from these authors seems to be that increasing processing temperature is beneficial to a point; thereafter, there may be negative consequences on broiler performance.

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LIGHTING FOR GROWTH AND FEED CONVERSION EFFICIENCY

P.D. LEWIS[1]

Summary

Photoperiod has minimal effect on feed intake, growth, and feed conversion efficiency (FCR) in broilers, but longer photoperiods are associated with higher mortality, in particular through Sudden Death Syndrome, and more leg problems. Three weeks of short photoperiods followed by long photoperiods have beneficial effects on liveability and leg integrity, and compensatory growth means that body weight and FCR are similar to conventionally illuminated birds at ≥ 42 d. Light intensity (≤ 200 lux) has minimal effect on feed intake, growth, feed conversion efficiency, liveability, or leg integrity. Feed intake is unaffected by light colour, but faster growth occurs under green light resulting in more efficient feed conversion than under longer wavelengths or white light. Photoperiod has little effect on feed intake and growth in turkeys up to about 12 weeks, but in males, longer photoperiods stimulate sexual development, and increased circulating testosterone induces faster growth and improved FCR.

I. INTRODUCTION

Traditionally, broilers and turkeys have been given long daylengths or continuous illumination to maximise feed intake and growth. In the early days of the broiler industry, body weight was 5 to 10% lower for birds exposed to 8 < 12-h photoperiods compared with birds given continuous illumination, but those given only 6-h days learned to eat in the dark and grew at a faster rate than birds given 14-h photoperiods. In the extreme, broilers transferred to continuous darkness at 7 d of age were reported to have a similar body weight at 63 d to birds given continuous illumination.

II. PHOTOPERIOD

Data in Table 1 show that whereas body weight gain in modern broilers increases by about 10 g/h of photoperiod up to 21 d, it decreases by about 6 g/h between 22 and 49 d for birds given ≥ 12 h. However, birds on 8-h daylengths continue to have the slowest growth (Renden et al., 1993). As a consequence, body weight at 49 d is similar for all daylengths > 8 h. This is because feed intake, which increases linearly by about 15 g/h during the first 21 d, is not significantly different after 21 d for ≥ 12 h daylengths, and a 1% per hour higher energy expenditure during the photoperiod results in birds on longer daylengths having less feed available for growth and poorer FCR. In contrast, photoperiod strongly influences bird health, with longer daylengths being associated with higher incidences of Sudden Death Syndrome, leg disorder, and general mortality.

Up to 21 d, the provision of 6-h photoperiods reduces body weight and cumulative feed intake compared with birds grown on 23-h photoperiods, but a transfer from short to long days results in compensatory growth so that body weights for the two groups become similar by 42 d (Table 2). However, the initial 3 weeks of 6-h days reduces total mortality and the incidence of leg disorders through 42 d, and produces comparable liveability figures to birds maintained on short days, but about half of those for birds given 23-h daylengths

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Table 1. Body weight, feed intake, and feed conversion efficiency for male and as-hatched broilers fed ad libitum and given various constant photoperiods between 8 and 23 h with differences between the male and as-hatched birds removed by least squares analysis (Renden et al., 1993, S.H. Gordon, personal communication).

	Photoperiod (h)					
	8	12	14	16	20	23
0 to 21 d						
Feed intake (g)	840	905	945	1010	1050	1070
Body weight (g)	660	695	720	760	790	815
Feed conversion (g/g)	1.36	1.39	1.40	1.41	1.41	1.40
22 to 49 d						
Feed intake (g)	3865	4045	3840	3930	3850	3905
Body weight gain (g)	1765	1835	1775	1780	1760	1750
FCR (g/g)	2.19	2.21	2.17	2.21	2.19	2.23
0 to 49 d						
Feed intake (g)	4705	4950	4785	4940	4900	4980
Body weight gain (g)	2425	2530	2495	2540	2550	2565
FCR (g/g)	1.99	2.02	1.99	2.01	2.01	2.01
Total mortality (%)	6.7	8.5	11.8	11.4	16.3	17.3
Sudden death (%)	1.6	2.6	*	4.0	6.2	*
Leg disorders (%)	0.4	0.3	*	0.8	1.1	*

Table 2. Body weight, feed intake, feed conversion efficiency, and mortality for as-hatched broilers maintained on 23 h from hatch or given an initial 6-h photoperiod prior to a transfer to 23 h at 21 d. Data are from 8 trials (Lewis, 2001)

	Constant 23-h	0-21 d, 6 h 22-49 d, 23 h	Paired *t* test *P* value
0 to 21 d			
Feed intake (g)	985	855	<0.001
Body weight (g)	705	635	0.002
FCR (g/g)	1.41	1.34	<0.001
0 to 42 d			
Feed intake (g)	3840	3745	0.036
Body weight (g)	2105	2095	0.662
FCR (g/g)	1.83	1.80	0.005
Total mortality (%)	10.8	6.3	0.001
Sudden death syndrome (%)	4.8	3.3	0.029
Leg disorders (%)	8.6	6.0	0.027

(e.g., Classen and Riddell, 1989). Skeletal development is related more to age than to body weight (Wise, 1970), and so retarding initial growth by the provision of short photoperiods

for the first 1 to 3 weeks results in smaller loads on the leg joints, and the skeleton is better able to carry the rapid growth that occurs when the birds are transferred to long photoperiods. Although the benefits are greater for males than for females, they are proportionally the same for both sexes. Improvements in the skeletal integrity of meat-birds exposed to shorter photoperiods are really responses to longer scotoperiods, and, as for the benefits to shell quality in laying hens, prolonged nocturnal melatonin secretion results in an extended release of calcitonin and parathyroid hormone, and an enhancement of calcium mobilisation, and advantageous modifications of osteoclast and osteoblast activity. However, ultra short daylengths may adversely affect skeletal health because long periods of inactivity could compromise the blood supply to the leg-bone growth plates (Thorp and Duff, 1988). Some of the compensatory body weight gain that follows a change to long daylengths, especially ≥ 5 weeks, may be in response to an increase in plasma testosterone, as indicated by male birds having larger and brighter combs than constant-photoperiod or naturally lit controls at 42-49 d (Classen and Riddell, 1989).

Although feed intake and body weight gain in turkeys during the first few weeks tend to be related to daylength, there is little effect of daylength on either trait at 6 weeks because, like broilers, turkeys on short days learn to eat in the dark. However, between 12 and 20 weeks, male turkeys maintained on ≥ 12 h daylengths start to develop sexually, and, in response to increasing circulating testosterone, convert feed more efficiently and have larger body weight gains, with growth proportional to the stimulatoriness of the photoperiod (Figure 1). Cumulative FCR in males to 20 or 22 weeks improves linearly by 0.014 kg/kg for each 1-h extension of the photoperiod between 8 and 23 h (Figure 2, Lewis et al., 1998). Mortality and the injurious pecking increase in proportion to photoperiod.

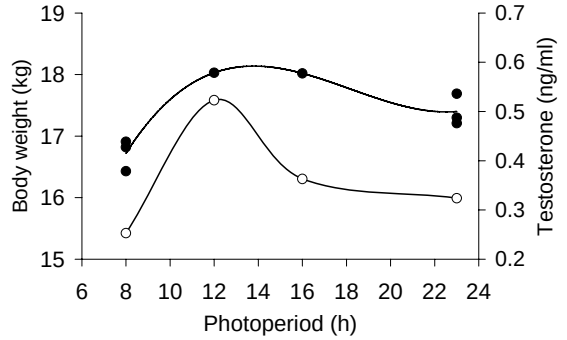

Figure 1. Plasma testosterone concentration at 15 weeks (○) and body weight at 20 weeks (●) for male turkeys given constant 8, 12, 16 or 23-h photoperiods

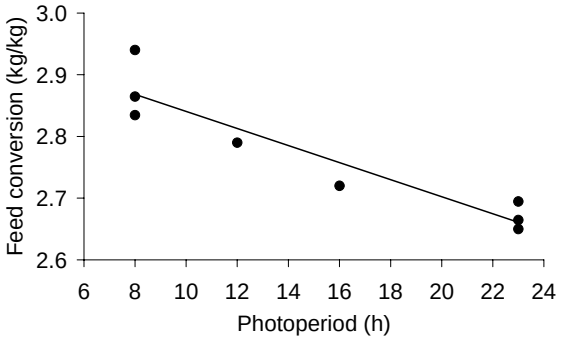

Figure 2. Feed conversion ratio to >20 weeks (kg feed/kg body weight) for male turkeys for male turkeys given constant 8, 12, 16 or 23-h photoperiods

III. LIGHT INTENSITY (ILLUMINANCE)

A meta-analysis of data sets from six broiler trials reported since 1988 shows that despite a small but significant 20-g depression of growth and strong tendency for a 30-g reduction in feed intake as light intensity increases from 1 to 100 lux, illuminance generally has minimal influence on growth (Figure 3). Although these changes concur with earlier findings (Morris, 1967), they are unlikely to be commercially significant. Surprisingly, despite the stimulation of activity under brighter light intensities, experimental evidence indicates that illuminance has no effect on overall mortality or on the incidence of leg problems (Newberry et al., 1986). Cycling light intensities (4 and 40 lux or 5 and 100 lux)

alter activity patterns, but appear to impart no beneficial effects to bird welfare (e.g. Gordon and Tucker, 1996).

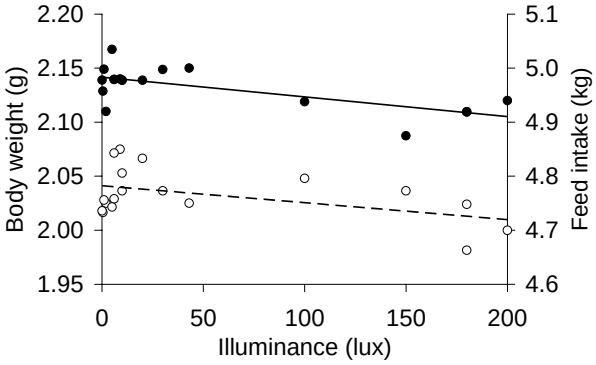

Figure 3. Effect of illuminance on body weight at (●, solid line), and feed intake to (○, dotted line) 49 d in broilers. Meta-analysis of 6 data sets.

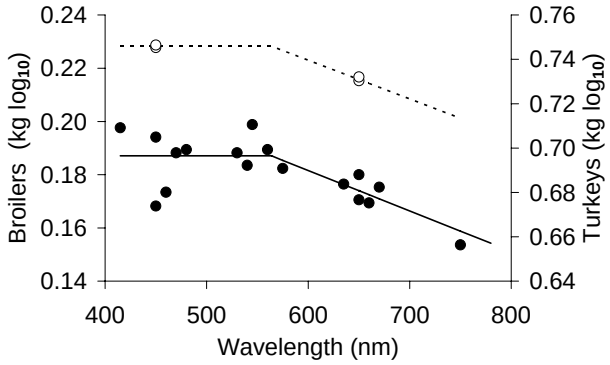

Figure 4. Effect of light colour on body weight (kg x $\log_{10}$) in broilers (●) and turkeys (○). Meta-analysis of 7 broiler and 2 turkey data sets.

In turkeys, illuminance generally has minimal effect on growth, feed intake or FCR, provided the light intensity during the first 1-2 weeks is at least 10 lux. Low intensity light (1 lux) up to 14 d, depresses feed intake, reduces body weight gain, markedly increases mortality, and induces eye and adrenal gland enlargement compared with birds exposed to brighter (≥ 10 lux) intensities.

IV. LIGHT COLOUR (WAVELENGTH)

When broilers have been exposed to violet, blue or green (415 to 560 nm) monochromatic light at the same irradiance or illuminance, body weight gain up to 11 weeks has been greater than for birds given red (> 635 nm) or white light (Figure 4, Lewis and Morris, 2000). Similarly, body weight gain has been reported to be faster to 16 weeks in male and to 18 weeks in female turkeys when they have been exposed to blue light, compared with red or white light (Figure 4). It is likely that growth is suppressed by longer wavelengths rather than enhanced by shorter wavelengths of light.

After 18 weeks, growth in turkeys is faster under red and white than under blue light. At this age, turkeys maintained on long daylengths commence rapid gonadal development, and long wavelengths are more sexually stimulatory than short wavelengths, so the improved growth under red light is likely to be a response to increased concentrations of plasma sex steroids and not a direct effect of wavelength per se.

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ROLE OF BROILER BREEDER GENETICS ON BREEDER CHICK QUALITY AND SENSITIVITY TO OVERFEEDING

R. A. RENEMA[1], F. E. ROBINSON[1], and M. J. ZUIDHOF[1]

Summary

Broiler breeders must have the genetic potential for efficient growth as well as the ability to effectively reproduce. However, the interaction between nutritional and reproductive traits is complex and continually changing with the introduction of new genetic lines. These studies were designed to gain a better understanding of how selection for growth traits and variability among strains affects growth traits, reproductive morphology and production traits of broiler breeders. Breeder strain influenced sensitivity to photostimulation, to excess feeding and to pullet growth profile. By understanding how sensitivity to changes in nutritional status differs among strains differing in muscling, then feeding and management strategies can be refined to maximise the production efficiency of the hen.

I. INTRODUCTION

Broiler breeders are a moving target. While broiler 42-day body weight is increasing each year, the target body weight for mature male and female broiler breeders has changed little (Rustad and Robinson, 2002). In 1979, Hubbard male and female breeders were approximately 50% of the 42-day broiler weight. In 2001, this percentage had decreased to 36.1 for males and 30.3 for females. The situation for knowing what nutrients these birds need is compounded by the development of "yield" lines, carrying increased amounts of breast muscle yield, often on a smaller carcass frame. This increased growth efficiency is expressed partly in the greater capacity for muscle growth. Pym *et al.* (2004) indicated that differential fractional rates of protein deposition, breakdown and synthesis have resulted in an increased protein retention in a high compared to a low efficiency line.

The reproductive efficiency of broiler parents is increasingly dependant on very specific feed restriction and lighting programs to optimise reproduction. Broiler breeders must have the genetic potential for efficient growth as well as the ability to effectively reproduce (Robinson *et al.,* 1993). Excess body weight can result in reduced egg production, hatchability, livability, egg weight, feed efficiency and increased shell porosity (Wilson and Harms, 1986; Robinson *et al.,* 1993). Overfeeding can accelerate the sexual maturation process and elevate ovarian large yellow follicle (LYF) numbers in birds of similar BW (Renema *et al.,* 1999). Furthermore, feeding programs during rearing and early lay can change frame size and breast muscle fleshing in the birds (Wilson *et al.*, 1995).

These studies were designed to gain a better understanding of how selection for growth traits and variability among strains affects growth, conformation, composition, reproductive morphology and production traits of broiler breeders.

II. STRAIN VARIATION IN THE ACCELERATION OF SEXUAL MATURATION

Broiler breeder strains vary in the extent to which sexual maturation is influenced by nutrient intake (Robinson *et al.,* 1998). Four commercial strains were reared on a common body weight target, and fed one of three feeding programs from photostimulation (22 wk): *Ad libitum*; Fast-Feed (weekly feed adjustments based on a 5 g increase for every 5%

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increase in production); and Slow-Feed (daily adjustments of 1 g/d between 22 and 26 weeks of age, and 0.5 g/d until 31 weeks of age).

There was a 1 wk range in the mean age at first egg among the strains. *Ad-libitum* feeding did not accelerate sexual maturation in two of the strains, suggesting these birds have a later maturation of the hypothalamo-pituitary axis. This is a significant finding that serves to show a basis for genetic differences in photo-sexual response among commercial stocks. In these late-maturing strains, it would seem to be pointless to subject these birds to increasing day lengths and feed allocations as soon as you would more traditional early-maturing strains. In the strains responding to overfeeding, maturation was accelerated by 6 to 7 d, on average.

The number of large yolky follicles varied among strains, with the two strains that reached sexual maturity first having the fewest large follicles. These data strongly suggest that it is essential to follow management recommendations specific to a breeder genotype.

III. IMPACT OF GROWTH SELECTION ON SENSITIVITY TO OVERFEEDING

A study was designed to show the effects of degree of selection for yield traits on the ability to cope with a feeding challenge. The strains were: Random-bred (unselected since 1977), Ross 308 (a high-yield bird suited for the whole-bird market), and Ross 508 (a very high-yield bird suited for the cut-up and further processing market). Each strain was raised to the same target body weight at 20 wk of age, when they were individually caged. Beginning at photostimulation (22 wk of age), pullets were fed 100% (control), 120%, and 140% of the feed needed to maintain the Ross 508 growth curve. A total of 90 birds were dissected at sexual maturity and 144 were kept to 58 wk of age for measurement of production traits.

The timing of sexual maturity was affected by strain, with the RB20, Ross 508 and Ross 308 birds laying eggs 16.5, 20.2, and 27.4 d after photostimulation (Table 1). Birds of all strains appear to have acquired the appropriate level of growth and composition to support rapid sexual maturation. At sexual maturity (onset of lay), the Ross 508 birds had the highest proportion of breast muscle. Conversely, the Random-bred hens were the fattest – reflecting the less efficient growth of their older genetics. The 120 and 140% treatments only added an additional 5.3% and 9.7% to BW at sexual maturity, respectively (Table 1).

Table 1. Time to sexual maturity (SM) from photostimulation (PS), weight at SM, breast muscle and ovary weight at SM and settable egg production to 58 wk of age in three broiler breeder strains (RB20=1977 Random-bred; Ross 308 and Ross 508) on a 100%, 120%, or 140% feeding regimen from photostimulation.

Source	Days from PS to SM	Body weight at SM	Breast weight	Ovary weight	Settable egg production
	(d)	(g)	(% of BW)	(g)	(#)
Strain[1]					
RB20	16.5^{c}	2842^{c}	13.8^{c}	46.3^{b}	141^{b}
Ross 308	27.4^{a}	3565^{a}	16.8^{b}	59.0^{a}	167^{a}
Ross 508	20.2^{b}	3289^{b}	17.9^{a}	51.0^{b}	153^{ab}
Feed					
100	21.8	3078^{c}	16.4	47.8	166^{a}
120	20.7	3240^{b}	16.4	52.9	159^{ab}
140	21.7	3377^{a}	15.7	55.3	137^{b}

Feeding regimen had a big impact on egg production, with 166, 159, and 137 settable eggs produced by the 100, 120, and 140% groups, respectively. Interestingly, the modern, high breast-yield Ross 508 birds were the most sensitive to overfeeding, producing 177 eggs with the 100% feed allocation compared to only 123 eggs on the 140% feed allocation. When coupled with decreased rates of fertility and hatchability in the 140% treatment, overfeeding had a devastating effect on chick numbers. The hatchability of the 140% Ross 508 hens near the end of the study ranged between 20 and 30%. At the end of the trial, 94% of the 100% feed allocation birds were still in active lay compared to 86% in the 120% allocation group and only 63% in the 140% allocation group. The productivity of the Ross 308 hens was least impacted by feed allocation, demonstrating a better tolerance to a range of feeding profiles than either the Random-bred or the Ross 508 hens in this study.

IV. EFFECT OF STRAIN AND GROWTH PROFILE ON PRODUCTION TRAITS

An experiment was performed to test how the interactions between genetic strain, age at photostimulation and target body weight profile impact growth rate and efficiency, nutrient partitioning, sexual maturation and reproductive efficiency. The strains used were: Hubbard Hi-Y, Ross 508, and Ross 708. The 4 body weight profiles separated at 5 wk and converged at 32 wk of age as follows: STANDARD (control); LOW (12 wk body weight target = 25% lower than STANDARD followed by rapid gain to 32 wk); MODERATE (12 wk body weight target = 150% of STANDARD followed by lower rate of gain to 32 wk); and HIGH (12 wk BW target = 200% of STANDARD followed by minimal growth to 32 wk).

One of the primary effects of the growth profiles was on frame size. The long-term concern would be that feeding a small-framed to the same body weight target as a larger-framed bird will result in increased fatness and the triggering of reproductive disorders associated with overfed hens. During the period immediately after photostimulation at 18 wk, the LOW birds had a very high feed allocation relative to that of the other growth curve treatments to allow their weight profile to converge with the others by 32 wk. Despite what would normally be considered excess feed, sexual maturation was still delayed in the LOW birds. In contrast, the HIGH birds had a very low feed allocation during this period, which delayed sexual maturation the Ross 708 hens and suggesting that these birds do not tolerate nutrient shortages well. These birds carried a greater proportion of breast muscle and less fat than the other strains, which may contribute to their inability to cope with reduced feed at this critical time. Photostimulating birds at 22 wk of age alleviated most of these problems.

Body weight at sexual maturity was 3.40, 3.21, 3.01, and 2.84 kg for HIGH, MODERATE, STANDARD, and LOW birds, respectively. The body weight differences impacted shank and keel length, indicating differences in frame size. Interestingly, ovary weight in the later maturing LOW birds (55 g) was 6 g heavier than in the other groups. The number of large yellow follicles on the ovary did not change with photostimulation age (average of 7.5 follicles), except in the HIGH birds, where it dropped from 8.1 in birds photostimulated at 18 wk to 6.5 in those photostimulated at 22 wk. Feed allocation to the HIGH birds was quite low during this period to keep the body weight on target, which likely impacted ovary development.

The reduced feed on the MODERATE and HIGH profile also reduced early egg size and stunted the length of the prime sequence (the characteristically long daily egg laying sequence occurring early in lay) (Table 2). Although these birds were larger, their early production traits were similar to that of a much smaller bird. This illustrates how recent feeding level may have a greater impact on production traits than body or growth pattern does.

Ultimately, the 18 wk PS-age birds yielded 8 more eggs (170) than 22 wk PS-age birds to 58 wk of age, with no affect on unsettable egg production. On average, total egg production was similar among growth profile treatments. However, there was variability in the productivity of specific strains grown on some profiles. The Ross 708-HIGH hens, for example, under-performed (138 eggs) compared to the other profiles (mean = 166.3). Alternatively, Ross 508-HIGH birds laid the same number of eggs as Ross 508-STANDARD birds (mean = 178.7) (Table 2).

Table 2. Egg production parameters (to 58 wk of age) of three broiler breeder strains reared on varied BW profiles and photostimulated at 18 or 22 wk of age.

	Egg production[1]			Laying sequence analysis	
Source	Total (#)	Normal (#)	Settable (#)	Prime sequence (d)	Mean Length[2] (d)
Strain X BW profile					
HiY-Low	177.8^{abc}	175.4^{abc}	159.8^{a}	27.1^{a}	5.22^{a}
HiY-Standard	164.9^{c}	160.9^{d}	147.2^{abc}	20.8^{abc}	3.35^{d}
HiY -Moderate	175.6^{abc}	173.8^{abcd}	149.0^{abc}	22.0^{ab}	4.16^{bc}
HiY -High	171.5^{abc}	169.3^{abcd}	137.7^{c}	17.7^{bc}	3.45^{d}
508-Low	172.5^{abc}	168.5^{abcd}	153.8^{ab}	21.5^{ab}	3.80^{bcd}
508-Standard	178.3^{ab}	176.2^{ab}	158.4^{ab}	27.8^{a}	4.27^{b}
508-Moderate	165.8^{c}	163.8^{bcd}	143.4^{bc}	14.2^{c}	3.16^{d}
508-High	178.7^{a}	176.6^{a}	153.3^{ab}	17.3^{bc}	3.60^{bcd}
708-Low	164.9^{c}	163.1^{cd}	147.9^{abc}	19.4^{bc}	3.55^{cd}
708-Standard	166.3^{bc}	163.6^{cd}	148.7^{abc}	19.2^{bc}	3.23^{d}
708-Moderate	168.1^{abc}	166.6^{abcd}	146.7^{abc}	13.6^{cd}	3.14^{de}
708-High	142.8^{d}	141.5^{e}	107.8^{d}	6.5^{d}	2.40^{e}
SEM	5.0	5.1	6.0	2.8	0.28

[a-f]Means within a column with no common superscript differ significantly (P<0.05).

[1]Normal = no shell defects; Settable = normal egg ≥52 g; defective = soft shell, shell-less, double yolked, or abnormal shell eggs.

[2]Mean length calculated as mean of all sequences occurring in each bird.

Examination of individual growth profiles revealed strain-based strategies for managing reproduction. The Ross 708 tied up nutrients deposited during the pullet phase tightly, and was unable to mobilise nutrients from storage as they were needed under conditions of dietary deficiency. This may be partly due to their increased breast muscle mass. Under more normal feeding conditions these birds performed very well. The Hubbard Hy-Y hens appeared much more able to mobilise nutrient stores, and were not hindered by the very low feed allocations provided to the HIGH birds during sexual maturation. Economic analysis of production traits revealed that using a STANDARD feeding profile and photostimulating pullets at 22 wk of age was most often the best management practice on a cost/chick basis. Photostimulating birds at 18 wk of age resulted in higher total egg production, although much of this advantage was lost in small, unsettable eggs (<52 g) and a higher degree of production variation among hens. Ultimately feeding profiles affected egg production traits differently among strains, with little effect of photostimulation age.

VI. CONCLUSIONS

The newer broiler breeder genetic strains are becoming more specialised and appear to have more specific management methods associated with them. Both genetic strain and feeding treatment affected how the birds came into production and had some influence on carcass fleshing traits. However, this did not have a consistent effect on egg production traits. The negative effects of overfeeding were more pronounced in the highest breast-yield strain. These studies indicate some of the complexity in the interaction between nutritional and reproductive parameters and demonstrate the need for strain-specific management strategies.

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OPTIMUM BROILER FEEDS; THE AVIAGEN PROTEIN CALCULATOR

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Summary

A method is described for calculating the level of balanced protein in broiler feeds which will maximise economic returns under different production and price scenarios. Empirical estimates of bird response derived in a series of experiments are used as the basis of the calculations. The results obtained are specific to the input data used but in general the method emphasises the importance of considering the whole enterprise in making nutritional decisions and the significant effects of economic circumstances on optimum nutrient levels.

I. INTRODUCTION

Two developments make it appropriate to keep protein and amino acid levels in broiler diets under review. Firstly, continued genetic improvements in broiler growth, efficiency and body composition mean that dietary nutrient levels may require adjustment. This is especially true for protein because of the emphasis on body composition and the yield of saleable product. Secondly, it is now widely accepted that the choice of dietary protein and amino acid levels should be an economic decision to be made for each company or enterprise. This idea replaces the concept that chickens in general have characteristic 'requirements' which should be met under all conditions.

Determining economically optimum protein levels requires a lot of information but this is readily available in a modern broiler enterprise. Complexity is handled by the use of computers. Here we describe an empirical model approach to optimising protein levels in broiler feeds.

II. PRINCIPLES AND LIMITATIONS

As with any empirical model the calculations are limited by the trials used to determine bird responses. The data presented are for the Ross 308 breed and other conditions are as described below.

'Protein' is defined as Balanced Protein (BP) with ratios between the minimum levels of digestible essential amino acids and digestible lysine as defined in the Ross Broiler Manual (2002). With the ingredients used, including crystalline lysine, methionine and threonine, levels of LYS, MET, MET+CYS and THR were held at the minimum balanced ratios and one other essential amino acid (usually ARG or VAL) was also at the minimum ratio and in addition determined the crude protein level. Small excesses of other amino acids and total crude protein are assumed to have no effect on bird performance. To permit calculation of optimum BP levels throughout the life of the birds all nutrient scales are related to the recommendations in the Ross manual. Thus BP level 100 represents the manual amino acid recommendations in starter (0-10d), grower (11-28d) and finisher feeds.

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III. TRIAL PROCEDURES

Four trials were completed with commercial Ross 308 broilers during 2003-2004. Trial facilities provided replicated litter-floored pens in each of which about 90 day-old male or female chicks were placed at 16 birds/m^2 and reared to 49 days. Birds were removed for determination of body composition or by thinning to prevent the stocking density exceeding 34 kg/m^2. Light, about 10-15 lux, was either provided for 23 hours each day or according to a light restriction programme. Feed and water were available *ad libitum*.

Trial diets were designed to provide different levels of BP as described above. Across all trials, BP levels varied from 70 to 120% of the standard used and in all treatments the same relative level was used in the starter, grower and finisher phases. Energy and other nutrient levels were as in the Ross Broiler Manual (2002).

Feeds were formulated from ingredients available in a commercial feed mill using a high quality commercial database to describe ingredient compositions. The same feed specifications were used in all trials but diets were re-formulated on each occasion to take account of changes in the ingredient database. The main protein contributing ingredients were wheat or maize, highpro soybean meal, full fat soybean meal, fish meal, DL-methionine, L-lysine HCl and L-threonine. Vitamin and mineral supplements were according to UK practice. All feeds contained xylanase enzymes but no growth promoters, coccidiostat or other additives. Birds were vaccinated against coccidiosis at day-old. All starter feeds were presented as sieved crumbs and grower and finisher feeds as 3mm pellets. All feeds were heat treated for a high standard of biosecurity.

Accuracy of feed preparation was assured by the ingredient database and by well-controlled weighing and mixing routines. Levels of crude protein and calcium were checked on all batches for conformity to expectation and feeds were re-mixed if suitable standards were not reached. Nutrient levels in feeds are described here by the theoretical values.

Bird weights and feed intake were measured for each pen at frequent intervals of about 7 days. Dead birds and birds culled because of leg defects were weighed and recorded daily. Birds were removed at about 32 and 46 days for processing and determination of body composition. Processing on a small-scale commercial line provided the following observations on individual birds; live weight, eviscerated carcass weight, breast meat weight, thigh meat weight, drum meat weight, thigh, drum and wing portion weights and abdominal fat pad weight. The following subjective scores were made during each trial; clinical leg defects, feather score and litter condition by pen. The results of the four trials were combined by expressing all data relative to the common treatment BP=100.

IV. ESTIMATES OF BIRD RESPONSE TO BALANCED PROTEIN

Performance data for males and females separately were calculated at 1.7, 2.0, 2.5 and 3.0kg by suitable regression analysis and interpolation. Age at each bodyweight, feed conversion ratio corrected for mortality, mortality, eviscerated yield and weights of breast, thigh, drum and wing portions, weight of breast and dark meat and abdominal fat were determined. Responses to BP in the pooled, scaled data for all experiments were determined by linear or exponential regression.

Figures 1a and 1b show typical response data derived from these experiments and as used in the calculation of optimum BP levels.

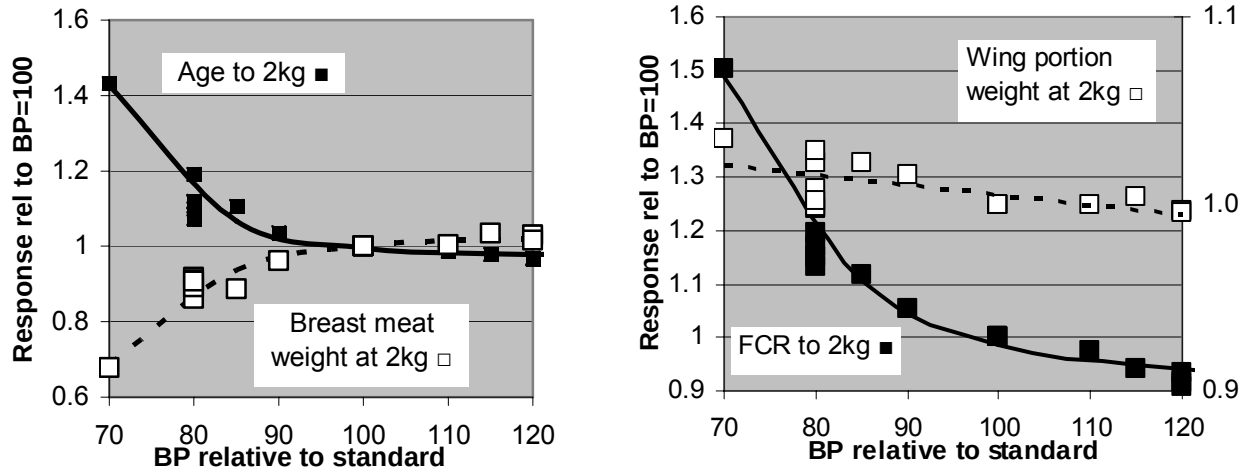

Figure 1. The responses in Ross 308 males of age (■) and breast meat weight (□) at 2kg (left-hand figure) and of FCR (■) and wing portion weight (□) at 2kg (right-hand figure) to variations in BP. Data points derived from 4 experiments as described in the text. Regression lines were fitted by least-squares procedures using the NLREG computer program.

V. CALCULATION OF OPTIMUM BALANCED PROTEIN LEVELS

All calculations are set up in a spreadsheet. The bird performances at each bodyweight are calculated by solving equations for lines similar to those shown in Figure 1. Feed input costs are determined by formulating feeds containing 70 to 130% of the amino acid levels used as standard. Feed manufacturing and transport costs are added. Other production and processing costs are entered from data for the individual enterprise. This part of the calculation can easily be adapted for individual companies. Costs are distinguished as fixed, and therefore proportional to the time taken to reach a given bodyweight, or variable, and therefore unaffected by time. Revenue is determined by the range of products sold by the company. Revenue rates can be determined at the point considered appropriate for guiding nutritional decisions (e.g. contract grower prices, inter-department transfers, customers sales etc).

In the current version of the spreadsheet the data are combined to determine margins over all costs corrected for, or ignoring, mortality and downgrades. Margin is determined at the farm gate (revenue from live weight only), after primary processing to eviscerated carcasses or after full processing to portions and/or processed products. Again these aspects of the calculations are readily adapted to the individual business. In addition indices of performance such as the Production Efficiency Factor (PEF) or feed costs per kg of product can be calculated easily.

VI. RESULTS

The results obtained in these calculations are obviously specific to the conditions set. However some general trends emerge and data such as those in Table 1 are frequently obtained. These results show that higher levels of BP are required to yield maximum profit after processing than at the farm gate. Males are more responsive than females in this respect at 3kg but not at 2kg.

Table 1. Protein levels yielding maximum profit for 2kg and 3kg male and female broilers. Profit is assessed for the grower (revenue live weight), as processed whole birds (revenue eviscerated carcass) and after processing (revenue breast meat plus portions). Economic conditions approximate to those in the UK at December 2004. Values are relative to the standard digestible amino acid levels recommended by Ross Breeders (2000) which = 100.

	2kg birds		3 kg birds	
	M	F	M	F
Grower	103	102	109	99
Whole birds	104	105	113	107
Processed	110	112	123	114

The values shown in Table 1 are at the maximum calculated margin. No account is taken of very small differences in margin which might be available at lower protein levels. For example the detail for processed 2kg male birds shows that although the maximum margin (€1.2916) occurs at 110% BP as in Table 1, even down to 100% BP the margin is only reduced to €1.2706, i.e. by about 1.6 percent. If risk is discounted to some extent, a feeding level somewhere in this range might rationally be selected. Methods for doing this more sophisticated economic analysis have not yet been devised.

The method provides a powerful method of evaluating the effect of feed prices on optimum feeding strategies. For example the figures given in the column for 2kg males birds in Table 1, namely 103, 104 and 110, are obtained when changing the BP level costs €8.81 per tonne per 10 BP units. When this relative cost of protein is reduced to €3.31 per tonne these values change to 117, 118 and 121. When protein cost increases to €13.31 per tonne the resulting optimum BP levels are 98, 99 and 107 relative to the standard used. The relative cost of protein is found to vary widely in different parts of the world.

VII. DISCUSSION

Although the method is constrained by the trials, it is believed that the results are applicable under a wider range of conditions. For example moderate deviations from the feeding programme used are assumed to be acceptable and different energy levels are accommodated by assuming that the responses to BP : energy ratios are independent of energy level. This conclusion is encouraged by the experiment described recently by Lemme et al. (2005). However bird responses to BP may be different in other strains of broiler as shown by Kemp et al. (2005) and this emphasizes the need to keep the effect of genetic changes in broilers on optimum nutrition under continuous review.

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RELATIONSHIP BETWEEN GUT MICROBIAL SPECIES AND ENERGY METABOLISM IN BROILER CHICKENS

V.A. TOROK[1], K. OPHEL-KELLER[1] and R.J. HUGHES[2]

Summary

The gastro-intestinal tract contains a complex population of bacteria, which can have both negative and positive effects on their host. However, the complexity of these interactions is not yet fully understood. This report describes the application of T-RFLP, a microbial profiling technique for examining the chicken intestinal microflora based on high-throughput, high resolution fingerprinting of bacterial gene regions. This tool is capable of providing a "snap-shot" of the complex bacterial population at any particular time. T-RFLP combined with multivariate statistical analysis has enabled relationships between gut microflora and bird performance to be investigated for the first time. These tools are being used to examine diet-induced changes in the microbial community of the chicken gut and will contribute to an increased knowledge of the chicken gut microbiota. We suggest that there may be a link between the presence and/or absence of particular gut bacterial species and improved energy metabolism in broiler chickens.

I. INTRODUCTION

Gut microbiology and its role in animal health has become increasingly important, particularly now that the use of antibiotics in animal feeds to promote growth is limited due to legislation in some countries and consumer pressure generally. The microorganisms that colonise the gastrointestinal tract during the early post-hatch period form a synergistic relationship with their poultry host. Gastrointestinal microorganisms have a highly significant impact on uptake and utilisation of energy (Choct *et al*., 1996) and other nutrients (Smits *et al*., 1997; Steenfeldt *et al*., 1995), and on the response of poultry to anti-nutritional factors (such as non-starch polysaccharides), pre- and pro-biotic feed additives and feed enzymes (Bedford and Apajalahti, 2001). Microorganisms can also directly interact with the lining of the gastrointestinal tract (Van Leeuwen *et al*., 2004), which may alter the physiology of the tract and immunological status of the bird (Klasing *et al*., 1999).

Current methods for analysis of intestinal flora rely on culturing, which is not only laborious but may miss a large part of the uncultivable microflora. Alternatively, DNA techniques have the advantages of being rapid, relatively inexpensive and capable of monitoring gene regions of complex populations. To this end, terminal restriction fragment length polymorphism (T-RFLP) (Osborn *et al*., 2000), a microbial profiling technique for examining the chicken intestinal microflora based on high-throughput, high resolution fingerprinting of bacterial gene regions has been developed and is being used to monitor diet-induced changes. This tool will help provide insight into how gut microflora may impact on poultry health and nutrition. We used T-RFLP to examine changes in gut microbial communities in response to addition of enzyme product to a barley-based diet to increase apparent metabolisable energy (AME).

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II. MATERIAL AND METHODS

Total nucleic acid was extracted from chicken gut samples by a modification of a SARDI proprietary extraction method. Bacterial ribosomal DNA was amplified with universal 16S bacterial primers, one of which was 5′-labelled with 6-carboxyfluorescein. Amplicons were cut with a four base pair recognition sequence restriction enzymes and separated on a capillary DNA sequencer (ABI 3700, Applied Biosystems). Data were analysed using GeneScan 3.7 (Applied Biosystems) to determine positions of terminal restriction fragments (TRF). Prior to statistical analysis the TRF profiles were analysed by a modified method of Dunbar *et al*., 2001 and resulting TRF treated as operational taxonomic units (OTU). OTU were analysed using multivariate statistical models (Primer 5, Primer-E Ltd., Plymouth UK).

Forty-eight Cobb 500 broiler chickens were separated into two equal groups of twenty-four and raised on a barley diet and a barley diet supplemented with exogenous enzyme product (Avizyme 1100 at 1 kg/tonne inclusion rate). Digesta and gut sections were taken from the ilea of each chicken and the microbial community analysed by T-RFLP. The apparent metabolisable energy (AME) values of barley-based diets with and without feed enzyme product were determined in a classical AME study involving measurements of total feed intake and total excreta output and subsequent measurement of gross energy values of feed and excreta by isoperibol bomb calorimetry.

III. RESULTS AND DISCUSSION

Operational taxonomic units (OTU) represent taxonomically related groups and/or species of bacteria found in the chicken ileum (Figure 1). Peak heights are a rough representation of the proportion of different bacterial groups found in the population. Differences in the overall bacterial populations between the two treatments were noticeable.

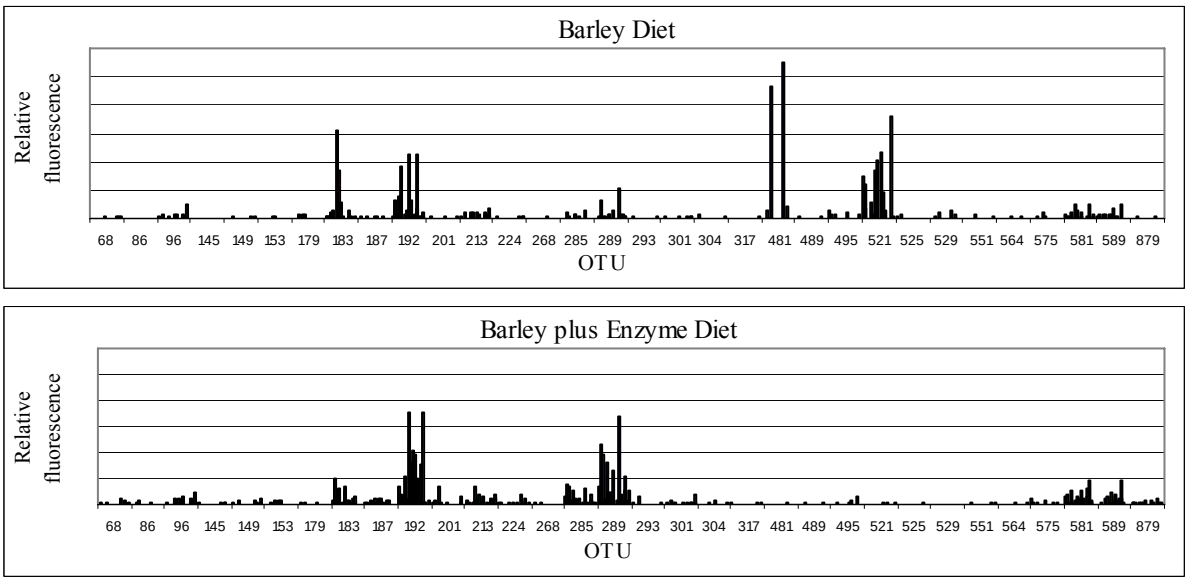

Figure 1. OTU obtained from T-RFLP analysis of the bacterial ribosomal DNA genes amplified from ileal samples from chickens fed a barley diet (n=24) or a barley plus enzyme diet (n=24).

The most significant difference between the two treatments is the presence of a taxonomically related group and/or species at an OTU of 521 in the barley diet group, which is insignificant in the barley plus enzyme diet group. Using sequence data obtained from

chicken gut bacterial isolates it is possible to extrapolate that the OTU at 521 represents *Clostridium perfringens*. This would be consistent with other research indicating that the addition of exogenous enzyme to the diet reduces *C. perfringens* growth (Bedford, 2000). The increased presence of the OTU at 289 in the barley plus enzyme diet is also significant, although the possible identity of this bacterial species or taxonomic related group is yet unknown.

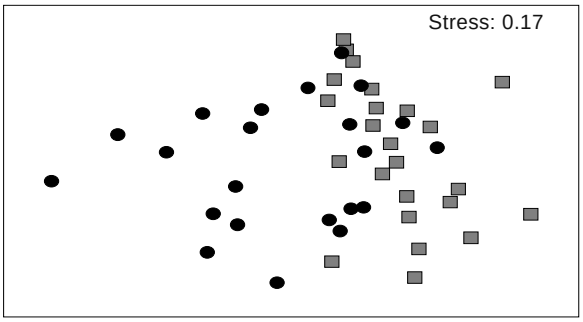

Figure 2. Multidimensional scaling (MDS) of OTU's from T-RFLP analysis of the bacterial ribosomal DNA genes amplified from ileal samples of chickens fed a barley diet (n=24)● or a barley plus enzyme diet (n=24)■ .

Figure 3. Comparison of AME of individual chickens fed a barley diet (n=24) and barley plus enzyme diet (n=24).

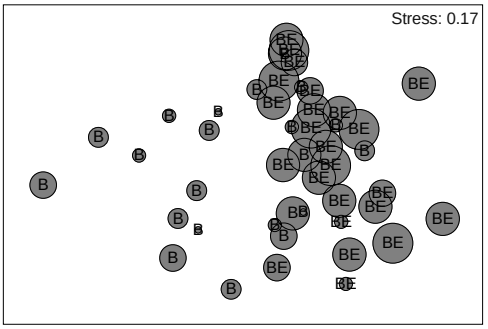

Figure 4. Multivariate statistical analysis of OTU's from T-RFLP analysis of gut microbial communities from chickens fed a barley diet (B) or a barley plus enzyme diet (BE). Bubbles represent AME of individual birds. Sizes of bubbles are proportional to AME values.

Multivariate statistical analysis of OTU's from the two treatments (Figure 2) showed that there is a significant difference in the overall ileal microbial communities of chickens fed a barley versus barley plus enzyme diets. Each point in the plot represents the overall ileal

gut microbial profile of individual chickens and their similarity to the ileal microbial profiles of all other chickens in the experiment (n=48).

Classical growth/performance analysis showed that chickens fed a barley plus enzyme diet had a significantly higher AME (13.87 ± 0.22 MJ/kg dry matter) than chickens on the control barley diet (13.47 ± 0.29 MJ/kg dry matter) (Figure 3). Overall, metabolic energy was increased and variability (expressed as the standard deviation) was reduced in the diet containing exogenous enzyme, which are consistent with many previous reports (see review by Bedford, 2000).

Correlations could be made between changes in overall bacterial communities of chicken fed a barley versus barley plus enzyme diet and an increased AME for individual chickens on the barley plus enzyme diet (Figure 4). The presence of beneficial bacterial species (e.g., lactobacillus; Guan *et al*., 2003) and/or the absence of detrimental bacterial species (e.g., *C. perfringens*; Bedford, 2000) may be indicators of improved energy metabolism in these chickens.

IV. CONCLUSSION

T-RFLP has been used to monitor shifts in the chicken gut microbial population associated with dietary changes. Diet has been shown to affect AME and the ileal microbial community of chickens independently, however this is the first report that correlates a higher AME to a specific microbial profile associated with diet change. We are pursuing the identity of organisms which may be indicators of better poultry performance.

The T-RFLP tool will contribute to an increased knowledge of the chicken gut microbiota, and hence, a better understanding in its role in chicken nutrition.

ACKNOWLEDGEMENTS

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INFLUENCE OF AGE ON THE APPARENT METABOLISABLE ENERGY OF DIETS CONTAINING MAIZE OR WHEAT FOR BROILERS

D.V. THOMAS[1] and V. RAVINDRAN[1]

In a previous study (Thomas and Ravindran, 2005), the apparent metabolisable energy (AME) of diets based on wheat, sorghum or maize for broiler chicks was observed to decline from day 5 to day 7 post-hatching and then to increase to day 14. The present study was conducted to confirm these results and to investigate the changes in AME over the first 21 days of life. Two experimental diets containing either wheat or maize as the cereal base were formulated to contain recommended levels of major nutrients for broiler starters. The wheat-based diet was supplemented with a commercial xylanase. Each diet was offered to six replicate groups (8 birds/replicate) from days 1 to 21 post-hatching. On days 3, 5, 7, 14 and 21, total excreta collection was carried out for 48 hour periods and feed intake recorded for the determination of AME. The AME results were subject to repeated measures analysis. The results are summarised below.

Least square means for AME (MJ/ kg DM) of wheat and maize-based diets

Day	3	5	7	9	14	21	SEM
Wheat	12.75^{a2}	11.05^{a1}	11.27^{a1}	11.62^{a1}	13.12^{a2}	13.17^{a2}	0.24
Maize	13.95^{b2}	12.95^{b1}	13.02^{b1}	12.95^{b1}	14.14^{b2}	13.99^{b2}	??

[a,b] Different superscripts in a column are significantly different ($P < 0.05$).
[1,2] Different superscripts in a row are significantly different ($P < 0.05$).

Cereal effects on AME were significant ($P < 0.05$) at each time point over the 21-day study, with the AME of maize-based diet being higher than those of wheat-based diets. Significant ($P < 0.0001$) age effects were also observed. In both diets, the AME values were higher at day 3 and then declined during 5 to 9 days, before increasing at day 14 post-hatching. The decreases between days 3 and 5 were greater in wheat-based diets. No differences ($P > 0.05$) in AME were observed between 14 and 21 days of age. These data confirm our previous findings and those of Zelenka (1968). The higher AME values determined at day 3 may be due to favourable effects from yolk utilisation and a relatively sterile gut environment. The subsequent drop between days 5 and 9 may be attributed to changing gut flora, declining influence of the yolk sac, inadequate secretion of digestive enzymes, or inadequate digesta mixing. In general, the present data are suggestive of digestive inefficiency during the first week of life in modern fast-growing broilers. Supplementation with exogenous enzymes may be useful in this context and further research is required to explore this strategy.

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APPARENT ILEAL AMINO ACID DIGESTIBILITY OF AUSTRALIAN SORGHUM

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Sorghum is one of the most common cereal grains used by the Australian poultry industry. It can be included up to 60-70% of a broiler diet and may contribute most of the energy and a great portion of amino acids in the diet. Small differences in nutrient content or digestibility can affect the bird performance. Considerable research has been reported on the apparent metabolisable energy (AME) values of Australian sorghum and the values have been found to vary from 15.3 to 16.7 MJ/kg DM (Black *et al*., 2005). However, data on the amino acid digestibility of Australian sorghum are limited. The objective of the present study was to determine the apparent ileal amino acid digestibility of locally grown sorghums and to examine the relationship between crude protein and amino acid contents in sorghum.

Male broiler chickens,that had been fed commercial broiler diets were individually weighed at day 34 post-hatching and the birds with body weights closest to the mean were selected and randomly allocated into pens (7 birds/pen). Seven locally grown sorghum samples were obtained from commercial sources and included in experimental diets (918 g sorghum/kg) as the only source of protein. Celite (20 g/kg) was added to all diets as a source of acid-insoluble ash (AIA) which was used as an indigestible marker. Diets were provided *ad libitum* and water was available at all times. Each assay diet was fed in a mash form to three pens from 34 to 41 days of age. On day 41, the contents of the lower half of the ileum were collected following a lethal injection of sodium pentobarbitone. Ileal digesta from birds within a pen were pooled, immediately frozen, freeze-dried and ground prior to chemical analysis. Amino acids and AIA were analysed as described by Li *et al.* (2005).

The crude protein content of sorghum samples varied from 80.2 to 117.5g/kg. The concentrations of amino acids were in the ranges which were reported by Ravindran *et al.* (1998). The correlation coefficient between crude protein and amino acid contents were positive. With increasing protein levels, the concentrations of all amino acids increased significantly ($r>0.90$, $P<0.01$) except for lysine ($r=0.743$, $P=0.056$). The average ileal digestibility coefficients of amino acids in different sorghums varied from 0.73 to 0.82. The differences in digestibility of crude protein and lysine between sorghum samples were approximately 11 percentage units. There were significant positive correlations between crude protein content and digestible amino acid content in sorghum. The amino acids in higher protein sorghums were more digestible than those in lower protein sorghums ($P<0.01$) except for methionine ($P=0.02$). Similarly significant positive correlations ($r>0.85$, $P<0.014$) between digestible crude protein and digestible amino acid concentrations have been found in sorghums. The digestibility of lysine varied among sorghum samples and was generally lower than other essential amino acids (except for threonine and histidine). However, digestibility of lysine tended to be higher in high protein cultivars compared to low protein cultivars.

The present results show that the concentrations of individual amino acids in sorghum are influenced by grain protein content. Amino acid concentrations in sorghum increased linearly with increasing levels of crude protein. The highly significant positive correlations indicate that it is possible to predict the total and digestible amino acid contents from the crude protein content of sorghum samples.

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NUTRITIONAL CHARACTERISITICS OF SORGHUMS FROM QLD AND NSW

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Summary

Sorghum grain provides a significant alternative to corn and wheat cereals used in animal diets. The value of feeding sorghum grain to poultry has been extensively studied with many reports of significant anti-nutritional factors (ANF) negatively effecting bird performance. The primary cause has been attributed to tannins, particularly the condensed tannins present in the grain testa and pericarp. This paper presents the preliminary results of the RIRDC project examining the nutritional characteristics of sorghum varieties from Queensland and New South Wales for meat chicken production.

I. INTRODUCTION

The development of new low tannin sorghum cultivars by plant breeders (Walker, 1999), are not expected to pose ANF when fed to poultry. More recent evaluations of low tannin sorghums have shown they possess similar nutritional values and, therefore, offer an alternative for the production of non-pigmented poultry products (Leeson and Summers, 1991; Nyachoti *et al.,* 1997). However, in areas where sorghum is used as the primary grain instead of wheat due to AME consistency and lower price, it has been noticed that there is significant carcass variability (breast yield) and depressed feed conversion ratio (FCR) for chicken meat production compared to wheat based diets. To address this problem studies into AME determinations, addition of essential amino acids with extra digestible lysine in the diets, and the addition of enzymes have been undertaken but have failed to resolve the problem.

This reduced FCR and carcass variability may be attributed to condensed tannins (CT) and other polyphenolic compounds. In general, condensed tannins (CT) are water soluble polyphenolic compounds of high molecular weight that have the ability to bind and form complexes with enzymes, proteins, starch, neutral detergent fibre and minerals (Perez-Maldonado, 1994; Walker, 1999). Due to these complexes the effect of CT can be either beneficial or detrimental depending on the type of CT and the host (human, ruminant or monogastric) consuming the plant material containing these compounds. CT and phenolic compounds present in sorghum grain can rapidly increase under adverse environmental conditions, including drought conditions as experienced in the last 5 years in Australia. Carbohydrate complexes can also be formed rendering them unavailable during digestion, with further complexes between free CT fraction and the host digestive enzymes reducing their activity and efficiency. Recent advances in the knowledge and effects of CT and their interaction will allow these theories to be investigated.

II. MATERIALS AND METHODS

Grain sorghum samples of approximately 600-1000 kg were collected from Qld and NSW identified by variety, harvest date and location (Table 1). Samples from PGLP group were included to test the accuracy of PGLP NIR calibrations for AME and feed intake.

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Sorghum samples were analysed to determine proximate composition, amino acid profile (including tryptophan), gross energy, starch, CT and ergot.

Table 1. Location and variety of sorghum grains.

Sample No.	Grain	Variety	State	Region
1	Sorghum	Hylan Dominator	Qld	Kingaroy
2	Sorghum	Hylan Liberty	Qld	Kingaroy
3	Sorghum	Hylan Liberty	Qld	Dalby
4	Sorghum	Pacific Buster	Qld	Biloela
5	Sorghum	Pacific Buster	Qld	Emerald
6	Sorghum	Pacific Buster	NSW	Moree
7	Sorghum	Pacific Buster	NSW	Yelarbon
8	Sorghum	Pacific Buster	NSW	Gunnedah
9	Sorghum	Pacific Buster	Qld	Pittsworth
10	Sorghum	Pacific Buster*	Qld	Dalby
11	Sorghum	Pacific Buster - micro*	Qld	Dalby
12	Sorghum	Pacific MR 43	Qld	Biloela
13	Sorghum	Pacific MR 43	Qld	Biloela
14	Sorghum	Pacific MR 43	NSW	Moree
15	Sorghum	Pacific MR 43	NSW	Yelarbon
16	Sorghum	Pacific MR 43	NSW	Gunnedah
17	Sorghum	Pacific MR 43	Qld	Clifton
18	Sorghum	Pioneer 85G83	Qld	Emerald
19	Sorghum	Pioneer Bonus	Qld	Dalby
20	Sorghum	Normal isoline*	Qld	Biloela
21	Sorghum	Waxy isoline*	Qld	Dalby

* PGLP connectivity samples

Male and female broiler chicks (COBB) from day old were reared from 0-22 d on litter under electrical heaters in six separate pens. A commercial starter diet was provided for the first 21 days. On day 22 birds were transferred to metabolism cages, 6 birds per cage. Diets for the AME assay were prepared as a cold pellet containing, per kg: 804 g sorghum, 155g casein, 11g limestone, 20g dicalcium phosphate, 3 g salt, 5 g vitamins and minerals, 2 g choline chloride. The assay diet was offered *ad libitum*. During the seven-day experimental period the air temperature was reduced in the range 26-24 to 24-22 ^{o}C.

AME methodology was provided by PGLP to ensure connectivity between research groups. The AME of the sorghums was determined from total collection of excreta and measurement of food intake (FI) over 4 days (d) made on four replicate cages (two female and two male) each of six broiler chickens (21-28 d old) and accustomed to the diets for 3 d. The experiment was a balanced incomplete randomised block design.

III. RESULTS AND DISCUSSION

The chemical analysis (Table 2) of varieties showed sorghum grains varied quite widely in chemical composition. Literature reports of crude protein (CP) levels vary significantly and can range from 7.2-15%, and are most likely to be between 10-13% (Gualtieri and Rapaccini, 1990; Nyachoti *et al*., 1997; Walker, 1999). CP levels in our experimental sorghums ranged from 9-14%. There was considerable variation within varieties between regions. Pacific Buster ranged from 9.1-12.9 %, while Pacific MR 43 showed lower variability with levels ranging from 11.6–13.5 %. Phosphorus levels ranged from 2–3.9 g/kg DM, while calcium was found in only trace amounts of less than 0.1%, these values were found to be similar to expected values. All sorghum samples are being analysed

Table 2. Chemical composition (g/kg DM) of experimental sorghum

Analysis	Sorghum Sample No.																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Dry matter (%)	87.7	87.1	88.6	88.8	87.1	88.1	88.2	87.0	87.6	89.2	91.9	90.2	88.3	88.3	87.0	87.8	89.2	87.3	88.2	88.6	88.6
Crude protein (%)	10.4	8.9	13.6	13.3	12.2	12.6	12.9	11.1	9.1	11.8	11.8	11.8	13.5	12.9	11.9	11.8	11.6	11.6	11.6	10.6	14.4
Phosphorus	3.3	2.5	3.9	3.9	3.1	3.3	3.4	3.4	2.5	2.5	3.1	4.9	3.7	3.4	3.3	2	2.6	3.3	3.2	2.6	4.1
Free condensed tannins	0.19	0.00	0.01	0.02	0.08	0.00	0.05	0.00	0.00	0.00	0.10	0.09	0.05	0.09	0.00	0.10	0.01	0.00	0.02	0.00	0.02
Bound condensed tannins	0.30	0.18	0.24	0.46	0.39	1.05	0.66	0.60	0.43	0.57	0.53	0.48	0.78	0.64	0.62	0.67	0.53	0.48	0.52	0.55	0.69
Total tannins	0.49	0.18	0.25	0.48	0.47	1.05	0.71	0.60	0.43	0.57	0.63	0.57	0.84	0.73	0.62	0.76	0.54	0.48	0.53	0.55	0.70
Alanine	10.8	8.7	14.6	11.6	13.7	12.4	12.6	12.3	10.3	12.5	11.0	11.0	12.2	11.7	13.1	12.1	12.0	12.4	11.6	10.6	12.7
Arginine	4.2	3.8	4.6	4.5	4.3	4.6	4.8	3.7	3.6	4.2	4.4	4.4	5.2	5.0	4.2	4.7	4.4	4.3	4.5	4.1	5.3
Leucine	12.8	10.4	16.6	16.1	15.4	16.0	16.4	13.6	10.5	13.8	14.3	14.3	16.6	16.7	14.7	14.2	13.9	14.1	14.0	12.8	17.9
Lysine	2.9	2.7	3.7	2.7	3.3	2.5	2.5	2.9	3.0	3.6	2.3	2.3	2.4	2.4	3.4	3.2	3.4	3.5	2.6	2.5	2.8
Methionine	1.0	1.0	1.8	2.7	2.0	1.3	1.4	1.5	1.4	1.4	2.5	1.8	1.1	1.4	1.7	1.1	1.7	1.4	1.4	1.1	2.5
Phenylalanine	3.7	3.0	4.6	5.4	4.1	5.0	5.1	3.6	2.8	3.6	4.8	4.8	5.8	5.2	3.9	4.1	3.9	3.7	4.4	3.8	5.9
Proline	7.5	6.2	9.8	9.8	9.2	9.3	9.4	8.0	6.2	8.2	8.5	8.6	9.7	9.6	8.6	8.1	8.2	8.4	8.2	7.6	10.5
Serine	4.2	3.5	5.2	5.2	4.7	5.0	5.1	4.1	3.4	4.4	4.7	4.7	5.3	5.4	4.6	4.6	4.5	4.4	4.5	4.1	5.5
Aspartic acid	8.5	7.2	12.4	11.0	12.5	8.7	9.9	10.2	9.3	12.2	9.6	9.5	9.6	8.3	11.0	10.0	11.3	12.7	8.5	7.3	12.1
Cystine	1.2	1.0	1.8	2.5	1.9	1.6	1.8	1.5	1.4	1.7	2.4	2.2	1.4	1.9	1.7	1.4	1.5	1.7	1.8	1.5	2.4
Glutamic acid	27.9	22.3	39.7	34.2	38.1	31.0	34.2	32.7	27.1	36.0	30.0	30.1	32.8	30.6	34.9	31.6	33.8	38.2	28.7	24.3	37.1
Glycine	2.6	2.3	3.0	3.1	2.7	2.9	2.9	2.4	2.2	2.7	2.9	2.8	3.3	3.1	2.7	2.9	2.8	2.6	2.7	2.5	3.2
Hisitdine	1.5	1.2	1.7	2.2	1.7	1.9	1.9	1.4	1.2	1.5	2.0	2.1	2.1	2.1	1.6	1.7	1.6	1.5	1.7	1.5	2.3
Isoleucine	3.8	3.2	4.9	4.6	4.4	4.5	4.6	4.0	3.2	4.1	4.1	4.1	4.8	4.7	4.3	4.2	4.1	4.1	4.1	3.7	5.1
Threonine	2.6	2.3	3.3	3.3	2.9	3.1	3.1	2.6	2.2	2.8	3.2	3.2	3.3	3.3	2.8	2.9	2.8	2.7	3.6	3.2	3.9
Tryptophan	1.2	1.1	1.7	1.4	1.5	1.5	1.5	1.3	1.0	1.5	1.3	1.2	1.6	1.6	1.4	1.4	1.5	1.4	1.4	1.3	1.5
Tyrosine	2.7	2.1	3.3	4.1	3.0	3.6	3.7	2.5	2.0	2.6	3.7	3.6	4.3	3.8	2.8	2.9	2.8	2.6	3.2	2.7	4.5
Valine	4.8	4.0	5.9	5.6	5.5	5.5	5.7	4.9	4.2	5.2	5.0	5.1	5.8	5.8	5.3	5.3	5.2	5.2	5.1	4.7	6.2
Broilers AME (MJ/kg DM)	15.94	16.13	15.92	15.64	16.23	15.82	15.74	15.86	15.61	15.70	15.24	15.58	15.65	15.45	15.91	15.60	15.82	15.87	15.86	15.64	15.89
Broilers AMEn (MJ/kg DM)	15.32	15.45	15.20	14.99	15.48	15.17	15.08	15.19	14.97	15.00	14.65	14.86	14.99	14.84	15.22	15.07	15.18	15.22	15.12	15.04	15.20

Note:

Calcium was available in trace amounts (<0.1%)

Ergot was not detected (<0.01)

for phytate-phosphorus. There was significantly more bound CT than free CT, with total CT levels ranging from 0.18 – 1.05 g/kg. There was no trend to indicate a variety effect on tannin levels with significant variation occurring within varieties between regions. This indicates that growing condition has a significant effect on tannin levels. The summary of reports of genotypic differences in sorghum chemical composition by O'Brien (1999) found all studies reported a significant difference in CP and also in tannins when it was measured.

Apparent metabolisable energy (AME) ranged from 15.24 - 16.23 (MJ/kg dry matter), and from 14.65 – 15.48 MJ/kg DM when corrected for N (Table 2). ME reported values for sorghum have ranged from 10.95-16.09 MJ/kg DM (Gualtieri and Rapaccini 1990; Walker 1999). There is minimal variation in our sorghum AME and AMEn values.

The amino acid (AA) profiles (Table 2) are similar to expected values (Ravindran *et al*., 1998; Rhône-Poulenc Animal Nutrition 1993). In comparison with corn, sorghum grains have similar lysine, methionine and cystine levels, higher isoleucine and tryptophan levels and lower protein digestible levels. However, as sorghum generally has a greater (2%) protein level, the essential AA availability from sorghum and corn are similar (Gualtieri and Rapaccini 1990; Nyachoti *et al*. 1997; Walker 1999). In comparison with wheat, sorghum has lower lysine, methionine, cystine and tryptophan, and similar isoleucine and threonine levels.

There use of sorghum grain as an animal feed source may provide benefits due to its adaptability to environmental conditions, economic feasibility and availability. Sorghum is already a significant grain crop of tropical and sub-tropical regions. However sorghum grain has had variable results when used as a feed source in poultry. This variability has been attributed to variety, region of growth and variety by region interactions affecting the grain chemical composition and tannin content. As shown in our results examining the nutritional characteristics of 21 sorghums, there is considerable variation within varieties that can be attributed to differences in growing conditions. A broiler performance experiment is currently evaluating each sorghum cultivar compared against wheat to complement the nutritional characterisation.

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NUTRITIONAL CHARACTERISATION OF SORGHUMS FROM QUEENSLAND AND NEW SOUTH WALES IN BROILER STARTER AND FINISHER DIETS

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Sorghum is an alternative grain to wheat due to AME consistency and lower price. However in broilers significant breast yield variability and poor feed conversion ratio (FCR) were observed on sorghum-based diets compared to wheat based diets. This study investigated production performance when crumbled starter (0-21 d old chicks) and finisher pelleted diets (21-42 d old) were formulated to achieve maximum production. There were 17 treatments offered to male broilers (Arbor Acres) grown in 96 mesh wire cages with 5 replicates per treatment, 8 birds per cage in a completely randomised design. The control wheat diet had 11 replicates and contained wheat (63%), soybean meal (19%), meat and bone meal (5%), canola meal (6%), sunflower meal (3%), vitamins, minerals, and amino acids. A xylanase enzyme was added to the control wheat diet. In the remaining treatments, sorghum from 17 different locations replaced wheat. Food, water, light and temperature were offered in an environmentally controlled house according to industry practice. Growth rates and feed intakes were recorded at 21 and 42 d of age.

Treatment	Feed intake (g/bird)			Growth rate (g/bird)			FCR (g/g)		
Age days	0-21	21-42	0-42	0-21	21-42	0-42	0-21	21-42	0-42
Wheat control	1270^{ab}	4021	5291^{b}	921^{a}	2210	3132	1.379^{j}	1.862^{b}	1.710^{bcde}
Sorghum 2	1234^{abcd}	4051	5285^{abc}	874^{bcd}	2236	3110	1.421^{ghi}	1.811^{bcd}	1.699^{cdef}
Sorghum 3	1229^{bcd}	4116	5346^{ab}	884^{b}	2295	3180	1.406^{hij}	1.810^{bcd}	1.692^{def}
Sorghum 4	1254^{abc}	3999	5252^{bc}	866b^{cd}	2175	3041	1.448^{defg}	1.853^{bc}	1.729^{bcd}
Sorghum 5	1217^{cdef}	4015	5232^{bc}	826^{efg}	2246	3073	1.468^{bcdef}	1.875^{b}	1.750^{b}
Sorghum 6	1182^{defg}	3961	5143^{bcd}	788^{gh}	2184	2972	1.500^{ab}	1.815^{bcd}	1.730^{bcd}
Sorghum 7	1180^{defg}	4148	5328^{ab}	837^{def}	2317	3154	1.410^{hi}	1.806^{bcd}	1.697^{cdef}
Sorghum 8	1230^{bcd}	4013	5243^{bc}	878^{bc}	2292	3171	1.403^{ij}	1.784^{cde}	1.668^{ef}
Sorghum 9	1190^{defg}	4119	5309^{ab}	839^{def}	2290	3129	1.437^{fgh}	1.821^{bcd}	1.710^{bcdef}
Sorghum 10	1011^{h}	3908	4919^{d}	689^{i}	2282	2971	1.467^{cdef}	1.731^{e}	1.665^{f}
Sorghum 11	1173^{efg}	3878	5050^{cd}	779^{h}	2162	2941	1.508^{a}	1.809^{bcd}	1.725^{bcd}
Sorghum 12	1234^{abcd}	4082	5316^{ab}	839^{def}	2246	3086	1.471^{bcde}	1.853^{bc}	1.745^{bc}
Sorghum 13	1252^{abc}	3995	5247^{bc}	857^{bcde}	2258	3115	1.489^{abc}	1.834^{bcd}	1.731^{bcd}
Sorghum 14	1162^{fg}	4040	5243^{bc}	805^{fgh}	2273	3079	1.443^{efg}	1.776^{de}	1.688^{def}
Sorghum 15	1290^{a}	4231	5521^{a}	875^{bcd}	2162	3038	1.478^{abcd}	1.966^{a}	1.813^{a}
Sorghum 16	1224^{bcde}	4018	5243^{bc}	840^{cdef}	2192	3032	1.463^{cdef}	1.832^{bcd}	1.724^{bcd}
Sorghum 17	1135^{g}	4131	5266^{bc}	786^{h}	2303	3090	1.455^{def}	1.811^{bcd}	1.713^{bcdef}
LSD (P=0.05)[1]	48	190	217	33	120	133	0.028	0.060	0.051
LSD (P=0.05)[2]	56	223	255	39	141	156	0.033	0.071	0.043

Values within a column with different superscript differ significantly (P<0.05); [1]= for comparing wheat control vs sorghums; [2]= for comparing between sorghums

Birds (0-21 d) on the wheat diet had superior (P<0.05) growth rate when compared with those on the sorghum treatments. Similarly, birds on the wheat diet converted more efficiently (P<0.05) and were matched only by the sorghum diets 3, 7 and 8. However, all differences in production performance disappeared (P>0.05) during the grower/finisher period (21-42 d), with all treatments supporting excellent growth rates and feed efficiency at day 42.

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INCREASE IN AME OF CORN AND CORN-SOYBEAN MEAL BY A MULTI-ENZYME LIQUID FORMULATION

A. LIM[1] and H.M. TAN[1]

Summary

RD20 is a multi-liquid enzyme formulation designed to improve the nutritional quality of corn-soybean meal diets for broilers. It contains a mixture of α-amylase, β-glucanase, protease, cellulase and xylanase and is targeted at feedmills using high pelleting temperatures. While *in vitro* studies have shown that RD20 is capable of releasing reducing sugars in corn-soybean meal mixtures, its biological efficacy has yet to be verified. This paper summarizes an *in vivo* trial that evaluated the effect of RD20 at 0.5 kg/tonne dosage, on the apparent metabolisable energy (AME) of corn and corn-soybean meal diets in 21-day old male Ross chicks. Results show that RD20 significantly increased the AME of corn by 0.33 MJ/kg DM ($P<0.01$) and corn-soybean meal by 0.27 MJ/kg DM ($P<0.05$). The positive energy sparing effect on corn-soybean meal diet, which is largely attributed to the synergistic effect of the glycosylase and protease activities in RD20, represents a cost-saving opportunity in broiler feeds.

I. INTRODUCTION

The use of liquid enzymes in post-pellet application on broiler feed is fast becoming a trend in major feedmills due to the high processing temperature (>90°C). Despite its clear advantage in delivering enzymes without the risk of thermal inactivation, this approach needs to overcome three hurdles: 1) stability of enzymes in non-physiological liquid media is much lower, 2) increased risk of proteolysis due to presence of protease and side protease activities, and 3) high degree of precision in applicators to ensure homogeneity of feed enzymes in the feed. Kemin Industries has developed a multi-liquid enzyme product, RD20, containing a stable mixture of α-amylase, β-glucanase, protease, cellulase and xylanase to improve the nutritional value of corn-soybean diets. This formulation differs from most commercial liquid enzyme products in that it contains a significant amount of protease, which has been shown to improve nutritional value of diets containing soybean meal (Ghazi *et al.*, 2003). The positive energy saving effects of RD20 in broiler chicks fed on corn and corn-soybean meal diets are presented in this paper.

II. METHODS AND MATERIALS

a) Recovery of enzymes

Two random samples from a one kg composite feed sample were assayed for β-glucanase activity using a plate diffusion assay (Walsh *et al.*, 1995). β-Glucanase activity in the feed samples was calculated from a calibration curve using RD20 at different dosages.

b) Broiler trial

The AME trial was carried out at the Monogastric Research Centre, Massey University (Palmerston North, New Zealand). A total of 220 day-old male broiler chicks (Ross) were obtained from a local hatchery and reared in floor pens on commercial starter/

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grower diets. At 21 days of age, the birds were transferred to colony cages that had facilities for total collection of excreta. Six treatments were tested and the details are shown in Table 1.

Table 1. Broiler trial treatments

Treatment code	Diet*	Liquid enzyme	Dose
A	Corn	-	-
B	Corn	RD20	0.5 kg/tonne
C	Corn-soybean meal (normal ME)	-	-
D	Corn-soybean meal (reduced ME)	-	-
E	Corn-soybean meal (reduced ME)	RD20	0.5 kg/tonne

* See Table 2 for diet formulation.

The corn-soybean meal diet was formulated to an ME of 13.39 MJ/kg (normal ME) as shown in Table 2. The Reduced ME diet was created by reducing the amount of soya oil from 53 g/kg to 30 g/kg. Both the corn-soybean meal diets (normal and reduced ME) were cold-pelleted (70 °C), while the corn diets were fed in mash form. RD20 was diluted at one part enzyme to 20 parts water and sprayed while the diets were being mixed in a horizontal mixer. Diets were offered *ad libitum* and water was available at all times. On day 25, birds were weighed individually and birds with relatively high or low body weights were discarded. A total of 180 birds were chosen and distributed into 36 groups (pens) of five birds each so that average weights per pen was nearly equal. Each of the six dietary treatments were then randomly assigned to six pens (six replicates per treatment).

The AME values were determined using a classical total collection method. The birds were fed the treatment diets for seven days (from day 25) with the first three days serving as an adaptation period. During the last four days, feed intake was monitored, and the excreta were collected daily, weighed and pooled within a pen. Pooled excreta were mixed well into slurry and two samples per pen were obtained and freeze-dried. Dried excreta samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at – 40°C for chemical analyses. The gross energy (GE) of the diets and excreta samples was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, UK) standardised with benzoic acid. The AME contents of the diets were calculated using the following formula:

$$AME, MJ/kg = \frac{Feed\ \ int ake \times GE_{Diet} - Excreta\ \ output \times GE_{Excreta}}{Feed\ \ int ake}$$

Appropriate corrections were also made for differences in dry matter content and the results are expressed as MJ/kg DM (dry matter) to enable comparison between treatments.

c) Statistical analysis

The data were analysed by the General Linear Models procedure of the SAS® (SAS Institute, 1990) with pen means as the experimental unit. Significant differences between means were separated using the Least Significance Difference (LSD).

Table 2. Diet formulation (g/kg)

Ingredients	Corn-soybean meal (Normal ME)	Corn-soybean meal (Reduced ME)	Corn
Corn	57.71	60.01	96.40
Soybean meal (480g/kg CP; ME 10.04 MJ	33.02	33.02	-
Soya oil	5.30	3.00	-
Limestone (38g/kg Ca)	1.22	1.22	1.30
Dicalcium phosphate (220 g/kg Ca, 180 g/kg P)	1.55	1.55	1.70
Salt	0.30	0.30	0.30
Sodium bicarbonate	0.17	0.17	-
DL-methionine	0.35	0.35	-
Threonine	0.13	0.13	-
Mineral premix	0.15	0.15	0.25
Vitamin premix	0.05	0.05	0.05
Sand	0.05	0.05	0.05
Calculated composition			
Crude protein	206	206	-
ME poultry, MJ/kg	12.99	12.46	-

III. RESULTS AND DISCUSSION

a) Recovery of β-glucanase activity in feed samples

β-Glucanase activity is high in RD20 and therefore it can be used to determine the homogeneity of RD20 in the feed. Table 3 shows that the β-glucanase activity in Treatment E is above the target activity of 1485.0 U/kg feed.

Table 3. Recovery of β-glucanase activity in feed samples

Treatment	Enzyme	β-glucanase activity (U/kg feed)			
		1*	2*	Average	Target
E	RD20	1742.9	1783.8	1763.3	1485.0

* Mean of two determinations.

b) AME of corn and corn-soybean meal diets

The AME of the corn used in the study was determined to be 15.20 MJ/kg DM. Addition of enzyme to corn resulted in significant ($P<0.01$) increase in AME by 0.33 MJ/kg DM (Table 4).

The AME determined for the corn-soybean diet, normal and reduced ME, were 14.23 and 13.82 MJ/kg DM respectively (Table 5). The determined difference 0.41 MJ/kg was lower than the expected difference of 0.54 MJ/kg. Addition of RD20 to the corn-soybean (reduced ME) resulted in significant ($P<0.05$) increase in AME by 0.27 MJ/kg DM (0.24 MJ/kg as-fed). Supplementation of exogenous enzymes did not completely recover the reduced AME of the Normal ME diet, most likely due to the relatively lower starch content in soybean meal.

Table 4. Influence of enzyme supplementation on the AME of corn of broiler chickens fed the corn diet.

Treatment	Description	AME [1,2] (MJ/kg DM)	AME (MJ/kg as-fed)
A	Corn	15.20 [a]	13.66
B	Corn + RD20	15.53 [b]	13.96
Difference		0.33	0.30
Pooled SEM		0.059	
Significance (P=)			
- Enzyme		0.01	
- LSD (P < 0.05)		0.186	

[a,b] Means in the same column with different superscripts are significantly (P < 0.05) different.
[1] Average of six pens.
[2] AME values of corn calculated based on the inclusion level of 965 g/kg of corn. Dry matter content of corn was 898.8 g/kg.

Table 5. Influence of enzyme supplementation on the AME of corn of broiler chickens fed the corn diet.

Treatment	Description	AME [1,2] (MJ/kg DM)	AME (MJ/kg as-fed)
C	Corn-soybean diet (Normal ME)	14.23 [a]	12.77
D	Corn-soybean diet (Reduced ME)	13.82 [c]	12.42
E	Corn-soybean diet (Reduced ME) + RD20	14.09 [b]	12.66
Pooled SEM		0.077	
Significance (P=)			
- Enzyme		0.01	
- LSD (P< 0.05)		0.228	

[a,b,c] Means in the same column with different superscripts are significantly (P<0.05) different.
[1] Average of six pens.
[2] Dry matter contents of Normal ME and Reduced ME corn-soybean diets were 898.0 and 898.6 g/kg, respectively.

IV. CONCLUSION

RD20 significantly increased the AME of corn by 0.33 MJ/kg DM (P<0.01) and corn-soybean meal by 0.27 MJ/kg DM (P<0.05). This may be due to the synergistic effect of the glycosylase and protease activities in the liquid enzyme product.

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HIGHLY VISCOUS BARLEY PLUS AVIZYME 1210 GIVES GOOD BROILER PERFORMANCE

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Summary

Barley of an extremely high viscosity was added at 4 inclusion levels (0, 150, 300, and 450g/kg) to a wheat/soybean based pelleted broiler diet with added enzyme. Diets were fed during the starter and grower production phase (1 to 42 days of age) to male broiler chicks housed in deep-litter pens in groups of 40 birds. At eleven days of age, birds on the highest inclusion level were unable to utilise the dietary nutrients through malabsorption and were withdrawn from the trial. Significantly higher bird growth and feed intake occurred to 21 days of age as the level of barley increased in the diet with the effect on growth continuing throughout the trial. All performance traits trended to improve as barley inclusion levels increased. The digesta viscosity collected at 21 days of age did not differ significantly despite the extremely high viscosity of the barley grain. It appears that Avizyme 1210 is effective in enabling up to 300g/kg inclusion of high viscosity barley in broiler diets.

I. INTRODUCTION

Water-soluble non-starch polysaccharides (NSP), are considered the major antinutritive factors in cereals and other feed ingredients (Bedford and Classen, 1992; Campbell and Bedford, 1992). The common NSPs include beta-glucans, arabinoxylans and fructans (Classen and Bedford, 1991). The effects of NSPs upon feed utilisation and poultry productivity have been reported by Salih *et al.* (1991) and others. Salih *et al.* (1991) reported that NSPs reduced feed intake and decreased broiler chicken performance. The most noticeable effect of NSPs in poultry diets is an increase in the viscosity of digesta and the excretion of sticky droppings. This is considered to be one of the greatest influences of NSP on broiler chicken productivity (Smits and Annison, 1996). Exogenous enzymes (xylanase/beta-glucanase) are now used to overcome these problems. Mechanisms associated with the effects of NSP on intestinal structure and function in broiler chickens were discussed by Bedford (1996) and Iji (1999).

Poultry do not digest NSP as well as other monogastrics such as pigs or rats (Huisman and Tolman, 1992; Jorgensen *et al.,* 1996). The endosperm cell wall of wheat is mainly composed of arabinoxylans, whereas beta-glucan is the major constituent of barley cell walls. The addition of exogenous enzymes to wheat- and barley-based diets increases digestibility of nutrients in cockerels (Carre *et al.,* 1990). It may also provide additional dietary energy as well as short chain fatty acids and oligosaccahrides (Iji, 1999). Such phenomena are associated with cellular proliferation and improved gut health (Iji, 1999).

The aim of the present study was to investigate further the effect of using a commercial enzyme preparation Avizyme 1210 containing xylanase and beta-glucanase on the performance and feed utilization of broilers fed diets based on wheat and high viscosity barley.

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II. MATERIALS AND METHODS

A sample of the barley and wheat were sent to SARDI for grain viscosity determination. Sufficient hatch-day 'Ross' broiler chicks vaccinated for Infectious Bronchitis, were obtained from Golden Cockerel Pty. Ltd (Mt Cotton) to provide 960 males. The experiment comprised 24 pens each containing 40 chickens. A randomized-block design was used comprising six blocks of four floor pens each. The four diet combinations were randomly assigned to the pens within each block. The dietary treatments were four inclusion levels of barley (0, 150, 300 and 450g/kg). Avizyme 1210 in liquid form was administered via the feed, post pelleting (65^{o}C), following thorough mixing of the appropriate quantities. Diet formulation and specificationis given in Table 1. The diets were presented to the trial birds in a crumbled form for starter birds (1-21 days) and as pellets in the grower/finisher stage (22-42 days). Diet and water were supplied *ad libitum*.

Bird liveweights were measured at 1, 21 and 42 days of age and bird feed intake was measured at 21 and 42 days of age. At 21 days of age three birds from each pen were euthanased by cervical dislocation and the contents of the lower half of the ileum was collected. The digesta sample was centrifuged at 2,000 g for 3 minutes and the resultant supernatant was evaluated for viscosity using a Brookfield Dial Viscometer. Data were analysed using Genstat statistical package, and Duncan's multiple range test for separating mean values.

Table 1. Diet Formulation and analysis of starter and Grower Diets, kg/tonne

Starter diets Ingredient	Diet A	Diet B	Diet C	Diet D
Barley[1]	0	150	300	450
Wheat[1]	712.9	551.1	382.5	213.9
Tallow	0	4	13	22
M&B Meal (50% CP)	60	60	60	60
Soybean Meal (47% CP)	208	216	226	236
Limestone	6	6	6	6
Salt	2.1	2.2	2.3	2.4
Sodium Bicarbonate	2	2	2	2
Choline 60%	1.3	1.2	1.1	1
Lysine HCL	3.2	2.9	2.6	2.2
DL Methionine	1.4	1.6	1.7	1.8
L Threonine	0.6	0.5	0.3	0.2
Vit/Min	2	2	2	2
Avizyme 1210	0.5	0.5	0.5	0.5
Coxistac	0.5	0.5	0.5	0.5
Total	1000.5	1000.5	1000.5	1000.5
Nutrients minimums				
ME, kcal/kg (MJ/kg)			3000 (12.55)	
Protein (actual), %			(23.9)	
Lysine total (actual), %			(1.35)	
Calcium, %			0.88	
Available P, %			0.44	
Digest. Lysine, %			1.15	
Digest. Methionine, %			0.426	
Digest. M+C, %			0.828	

Grower diets				
Ingredient	Diet A	Diet B	Diet C	Diet D
Barley[1]	0	150	300	450
Wheat[1]	754.3	592.5	428.7	263
Tallow	9	15	22	29
M&B Meal (50% CP)	42	42	42	42
Soybean Meal (47% CP)	177	184	191	200
Limestone	7	6	6	6
Salt	1.3	1.4	1.5	1.6
Sodium Bicarbonate	2	2	2	2
Choline 60%	0.4	0.3	0.2	0.1
Lysine HCL	2.9	2.6	2.3	2
DL Methionine	1.1	1.2	1.4	1.5
L Threonine	0.5	0.5	0.4	0.3
Vit/Min	2	2	2	2
Avizyme 1210	0.5	0.5	0.5	0.5
Coxistac	0.5	0.5	0.5	0.5
Total	1000.5	1000.5	1000.5	1000.5
Nutrients minimums				
ME, kcal/kg (MJ/kg)			3100 (12.97)	
Protein (actual), %			(22.2)	
Lysine total (actual), %			(1.20)	
Calcium, %			0.72	
Available P, %			0.36	
Digest. Lysine, %			1.02	
Digest. Methionine, %			0.389	

[1] Nutrient values for both wheat and barley were modified to take account of the effects of the Avizyme 1210. For wheat, ME was increased by 6% (from 12.833 to 13.598 MJ/kg), and digestible amino acids were increased by 10%. For barley, ME was increased by 10% (from 11.602 to 12.762 MJ/kg), and digestible amino acids were increased by 15%.

At eleven days of age, some birds on the highest barley inclusion level (450g/kg) were observed to have rotated legs, curling of the toes, inability to walk, diarrhoea and appeared stunted. After veterinarian inspection, the diagnosis was nutrient mal-absorption, and thus the treatment was withdrawn from the experiment.

III. RESULTS AND DISCUSSION

Viscosity of barley could not be determined as it was too viscous and a supernatant was unable to be obtained. Viscosity of wheat was 10.65 cPs. No significant differences in digesta viscosity (2.745 – 2.970 cPs) were detected between the three treatments despite the extremely high viscosity of the barley grain used. This suggests that the enzyme was effective in reducing gut viscosity to normal levels. Mean weight gain, feed intake and FCR are given in Table 2. Feed intake in the first 21 days increased significantly ($p<0.05$) when birds were offered barley when compared to the control diet particularly at the 300g/kg barley inclusion level. Feed intake during the period of 21 to 42 days of age and the overall intake from 0 to 42 days did not differ significantly, however the overall trend was for higher intake with increasing levels of barley in the diet. Both bird live- weight and live-weight gain were significantly higher at 21 days of age with the inclusion of barley in the diet. Live-weight gain from 21 to 42 days did not differ significantly although there was a trend towards higher values with increasing level of dietary barley. At 42 days of age, the highest level of barley inclusion (300g/kg) produced significantly higher bird live-weight and live-weight gain than

other treatments. This suggests that the nutrient modifications made to barley may have underestimated the actual enzyme effects. Feed conversion ratio did not differ significantly throughout the trial period, although the trend was for improvement in FCR as the level of barley increased.

Table 2. Mean weight gain, feed intake and FCR of broiler birds on barley-wheat based diets with added Avizyme 1210.

Treatment	g/kg Barley			P value	LSD, 5%
	0	150	300		
1-21 days					
Weight Gain (g)	757.2[a]	794.5[b]	827.0[b]	0.006	36.5
Feed Intake (g)	1085.1[a]	1128.3[ab]	1174.4[b]	0.040	66.0
FCR	1.450	1.440	1.433	0.685	0.044
1-42 days					
Weight Gain (g)	2622.3[a]	2677.2[a]	2786.0[b]	0.015	102.7
Feed Intake (g)	4878.9	4949.0	5103.4	0.052	180
FCR	1.861	1.842	1.823	0.283	0.0507

[ab] Means in a row with a common or no superscript are not different (P<0.05)

IV. CONCLUSIONS

The inclusion of 0.5 kg/t of Avizyme 1210 to up to 300g/kg of extremely high viscous barley in a wheat/soy bean meal based broiler diet had a significant positive effect on growth rate and feed intake during both the starter and grower phases. Feed conversion efficiency tended to improve as the level of barley increased in the diet.

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EXPANSION AND PECTINASE TREATMENT OF LUPINS FOR BROILERS

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Summary

We tested the hypothesis that expansion of lupins would improve the activity of pectinase (polygalacturonase, PG) by increasing the surface area available for PG to degrade the pectins in cell walls. This should increase the nutritive value of lupins and improve the growth of broilers. A 2x2x2x2 factorial experiment (no expansion or expansion, whole or dehulled lupins, 10 or 20% lupins, 0 or 0.8g PG/kg diet) was carried out for 11 days. Expansion broke down cell walls and pectin but this did not improve performance. The combination of expansion + PG led to greater breakdown of cell walls and pectin than either PG or expansion on their own but this was not reflected in the performance of birds. PG reversed the adverse effects of expansion more so for birds fed 10% whole and dehulled lupins than their corresponding 20% inclusion. These results highlight the significance of pectinase treatment of lupins for broilers because a 20% inclusion rate may be tolerated without significant losses in productivity and only slight increase in wet droppings.

I. INTRODUCTION

Feed enzymes such as pectinase (PG) can partially break down the indigestible cell-wall polysaccharides in lupins and increase their nutritional value for poultry. However, the response can be variable. Feed manufacturers and the lupin industry in Australia are seeking ways to increase the response to pectinase and make it less variable. One way to reduce the variability and increase the response is to subject lupins to mechanical and thermal processes before enzyme treatment. Extrusion is one possibility but it is a high-cost operation because of the extreme pressures and the low throughput. Expansion, often called low-cost extrusion, might be a possibility. Like extrusion, expansion has been shown to increase feed intake by 4%, feed conversion efficiency by 3%, nitrogen retention by 7%, metabolisable energy by 11%, and breakdown of β-glucan by 12 fold and arabinoxylan by 5 fold of wheat- and barley-based diets (Plavnik and Sklan, 1995; Vest, 1996; Fancher *et al*., 1996; Scott *et al*., 1997; Fasina *et al*., 1997; Chesson *et al*., 2002).

If expansion was used on its own without pectinase, it could be detrimental. Viscosity and water-holding capacity might increase if too much insoluble non-starch polysaccharide was solubilised (Fancher *et al*., 1996; Liebert and Wecke, 1998; Chesson *et al*., 2002). However, enzyme treatment (eg. xylanase or glucanase) after expansion counteracted the increase in viscosity (34 vs. 14%) and improved weight gain (7 vs. 4%), feed conversion ratio (3 vs. 2%) and metabolisable energy (11 vs. 9%) compared with either expansion or enzyme treatment on their own (Scott *et al*., 1997; Liebert and Wecke, 1998). Therefore, it seems that expansion should be used to increase the surface area and allow the enzyme to work more effectively and counteract any detrimental effects of the expansion. The hypothesis was tested that expansion of lupins would improve the activity of PG by increasing the surface area available for enzyme activity. This should increase metabolisable energy thereby increasing the growth of broilers and, at the same time, reduce the incidence of wet droppings.

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II. MATERIALS AND METHODS

Whole and dehulled lupins were expanded and included at either 10 or 20% in a commercial broiler diet based on wheat (64%) as the main ingredient. The experiment consisted of a 2x2x2x2 complete factorial (no expansion or expansion, whole or dehulled lupins, 10 or 20% lupins, 0 or 0.8g PG/kg diet). Diets were pelleted and fed to broilers for 11 days. Mixed-sex broilers (Cobb strain) 4 weeks old (576 birds) were randomly distributed into metabolism cages. Feed and water were supplied *ad libitum*. The powder pectinase consisted of polygalacturonase, PG (3000 unit/g) with traces of cellulase. Xylanase at 0.2g/kg (Rovabio^{TM}) was included across all diets. Feed intake, weight gain and water intake were recorded throughout the experiment. Excreta were collected twice a day for determination of water content of faeces and metabolisable energy. At end of the experiment, the birds were killed and the ileal digesta were extracted for measurement of viscosity. There were 12 replicates with 3 birds/replicate. Data were analysed by Analysis of Variance (ANOVA) and means, if significant, were tested using Tukey's Honestly Significant Difference.

III. RESULTS

PG on its own had no effect on food intake but it increased the digestibility of the diet by 10%, increased weight gain of birds by 5%, improved food conversion by 5%, reduced viscosity by 14%, water intake by 6% and faecal moisture by 6% across all lupin diets (Table 1 and 2). By contrast, expansion increased viscosity by 10%, water intake by 3% and faecal moisture by 3%. The combination of expansion and PG was the same as control, that is, it had no overall effect. PG more than doubled the breakdown of cell-wall polysaccharides and pectin by 4 fold. Expansion was more effective than PG because it broke down cell wall polysaccharides by 3 fold and increased pectin breakdown by 66%. There was a synergistic effect of PG plus expansion on the breakdown of cell walls by 3.5 fold and pectin by 5.5 fold. Increasing lupins from 10 to 20% reduced food intake by 4%, reduced digestibility by 5%, reduced weight gain by 11% and reduced the efficiency of food conversion by about 8%, increased viscosity by 32%, water intake by 10% and faecal moisture by 5%. In addition, breakdown of cell-wall polysaccharides was reduced by 20% and pectin by 28% when the proportion of lupins in the diet was increased. But PG reduced the faecal moisture of 20% lupin diets to the same amount as diets with 10% lupins. Broilers consuming dehulled lupins ate 5% more food, digested them better (5%), grew faster (16%), and better converted food into weight gain (10%) than broilers consuming whole lupin diets. Dehulling lupins reduced cell-wall polysaccharides by 26% and pectin by 5%. Hence, dehulled lupins were less viscous, reduced water intake of birds by 6% and faecal moisture by 3%.

IV. DISCUSSION

The hypothesis was rejected because expansion did not appear to increase the activity of PG and improve performance of the birds as was anticipated. When lupins were expanded and then treated with PG, growth performance and values for wet droppings were similar to the control. Yet expansion was very effective at breaking down both cell walls and pectin. Expansion may have had a negative influence on nutritive value of lupins and there are three possibilities. First, the heat of expansion may have solubilised the insoluble fractions of the cell-wall polysaccharides which in turn may have increased viscosity (Fadel *et al*., 1988; Vukic-Vranjes *et al*., 1994). This effect was more pronounced with whole than dehulled lupins because whole lupins contain 25% fibrous hulls of which 85-95% is insoluble fibre (Harris and Jago, 1985; Evans *et al*., 1993).

Table 1. Responses of broilers to expansion of lupins and PG supplementation.

Treatment	Whole lupins		Dehulled lupins		Mean
	10%	20%	10%	20%	
Weight gain (g/bird/day)					
Control	47.3	42.3	55.7	49.6	48.7[a]
PG	50.2	44.1	58.9	51.8	51.3[b]
Expansion	47.4	42.7	55.7	48.9	48.7[a]
Expansion+PG	48.7	43.2	56.7	48.7	49.3[a]
s.e.m.	1.1	0.9	1.2	1.1	1.1
Food conversion ratio (food g : gain g)					
Control	2.75	2.93	2.46	2.64	2.70[a]
PG	2.61	2.81	2.34	2.53	2.57[b]
Expansion	2.83	3.02	2.53	2.78	2.79[a]
Expansion+PG	2.75	3.01	2.47	2.77	2.75[a]
s.e.m.	0.03	0.04	0.03	0.04	0.04
Digestibility of dry matter (%)					
Control	51.7	49.6	53.5	51.6	51.6[a]
PG	57.9	52.6	60.7	55.0	56.6[b]
Expansion	50.1	48.3	51.6	51.0	50.3[a]
Expansion+PG	51.3	49.4	55.0	52.2	52.0[a]
s.e.m.	1.1	1.1	1.2	0.9	1.1
Apparent metabolisable energy (MJ/kg)					
Control	11.0	10.2	11.3	10.4	10.7[a]
PG	11.6	10.6	11.9	10.8	11.2[b]
Expansion	10.7	10.0	11.0	10.0	10.4[a]
Expansion+PG	11.1	10.1	11.5	10.3	10.7[a]
s.e.m.	0.2	0.3	0.2	0.2	0.2
Viscosity (m.Pas/sec.)					
Control	5.11	6.91	6.30	7.93	6.56[a]
PG	4.08	6.42	4.73	7.45	5.67[b]
Expansion	5.72	7.95	6.71	8.57	7.24[c]
Expansion+PG	5.16	7.02	6.43	8.04	6.66[a]
s.e.m.	0.2	0.2	0.2	0.2	0.2
Water intake (ml/bird/day)					
Control	305	330	321	350	327[a]
PG	283	319	299	336	309[b]
Expansion	314	342	333	363	338[c]
Expansion+PG	311	337	328	356	333[d]
s.e.m.	2	2	2	2	2
Faecal moisture (%)					
Control	65.4	68.7	68.5	70.3	68.2[a]
PG	60.1	66.6	62.7	68.2	64.4[b]
Expansion	67.4	71.1	70.6	72.9	70.5[a]
Expansion+PG	65.7	69.8	67.5	71.7	68.7[a]
s.e.m.	1.2	1.1	1.0	1.1	1.1
Breakdown of cell-wall polysaccharides (%)					
Control	3.2	2.1	4.6	3.0	3.2[a]
PG	7.3	5.8	9.2	7.3	7.4[b]
Expansion	9.7	7.4	12.4	9.6	9.8[c]
Expansion+PG	10.6	9.4	13.6	10.3	11.0[c]
s.e.m.	1.2	1.3	1.3	1.4	1.3
Breakdown of pectin (%)					
Control	2.5	2.2	2.5	2.3	2.4[a]
PG	11.1	8.1	12.1	8.9	10.1[b]
Expansion	5.3	3.0	4.6	3.1	4.0[a]
Expansion+PG	15.0	11.2	16.3	11.2	13.4[c]
s.e.m.	1.2	1.3	1.4	1.3	1.3

Means within columns with different superscripts differ significantly ($P<0.05$).

Second, expanded lupins have a higher absorptive capacity for water because of the "puffing" effect. This increases porosity of the final product and, consequently, increased water intake by the birds and increased wet droppings. Third, the heat generated during expansion could have damaged some of heat-sensitive nutrients such as lysine due to the Maillard reaction (Fadel *et al*., 1988).

PG offset the negative effects of expansion and, on its own, was very effective at reducing viscosity of digesta, water intake and wet droppings. It increased the digestibility of dry matter and growth rate but only at the 10% lupin inclusion not the 20% rate. The reason was that PG reduced food intake of birds fed 20% lupins but improved the digestibility of the diets. The net result was that birds received similar amounts of useable energy. Although PG did not increase growth rate of birds fed 20% lupins it did reduce wet droppings to a similar level to that seen in birds eating 10% lupins without enzyme treatment.

In conclusion, there was no synergistic interaction between PG and expansion. Breakdown of cell walls by expansion was not reflected by an increase in growth rate. PG on its own can improve the nutritive value of lupins for broilers and reduce wet droppings, particularly on diets containing 10% whole or dehulled lupins.

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THE RESPONSE OF BROILERS TO DIETARY DIGESTIBLE LYSINE LEVELS IN THE STARTER PHASE

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Adequate dietary lysine supply is crucial for the optimal performance of broiler chickens. In most published research total lysine value is used for diet formulation. It has been demonstrated that the performance of broilers fed diets formulated using digestible amino acid values was superior to that of birds fed diets formulated using total amino acid values (Li *et al*., 2002). The current study was to determine the optimal dietary digestible lysine level for production performance.

Day-old male Ross chicks and semi-purified diets with added synthetic amino acids were used in this study. All dietary digestible amino acids concentrations were based on the ideal amino acid concept (Baker, 1997) except lysine which was formulated at 6, 8, 10, 12 and 14 g/kg diet, respectively. Chicks were weighed, wing tagged and allocated to five dietary treatments with five replicates of seven birds in each diet. Individual body weight and pen feed intake were recorded weekly and feed efficiency was calculated.

Birds body weights were significantly increased from 378g to 812g ($p<0.0001$) at day 21 when dietary digestible lysine content was increased from 6g to 12g/kg. Feed intake from day 1 to 21 was also significantly increased ($p<0.001$) as the lysine content increased from 6g to 10g/kg. Feed conversion rates were substantially reduced from 2.06 to 1.41 ($p<0.0001$) when lysine content increased to 12g/kg. No beneficial effects were observed on production performance with further increase of lysine content to 14g/kg. The results from this study suggest that for optimal performance of broiler chicken during the starter phase, dietary digestible lysine content of 12g/kg is adequate.

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USE OF PREGERMINATION OF GRAINS AND OILSEEDS TO IMPROVE FEED VALUE AND GUT DEVELOPMENT IN BROILER CHICKENS

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Summary

It is hypothesised that the natural chemical processes that are activated when seeds germinate will have a beneficial effect on improving nutrient availability to broiler chickens and feed efficiency. The aims of optimal germination procedures of both grains and oilseeds were to maximise endogenous seed enzyme activity and reduce antinutritional factors. Male broiler chickens (288) were utilised to investigate the effect of a time-limited germination procedure (pre-germination) on bird performance, anatomical measurements and retention of dry matter and energy were taken. The data indicate that pre-germination lowered digesta viscosity, and improved digestibility and apparent metabolisable energy (AME). However, pre-germination of grains and oilseeds did not improve growth and feed efficiency. The variation in response observed with germination of grains (and oilseeds) warrants further investigation, recognising there are many factors that influence germination and, potentially, feed value.

I. INTRODUCTION

The use of exogenous enzymes, balanced diets, in-feed antimicrobials and feed processing techniques enable birds to ingest, digest, absorb and retain nutrients more effectively and efficiently. While these techniques are successful, the industry is interested in new and, in the case of in-feed antimicrobials, alternative additives and processes to increase feed value of ingredients and health of birds.

Previous research by Scott and Campbell (1998) demonstrated that pre-germination techniques applied to wheat and barley significantly improved feed intake and growth without a significant effect on energy availability. Work by Hughes and van Barneveld (2004) demonstrated a significant increase in AME in germinated barley (20 and 48h) but no observable effect with sorghum, triticale and wheat grain types. Optimal germination procedures in terms of increasing feed nutrient utilisation would therefore be considered as a viable 'natural' (green technology) alternative feed processing treatment for broiler chicken production.

II. MATERIALS AND METHODS

Preliminary studies were conducted to define optimum conditions for achieving radical emergence, defined as pre-germination by Bruggink (2004), of wheat, canola and sorghum seeds in sufficient quantity (dried) for use in broiler bioassay diets. Germination temperatures were set at 20 C for canola and wheat and 30 C for sorghum (Martin *pers comm*., 2005). Water was added to grain to provide final moisture levels of approximately: 45%, 36% and 44%, respectively for canola, sorghum and wheat. A cement mixer was used as the germination vessel and fixed with a timer to provide regular short mixes obtain even germination. Addition of water was specific for each grain to minimise leaching of nutrients out of the grain. All germinated grain was dried back (40 C for 48h) to less than 10% moisture level to inhibit microbial growth.

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The germinated (0 or 21 h) canola, sorghum and wheat were used to produce six diets; each was fed with (0.0 or 0.5 g/kg diet) supplemental enzyme (Phyzyme (phytase) and Avizyme 1302 (xylanase + protease); Finnfeeds Ltd, Marlborough, UK; supplied by Feedworks Pty Ltd). These 12 diets were balanced to supply nutrient requirements (NRC, 1994) for broiler chicks of 0 days to an age of 21 days (Table 1). The diets contained 1.0% Celite® as an acid insoluble ash marker for determining energy digestibility (AME kcal/kg diet; Scott *et al*., 1998) based on excreta collected at 19 d, which was freeze-dried. The trial was performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes .

The 12 diets and water were provided *ad libitum* to four pens of six male broilers from 0 to 21 d of age. Room temperature was monitored daily and reduced from 32 C to 24 C over the experimental period. Individual bird weights were recorded at 0, 7, 14 and 21 days. Feed intake was also recorded at these time intervals and used to determine FCR corrected for mortality.

At day 21 one bird was randomly selected per cage, weighed, humanely killed by cervical dislocation and digestive tract excised. Recordings included gizzard (empty and adhering fat removed), liver and breast weight, intestinal lengths (based on recording length of duodenal loop (not separated from pancreas), upper and lower (Meckels diverticulum to caecal junction) small intestine and caecum (total length)). Digesta was recovered from the upper small intestine (end of duodenum to Meckel's diverticulum) of each of the 48 birds to determine digesta viscosity.

The two middle toes from each of the sampled birds were collected and frozen for subsequent toe ash analysis. Total ash percentage was expressed as a percentage of total dry matter of the toes.

Data collected was analysed using Genstat® 8th edition (VSU International Ltd, UK.) and SAS statistical software (SAS/STAT® Software). Due to large results variation statistical analysis of individual body weight and digesta viscosity measurements required Log_e transformation. However, data is presented without transformation.

III. RESULTS AND DISCUSSION

Chickens fed canola-based diets recorded significantly higher relative gizzard, breast muscle and caecum length values than both sorghum- and wheat-based diets at 21 d. Additionally significantly higher body weight and feed intake and conversion values were recorded from chickens fed canola-based diets compared to both sorghum and wheat diets at 21 d. Enzyme addition had a significant positive effect on both body weight and feed conversion ratio at 21 d across all grain-type diets. There was a significant interaction effect observed between grain type and enzyme addition for viscosity measurements, with enzyme addition significantly reducing digesta viscosity in both canola and wheat diets but not sorghum-based diets. Digestibility, AME and ME efficiency values were all significantly lower in canola-based diets compared to both sorghum and wheat diets.

There was a positive effect of germination when canola seed was germinated and included in a broiler starter diet at 25% of the diet. Whereas, there was no significant effects of germination on sorghum diets (75% sorghum inclusion) and a reduction in growth of broilers fed germinated wheat-based diets (Table 2). This would indicate an overriding positive effect of germination of canola seed.

Table 1. Dietary ingredients (g/kg diet), estimated ME and actual ME values (MJ/kg diet)

Ingredients	Wheat	Sorghum	Canola
Estimated ME value	13.21	13.23	13.93
Actual ME values (ave.)	10.90	13.30	9.60
		g / kg diet	
Wheat (11%)	750.0	–	233.3
Sorghum (9%)	–	750.0	250.0
Canola seed	–	–	250.0
Corn gluten (60%)	50.0	72.0	–
Soy protein isolate	69.0	70.0	–
Soybean meal (48%)	–	–	183.9
Fishmeal (64%)	60.0	65.0	–
Tallow	28.5	–	–
Meat meal (52%)	–	–	40.0
Salt	1.3	1.2	1.5
Limestone	9.0	8.7	9.9
Dicalcium phosphate	13.8	14.5	9.9
Lysine	4.0	4.1	2.8
DL-Methionine	1.2	1.3	1.8
Threonine	0.6	–	–
Vitamin mineral premix	2.0	2.0	2.0
Choline chloride (60%)	0.6	1.2	5.0
Celite©	10.0	10.0	10.0
Ethoxyquin	0.5	0.5	0.5
Phytase	0.5^	0.5^	0.5^
Xylanase	0.5^	0.5^	0.5^

Note: % values indicate industry average crude protein levels

^ only added to enzyme positive diets

The lack of response of sorghum-based diets to germination treatment is perhaps indicative of a failure of the specific germination conditions applied here to invoke biochemical changes within this grain type in the time span allowed (21 hours). There is an extensive array of environmental variants (i.e., light, moisture, humidity, temperature, time, additives (soluble organic acids and germination enhancers) and grain type and variety) that can affect the process of germination and its nutritional outcomes.

The consistently negative influence of germination treatments on performance characteristics on broilers fed wheat-based diets conflicts with previous work performed by Scott and Campbell (1998). However, the germination procedure in their work utilised more sophisticated equipment to enable unimpeded water uptake, access to oxygen and thus a uniformly pre-germinated product. Additionally their germinated product was ground to a mash whilst wet (germinated) and then dried, possibly allowing greater enzymatic and phytochemical access (from the aleurone layer) to endosperm and thus more complete breakdown of antinutritional factors. It is possible that with the drying back of whole germinated wheat grain there was insufficient time and or access for endogenous enzymes to act upon endosperm substrates.

Table 2. Effects of germination period (0 and 21h) and enzyme addition (0.0 and 0.5g/kg diet) on mean body weight measurements at 21 d for canola-, sorghum- and wheat-based diets

	Germination (h) (P=0.23)		Enzyme addition (g/kg diet) (P=0.24)	
Canola-based diets	0	672.6 ± 120.3	0.0	665.3 ± 146.5
	21	704.3 ± 136.9	0.5	711.4 ± 106.5
Sorghum-based diets	0	674.7 ± 119.8	0.0	663.3 ± 100.4
	21	676.2 ± 104.2	0.5	687.8 ± 122.4
Wheat-based diets	0	686.7 ± 157.3	0.0	644.3 ± 147.2
	21	649.6 ± 143.9	0.5	694.4 ± 152.5

The time span over which germination is allowed to continue, the degree of sprouting progression and the conditions seeds are exposed to over that progression influences the biochemical changes within the seed itself (Finney, 1983). It is likely that the lack of specialised germination equipment to achieve optimum germination and thus an optimum feed product was a limitation in the ability of these trials to consistently demonstrate hypothesised responses to germination treatments. Significant changes in energy value or feed intake of germinated seeds/grains were not observed, possibly supporting the notion that our germination procedures and equipment did not enact sufficient biochemical changes within the seed to replicate observations of Scott and Campbell (1998). The potential for the natural process of germination to fulfil these improvements requires further exploration. The activity of endogenous seed enzymes in reducing anti-nutritional factors such as phytate in whole or ground wet germinated seed and or protein meals has to date not been examined closely.

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EFFECTS OF EXTENDED PREGERMINATION AND FORMIC ACID ON FEED VALUE OF WHEAT TO BROILER CHICKENS

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Summary

The effects of extended pregermination on improving wheat grain nutritional value to broiler chickens were explored in a broiler bioassay. It was hypothesised that longer germination times and the addition of formic acid would (1) allow greater time for endogenous enzyme activity to occur; and (2) inhibit microbial proliferation during the germination process. Male broiler chickens (288) were utilised to investigate the effect of a time-limited germination treatments on bird performance, anatomical measurements and retention of dry matter and energy. Results demonstrate that extended pre-germination time and the addition of formic acid lowered digesta viscosity, and improved digestibility and apparent metabolisable energy (AME). However, as demonstrated previously pre-germination of wheat grains did not improve bird growth and or efficiency.

I. INTRODUCTION

There is continued industry interest in developing alternative measures to inhibit and control in-feed microbial growth in addition to developing new processing techniques that enable birds to more efficiently retain nutrients from grain sources. Previous research was aimed at developing optimal germination procedures to improve feed utilisation (Tomkinson and Scott, 2006). These studies, particularly with the wheat source tested, raised concerns that the germination environment may favour microbial proliferation and negatively impact bird performance and health. In order to address this, the present study evaluated pregermination but also tested acidification as a means of minimising microbial contamination during germination. Farran *et al*. (2005) demonstrated diets prepared with the legume seed 'ervil' soaked in acetic acid (1% solution; 1:10 weight/volume) at 40°C for 24 hours led to broiler and layer performance improvements relative to other dietary treatments. To date there is little published data on the effect of adding organic acids during the germination process to acidify the feed product in an attempt to reduce feed microbial contamination and increase the feed value (impact on growth, efficiency, bird health and industry sustainability) of cereal grains.

II. MATERIALS AND METHODS

Germination procedures for preparing wheat diets are outlined in Tomkinson and Scott (2006). In the present study a total of eight dietary treatments were employed and each was fed to six cages of six male broilers from 0 to 21 d. The bioassay focussed on wheat germinated for 0, 21 and 42 h (under controlled moisture, lighting, aeration and temperature conditions described by Tomkinson and Scott, 2006); in addition a fourth treatment of 42 h germination with 3g/kg potassium diformate (Formi®; BASF) was fed to reduce pH and microbial contamination. Each diet was formulated with and without enzymes and identical measurements taken as described in Tomkinson and Scott (2006). Mortality in this study was high (~10%), however, there were no statistical treatment effects associated with mortality and it was assumed to be related to overall chick quality. The trial was performed in

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accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

III. RESULTS AND DISCUSSION

The addition of enzymes to all pregerminated wheat grain diets consistently resulted in significant improvements in body weight (g) at 7, 14 and 21 d and feed intake and conversion ratio 0-7, 7-14, 14-21 and 0-21 d (Table 1). Enzyme addition also significantly improved breast muscle weight (g) and significantly reduced gizzard weight (g), and all gut segment measurements expressed relative to body weight (Table 2). The extended germination time of 42 h (compared to 0 and 21 h) resulted in non-significantly higher digestibility and AME values recorded from chickens fed these pregerminated wheat-based diets (data not presented).

The addition of formic acid to wheat diets prevented germination as defined by radicle emergence. However, based on the changes to digesta viscosity it would still appear that the hydration process activated endogenous enzyme(s) and reduced digesta viscosity. Formate ions (formed by the dissociation of potassium diformate) have the potential to chelate essential minerals and proteins and remove their availability for use during germination (Newkirk *pers comm*., 2005). The formate ions would also be expected to decrease pH and alter enzyme function. This could be one such explanation for the grain failing to sprout when 3 g/kg of potassium diformate was added to the grain during germination. What appeared to be signs of microbial contamination of germinated wheat in the first study (Tomkinson and Scott, 2006) were not present in any of the germinated samples in the present study.

Table 1. Mean body weight (g) and viscosity (cPs) at 21 d [Mean ± Std Dev] and feed intake (g / bird / d) and feed conversion ratio (FCR; g feed : g gain) from 0-21 d for four different germination treatments (0h, 21h, 42h and 42h with formic acid (3.0 g/kg grain) and with and without exogenous enzyme addition (0.5g/kg diet)

	Body weight (g)	Viscosity (cPs)	Feed intake (g / bird / d)	FCR (g feed : g gain)
Germination		NS		
0 h	632 ± 141.5	23.6 ± 28.28	41.3 ± 5.63	1.54 ± 0.105
21 h	614 ± 144.3	16.7 ± 17.22	39.9 ± 5.01	1.53 ± 0.103
42 h	612 ± 146.7	19.1 ± 22.29	37.7 ± 6.26	1.53 ± 0.149
42 h + formic acid	625 ± 155.6	10.8 ± 8.81	38.2 ± 5.40	1.51 ± 0.118
Enzyme		**		
0.0 g/kg diet	553.4 ± 120.6	30.8 ± 21.73	36.6 ± 4.51	1.61 ± 0.077
0.5 g/kg diet	688.6 ± 138.7	4.3 ± 2.00	41.9 ± 5.38	1.45 ± 0.087

**, NS - represent P-values of P<0.01, P>0.05

Formic acid addition was beneficial in raising average body weights (not significantly) over and above 21 and 42 hours germination (without formic acid addition) but not the control diet (0 hours) at 21 d (Table 1). Birds fed diets containing germinated wheat with formic acid also recorded the lower digesta viscosity values with the least amount of variation than the other three germination treatment diets (Table 1). These figures would

indicate that in fact endogenous enzyme activity and germination related chemical reactions may have taken place within the grain even without growth of the embryonic axis.

Table 2. Gut measurements at 21 d expressed as a percentage relative to body weight [Mean ± Std Dev] for four different germination treatments (0h, 21h, 42h and 42h with formic acid (3.0 g/kg grain) and with and without exogenous enzyme addition (0.5g/kg diet)

	Breast muscle weight % (g)^	Gizzard weight % (g)^	Duodenal loop length % (cm)	Upper SI length % (cm)	Lower SI length % (cm)	Caecum length % (cm)
Enzyme	**	**	**	**	**	*
0.0 g/kg diet	16.3 ± 1.41	2.3 ± 0.30	2.0 ± 0.29	10.5 ± 1.41	9.5 ± 1.20	4.2 ± 0.64
0.5 g/kg diet	17.6 ± 1.90	2.0 ± 0.25	1.7 ± 0.29	8.3 ± 1.36	8.0 ± 1.10	3.8 ± 0.64

**, *, NS - represent P-values of P<0.01, P<0.05, P>0.05

^ denotes data not adjusted for missing values

The significant germination and enzyme interactive effect on relative lower small intestine length indicates that germination processes were possibly involved in reducing the NSP content of the diets (Figure 1). The significant reductions in relative lower small intestinal length at 0 and 21 hours germination with enzyme addition were not observed with 42 and 42 hours + formic acid germination treatments. This may indicate that the extended germination time might have allowed for more complete NSP breakdown and thus reduced the amount of fibre reaching the lower gastrointestinal tract. A similar trend (although non-significant) was observed in relative caecum length data.

IV. CONCLUSION

The time span over which germination is allowed to continue, the degree of sprouting progression and the conditions seeds are exposed to over that progression influences the biochemical changes within the seed itself (Finney, 1983). Although most other studies using germinated grains have not used organic acids to control microbial contamination, the present study indicates there may be an interesting possibility of arresting germination and related nutrient utilised to support grain embryo development while still achieving enzyme activation through high moisture exposure. The potential for the natural process of germination to fulfil these improvements requires further exploration. The activity of endogenous seed enzymes in reducing anti-nutritional factors such as phytate in whole or ground wet germinated seed and or protein meals has to date not been examined closely.

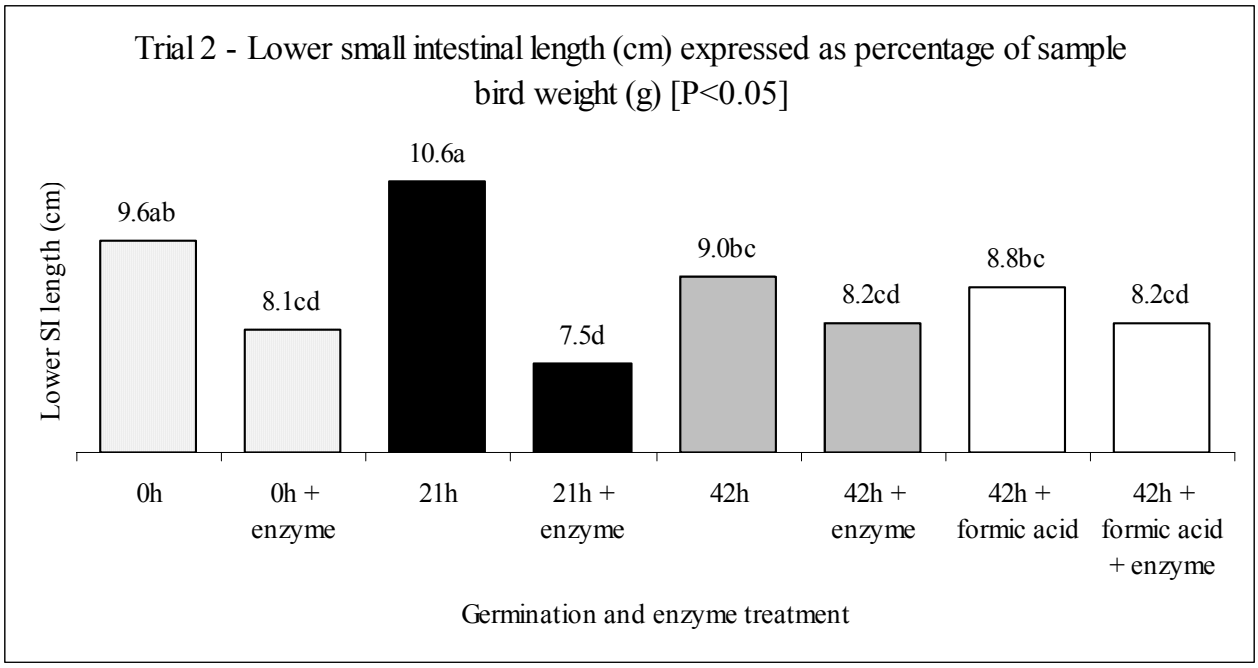

Figure 1. Mean lower small intestinal (cm) measurements at 21 d [Mean ± Std] expressed as a percentage of sample bird weight (g) for wheat-based diets germinated for 0h, 21h, 42h and 42h + formic acid with and without enzyme addition (0.5g/kg diet).

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PERFORMANCE OF LAYERS FED XYLANASE, PHYTASE OR BOTH IN LOW PHOSPHORUS DIETS

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Summary

This study was conducted to demonstrate the effects of xylanase and /or phytase in a standard P and a low P wheat and sorghum based layer diet. The two basal diets (standard P and low P) were fed as is and supplemented with xylanase, phytase or both, and offered to 27 weeks old ISA brown layers for a period of 27 weeks. Hen day and hen housed egg production were significantly lower for birds fed the low P diets than those fed the standard P diets. However, the egg production and feed conversion ratio of hens fed the low P diets were significantly improved by phytase supplementation. Average egg weight and feed intake were not significantly affected either by level of phosphorus in the feed or by enzyme supplementation.

I. INTRODUCTION

Due to the lack of endogenous phytase enzyme in chickens to hydrolyse dietary phytate bound phosphorus, only 30-40 per cent of the phosphorus from plant sources is freely available for digestion and absorption by poultry (Nelson, 1976). The current practice to make up the phosphorus needs of chickens is to include either meat and bone meal or inorganic phosphorus in the feed. The use of meat and bone meal in poultry diets or in stock feed is restricted or prohibited by public health authorities in some European countries and this may happen in other countries in the near future. Another conventional source of phosphorus for chicken feed is inorganic phosphorus in the form of dicalcium or mono-calcium phosphate.

Undigested and unutilised inorganic phosphorus and phytate phosphorus residues are excreted. High phosphorus levels in excreta cause a major environmental problem when animal waste is used as a manure for growing crops (Ravindran *et al*., 1998). It has been shown that phytase supplementation of the feed improves phytate phosphorus utilisation in laying hens (Gordon and Roland, 1997; Van der Klis *et al*., 1997; Carlos and Edwards 1998; Um and Paik,1999; Jalal and Scheideler, 2001; Scott *et al.,* 2001). In addition to the phytic acid, soluble non-starch polysaccharides are one of the factors inhibiting the growth and production performance of broilers and layers fed wheat or barley based diets. It has been demonstrated that anti-nutritional effects of non starch polysaccharides in broiler and layer diets can be counteracted by supplementing the diet with xylanase or beta-glucanase enzymes (Annison and Choct, 1991).

This study was undertaken to compare the effects on egg production of adding xylanase or phytase or both to a standard phosphorus or a low phosphorus sorghum-wheat-soybean meal based layer diet.

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II. MATERIALS AND METHODS

A total of 504, twenty seven weeks old, ISA Brown layers, were allocated three to a cage in a standard layer housing facility. There were two basal diets, a standard phosphorus diet and a low phosphorus diet. Each basal diet contained sorghum, wheat and soybean meal as major ingredients to provide 11.28 MJ, ME/ Kg, 15.53 % crude protein, 0.5 % methionine plus cystine, 0.7 % available lysine and 3.5% calcium. The major difference between these two diets was the available phosphorus content. The standard-P and low-P diet had an available phosphorus content of 0.31 and 0.12 %, respectively. Each basal diet was supplemented with no enzyme, xylanase, phytase or both, and the enzyme activity of each experimental diet was analysed. Each diet was offered to 21 replications of three hens per cage. The eight experimental mash diets were fed for a period of 27 weeks.

Daily egg production was recorded for the twenty seven weeks of the trial and all eggs laid in the day were weighed once every four weeks. Feed intake was measured during the trial. Yolk colour and albumen qualities were measured using a Roche colour fan and Haugh Unit meter respectively, in the last week of the trial.

Data were subjected to analysis of variance to test for the probability of significant differences among the means and least significant differences (LSD) were used to test for significant differences between means.

III. RESULTS AND DISCUSSION

The production performance of the hens fed the standard phosphorus diets and the low phosphorus diets with or without enzyme supplements is presented in Table 1. Hen housed and hen day egg production of hens fed the standard-P diet was significantly higher than those fed the low-P diets. Supplementation of the standard-P diet with xylanase, phytase or both had no significant effect on hen housed or hen day egg production. In contrast, supplementation of the low-P diet with phytase significantly improved hen housed and hen day egg production compared with those fed the unsupplemented low-P diets. However, the addition of xylanase to these diets did not significantly improve the hen housed or hen day egg production. Hens fed low-P diets supplemented with xylanase alone did not have a significantly improved egg production over those fed the unsupplemented low-P diets.

Egg weight was not significantly affected either by level of phosphorus in the diet or supplementation of xylanase and/ or phytase. Egg mass was significantly ($P<0.05$) higher in groups fed the standard-P diet compared with those fed the low-P diets without enzyme supplements. Egg mass produced by the hens fed the low-P diet supplemented with phytase was significantly higher than those fed the unsupplemented low-P diets. There was no significant improvement in egg mass produced by adding xylanase to phytase supplemented low-P diets.

Feed intake was not significantly influenced either by phosphorus concentration in the diet or enzyme supplementation of these diets. Feed conversion ratio was not significantly different between the groups fed standard-P or low-P diets. Xylanase or phytase supplementation of the standard-P diet had no significant effect on FCR. However, birds fed the phytase supplemented low-P diets significantly improved their feed conversion ratio compared with those fed the unsupplemented low-P diets. Excreta moisture was significantly lower in groups fed the low-P diets than the other groups in the trial.

Yolk colour was significantly higher in groups fed the low-P diets supplemented with both xylanase and phytase than in the other groups. The Haugh unit value was significantly lower in groups fed the standard-P diet than in the other groups. However, the Haugh unit value was significantly improved by supplementation of the standard-P diet with xylanase

and phytase. Neither phytate phosphorus concentration in the diet nor supplementation of the diets with xylanase and/or phytase had any significant effect on shell thickness or mortality.

Reduced available phosphorus concentration in sorghum-wheat-soybean meal based layer diets significantly reduced egg production performance of ISA brown layers. However egg production performance of the hens fed these low-P diets can be significantly improved by supplementing with phytase at 450U per kg of feed. Similar results were reported in phytase supplemented groups by Gordon and Roland (1997), Van der Klis et al. (1997), Carlos and Edwards (1998), Um and Paik (1999), and Jalal and Scheideler (2001). Supplemented phytase might be breaking down or hydrolysing phytate bound phosphorus resulting in increased available phosphorus for egg production performances (Um and Paik, 1999; Jalal and Scheideler, 2001). There was no synergistic effect on egg production performance of supplementing low-P diets with xylanase and phytase. Supplementation of a standard-P diet with xylanase, phytase or both had no significant effect on egg production performance.

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Table 1: Effects of xylanase and phytase in standard-P and low-P diets on layer performance

Measurements	Control Standard P (CSP)	CSP + Xylanase	CSP + Phytase	CSP + Xylanase + Phytase	Control Low P (CLP)	CLP + Xylanase	CLP + Phytase	CLP + Xylanase+ Phytase	LSD
Production performance									
Egg production (HH) (%)	95.60^{a}	95.61^{a}	94.74^{ab}	94.07^{ab}	92.72^{b}	94.23^{ab}	95.43^{a}	95.67^{a}	2.38
Egg production (HD) (%)	95.60^{a}	95.61^{a}	94.74^{ab}	94.07^{ab}	92.72^{b}	94.71^{ab}	95.43^{a}	95.67^{a}	2.21
Egg weight (g/egg)	64.41^{a}	64.73^{a}	63.86^{ab}	64.06^{a}	63.73^{ab}	63.87^{ab}	63.93^{ab}	62.98^{b}	1.04
Egg mass (g/hen/day)	61.58^{a}	61.90^{a}	60.52^{ab}	60.25^{ab}	59.07^{b}	60.18^{ab}	61.00^{a}	60.27^{ab}	1.80
Feed intake (g/hen/day)	115.06	114.80	114.65	114.74	112.83	112.79	112.67	112.72	3.598
FCR (g feed/g egg)	1.869^{abc}	1.854^{bc}	1.894^{abc}	1.904^{ab}	1.912^{a}	1.863^{abc}	1.847^{c}	1.870^{abc}	0.057
Mortality (%)	0	0	0	0	0	3.14	0	0	3.159
Excreta Moisture (%)	66.19^{ab}	69.18^{ab}	69.76^{a}	70.47^{a}	63.32^{b}	71.22^{a}	71.56^{a}	68.83^{ab}	6.22
Egg quality parameters									
Yolk colour (Roche scale)	10.23^{b}	10.66^{b}	10.61^{b}	10.57^{b}	10.38^{b}	10.52^{b}	10.28^{b}	11.76^{a}	0.44
Haugh unit	70.42^{b}	73.81^{ab}	75.52^{a}	72.33^{ab}	75.52^{a}	74.76^{ab}	73.04^{ab}	73.71^{ab}	4.68
Shell thickness (mm)	0.308	0.307	0.312	0.303	0.313	0.313	0.320	0.311	0.020

Means with the same superscripts are not significantly different ($P<0.05$)

THE RELATIONSHIP OF PER AND AME MEASUREMENTS WITH THE GROWTH PERFORMANCE OF BROILERS FED WITH THREE DEHULLED SOYBEAN MEALS OF DIFFERENT ORIGINS

S.B. NEOH[1] and L.E. NG

Summary

Protein efficiency ratio (PER) and apparent metabolizable energy (AME) may be good indicators to differentiate between the performance of different soybean meals (SBM). Broiler feeding trials were conducted to determine the PER, AME and growth performances of three different dehulled SBM. The results show significant differences in PER and AME between the three different SBM. Correlations between PER, AME and growth performances were observed, suggesting that AME and maybe PER measurements can be used to differentiate between the broiler growth performances of different SBM.

I. INTRODUCTION

Among protein sources, soybean meal has an excellent reputation for high amino acid quality. Dehulled SBM is preferred by animal producers due to its better balanced protein, lower fiber and higher energy levels. However, there still remains a large variation in the nutritive value of the SBM products available commercially. Due to the high variability of performances among SBM, there continues to be a great need for assays that are relatively simple, yet sensitive enough to differentiate between SBM of different quality. Proximate analysis, *in vitro* amino acid digestibility, trypsin inhibitor activity (TIA), potassium hydroxide protein solubility (KOHPS), protein dispersibility index (PDI) and urease activity do not accurately predict the actual performance of different normally processed SBM in poultry and swine nutrition (Neoh, 2003; Parsons *et al*., 1991; Vohra and Kratzer, 1991). However, these tests can detect poorly processed SBM, eg. a SBM with KOHPS of less than 70% is considered over processed and will not perform well (Araba and Dale, 1990). Unfortunately, most SBM available in the market will have very similar proximate and other chemical analysis. The challenge is to find a simple assay that can differentiate between the qualities of normally processed SBM and correlate it to animal growth performance.

The protein efficiency ratio (PER) assay has historically been used extensively for predicting the protein quality of foods for human consumption (Howe *et al*., 1965). The PER assay is simple in that it consists of feeding the test ingredient as the sole source of dietary protein. The classical PER assay is conducted with rats and to a lesser extend it has been used for poultry to evaluate various feed ingredients (Johnson and Parsons, 1997). The energy content of the diet has a large influence on the growth rate and feed utilisation of animals. Metabolizable energy (ME) is a reliable tool to determine the energy that is available in the diet for maintenance and production (MacLeod, 2002). Logically, biological determinants such as PER (Swick, 2003) and AME should be reliable indicators to differentiate the performance of different SBM. The purpose of this study is to determine the PER and AME of three dehulled SBM of different origins and correlate them to the growth performance of broiler chickens.

[1] Soon Soon Oilmills, Malaysia

II. MATERIALS AND METHODS

Seven hundred and thirty two day-old Arbor Acre male broiler chicks of the same hatch were fed a starter diet (Baker and Han, 1994) before the commencement of the PER and AME bioassays. For the PER study, a total of 432 chicks were housed in 24 floor pens. Eighteen eight-day-old birds were randomly allocated to each pen with six replicates for each treatment. The PER diets (Johnston & Coon, 1978) consist of 300g/kg of SBM as the only protein source were prepared from three dehulled SBMs originating from Soon Soon Oilmills of Malaysia (SS), United States (US) and Argentine (ARG). The diet with SS SBM was prepared in two forms, one in mash form the other in pellet form. The US and ARG diets were in pellet form. These test diets were fed to the chicks from day 8-17 for the PER determination. Data on initial, day 8 and 17 body weights, feed intake, feed conversion ratio (FCR) and PER were recorded. PER was calculated as the amount of weight gain per unit of protein consumed (Johnston and Coon, 1978; Johnson and Parsons, 1997).

For the growth performance trial and AME trial, 3 x 100 day-old chicks were fed respectively with three starter diets (Baker and Han, 1994) which were isonitrogenous and isocaloric and formulated with the above mentioned three SBMs. The chicks were then reared in floor pens until day 21. The weight gains and FCRs were recorded for each treatment. On day 22, five birds were assigned into each metabolism cage with six replicates for each AME diet (Annison *et at*, 1994). From day 25-28, excreta samples were collected daily and oven dried for AME determination. AME was calculated as intake energy minus excretory (fecal and urinary energy) losses (Sibbald, 1980). AME_n = [(GE feed x g feed consumed) - (GE excreta x g excreta) - (NR x K)] / (g feed consumed)]. GE is the gross energy while NR is the nitrogen retention which is assumed to be 6.25 divide by 20% of body weight gain or loss and K is the constant which equals to 34.35MJ/kg nitrogen gain/loss (Jiang and Pruekvimolphan, 2003).

III. RESULTS AND DISCUSSION

The results (Table 1) show that the 10-day PER bioassay is able to distinguish between the qualities of the three dehulled SBM. Significant improvements in FCR and PER were observed from the birds fed diets using SS SBM when compared to their counterparts. The FCR of SS SBM fed birds was significantly improved by 6.2%, 6.7% and PER was increased by 7% and 5% when compared with birds fed with diets using US and ARG SBM, respectively. The final body weight and body weight gain of the broilers fed with SS SBM were also significantly higher than those fed with ARG SBM. The improvements were 8.6% and 15.2%, respectively. Broilers fed with US SBM also had significantly higher final body weight and body weight gain than those fed with ARG SBM. These results are in agreement with similar findings reported by Mateo and Swick (2004). In comparison, the mash diet using SS SBM gave a lower body weight gain but significantly better FCR and PER than the equivalent pelleted diet. This suggests that the protein may be damaged during the pelleting process thus making it less available to the animal. The answer to the question of whether the PER study can be used to predict the 1-21 day growth performance of broilers fed with the three different SBM is shown in Figure 1 and 2. These results show that there maybe a correlation between PER and body weight gain as well as FCR for broilers grown to 21 days. More studies have to be done as there is insufficient data to draw a conclusion.

Table 1. Growth performance of broiler chicks fed different SBMs

Parameters	PER trial (day 8-17)						Growth trial (day 1-21)				
	SS(M)*	SS	US	ARG	SE	CV (%)	SSHE	US	ARG	SE	CV
Initial body weight, g	165.3	165.4	165.3	165.3	-	-	39	39	39	-	-
Final body weight, g	397.9[a]	411.8[a]	407.1[a]	379.2[b]	5.07	3.11	874	850	817	-	-
Body weight gain, g	232.6[a]	246.5[a]	241.8[a]	213.9[b]	5.05	5.30	836[a]	811[ab]	779[b]	8.45	2.38
FCR	1.843[c]	2.023[b]	2.149[a]	2.159[a]	0.04	4.55	1.294	1.319	1.35	-	-
PER	3.62[c]	3.37[b]	3.15[a]	3.21[ab]	0.06	4.41	-	-	-	-	-

[a,b] Means with different superscript within the same row differ significantly, P<0.05

* Mash form , others in pellet form

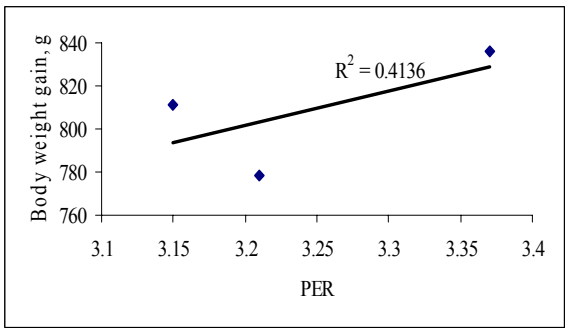

Figure 1. Relationship between body weight gain and PER

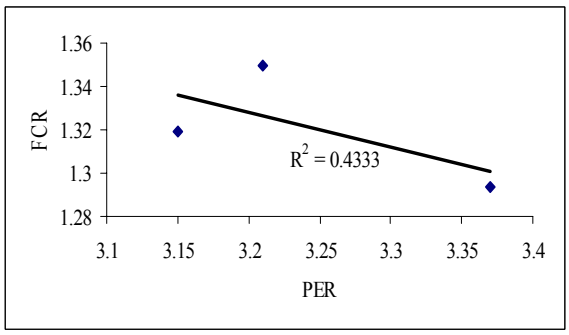

Figure 2. Relationship between FCR and PER

The results of the AME trials are presented in Table 2. Broilers fed with the diet using SS SBM had significantly the highest AME and AME_n when compared to their counterparts. The AME_n of SS SBM was 7.1% and 7.9% higher than US and ARG SBM respectively. The body weight gain (Figure 3) and FCR (Figure 4) for broilers grown to 21 days were plotted against AME_n. Increasing AME_n values led to improved body weight gains and FCRs. MacLeod (2002) claimed that ME is an index indicating what is available to the bird for maintenance and production, but not a predictor of how efficiently the birds then utilizes what is available. However, correlations shown in Figure 3 and 4 imply that the efficiency of feed utilization was improved as more energy was available for growth and production. Interestingly, there appears to be good correlation between PER and AME_n (Figure 5). In conclusion, it appears that AME and PER measurements can be used to distinguish between SBM of different qualities, and they may also be able to predict the broiler growth performances of different SBM.

Table 2. AME and AME_n of different SBM diets

	SSHE	US	ARG	SE	CV (%)
AME, MJ/kg DM	11.95[a]	11.22[b]	11.13[b]	0.168	3.59
AMEn, MJ/kg DM	10.60[a]	9.90[b]	9.82[b]	0.164	3.97

[a,b] Means with different superscript within the same row differ significantly, P<0.05

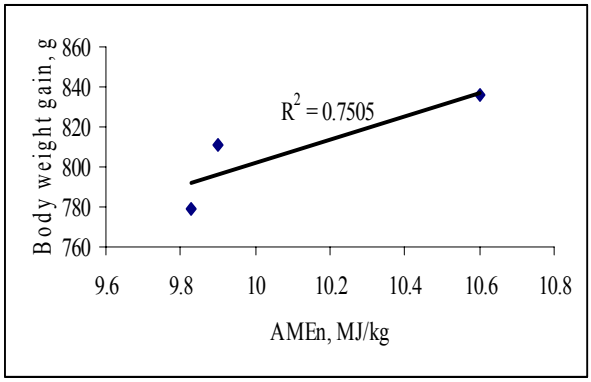

Figure 3. Relationship between AME_n and body weight gain

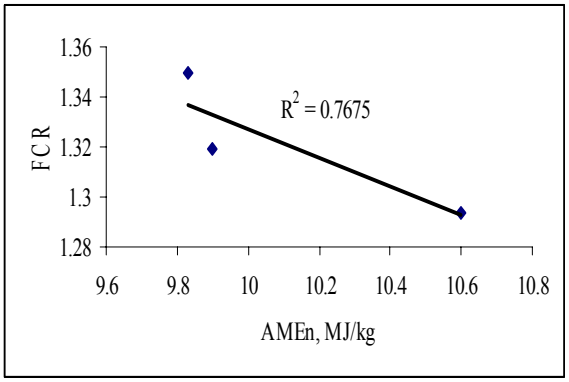

Figure 4. Relationship between AME_n and FCR

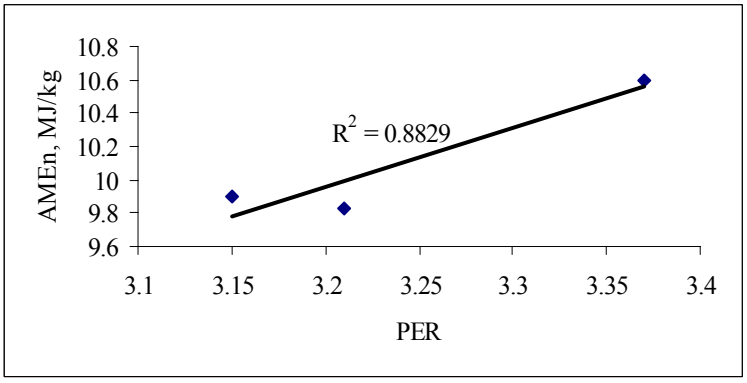

Figure 5. Relationship between AME_n and PER

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FEED VALUE OF WHEAT-, TRITICALE- AND SORGHUM-BASED DIETS SUPPLEMENTED WITH AND WITHOUT ENZYMES FOR BROILERS

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Summary

Defining the feed value of cereal grains and the contribution of supplemental enzymes for feeding broilers continues to evolve. The present study supports earlier work that for broiler chickens it is no longer sufficient to measure nutrient (i.e. energy) availability of cereal grains as an assessment of feed value/performance. We must also determine the variation in intake and its retention for growth, ideally as high-value muscle. Variation in feed intake (~24% in 34 wheat-based diets with enzyme) was positively associated with body weight (r^2>0.70; P<0.01) and FCR (r^2=-0.30; P<0.05); however, correlations with AME of diet were positive and not negative. Fifteen of the 34 wheat samples evaluated in this study were previously tested by the Premium Grains for Livestock Program. The present and past production trait measurements, but not AME levels, were significantly correlated. This indicates that factors within these common grains consistently influenced broiler performance in a like manner, but not AME determination. Further work is required to minimise variation in intake and growth.

I. INTRODUCTION

Defining differences in feed value of wheat due to genetics and environment (Scott *et al.,* 1998; McCracken and Quintin, 2000) will allow broiler producers to gain maximum benefit from a given grain sample. In the past, the principal goal of determining the feed value of wheat for broilers has been to measure the energy retention (AME). However, Scott *et al*. (1998) suggested that that determination of feed value of wheat-based diets should include nutrient intake as well as nutrient retention. Growth rate was directly related to voluntary intake and neither growth nor feed intake was strongly related to measurements of AME (Scott, 2005ab). Although supplemental enzymes resulted in increased intake of the diets, this supplementation did not negate differences in growth rate between sources of wheat or its relationship to feed intake. Black *et al.* (2005) summarised an extensive study to determine variation in feed value of Australian cereals for broiler chickens. Although the broiler bioassay (Black *et al.,* 2005) was different from Scott's there were very similar concerns regarding limitations in feed intake and its relationship to growth.

II. MATERIALS AND METHODS

In the present study, 45 grain (34 wheat, 8 triticale and 3 sorghum) samples were ground and included (750 g/kg) in mash diets (Table 1) formulated to meet or exceed the nutrient requirements for starting broilers (NRC, 1994). Each diet was split and supplemented with or without xylanase and phytase (0.5 g/kg diet Avizyme 1302 and Phyzyme; Danisco Animal Nutrition, Marlborough, UK; Feedworks Pty Ltd, QLD). All diets contained 10g/kg Celite™, an acid insoluble ash marker. The 90 diets were each fed *ad libitum* to one cage of six male broilers (Cobb 500; provided by Baiada Poultry Pty Limited, Cordina Hatchery; 4 to 17 d of age) in four consecutive bioassay series (i.e., four replicates/diet). Six control pens of birds were maintained on a commercial broiler starter in each of the four bioassay series to assess variability due to series. Although variation

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between series existed, it was deemed unnecessary to correct for these differences. At 16 d of age, a six hour excreta collection was conducted, excreta (free of feathers and feed contaminants) was frozen, freeze-dried and ground for determination of acid insoluble ash (Scott *et al.,* 1998) and gross energy to calculate AME (MJ/kg of diet). At the end of the study we determined body weight, feed intake (g/bird/d) and feed conversion ration (FCR) corrected for mortality. The study was approved by Sydney University Animal Ethics Committee.

A total of 15 of the wheat samples had been previously tested in a broiler bioassay conducted by Premium Grains for Livestock Program (PGLP). The PGLP samples were evaluated in a different broiler chick bioassay (Black *et al.,* 2005) with diets not supplemented with enzymes and fed to five-week of age broilers. AME in these studies were determined by total collection (3 days). All of the present grain samples were provided by PGLP and were maintained by the University of Sydney (I A Watson Wheat Research Centre, Narrabri NSW 2390). Data from the two bioassays were combined and used to determine repeatability of the measurements for growth, feed intake, FCR and AME.

II. RESULTS AND DISCUSSION

The study was principally designed to identify variability in feed value as previously described by Scott *et al.* (1998) to include measurements of nutrient level, availability / digestibility, intake and retention (i.e. growth and FCR). Further analysis will determine if these differences are influenced by gut physiology (immunological/ allergic response) and anatomy (villi development/ size).

Table 1. The dietary ingredient profile (and calculated AME and crude protein) of wheat-, triticale- and sorghum based diets. Diets were fed with (+) or without (-) enzyme.

Ingredient (g/kg diet)	Wheat- Triticale	Sorghum
Grain source	750.00	750.00
Vegetable oil	28.50	0.00
Isolate soy protein	69.00	70.00
Corn gluten meal	50.00	72.00
Fish meal (64%)	60.00	65.00
L-Lysine	4.00	4.10
DL-Methionine	1.20	1.30
Limestone	9.60	8.70
Dicalcium phosphate	13.80	14.50
Salt	1.30	1.20
Choline chloride	0.60	1.20
Vitamin and mineral premix	2.00	2.00
Celite™ (acid insoluble ash marker)	10.00	10.00
Phyzyme / Avizyme 1302	+/- 5.00	+/- 5.00
Nutrient profile – calculated		
AME (MJ/kg diet)	13.21	13.23
Crude protein (g/kg diet)	222.00	219.30

There were only three sorghum grains tested in the present study. Therefore, it is difficult to discuss variation between samples; however, variation in body weight, FCR and AME was numerically greater when diets were supplemented with enzyme. There was an unexpected significant ($P<0.05$) decrease in AME of sorghum diets due to enzyme supplementation. This may be a remnant of the small number of sorghum grains tested.

Variation in production traits and AME for the eight triticale diets supplemented with enzyme was high; body weight, feed intake and FCR varied by 30, 24 and 23%, respectively. Differences in AME of the eight diets was 14%, and as reported previously for other cereals (Scott, 2005), AME was not well correlated with feed intake, FCR or body weight (data for triticale not shown). The production traits and AME of diets were lower when diets were not supplemented with enzymes. However, the range in AME was less for the eight triticale diets when no enzymes were used. FCR of enzyme supplemented triticale diets was 1.46 for broilers 4 to 17 d of age; this is significantly higher than the FCR of 1.36 for the 34 wheat-based diets with enzyme. This is mentioned as the calculated AME for tritcale diets was 12.9 as compared to 12.0 MJ/kg diet for wheat.

Table 2. The mean, standard deviation and range for body weight (17d; g), feed intake (g/b/d 4-17d of age) and FCR (4-17d of age) of four cages of six male birds for each grain source with or without enzyme (Avizyme 1302 and Phyzyme).

Enzyme	Grain	Variable	Mean	Std dev	Minimum	Maximum	%Diff
5g/kg Avizyme 1302; 5g/kg Phyzyme	Sorghum (n=3)	Body wt 17d (g)	496	24.8	473	523	10.6
		Feed intake (g/b/d)	41.3	1.28	40.1	42.6	6.2
		FCR	1.42	0.033	1.39	1.45	4.3
		AME MJ/kg diet	12.6	0.98	11.5	13.5	17.4
	Triticale (n=8)	Body wt 17d (g)	493	39.5	417	542	30.0
		Feed intake (g/b/d)	40.9	2.90	35.6	44.1	23.9
		FCR	1.46	0.108	1.32	1.62	22.7
		AME MJ/kg diet	12.9	0.68	12.1	13.8	14.0
	Wheat (n=34)	Body wt 17d (g)	533	24.2	476	574	20.6
		Feed intake (g/b/d)	43.4	2.19	40.0	49.5	23.8
		FCR	1.37	0.061	1.30	1.54	18.5
		AME MJ/kg diet	12.0	0.86	9.6	14.0	45.8
No Enzyme	Sorghum (n=3)	Body wt 17d (g)	487	15.8	471	502	6.6
		Feed intake (g/b/d)	40.6	1.97	39.0	42.8	9.7
		FCR	1.41	0.024	1.39	1.43	2.9
		AME MJ/kg diet	13.6	0.52	13.0	14.1	8.5
	Triticale (n=8)	Body wt 17d (g)	468	30.7	411	496	20.7
		Feed intake (g/b/d)	38.2	2.10	35.3	41.3	17.0
		FCR	1.44	0.072	1.36	1.60	17.6
		AME MJ/kg diet	12.4	0.58	11.4	13.3	16.7
	Wheat (n=34)	Body wt 17d (g)	481	34.2	409	546	33.5
		Feed intake (g/b/d)	39.9	2.83	34.9	45.7	30.9
		FCR	1.45	0.085	1.27	1.60	26.0
		AME MJ/kg diet	11.6	1.25	8.5	14.3	68.2

The variation in body weight, feed intake and FCR for wheat-based diets with enzyme was 20.6, 23.8 and 18.5%, respectively. This variation reflects that reported earlier by Scott *et al.* (1998), Black *et al.* (2005) and Scott (2005ab). Variation in AME for enzyme supplemented wheat-based diets was over 45%. Also as reported earlier, there are high positive correlations between body weight gain and feed intake, supporting Scott's (2005ab) concerns that limitation in feed intake are limiting body weight (Table 3). The bird's feed intake was positively correlated (r^2=0.30; P<0.05) with AME of diets. If broilers were

capable of eating for their energy requirements one would expect a significant negative correlation with AME. Scott (2005b) associates the positive correlation between feed intake and AME to be due to those diets having higher AME also having fewer factors that limit digesta passage and higher consumption of feed and thereby limit growth.

Although only 15 wheat samples were previously evaluated by PGLP in the present study due to availability of sufficient grain, there were significant positive relationships between studies for respective measurements of body weight (r^2=0.51; P<0.05), feed intake (r^2=0.52; P<0.05) and FCR (r^2=0.73; P<0.01). The negative correlation (r^2=-0.31; P<0.05) between feed intake and FCR indicates that diets that enabled higher intake resulted in significantly higher growth and improved FCR. However, there were no significant correlation between measurements of AME (r^2=-0.27). This lack of relationship between AME was also present when the wheat-based diets tested in this study were fed without enzyme, as for the original PGLP analysis. The comparison of production variables between studies with diets with no enzymes resulted in almost identical to those reported for diets with enzyme (data not shown). This indicates that the performance was consistent over time for the 15 grain sources; however, assessment of AME was not. In the PGLP, AME was determined by total collection and an acid insoluble ash marker was used in the present study.

Table 3. Correlations between wheat (n=34) diets with enzyme (present study) and 15 same source wheat samples measured in Premium Grains for Livestock Program (PGLP).

Wheat + enzyme	Body wt 17d	Feed intake	FCR	AME
Feed intake g/b/d 4-17d (n=136)	0.71**			
FCR 4-17d (n=136)	-0.67**	-0.30**		
AME MJ/kg diet 16d (n=136)	0.45**	0.30*	-0.44**	
PGLP body wt (5wk) E- (n=15)	0.51*	0.44*	-0.53*	-0.21
PGLP feed intake E- (n=15)	0.37	0.52*	-0.23	-0.01
PGLP FCR E- (n=15)	-0.62*	-0.29	0.73**	0.23
PGLP AME (MJ/kg diet) E- (n=15)	0.19	-0.23	-0.44*	-0.27

This report reinforces the importance of developing processes for or selection of feed grains that will facilitate higher nutrient intake and thereby increase retention and growth. This will be ultimately important in reducing variability in broiler performance and enabling optimum growth and efficiency. We strongly recommend further research in this area.

ACKNOWLEDGMENTS

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EFFECT OF DRYING TEMPERATURES ON FEED VALUE OF MAIZE FOR BROILERS

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Summary

Apart from slight changes in starch contents, maize grain quality was largely unaffected by drying at different temperatures between 85^{o} and 105^{o}C. Over a feeding period of 28 days feed intake was increased ($P<0.05$) as a result of heat-treating the maize up to 95^{o}C. Including a microbial enzyme, Avizyme 1500, resulted in an increase ($P<0.05$) in final body weight of chicks but there was no response when growth was assessed at an earlier age. Over the entire feeding period, feed conversion efficiency (FCE) declined ($P<0.01$) with increasing oven temperature, regardless of supplementation with the microbial enzyme. The weight of visceral organs, protein content and activities of pancreatic and jejunal digestive enzymes were unaffected by grain heat treatment or microbial enzyme supplement. The ileal digestibility of calcium was reduced ($P<0.01$) on diets based on fresh maize and maize that was oven-dried at 105^{o}C. Heat-treatment improved ($P<0.05$) the ileal digestibility of phosphorus in chicks on the diets without the enzyme supplement. No effects of grain heat treatment or microbial enzyme supplementation was observed on ileal digestibility of energy, protein, Ca and amino acids. The results indicate some variations in grain quality as a result of heat treatment but the differences were not significant enough to stimulate major responses to the microbial enzyme.

I. INTRODUCTION

Maize is a premier cereal that is relatively devoid of viscous non-starch polysaccharides (NSP), the principal antinutritive factors present in most temperate cereals (Bedford, 1995). However, maize grain quality may also vary widely, as a result of climatic conditions during growth and harvest as well as post-harvest processing and storage (Leeson *et al.*, 1993).

The digestibility of maize starch is greatly influenced by the ratio between amylose and amylopectin, the latter being more readily digested due to its amorphous nature. About 15 % of maize starch is known to remain undigested up to the terminal ileum, which presents an opportunity for use of microbial enzymes, as is done with wheat and other temperate cereals (Noy and Sklan, 1994). Resistant starch is formed during seed formation and also during feed processing and storage. While most maize is sun-dried *in situ*, wet weather may necessitate the use of artificial drying techniques. Artificial drying could result in the annealing of starch, while long periods of sun-drying have been known to cause stack-burn (loss of quality in the field).

This study was conducted to examine the effects of varying temperature during drying on the nutritive value of maize. The gross response and digestive function of broiler chickens raised on diets based on these grains were also evaluated.

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II. MATERIALS AND METHODS

The maize grain tested was received at a moisture content of 11.3 %. Some of the maize was used as received (Fresh) while other batches were obtained through drying in large draught-ovens at 85°, 95° or 105°C. Each batch was dried over 24 hours to yield varying moisture contents, followed by milling and storage in sealed polythene bags at 5°C. Four diets were prepared, based on these grain batches and a commercial soybean isolate. Three hundred and twenty day-old female broiler chicks (Ross) were randomly allocated to the treatments, in cages (without bottom) on litter, in groups of 10. Each of the four diets, in mash form was fed with or without a microbial enzyme supplement, Avizyme 1500, included at 1 g/kg diet. There were four replicates for each of the eight general treatments. The diets were fed for 28 days. Feed and water were supplied *ad libitum* and light was provided over 23½ hours. The room temperature was managed according to the requirements for chicks between hatch and 28 days.

Feed intake and body weight were measured at the end of each week. At the end of the feeding period, six chicks, randomly selected, per cage were killed through asphyxiation with CO_2 and dissected. Samples of ileal digesta were collected and frozen, and used to determine nutrient digestibility. The weight of visceral organs was obtained. The pancreas and a sub-sample of the jejunum were also taken and snap-frozen in liquid nitrogen. These tissues were later processed and used to determine protein content and the activities of digestive enzymes, targeting various nutrients.

III. RESULTS AND DISCUSSION

As expected, dry matter (DM), crude protein (CP), lipid and phosphorus (P) contents increased with increase in drying temperature (Table 1). The amino acid contents of the oven-dried maize were also generally higher than those of the sun-dried maize (Fresh), especially up to 95°C of heating. Compared to the fresh maize, oven-drying at 105°C improved the total starch and amylopectin contents by 8 and 5 %, respectively while amylose content was reduced by about 12.4 %. The ratio of amylose:amylopectin also declined with increasing drying temperature. This reduction in amylose signifies an improvement in quality but is contrary to the classical response of wet starch to heating (Ito *et al.*, 1999). The moisture content of the samples used in this study may be too low to cause retrograding of starch through heating. Although the samples were stored at low temperature prior to use, this was not done immediately after oven-drying. Therefore, the grains would have cooled slowly, which would reduce the chances of annealing.

Feed intake during the entire trial period was increased ($P<0.05$) as a result of heat-treating the maize up to 95°C (Table 2). Further heating did not have any significant effects on intake of the diets. There were no significant effects of microbial enzyme supplement on feed intake at any period of assessment. The 7-day body weight of chicks was reduced ($P<0.05$) in chicks on the control (without Avizyme) diet in which maize had been heated at 85°C. At 14 days of age, body weight was also reduced ($P<0.05$) by oven-drying at up to 95°C but this was observed only on diets supplemented with the microbial enzyme. The final body weight of chicks on the diet based on fresh maize was improved ($P<0.05$) by the microbial enzyme supplement. There was no effect of the enzyme supplement on body weight assessed at earlier ages. The response of birds to drying was not consistent. For example, the increase in total feed intake, was not accompanied by an increase in final body weight although body weight at other points of evaluation was improved on diets based on oven-dried maize. There was also no pattern in the effect of the microbial enzyme supplement on the gross biological response of the chicks. These inconsistencies may

probably be due to lack of larger differences between the samples. The effects of Avizyme, as is the case for most microbial enzymes, are known to be most pronounced in low-quality maize and sorghum or where there are wide differences between samples (Bedford, 2000).

Table 1: Chemical composition of maize batches under varying drying conditions.

A. Dry matter (DM), crude protein (CP), crude fibre and mineral contents *(g/kg DM)*

Maize	DM	CP	Crude Fibre	Lipid	P	Mg
Fresh	887.0	77.9	227.5	34.8	1.9	1.1
85°C	915.0	79.9	23.9	43.4	2.2	1.2
95°C	924.5	80.4	20.7	40.7	2.1	1.1
105°C	928.5	80.8	19.7	41.0	1.9	1.1

B. Starch content and components (g/kg DM)

	Starch	Resistant starch	Amylopectin	Amylose	Amylose: Amylopectin
Fresh	699.1	177.2	529.5	169.6	0.32
85°C	701.8	183.7	546.2	155.6	0.29
95°C	700.5	167.7	541.8	158.7	0.29
105°C	704.8	158.9	556.2	148.6	0.27

C. Gross and metabolisable energy (MJ/kg DM)

	GE	AME_n	TME_n
Fresh	18.5	15.7	16.1
85°C	18.6	16.0	16.4
95°C	18.4	15.7	16.1
105°C	18.2	15.6	16.0

Table 2: The effect of maize drying temperature, microbial enzyme supplementation and bird age on feed intake and body weight.

		Feed intake (g/bird)				Body weight (g)			
Maize	Avizyme	0-7d	0-14d	0-21d	0-28d	7d	14d	21d	28d
Fresh	-	99.4	353.4	841.6	1508.9^{b}	113.3^{a}	249.5^{ab}	476.5	770.7^{bc}
	+	89.6	351.4	839.3	1525.3^{b}	112.0^{ab}	263.1^{a}	539.8	851.4^{a}
85°C	-	86.2	340.2	843.9	1550.5^{b}	101.5^{c}	227.3^{b}	433.9	709.2^{c}
	+	81.3	332.8	854.0	1649.0^{ab}	103.5^{bc}	233.9^{ab}	470.6	766.1^{bc}
95°C	-	87.3	343.5	852.6	1633.3^{ab}	107.4^{abc}	241.3^{ab}	480.1	777.0^{abc}
	+	84.5	345.1	874.5	1732.3^{a}	105.4^{abc}	231.4^{b}	473.7	791.6^{ab}
105°C	-	87.6	355.0	936.8	1631.4^{ab}	112.3^{ab}	256.0^{ab}	495.8	764.5^{bc}
	+	92.8	344.0	913.5	1645.6^{ab}	109.3^{ab}	258.5^{ab}	492.4	775.1^{abc}
	SEM	6.77	19.17	46.94	68.76	4.73	15.33	29.32	38.18
Source of variation									
Maize		NS	NS	NS	*	*	*	NS	NS
Enzyme		NS	NS	NS	NS	NS	NS	NS	*

[a,b] Mean values on the same column not sharing a superscript are significantly different (*P<0.05) for the factors shown. NS – not significant. SEM is standard error of difference between mean values.

Over the entire feeding period, FCE declined ($P<0.01$) with increasing oven temperature, regardless of supplementation with the microbial enzyme. There were no significant effects of the microbial enzyme supplement on FCE at any of the periods investigated.

The ileal digestibility of calcium was lowest ($P<0.01$) in chicks on diets based on fresh maize and maize that was oven-dried at 105^{o}C. Heat-treatment also improved ($P<0.05$) the ileal digestibility of phosphorus in chicks on the Fresh diets. In chicks on the enzyme-supplemented diets, there was a linear increase ($P<0.05$) in ileal digestibility of P as a result of oven-drying. The change in digestibility of the two mineral elements as a result of heat-treating the maize is unclear. The results appear to contradict those reported by de Schrijver *et al.* (1999) who did not observe any significant effects of high levels of retrograde starch on the faecal digestibility of minerals, including Ca, P and Mg. There was no effect of heat treatment on ileal digestibility of energy or protein.

IV. CONCLUSION

Heat treatment had some effects on the chemical composition of maize grain but this did not impart enough variation in the samples to stimulate a response to the microbial enzyme supplement tested. Heat treatment tended to also improve the starch quality of the grain, thereby obviating the need for microbial enzyme supplement. It may be necessary to test samples obtained from actual commercial processes or more closely simulate the commercial conditions in future studies.

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Starch content and components were measured at the Department of Agricultural and Environmental Science, Queen's University, Belfast, UK.

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CITRIC ACID ENHANCES ENZYMATIC HYDROLYSIS OF PHYTATE

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A series of investigations, designed to assess the feasibility of eliminating phytate ('dephytinisation') from sorghum (2.019 g/kg phytate-P DM) and soyabean meal (4.486 g/kg) by *in vitro* pre-treatment with exogenous *Aspergillus niger*-derived phytase, was completed. The purpose for this is to define the anti-nutritive properties of phytate, the mixed salt of *myo*-inositol hexaphosphoric acid (IP_6), in broilers via the experimental use of dephytinised feed ingredients.

Test feed ingredients were finely ground and mixed with liquid phytase (50,000 to 250,000 FTU/kg) and distilled water, heated to 55°C, to produce a slurry, and dried (~ 5% DM) at 40-70°C for 48 hours. Phytate-P concentrations in untreated, sham-treated and treated feedstuffs were determined by a ferric chloride-precipitation method (Miller *et al*., 1980). However, these standard methods for phytate analyses have limitations (Frolich *et al*., 1986); therefore, the intention is to use an HPLC method in future. Because dephytinisation did not approach the objective of > 90% phytate removal, citric acid was included in the slurry, which reduced average pH from 6.4 to 5.4, which is more optimal for the phytase used (Engelen *et al.,* 1994). On average, (n = 37), incorporation of citric acid into the dephytinisation (exogenous phytase and water) procedure substantially increased hydrolysis of phytate in sorghum (54.8 to 94.6%) and soyabean meal (60.4 to 94.1%).

Similar citric acid induced increases in *in vitro* dephytinisation have been reported in soyabean meal (Cain and Garling, 1995). While the buffering capacity of citric acid would be conducive to phytase activity, it is probable that citrate's chelating potential is more important. It is likely that citric acid strips cations (eg. Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}) from mineral-phytate complexes, rendering phytate more soluble and susceptible to phytase degradation (Maenz *et al.,* 1999). Moreover, the addition of citric acid to broiler diets has been shown to enhance *in vivo* phytate-P utilisation in several experiments (eg. Rafacz-Livingston *et al*., 2005). This probably reflects a reduction in the formation of insoluble Ca-phytate complexes in the gut (Wise, 1983). In the context of the dephytinisation project, the addition of citric acid to the slurry will permit adequate elimination of phytate at lower phytase inclusion levels. This, in turn, should result in satisfactorily low levels of residual phytase activity in the treated feed ingredient.

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INITIAL INVESTIGATION OF MORTALITY CAUSES IN FREE RANGE LAYER FLOCKS

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An investigation was undertaken in the Gatton free range layer facility at the University of Queensland to estimate the major causes of mortality for laying hens kept in free-range flocks. The aim was to develop better strategies for improving health and welfare in free range housing systems. Data on mortality were collected from the start of lay to 45 weeks of age. Mortality causes were diagnosed from gross necropsy only and the examinations were performed for the period from 30 to 45 wks of age. All dead/culled birds were collected daily, immediately refrigerated, and necropsied weekly. Samples for microbiological and pathological examination were not collected because of the delay between death and necropsy in many cases. This study is ongoing.

The results show that from start of lay until 45 weeks of age the average mortality rate in the free range flocks was 2.7% (1.7%, 1.7%, and 4.8%, respectively for each replicate). Of the birds necropsied 85% showed fresh and profound lesions/wounds; evidence of cannibalism. Additionally, there were dead birds (12% of examined birds) that did not exhibit external wounds and died due to cachexia (from stress and/or malnourishment). Examination of the reproductive tract of cachectic birds confirmed the presence of an undeveloped ovary and lack of large yellow follicles. The cause of death was also unclear in two cadavers (no signs of infectious or non-infectious diseases were found). The gross pathology findings indicated that cannibalism was the cause of death in 85% of all cases, which took the form of body pecking (50% of all cases, with a significance of pecking more on the left side than on right side of the tail), vent pecking (35% of all cases), and head pecking (15% of all cases).

Feather picking and cannibalism, which frequently occurs in free-range poultry, were among the major causes of mortality in three free range flocks. A wealth of factors such as environmental, ontogenetical and genetical may have played a role in the development and spread of feather-pecking and cannibalism behaviours in the flocks. Pecking started during rearing when mortality rate was very low, feather pecking was insignificant and no cases of death due to cannibalism were recorded. Cannibalism started to appear as feather pecking with the start of lay, probably developed and spread as an abnormal pecking behaviour emphasising changes in the hormonal status of the hen, high light intensities and social stress. The literature indicates that selective breeding of chicken for gentler behaviour could provide an alternative approach to address these problems and improve the welfare of free-range layers (Cheng *et al.,* 2001). However, the development of cannibalism is still unclear. More research is needed on the motivational and learning components in selective breeding for high group productivity and survivability that would result in reduced mortality from cannibalism and improved productivity.

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REARING PULLETS FREE RANGE: HEALTH AND WELFARE IMPLICATIONS

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The experience of brooding and rearing pullets in free range conditions at the University of Queensland, Gatton is described with the aim to emphasise the importance of good management during these critical periods which assures that pullets succeed through the growing period and reach their genetic potential during the laying period. From day one to 16 weeks old, three Hy-Line Brown layer pullet flocks were brooded and reared free range for free-range egg-production. Guidelines for general brooding and rearing of chicks were available from the Australian Model Code of Practice for the Welfare of Animals (2002) and Hy-Line Brown Chick Management Guide (2002-2004). Brooding commenced in spring and birds were infra-red brooded and reared on non-treated pine litter. Prior to chick delivery the free range area was prepared (cleaned and disinfected), and modified with waters and feeders, with birds having access to nipple drinkers and a feed trough system from day one. Brooder rings were set up in the pens to confine the birds to a smaller space to make it easier for them to find feed and water and to maintain ambient temperature. Birds were maintained at a stocking density of 50 birds/m^2. Rings were removed when birds were 7 days old, consequently decreasing the stocking density indoors to 5 birds/m^2 and outdoors at 1500 birds per hectare. Infra-red heating was provided until birds were 3 wks old, thereafter sheds were naturally warmed and ventilated. Pullets were inspected three times every 24 hours during brooding, and twice every 24 hours during rearing. The lighting program was planned in conjunction with recommendations for open ventilation housing and changes in the natural day length. Fresh feed and water were provided daily. Pullets were fed a mash starter diet from day one to 8 weeks old and a pullet grower diet from 9 weeks to 16 weeks old. Birds were given access to the outdoor area from 4 weeks of age. Shaded areas were provided on the north and south side of the shed. Flock weights were established every two weeks and mortality were recorded daily.

The results showed that pullets grew consistently and reached an average body weight of 1376g at 16 weeks of age. The health and well-being of three flocks was consistent as demonstrated by low mortality rates. The average mortality (cumulative at the end of 16 weeks of age) was 2.4%, with a spread of 2.2%, 2.4% and 2.7% for each flock, respectively. Applying a program of vaccination suitable for pullets in free range assured that the bird's immune system could responded appropriately to disease challenge i.e., mortality was minimized. There were few losses due to a postvaccinal reaction that occurred during a hot day (December). No losses from predators were recorded.

It was demonstrated that from week 1 to week 16 flocks showed a consistent trend of increasing body weights and a lower mortality than expected from the breeders standard. This experience showed that rearing chicks appropriately in a free range system (in the housing system into which they will be introduced before laying) can minimize losses and subsequently help reduce the stress of the movement to the laying range (house).

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INTEGRATION OF HENS INTO A CROP AND PASTURE ROTATION SYSTEM IN AUSTRALIA - PRODUCTION AND AGRONOMIC ASPECTS

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Summary

Hens, housed in eco shelters, were integrated into a pasture crop-rotation to establish if free-range hen operations associated with organic grain production could be used on a niche scale in the wheat belt of Australia. Over a three year period, sheep were compared with hens to determine the effect of grazing on herbage availability, weed control and soil fertility in the crop-pasture rotation. The free-range system used in our study allowed hens to move freely around the paddocks and forage on crop stubble or medic pasture. Hens foraged extensively in the wheat stubble with egg production averaging 90% (28 to 36 weeks-of-age), but overall production of free-range hens was lower than the Hyline cage standard. Paddocks foraged by hens had more herbage and stubble remaining after grazing compared to paddocks grazed by sheep. Hens and sheep had a similar ability to control broad leaf weeds, but sheep preferred wire weed. Soil nitrate nitrogen increased after grazing by hens and sheep. The results from our study indicated that integrating hens into crop and pasture rotation system can assist in weed control and there is potential to reduce herbicide input in these systems. A concern was the attack on hens by foxes in the third year of the trial.

I. INTRODUCTION

Free-range hen farming gives the opportunity to develop integrated farming systems, where there is minimal chemical input. Farm products can be produced utilising the animal waste as fertiliser and the animals can be used to control weeds and pests without the use of insecticides and weedicides. Under natural conditions, a bird's diet comprises seeds, fruits, herbage and invertebrates and could partly achieve a reduction in problem insects and weed seeds (Tadelle and Ogle, 2000; Lomu *et al*., 2004). Therefore, utilising hens in these areas on a small scale could gradually rectify the soil condition and eliminate weeds and pests, which have caused the overuse of chemicals. In addition, consumers are also beginning to demand products from free-range animal production systems (http://www.free-rangepoultry.com/compare.htm). Furthermore, for the best welfare outcome, hens should be able to express normal behaviours (Brambell, 1965) which is possible in free-range systems. This paper examines the integration of hens (housed in eco shelters) into a pasture and crop rotation system to establish if free-range operations associated with organic grain production could be used on a niche scale in the wheat belt of Australia. Comparison was made with sheep, which are traditionally used in crop and pasture farming systems.

II. MATERIALS AND METHODS

a) Paddock and facility

A 4 ha paddock located at Roseworthy Campus was used for this project over a 3 year period. The paddock was fenced into 8 plots with 0.5 ha/plot. Hens grazed in 4 plots with a stocking density of 110 hens/ha, and sheep grazed in the other 4 plots with a stocking density

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of 12 sheep/ha. Medic pastures were established in May 2000 for the first year of the trial. The pasture production, hen production, soil fertility, weed and insect population were evaluated. In the second year, the paddock was sown with wheat. After harvesting the wheat, free-range hens and sheep were moved into the paddock to forage the insects, crop residue and weeds. The population of weeds and insect were monitored. In the third year, regenerated annual medic pasture was foraged by hens and sheep.

b) Housing

An eco-shelter (3m x 3m) was built in the centre of the 4 ha paddock. The eco-shelter had four internal pens of equal size each housing 55 hens (Hyline Brown). Throughout the grazing trial, hens were fed twice daily. Half of the layer ration (55 g/hen) was fed in the morning and the other half (55 g/hen) in the evening. Hens were locked in the shelter over night. Sheep were given access to the paddock only.

c) Measurements

Detailed information on measurements undertaken including hen production, sampling soil and forage (pasture, weeds, seeds and pods), soil pH (in water and in 0.01M CaCI), soil nitrate-N, ammonia-N and penetrometer readings are provided in Glatz *et al*. (2005).

d) Experimental design and statistical analysis

The treatments in the experimental paddocks were arranged in a randomised design. Animals were the main treatment factor. Sampling date, pasture type, zone and interactions were also analysed. Production performance could not be analysed statistically. Comparison was made, however, with the Hyline standard for cage birds. The treatment effects were assessed with ANOVA in Systat software (Wilkinson, 1996). Bonferroni's post hoc was used to separate means only if significant main effects were detected by analysis of variance. Bonferroni's post hoc test is a multiple comparison test based on Student's t statistics and adjusts the observed significance level when multiple comparisons are made.

III. PRODUCTION OF FREE-RANGE HENS

In the first year the production performance of egg layers (Hyline Brown) in the free-range system was compared with the production specifications published by the Hyline Company for the strain housed in a cage system. The free-range hens had higher levels of mortality and lower rates of lay, egg weight and body weight over the period 18-40 weeks (Table 1). The reduction in performance of birds relative to the benchmark was expected considering the heat wave conditions experienced during this period. In the second year the performance of the hens was excellent with production during March-May averaging 90%, close to industry standards. This may be because the summer conditions during the experimental period on this occasion were mild and also the hens probably consumed considerable quantities of spilt grain from the wheat harvest. In the third year production of hens was comparable with the rate of lay recommended for this strain of birds in cages. However, two fox attacks in the last 4 weeks of lay while hens were foraging during the day resulted in a sharp decline in production (15%) relative to the standard performance expected for these birds if housed in cages (Table 2, 3).

Table 1. Production performance of free-range birds foraging on medic pasture in Year 1 compared to the cage standard over 18-40 weeks.

Treatment	Mortality and culls (%)	Rate of lay (%)	Egg weight (g)	Live weight (kg)
Free-range	9.1	80	57.2	1.9
Cage standard	1.2	87	63.9	2.2

Table 2. The liveweight and egg weight of laying hens foraging on wheat stubble in Year 2 and regenerated medic pasture in Year 3.

	Liveweight (kg)			Egg weight (g)		
Treatment	Age of hens (wks)	Free-range	Standard	Age of hens (wks)	Free-range	Standard
Wheat stubble	24	1.9	1.8	24	54.3	57
	28	1.9	1.9	28	55.7	61.3
	32	2.1	2.0	32	58.2	62.7
Regenerated	46	2.4	2.0	45	64.1	64.8
medic pasture	51	2.2	2.0	49	64.7	65.2
	55	2.2	2.0	53	64.0	65.6

Table 3. The hen-day production and mortality of laying hens foraging on wheat stubble in Year 2 and regenerated medic pasture in Year 3.

		Hen-day production (%)		Mortality (%)	
Treatment	Age of hens (wks)	Free-range	Standard	Free-range	Standard
Wheat stubble	20	7.6	26	0	0.1
	24	74.4	93	0	0.3
	28	89.3	95	3	0.5
	32	91.6	94	4	0.7
	36	90.9	93	0	0.9
Regenerated	48	76.9	89	2	1.5
medic pasture	52	79.5	87	11	1.8
	56	81.0	85	72	2.0
	60	67.8	83	56	2.3

Production of free-range hens in our study was lower compared to cage birds. However, Gibson *et al.* (1984) reported that egg production was similar for free-range hens and caged hens (283 vs 280) over 20-72 weeks and feed intake was higher for free-range hens at 36 weeks (152.4 vs 119.8 g/day/bird) and at 70 weeks (142.9 vs 123.0 g/day/bird). In the first year of our study, hens showed higher levels of mortality and lower rates of lay over 18-40 weeks. This may be because the weather was extremely hot which reduced the feed intake and hence the production of the hens. Portsmouth (2000) stated that high temperature in summer often reduces feed intake, rate of lay and egg size. In the third year of our trial, fox attacks in the last 4 weeks on free-range hens reduced egg production. In addition to death from injuries, a large number of the hens appeared to die as a result of the severe stress of being chased by the fox.

VI. AGRONOMIC ASPECTS

a) Herbage availability and weed control

In the first year, herbage availability in hen paddocks after grazing was greater ($P<0.05$) than the sheep paddocks. This was expected as sheep ingest more herbage than

hens. Sheep, however, were very effective (P<0.05) in grazing the wire weed, which contaminated the paddocks whereas hens avoided this weed. In contrast, the number of unidentified weeds (mainly broad leaf weeds) in the sheep paddock was greater (P<0.05) than the hen paddocks. This raises the possibility that sheep and hen could be grazed together in some circumstances, to provide a method for reducing weed build up. Sheep could be used to graze weeds they prefer and hens to consume weed seeds that sheep avoid. Hens foraged extensively on the wheat stubble throughout the experimental period in the second year (January-May 2002). Again after grazing, hen paddocks had a significantly (P<0.05) higher amount of herbage remaining compared to the sheep paddocks (Table 4). In the third year, there was a reduction in insect numbers before and after grazing in the hen paddocks indicated by a related study (Lomu *et al*., 2004). Insects consumed by the hens were observed in the crop contents together with seeds, grass, soil and small stones. There was no difference in the sheep and hen paddocks in the number of insects although after grazing the number of insects was lower than an ungrazed area (Glatz and Ru, 2004). This shows that hens have an ability to reduce insects by consuming them or deterring them from a grazing area.

Table 4. Herbage availability and weed content in paddocks after foraging by hens and sheep.

Pasture	Year	Animal	Herbage only (g/m^2)	Wire weed ($no./0.1m^2$)	Other broad leaf weeds
Medic pasture	1	Hen	456.4	2.3	0.5
		Sheep	118.1	0	1.6
P value			*0.02*	*0.01*	*0.02*
SEM			*73.1*	*0.4*	*0.24*
Wheat stubble	2	Hen	389.2	2.4	1.3
		Sheep	235.6	0.1	0.4
P value			*0.06*	*0.00*	*0.2*
SEM			*47.2*	*0.2*	*0.4*
Regenerated medic pasture	3	Hen	419.0	0	0.1
		Sheep	139.8	0	0
P value			*0.00*	*1*	*0.36*
SEM			*32.2*	*0*	*0.1*

Our results indicate that birds consumed a small quantity of forage, which is supported by other work (McBride *et al.*, 1969; Savory *et al*., 1978; Lomu *et al*., 2004). Compared to sheep, hens appeared to have a preference for wheat and rye grass and less preference for herbage, medic pods and other pasture seeds (Glatz and Ru, 2004). The results from this study suggest that integrating hen into a crop and pasture rotation system can assist in weed control and has the potential to reduce herbicide input.

b) Soil fertility and penetrometer readings

In the first year, soil fertility was not different (P>0.05) between the sheep and hen paddocks. Nitrate nitrogen content in both paddocks increased after grazing by both animals. In second year, there was no effect (P>0.05) of grazing on soil pH given the low stocking density of both sheep and hens but soil nitrate nitrogen levels were higher after grazing, suggesting that animal droppings were contributing to the increase (Glatz and Ru, 2004). After sheep had grazed the wheat paddocks there was an increase in the penetrometer readings in the paddocks. This reflects the trampling effect that sheep have on forage and

soil with continued grazing whereas hen paddocks showed no change in penetrometer readings. In the third year, soil fertility in the hen paddock did not change during the growing season for the regenerated medic pasture. However penetrometer readings increased ($P<0.05$) in the hen paddocks as the soil dried out towards the end of the pasture growing season. This was also observed in the sheep paddocks where the increase ($P<0.01$) in surface hardness was more apparent (Table 5).

Table 5. Soil fertility and penetrometer readings in paddocks after grazing by hens and sheep.

Pasture	Year	Animal	Nitrate_N (mg/L)	Ammonia_N (mg/L)	pH_water	pH_0.01M (CaCI2)	Penetrometer reading
Medic pasture	1	Hen	18.4	0	7.2	6.2	4.0
Medic pasture		Sheep	24.2	0.1	6.8	5.8	3.5
P value			*0.20*	*0.36*	*0.42*	*0.51*	*0.31*
SEM			*2.9*	*0.1*	*0.1*	*0.1*	*0.2*
Wheat stubble	2	Hen	33.0	2.2	7.3	6.4	1.9
Wheat stubble		Sheep	39.9	6.1	6.3	5.5	2.4
P value			*0.27*	*0.21*	*0.28*	*0.23*	*0.19*
SEM			*4.0*	*2.0*	*0.6*	*0.5*	*0.2*
Regenerated medic	3	Hen	0.4	4.7	7.3	6.5	4.8
Regenerated medic		Sheep	1.2	10.7	7.0	6.1	9.1
P value			*0.17*	*0.26*	*0.52*	*0.46*	*0.00*
SEM			*0.4*	*3.4*	*0.3*	*0.4*	*0.8*

There was no significant ($P>0.05$) difference in the soil nitrate and ammonia content in paddocks grazed by hen or sheep. Generally the soil nitrate increased in the first two years. This increase probably resulted from dung and urine excretion of animals, as ammonium-N and organically bound N. The organic N will mineralise gradually over time to produce ammonium-N which will be converted to nitrate-N and contribute to the soil mineral nitrogen pool and will be available for plant uptake (Worthington and Danks, 1992). Integrating birds into this farming system will be beneficial for the soil fertility.

VII. CONCLUSION

Integrating hens into a traditional crop and pasture system was beneficial for this system. Hens obtained feed resources from the paddocks and production performance of hens approached industry standards. The disappointing aspect was the fox attacks on hens. The hens were locked up at night and this avoided any attacks except for the last month of the production trial. It is recommended in free-range systems that fences of sufficient height and strength are built to prevent foxes entering. The fox attacks occurred during the day, which was not expected.

The shelters constructed were ideal for hens. Even under extreme weather conditions the shelters provided adequate protection from the elements for the hens. The

only concern was the very strong winds, which caused the blinds to flap loudly and driving rains, which sometimes entered the shelter.

Hens were able to utilise the forage sources in the paddocks and graze weeds. The stocking density used in the trial was very low. Use of a large number of hens on pasture heavily infested with weeds offers an alternative approach to controlling weeds and avoiding the use of chemicals. The use of strip grazing to clean up weeds and moving the animals frequently to new areas is a strategy which could be employed particularly on farms where organic grain is being produced. The use of sheep with other species to selectively graze weeds and grasses has potential. The advantage of the low stocking density is that the production system is environmentally sustainable. Traditional fee-range systems can cause environmental problems especially where land has been denuded and animals continually utilise the same area of land for extended periods.

The use of animals in a cropping pasture system has potential and the system established at Roseworthy attracted considerable interest. Most people were pleased to see the animals utilising the free-range facility and their perception was that it was a good system of production albeit with some of the problems that resulted many years ago in the commercial industry moving to intensive systems of production.

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MEASURES OF FEAR AND THE RESPONSE TO STRESS IN LAYING HENS

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Chronic stress is associated with poor welfare. Corticosterone levels are used as a physiological measure of stress in hens. Introducing hens to an unfamiliar environment is fear provoking and the response depends on a range of factors (Jones, 1996). The tonic immobility test and the time taken to approach a novel object have been used as measures of fearfulness in poultry (Jones, 1996). If there was a relationship between fearfulness and a hen's response to stress, it might be possible to identify hens whose welfare is most at risk in particular production systems.

At 30-33 weeks of age, 150 individually caged Isa Brown hens were subjected to tonic immobility tests on a least 3 separate occasions. Two groups of hens were selected. Hens in group 1 had mean tonic immobility times of less than 70 sec and group 2 hens, mean tonic immobility times of greater than 300 sec. At 0500h on day 1 of the study, hens were moved to transport crates and remained there for 4 h before being moved to new cages in a different part of the layer shed. The day before moving and on days 2, 3, 5 and 7 after moving, all eggs laid were collected and weighed. The egg albumen was then removed, weighed and stored at -20^{0}C. Between 15:30 and 16:30h on the day the hens were moved a 1 ml blood sample was taken by jugular venipuncture and the plasma collected and stored at -20^{0}C. The corticosterone concentrations in plasma and albumen were determined by radioimmunoassay. One month later, hens from both groups were subjected to a novel object test. For this test a multi-coloured rod was placed in the feed trough and the time taken for the hens to approach the rod was recorded.

Treatment	Tonic immobility (sec)	Novel Object (min)	Plasma corticosterone (ng mL^{-1})	Albumen corticosterone concentration (ng g^{-1})	Total albumen corticosterone (ng)
Group 1	47 ± 3^a	4.3 ± 1.6	1.02 ± 0.16	1.33 ± 0.08	50.0 ± 3.0
Group 2	575 ± 56^b	1.8 ± 0.5	0.96 ± 0.16	1.38 ± 0.08	49.0 ± 3.0

Values with different superscripts are different (P<0.05)

The increase in egg albumen corticosterone concentration after moving was small (group 1, 1.16 to 1.33 and group 2, 1.22 to 1.38 ng/g). This suggests that hens were not unduly stressed. For individual hens, there was no relationship between the measures of fearfulness and the corticosterone response. Also, no relationship between the tonic immobility test and the novel object test was observed. For this strain of hen, the measures of fear were not a good indicator of how individuals responded to the stressor.

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POSITION EFFECTS ON THE FEAR RESPONSE OF LAYING HENS IN COMMERCIAL CONVENTIONAL CAGE SYSTEMS

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Summary

The withdrawal response of laying hens to an unfamiliar human was assessed at five commercial farms using two behavioural tests. The proximity of the hens' cage to the main entrance of the shed had no significant effect on the withdrawal response, however birds housed in the inside aisles displayed less withdrawal than hens in the outside aisles. This may be due to an increased amount of visual human contact experienced in the middle aisles reducing the fearfulness of the hens to humans.

I. INTRODUCTION

Fear is generally considered a powerful emotional state that normally gives rise to defensive behaviour or escape behaviour. In concert with these behavioural effects, fear normally activates the autonomic nervous system and the neuroendocrine system, which in turn through their effects on regulatory mechanisms such as energy availability and use, and cardiac and respiratory functions, assist the animal to meet physical or emotional challenges (Hemsworth and Coleman, 1998). Gray (1987) recognises that fear may be triggered by environmental stimuli which are novel, have high intensity such as being loud or large, have special evolutionary dangers such as height, isolation and darkness, arise from social interaction such as contagious learning or have previously been paired with aversive experiences.

Farm animals, such as poultry, may frequently interact with humans and through conditioning, may associate humans with rewarding and punishing experiences that occur at the time of these interactions and thus conditioned responses to humans may develop. Extensive studies in the livestock industries have shown marked between-farm variation in the fear responses of farm animals, including poultry, to humans. For example, Barnett *et al.* (1992) used the behavioural response of the laying hen to an experimenter to assess the hen's fear of humans; they found a negative inter-farm correlation between fear of humans and productivity of the hens. Such negative correlations, based on farm averages, indicate that high levels of fear of humans may be an important factor limiting the productivity and welfare of livestock.

Studies in the dairy and pig industries have shown significant sequential relationships between the stockperson's attitudes and behaviour towards animals and the fear of farm animals toward humans (see Hemsworth and Coleman, 1998). The existence of these sequential relationships between human and animal variables in the livestock industries indicates that the opportunity exists to modify stockperson attitudes and behaviour in order to improve livestock welfare and productivity, and such opportunities may also exist in the poultry industries.

The data reported in this paper on the effects of position in the shed are initial data for a larger study that is examining human-animal interactions in the egg industry.

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II. MATERIALS AND METHODS

Five Victorian farms, with Hyline Brown or ISA Brown strains of laying hen were used in this study and all birds were tested between 40 and 60 weeks of age. Details of the farms are provided in Table 1.

Table 1. Summary of commercial farms used in this study.

Farm	Shed Type[1]	No. of Birds	No. of Tiers	No. of Aisles	Birds/cage
1	Closed	14000	3	5	3
2	Open	13000	3	4	3
3	Closed	23300	4	5	5
4	Open	2000	1	6	3
5	Closed	1100	1	3	3

[1] 'Closed' refers to environmentally controlled sheds and 'Open' to open-fronted sheds

Two behavioural tests were used to assess the withdrawal response of the birds to an unfamiliar human, the Stroll Test (adapted from Cransberg *et al.*, 2000) and the Approaching Human Test (adapted from Hemsworth *et al.*, 1993). These tests have been successfully used in the past to assess the fear response of poultry, and do not require the birds to be handled and thus avoiding any handling stress on bird behaviour. At each farm, both tests were conducted on the same morning and the sequence of testing within localities in each shed was the same. Aisles were numbered from left to right of the door, and the tests began at the first cage in Aisle 1. Only the right hand side of each internal aisle was tested to avoid exposing untested birds to additional visual contact with the observer. The same observer was used as the human stimulus in each test. Due to the varying fear response of birds in different tiers of cages (Hemsworth and Barnett, 1989; Hemsworth *et al.*, 1993), only birds in the second tier were tested, except for those farms with single tier sheds where birds in the single tier were tested.

a) The Stroll Test

This test had two phases, a 'Movement Phase' and a 'Stationary Phase'. A video camera, equipped with infra red night vision for recording under low light conditions, was used to record the withdrawal response of the birds as the observer moved through the shed in a standard manner. Only data recorded from Movement Phase are reported in this paper. This phase consisted of the observer (an unfamiliar human) commencing in a standardised starting position and moving at a speed of one step/second along each aisle. The video camera was held level with the second tier in multi-tiered sheds or the single tier in single-tiered sheds to record the behaviour of birds in the first four cages on the right-hand side immediately in front of the observer as the observer moved through the shed. During video read-out, the number of birds with their heads extended through the front of the first four cages were counted instantaneously at 5 second intervals (variable labelled Birds_2m).

b) The Approaching Human Test

The second test assessed the response of the birds to an unfamiliar human directly approaching the cage front and was conducted at every tenth cage along the aisle. The observer approached the focal cage from the opposite side of the aisle and remained at a distance of 0.5 m away from the front edge of the focal cage for a period of 5 sec to allow the birds to adjust to the observer's presence. The observer then stepped sideways (facing towards the cage) directly in front of the cage and recording commenced. After 5 sec, the

observer stepped forward so that the torso contacted the feeder of the focal cage. After another 5 sec the observer stepped back to the opposite side of the aisle, and in the following 5 sec stepped forwards to the cage front again. The observer thus spent two 5-sec periods close to the cage and two 5-sec periods on the opposite side of the aisle from the cage. During each 5-sec period the number of birds with their heads extended through the front of the cage were recorded (variables labelled AHThead5 to AHThead20). Also, a point count of the number of birds with their beak in the front 5 cm of the cage was made at the end of each 5 sec period, producing the variables AHT5, AHT10, AHT15 and AHT20.

An analysis of variance with data blocked on farm was used to examine the effects of rows location (inside rows and outside rows) and cage location relative to entrance (first four focal cages nearest the main entrance to the shed and the last four cages in the aisle furthest from the main entrance.

III. RESULTS

The cage position in the shed relative to the entrance had no significant effect on the withdrawal response of the birds in any of the tests (AHT5, $F_{1,34} = 0.17$, $P = 0.680$; AHT10, $F_{1,34} = 0.33$, $P = 0.571$ and Birds_2m $F_{1,34} = 2.83$, $P=0.102$). However, birds housed in the outside aisles showed more avoidance of the unfamiliar human. As shown in Table 2, fewer birds in the outside aisles had their heads in the front 5cm of the cage during the Approaching Human Test at both 5 (AHT5) and 10 (AHT10) seconds when the observer was opposite and close to the cage, respectively. Also, during the Stroll Test fewer birds in the outside aisles had their heads extended outside the cage than birds in the inside aisles ($P<0.05$).

Table 2. The results of the analysis of variance on the effects of row location on bird behaviour.

Test and Variable	Mean Number of Birds		LSD (P=0.05)	F-ratio	P value
	Inside Aisles	Outside Aisles			
Approaching Human (AHT5)	1.6	1.4	0.15	4.10	0.044
Approaching Human (AHT10)	0.9	0.7	0.15	5.96	0.015
Stroll (Birds_2m)	3.9	3.6	0.29	4.56	0.033

To examine the possibility that order of testing birds may have affected the behavioural responses of birds, the responses of birds in the first and last aisles were compared. There was no significant difference ($P > 0.05$) in behaviour between birds in the first and last aisles. For example, the mean number of heads at the cagefront in the AHT5 were 2.1 and 2.0 in the first and last aisles respectively ($P = 0.459$, LSD (P=0.05) = 0.31).

IV. DISCUSSION

These results provide an interesting basis for the study of human-animal interactions in the laying hen. The lower withdrawal response of the hens in the inner aisles compared to the outer aisle could be due to the higher level of human contact, particularly visual contact that the former birds receive. Greater social stimulation available to birds in the inner aisles may also be implicated. Aisles on the outside of the shed only contain a single row of cages, whereas inside aisles have cages on both sides. The hens located in the outside aisles generally only have visual contact with the hens in adjacent cages, depending on the cage design, and face a solid wall. The location of cages on both sides of the aisle in the inside

rows also means that stockpeople will make twice as many passes along these aisles, particularly when routinely inspecting the birds and perhaps during other tasks, thereby exposing these birds to about twice the amount of human contact.

There were no significant effects of location relative to the main entrance. Farms 1, 2, 3 and 5 had doorways at both ends of the sheds that were observed in use during the study, and in the cases of Farms 1, 2 and 5, stockpeople often walked through the shed to access these exits, especially during the cleaning and maintenance of the shed. The presence of the additional door in each of these farms may result in similar human contact at both ends and corresponds with the data in this study of no effect of the cages being position either close to the main entrance or at the far end of the shed on withdrawal/approach response. However, the additional entrance in sheds was not used as often as the main entrance. While more human contact may be available to birds close to the main entrance, it may also involve greater startling responses that may occur with the sudden entrance of stockpeople, perhaps negating any positive effects of increased human contact on the hens' fear response of humans. Thus, the nature of the human contact is likely to be an important factor in the level of fear of humans by laying hens and this factor will be examined in the larger study on human-animal interactions in the egg industry.

Within the context of this larger study, targeting human-animal interactions requires understanding the stockperson behaviours regulating these interactions and in turn the stockperson attitudes leading to these stockperson behaviours. Such knowledge may provide industry with an opportunity to reduce any limitations on animal welfare and productivity imposed by these interactions.

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EVALUATION OF A BANTAM CROSS EGG LAYER

G.B. PARKINSON[1] and P.H. CRANSBERG[1]

Summary

This paper reports on two experiments in which a bantam White Leghorn male (Line A) with commercial egg production traits was crossed with a conventional female parent White Leghorn line (Line D). The offspring of this experimental cross were then compared to offspring of the female parent line (Line D) crossed with a conventional White Leghorn male line (Line B).

The average weight of the bantam cross (Line A x D) was 1518g at 50 weeks of age, whilst the body weight of the commercial White Leghorn (Line B x D) was 1712g. Production in the bantam cross White Leghorn peaked at similar levels to the conventional White Leghorn, 91-94% at 22-26 weeks of age, and remained above 80% from weeks 20 to 31 in experiment one and from weeks 21 to 40 in experiment two. Average egg production from the bantam cross was 4.7% lower between 18-50 weeks than the conventional White Leghorn. Average feed consumption in the bantam cross was approximately 11.3% lower than the commercial White Leghorn to 50 weeks. Average egg weight of the commercial White Leghorn at 50 weeks of age was 60.8g, whilst the bantam cross was 62.4g.

Overall the results are very promising, and provide the industry with an opportunity for a quantum efficiency leap by reducing body weight and maintenance requirements whilst holding egg mass output relatively constant. In the future, feed efficiency in egg production can be significantly improved by using a bantam male line crossed with a regular sized commercial female line. It will not be difficult to recover the small to moderate discounts in egg mass that have been observed in this preliminary cross. A number of alternative genetic approaches could be adopted, such as crossing the bantam line with a superior female parent line, further selection of the pure bantam line, or a program of introgression of the bantam genes into elite parent stocks.

I. INTRODUCTION

Genetic selection over the last 40 years has seen massive improvements in the performance of laying hens. It is likely, however, that the rates of feed efficiency gain are going to slow as egg production approaches the threshold of 365 eggs per annum. Two alternative genetic approaches have been suggested to accelerate body weight reduction in commercial laying strains and improve feed conversion. These two approaches have been the introduction of dwarf or bantam genes into commercial lines. Research with dwarfing genes has been generally unsuccessful mainly due to lower peaks of production, reduced persistency of production and low egg weights (Polkinghorne and Lowe, 1973).

Unlike the introduction of dwarf genes, bantam genes have shown more promise as a mechanism to alter the relationship between body weight and egg weight without affecting egg production. Yoshida and Saito (1983) found that the introduction of Sebright bantam genes into various strains of fowl reduced adult body weight by 8-17%, reduced egg weight by only 3-4% and had no effect on egg production. Additionally, Parkinson and Cransberg (2000) introduced bantam genes into a commercial White Leghorn x New Hampshire cross with equally promising results. These bantamised hybrids had unusually high egg weight to body weight ratios and maintained competitive rates of egg production when compared in controlled experiments to commercial brown egg laying stocks.

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The experiment reported here assesses the performance of a cross between a bantamised WL male and a commercial WL female for production characteristics and general viability. The experiment directly compares these results to a commercial WL cross that uses the identical commercial female line.

II. MATERIALS AND METHODS

The strains used in these experiments were a commercial WL (Line B x D), and a cross between a bantam WL male (mature body weight of bantam male approximately 1500 grams) and the commercial WL female (Line A x D). Birds in experiment one were monitored to 50 weeks of age, while birds in experiment two were monitored through to 70 weeks of age. Both experiments were conducted with identical inputs under identical conditions. All birds were reared in cages in a controlled environment shed, fed a commercial diet and were exposed to natural day-length at a light intensity of approximately five lux to 14 weeks of age. At 14 weeks of age, birds were placed into five bird cages in an environmentally controlled shed with temperatures ranging from 16-27^{o}C with an average of 21-23^{o}C. Light was constant at 16 hours per day, with a light intensity of 5-10 lux.

All birds were fed a commercial grower ration (ME 12.1 MJ/kg and crude protein 160 g/kg) between 9-18 weeks of age, followed by a commercial layer diet (ME value 11.61 MJ/kg, 18.5%crude protein, 3.75% calcium) for the remainder of the experimental period. Feed and water were available *ad libitum.*

Birds were weighed intermittently, starting at 16 weeks of age and continuing through to the end of each experiment. Egg production was recorded daily and accumulated to provide weekly figures. Egg weight was measured weekly (all eggs from a particular day were weighed) while feed consumption was calculated at 50 weeks of age.

III. RESULTS

a) Comparison summary of bantam cross with commercial WL

Table 1. Summary of production parameters of the bantam cross and the commercial WL to 50 weeks of age (results are an average of experiments one and two).

Parameter	Layer strain	
	Commercial WL	Bantam cross
Average body weight @ 50 weeks (grams)	1712	1518
Ave. feed consumption (gm/bird/day) @ 50 weeks	115.5	102.4
Ave. egg production (%) from 18-50 weeks	81.3	76.6
Average egg weight (grams) 20-50 weeks	55.7	55.9
Average egg weight (grams) @ 50 weeks	60.8	62.4
Average egg mass (grams) 20-50 weeks	47.7	45.0
Egg weight: body weight ratio (%) @ 50 weeks	3.55	4.11

b) Body weight – Experiment 2

At 16 and 70 weeks of age the bantam cross had an average body weight of 1202 and 1605g respectively, whilst the commercial white leghorn was 1256 and 1809g at the same ages (Figure 1). The bantam cross was significantly smaller ($P<0.01$) than the commercial WL from 16-70 weeks (Figure 1).

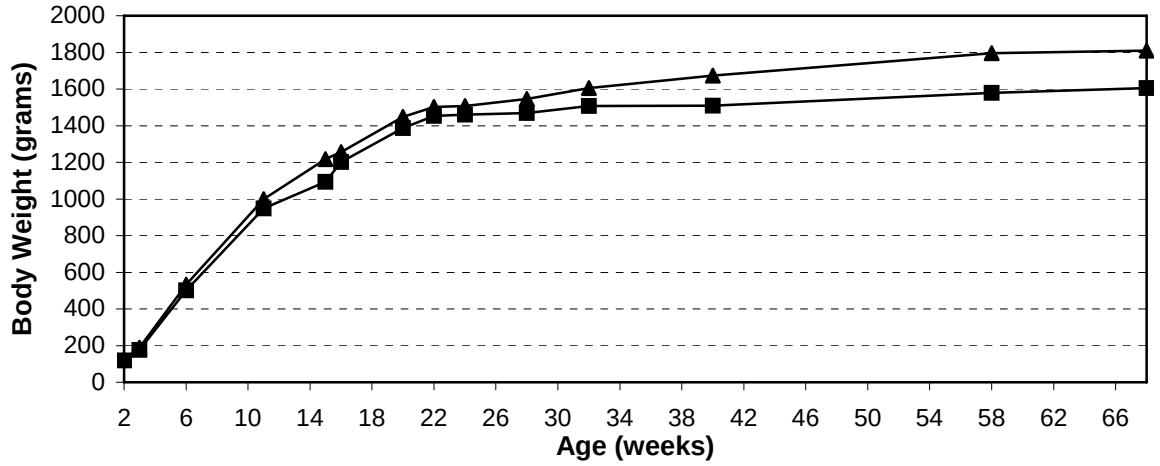

Figure 1. Average body weight of the bantam cross (■) and commercial WL (▲) between 2 to 68 weeks of age.

c) Egg production – Experiment 2

The bantam cross had high levels of egg production, peaking at 91.1% at 22 weeks of age (Figure 2). In comparison the commercial line peaked at 94.2% in week 26 (data point at 34 weeks is likely to be erroneous). Average egg production from 17 to 70 weeks of age was 70.9% in the bantam cross and 76.1% in the commercial WL.

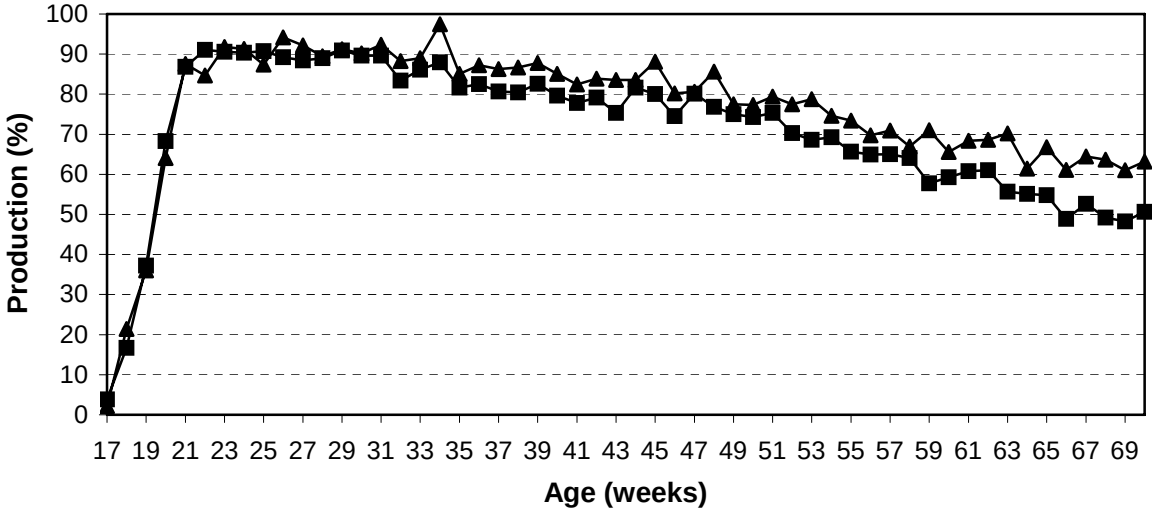

Figure 2. Average egg production of the bantam cross (■) and commercial WL (▲) between 17 to 70 weeks of age.

d) Egg weight – Experiment 2

The average egg weight of the commercial WL is superior to the bantam cross up to 31 weeks of age. At all data points after 31 weeks of age, egg weight of the bantam cross was superior to the commercial WL. Average egg weight from week 21 to 70 was 58.6g in the bantam cross and 57.2 in the commercial WL. The egg weight to body weight ratio at 70 weeks was 4.05 and 3.48 in the bantam cross and the commercial WL respectively.

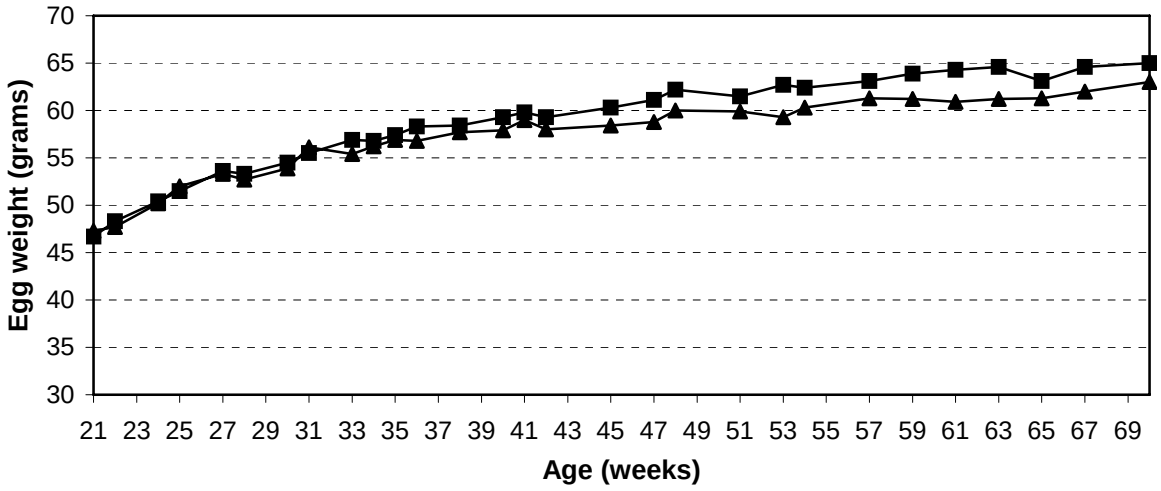

Figure 3. Average egg weight of the bantam cross (■) and commercial WL (▲) between 21 to 70 weeks of age.

IV. DISCUSSION

The production results achieved by these birds are extremely encouraging in comparison to the commercial WL. Overall there is a superior average egg weight in the bantam cross and production is only 6% lower despite the large reduction in body weight (11.3 % at 50 weeks). It is anticipated that the production performance and egg mass output can be further improved by crossing the bantam line with a superior female parent line, further selection of the pure bantam line, or a program of introgression of the bantam genes into elite parent stocks.

The ratio of egg weight to body weight in the bantam cross was markedly superior to the commercial WL and illustrates the large egg size relative to body size.

Mathematical simulations indicate that feed consumption will be reduced by approximately 5-6% if the egg mass of the bantam cross and commercial WL were equivalent. In others words, the lower egg mass recorded in the bantam cross accounts for a reduction in feed intake of about 5%, whilst the reduction in metabolic body size accounts for a further 5-6% reduction in feed intake.

The global trend towards out of shell egg products will increase the emphasis on feed conversion efficiency at the expense of shell colour and probably very large eggs, and lends strong support to the development of commercial lines as described in this experiment. Additional research will be directed at evaluating bantam crosses with lower body weights. Experimental bantamised White Leghorn crosses have been produced with mature weights as low as 1.37 kg with acceptable average egg weight and production performance.

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THE CHICKEN AND THE EGG SLIMMING DIET: PRELIMINARY RESULTS

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Dr Atkins' main contribution to slimming was the recognition that protein has a high satiety effect; much higher than fat and carbohydrate on a kJ basis (Astrup and Raben, 1996). Chicken meat is low in fat (skin off), while eggs are the 'complete food' containing about 50% protein on a dry weight basis. The diet used here over 31d was designed to provide 6300 kJ (1500 kcal)/day and a weight loss of 1 kg/week. Food included 2 eggs on most days, chicken (skin off) four times/week, fish twice a week and free-choice on day 7. One serving of starchy foods (potatoes, pasta) may be eaten weekly. White flour products, salt and cane sugar were avoided. Wholemeal bread and high-fibre savoury biscuits were on the menu, as was cheese but sparingly. Butter was spread thinly and only olive oil recommended. Meat and fish were grilled or barbecued and a non-stick pan with spray-on olive oil for frying. Low-fat foods (eg. yogurt, 2% milk and salad dressing) were favoured. At least four servings of vegetables, including a vegetable juice, and 3-4 servings of fruit/d, including fruit juice, were on the menu. A hearty green or bean salad was eaten each day. Eggs could be cooked in any way and fat-trimmed bacon was eaten twice weekly. Two1g fish oil capsules and 4-5g wheat bran/d were consumed for peak health. Alcohol was allowed (200ml wine or 375ml beer or 40ml spirits/d). All food, and uneaten food, were weighed to the nearest 2g. Activities were recorded to the nearest minute and divided into four categories for calculating energy expenditure. Exercise was taken daily. A fasting blood sample was drawn before the start and at the completion of the diet by a medical practitioner who also recorded body weight. The main results are shown in the table.

Table. Body weight, food energy in (I), energy expenditure (E) and energy balance (B) all in kJ/d over a 31d period on the Chicken and the Egg Diet (n=1)

Day	0	7	14	21	28	31
Weight kg	75.5	74.4	73.8	72.7	71.8	71.6
Energy I		6526	5996	5928	6435	6754
Energy E		10120	10391	10440	10510	10020
Energy B		- 3594	- 4395	- 4512	- 4075	-3446

Weight loss and energy intake were on target. Egg consumption was 56 and edible chicken meat 3.13 kg/31d. Mean cholesterol intake was 460 mg/d. Blood profile and blood pressure (120/75 at start and finish) were unaltered. D 0 and d 31 blood lipid values (mmol/L) were: cholesterol 6.2, 5.7; HDL 0.8, 0.7; LDL 3.4, 3.4; triglycerides 0.8. 0.7. One kg of weight loss is about 31,130 kJ/kg or 124,520/ 4 kg which agrees well with a total energy deficit of 126,370 kJ. The results demonstrate that following this diet allows a person to lose substantial weight. The food and exercise regimen can be modified to decrease rate of weight loss, which was rapid here. The high satiety value of eggs and meat, and emphasis on high-fibre foods, did not impose any noticeable hardship.

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DUST-BATHING BEHAVIOUR BY HENS IN FURNISHED CAGES

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Summary

The use of the dust bath and the behaviour of laying hens in the dust bath of 8-bird furnished cages with and without litter were measured from video records of hens around the peak of lay (period 1) and later during the laying cycle (period 2). All 18 cages contained a perch. Average occupancy of the dust baths was relatively low at both periods (<30% of the available time). There were few effects of litter on hen behaviour, although dust baths were used during more of the time ($P>0.05$) if litter was present compared to absent. The increased duration of dust bath use with litter was due to longer bouts of occupancy rather than more frequent visits by hens. A comparison of focal dust bath visits (n = ~6 per cage) found that in the presence of litter hens had more bouts of stand and sit posture in the dust bath per visit at period 2 ($P<0.05$), and were more likely ($P<0.05$) to 'shake-out feathers' at period 1. The frequency and duration of visits suggest that although the dust bath was not used to capacity, the presence of litter increased its use.

I. INTRODUCTION

The EU Council Directive 1999/74/EC requires that European cage egg producers phase out so-called 'unenriched' layer cages by 2012. These furnished cages will then be the only acceptable form of hen housing for the production of cage eggs in the EU, and are designed to obviate the barren and non-stimulating environment that laying hens are reported to experience in conventional cages, through opportunity for hens to perform several of their 'natural' behaviours (Abrahamsson and Tauson, 1997). In furnished cages hens can perform behaviours such as perching, egg laying inside a nest box and dust bathing in litter (Nicol, 1992). Although Barnett and Cronin (2005) found no clear welfare advantages to hens from items of furniture in the cage, apart from improved bone strength due to a perch, the detailed study of how hens utilise furniture may help explain why furniture did not 'improve' hen welfare, which is the intended outcome of housing hens in such cages.

From a functional perspective dust bathing facilitates maintenance of feather integrity (van Liere, 1992). Deprivation of a dust bath / dust bathing behaviour generally results in a rebound in dust bathing behaviour implying, according to Widowski and Duncan (2000) amongst others, that hens are therefore motivated to dust bathe. However, according to Petherick *et al.* (1993), hens are not necessarily highly motivated to dust bathe. For example, Widowski and Duncan (2000) found that while most hens were willing to work to obtain a dusty substrate when they could see it, they were not necessarily willing to work harder to access dust when in a state of deprivation. It is possible that dust bathing is stimulated by visual cues such as dust, litter or the sight of other hens dust bathing rather than the level of motivation or presence of the dust bath *per se*. In the Victorsson 8-bird furnished cage, the dust bath is situated above the nest box. Hens access the dust bath almost exclusively by jumping up from the perch (Barnett and Cronin, 2005) implying that the utilisation of the dust bath in this design of furnished cage may not allow hens to easily see the litter in the dust bath (unless standing on the perch). Widowski and Duncan (2000) have suggested that dust bathing can be stimulated by the sight of dust and of other hens dust bathing. The aim of the

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present experiment was to measure hens' use of the dust bath in the presence or absence of litter and their behaviour in the dust bath.

II. MATERIALS AND METHODS

As described by Barnett *et al*. (2006), Hy-Line Brown hens were housed in Trivselburen Furnished Cages (AB Bröderna Victorsson, Sweden) to examine the effects of individual items of furniture on hen welfare, in a factorial experiment with 8 birds per cage. This paper reports a component of this experiment related to utilisation of the dust bath, which was located on top of the nest box; the cages studied all included a perch. The dust bath was situated on the right side of the cage (viewed from the front) and consisted of a tray base made from 2-mm-thick black plastic measuring 470 mm x 240 mm. Hens entered the dust bath from one 470 mm side, which together with the front of the dust bath, was bordered by an 80-mm-high lip. The side lip was made of metal and was 210 mm above the perch and 305-350 mm above the floor (ie from rear to front of cage). The rear and far-side walls of the dust bath were formed by the metal walls of the cage. The present experiment involved 2 of the Dust Bath treatments (dust bath with and without a dust bathing substrate). The treatment with litter received about 2 cups (500 mL) of hardwood sawdust twice weekly, and to ensure that the 'no litter' dust bath did not have litter generated from settled air borne dust, the litter tray was cleaned using a vacuum cleaner at least once per week. The cages were 1206 mm wide, 498 mm deep and 455 mm high at the rear of the cage and were located in a controlled climate shed. Lights were on for 16 h from 0500 h daily from 26 weeks of age and the dust bath was open from around midday for 6.3 h/day.

There were two, 3-week periods of video recording, commencing at 29 (period 1) and 59 (period 2) weeks of age, during which 24-h time-lapse video records were made of all treatment cages in the large experiment. Video recording in dark/low-light conditions was assisted with low-light, black and white cameras and the use of infra-red light. Hen behaviour in the dust bath was monitored from a mini-camera positioned within the cage at cage ceiling height. Dust bath use was analysed as described by Barnett *et al*. (2006). In addition, detailed analysis of hen behaviour was conducted for up to 8 dust-bath hen visits per cage (average 6 per cage); focal visits selected were at least 20 s in duration. Differences in hen behaviour per cage were tested using one-way analysis of variance (Genstat 8.1). Cages in which hens did not visit the dust bath (n=2) were treated as missing values in the focal visit analyses.

III. RESULTS

On average, dust baths were occupied during 27.8% and 23.5% of the available time at periods 1 and 2, respectively, and the mean (± SD) duration of dust bath visits at these times was 8.0 (± 10.9) and 14.3 (± 20.8) min, respectively. Although the dust bath was used more if litter was present compared to absent, there were no significant differences at either period on the frequency or duration of dust bath visits, the latency for the first hen to enter the dust bath after it opened, or the duration of the first visit after opening (Table 1). There were large within and between cage variances for all parameters.

Examination of hen behaviour in the focal dust bath visits indicated that all birds entered the dust bath by first jumping from the perch to the edge of the dust bath (80 mm high lip). A visit to the dust bath was considered to commence from the time the hen alighted on the edge of the dust bath. Table 2 shows the characteristics of the different hen behaviour parameters. Although the frequency of bouts of standing and sitting posture in the dust bath per visit was greater for hens with litter at both periods, the effect of litter was only

significant at period 2. Litter in the dust bath increased the proportion of visits in which the behaviour 'hen shakes out' feathers occurred at period 1 ($P<0.05$) but not period 2.

IV. DISCUSSION

Hens in this experiment used the dust baths for less than 30% of the available ~6.3 h per day when dust baths were open. On average, hens visited the dust bath 1.6 and 0.8 times per hen at the two observation periods. Using the same design of furnished cage, Tauson and Holm (2002) reported data from two commercial farms in Sweden where the farmers scanned their cages 30-min after opening of the dust baths; the dust bath was occupied in about 45% of cages. It is difficult to compare these data with our observations due to differences in the method of data collection. However, in another study observing dust bathing by hens in an enriched cage design in which the dust bath was located above the nest box and access to the dust bath was restricted, Lindberg and Nicol (1997) found that hens visited the dust bath about 1.6 times per hen per 12 h. While this rate per hour is double that found in our observations, it is nevertheless similar to our results in the absolute frequency of use. Further, in Lindberg and Nicol's study, hens performed an average of 2.9 dust bathing bouts/12 h, with bouts (equivalent to visits recorded in our observations) lasting about 346 seconds, compared to about double this in our study at period 1 in the presence of litter.

Another finding of our study was the large variability between cages in dust bath use. In addition, as the experiment did not involve identifying individual hens for video observations, we cannot report on whether all hens used the dust bath. For 5 and 13 cages at periods 1 and 2, respectively, there were fewer than 8 visits to the dust bath per day, indicating that one or more hens did not use the facility; at period 2 there were 2 cages in which no hens visited the dust bath, despite the presence of litter. Litter increased the use of the dust bath, although the differences measured were not significant, probably due to the large variation between and within treatments. Nevertheless, the increased use supports other findings in the literature that litter (dust) is attractive to hens (Widowski and Duncan, 2000). One physical response to litter was that hens were more likely to perform a behaviour in which they appeared to 'shake out' the litter from their feathers. In conclusion, our findings suggest the dust bath was of low value to hens (relative to other furniture or hen activities; see Barnett *et al*., 2006). While the location of the dust bath in this cage design may be a contributing factor associated with the low use, the provision of litter increased the use of the facility, possibly through increased motivation. While this experiment describes the use of the dust bath by hens, the implications of a dust bath for welfare, based on these data, remain equivocal.

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Table 1. Dust bath use behaviour parameters in the presence and absence of litter.

Behaviour parameter	Units	Period 1		Period 2	
		Litter	No litter	Litter	No litter
Total time used (per cage)	min	122.7	88.6	91.3	71.4
Min. and max. values (between cages)		39-245	15-166	0-268	4-172
Bouts of use	freq.	12.4	14.1	6.1	5.8
Min. and max. values (between cages)		3-35	1-27	0-20	1-11
Latency to 1st use after opening	min	16.5	27.2	7.6	19.7
Duration of 1st bout after opening	min	19.2	15.9	29.3	6.7

Table 2. Hen behaviour associated with dust bath (DB) use in the presence (L) and absence (No L) of litter, in focal bout observations. Values shown are means; % refers to proportion of visits in which the behaviour occurred; s = seconds.

Behaviour parameters	Period 1				Period 2			
	L	No L	sed	P value	L	No L	sed	P value
1) Entry to DB-hen is on perch								
reaches into DB and pecks floor (%)	66	62	11.5	0.71	57	41	18.7	0.41
2) Entry to DB-hen is on edge of DB								
pecks at floor of DB (%)	68	85	9.2	0.08	47	58	18.5	0.54
latency to step into DB from edge (s)	24	45	22.5	0.36	37	60	27.2	0.42
3) Hen is in the DB								
duration of visit (s)	582	357	137.3	0.12	1220	507	551.3	0.22
frequency of standing bouts/visit	2.4	2.0	0.30	0.16	2.6	1.8	0.35	0.031
duration of standing bouts/visit (s)	202	169	42.6	0.45	140	119	36.2	0.57
frequency of sitting bouts/visit	1.5	1.0	0.32	0.14	1.6	0.9	0.33	0.047
duration of sitting bouts/visit (s)	406	188	128.8	0.109	1080	388	544.4	0.22
pecks at floor of DB (%)	100	98	1.9	0.33	98	88	5.7	0.11
flutters / fluffs feathers (%)	63	54	11.4	0.41	69	58	15.6	0.51
'shakes out' feathers (%)	50	19	11.4	0.013	54	39	17.6	0.43
4) Hen exits the DB								
Time spent perched on DB edge (s)	41	107	54.9	0.24	104	143	63.3	0.55

THE EFFECTS OF FURNITURE IN FURNISHED CAGES ON HEN WELFARE

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Summary

This factorial experiment with laying hens in furnished cages examined the effects of a dust bath, a perch and a nest box, alone and in combination, on welfare related criteria in hens in 8-bird cages. Although all items of furniture were used, particularly the perch and nest box, other than the benefits of a perch on bone strength, the effects of the items of furniture, either alone or in combination, on the welfare traits used were relatively minor and difficult to interpret in terms of welfare.

I. INTRODUCTION

Furnished cages are due to replace conventional cages for laying hens in the EU in 2012. Despite the relatively few in-depth studies on furnished cages, there is considerable support for such designs of cage, particularly in Europe and particularly based on the increased behavioural repertoire they permit. This experiment examined the welfare of hens housed in furnished cages and the effects of the individual items of furniture and follows a report of a preliminary study in the same experiment that included the effects of space allowance and group size (Barnett *et al*., 2005).

II. MATERIALS AND METHODS

Hy-line Brown hens (floor reared) housed in furnished cages at 15.5 weeks of age were used to examine the effects of individual items of furniture in a factorial experiment with 8 birds per cage. The cages were complete or modified Trivselburen Furnished Cages (AB Bröderna Victorsson, Sweden; the cage is pictured in Tauson and Holm, 2002). Each cage had a side nest-box, a dust bath located on top of the nest box and a wooden perch (see Barnett *et al.*, 2005 and Cronin *et al.,* 2006 for details). There were 2 Perch (present or absent), 3 Nest Box (present, blocked off or no nest box but the space it occupied was available to the hens) and 3 Dust Bath (present, blocked off or the space it occupied was available, but without a dust bathing substrate {hardwood sawdust}) treatments. The cages were 1206 mm wide, 498 mm deep and 455 mm high at the rear of the cage. Two additional external control treatments examined the effects of Space Allowance (8 birds in double-width cages) and Group Size (16 birds in double-width cages) in cages without furniture (see Barnett *et al*., 2005). The cages were in a controlled climate; lights were on for 16 h/day from 0500 h from 26 weeks of age and the dust bath was open from around midday for 6.3 h/day.

There were two 8-week periods of measurement, commencing at 29 and 59 weeks of age, respectively. In order of sampling these were: video observations of behaviour

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(including feeding behaviour and head movement, both overall and during lights-on, as indicators of activity, and use of the perch, nest box and dust bath during lights-on, lights-off and overall), blood sampling for assessment of immune function, collection of eggs for determination of corticosterone concentrations, blood sampling for determination of corticosterone concentrations, scoring of feather damage and cover, feather cleanliness, foot condition, pecking injuries on the comb, around the cloaca and on the back and keel bone deformation, measures of body weight and claw length and blood sampling 60 min after injection of ACTH for determination of the maximum corticosterone response. At 67 weeks of age, 3 birds from each cage were euthanased for bone strength determination. Methods have been described (see Barnett *et al.*, 2005).

Measurements were analysed as a 3 Nest Box by 3 Dust Bath by 2 Perch treatment factorial plus 2 added group size and space allowance controls, in a 3 replicate rectangular lattice, using restricted maximum likelihood (REML). A likelihood ratio test for fixed effects (Welham and Thompson, 1977) was used in the models to test for the main effects of items of furniture. The main effects that we consider having the greatest evidence of occurring (based primarily on $P<0.05$, but also considering consistency between results, residual error, discreteness of data and plausibility) are presented (Table 1).

III. RESULTS

Mortality in the experiment was 7% and a further 5.8% of hens were culled; hen day production during periods 1 and 2 was 93 and 80%, respectively. There was some evidence of an increased overall feeding activity ($P=0.034$) and increased feeding activity in the light period ($P=0.025$) in the Nest Box Space treatment during period 2 (Table 1). When a nest box was available for use, the majority of eggs were laid in the nest box (57 and 67% in periods 1 and 2, respectively). This varied with the presence of a perch (67 and 47% of eggs were laid in the nest box when a perch was either present or absent at period 1 ($P=0.0089$) and 78 and 61% respectively at period 2 ($P=0.085$)).

There were no overall effects of treatment on the use of perches by hens ($P>0.05$). There was a general lack of use of the dust bath in the absence of a perch (there was a perch effect on dust bath use at both periods ($P<0.001$ using a permutation test)) and the dust bath data were analysed for only those treatments that included a perch. In general, hens used the perches less in period 1 than in period 2 (means were 27% and 43% of observations over 24 h) and less in the light than dark (means were 21% and 30% of observations in the light and 37% and 66% in the dark, in the 2 periods, respectively). The number of bouts of dust bath use was least (both periods), total duration of dust bath use was least (both periods) and latency of first use of the dust bath after it opened was greatest (period 2), when the nest box was unavailable but the floor space that it occupied was available (Table 1).

Despite reasonably good precision, there was little evidence for effects of furniture on total and differential white cell counts, cell population ratios, immunological responsiveness or physiological measures of stress, except for a higher white blood cell count in period 1 in the Dust Bath Space treatment (Table 1; $P=0.021$). Stronger bone strength of the tibia, humerus and coracoid was found in the Perch treatment (Table 1; $P<0.05$). While feathers were dirtier in the Perch treatment at both sampling periods ($P=6.1 \times 10^{-6}$ and 0.002, respectively), the differences were <0.25 on a 4 point scale. Feather damage and cover score was slightly worse in the Perch treatment in period 2 ($P=0.012$). A perch improved foot condition at both sampling periods ($P=0.003$ and 0.0004, respectively) (Table 1).

Table 1. Effects of furnished cage treatments on hen behaviour, white cell count, feather condition and cover, foot condition (a high score indicates increased damage or poorer cleanliness) and bone strength. The values are predicted means with back-transformed mean values in parentheses.

Parameter	Factor[1,2]	Mean values			SED	P value
		Yes	No	Space		
Sampling period 1 from 29-36 weeks of age						
Number of bouts of dust bath use[3]	Nest box	3.64 (13.3)	4.24 (18.0)	2.39 (5.7)	0.584-0.590	0.023
Total duration of dust bath use/day *(min)*[4]	Nest box	2.02 (105)	2.12 (131)	1.66 (46)	0.142-0.143	0.018
White cell count *(10^6cells/mL)*	Dust bath	16.7	15.8	19.2	1.21-1.22	0.021
Feather cleanliness	Perch	1.45	1.23	-	0.042	$6.1x10^{-6}$
Foot condition	Perch	0.61	0.79	-	0.058	0.0030
Sampling period 2 from 59-66 weeks of age						
Hen feeding activity[5,6]	Nest box	0.92	0.97	1.14	0.084-0. 085	0.034
Hen feeding activity[5,6] in the light period	Nest box	1.30	1.40	1.63	0.117-0.118	0.025
Number of bouts of dust bath use[3]	Nest box	1.97 (3.9)	3.08 (9.5)	1.21 (1.5)	0.528-0.552	0.019
Total duration of dust bath use/day *(min)*[7]	Nest box	1.52 (32)	1.85 (69)	1.08 (11)	0.269-0.299	0.082
Latency to first use dust bath after opening *(s)*[4]	Nest box	0.63 (4.3)	0.21 (1.6)	1.04 (11.0)	0.212-0.242	0.014
Feather cover and damage score	Perch	2.32	2.13	-	0.072	0.012
Feather cleanliness	Perch	2.05	1.83	-	0.068	0.0020
Foot condition score	Perch	1.85	2.20	-	0.091	0.00042
Bone strength (N) at 67 weeks of age						
Tibia	Perch	162.7	151.0	-	4.57	0.0090
Humerus	Perch	116.6	102.1	-	5.11	0.0063
Coracoid	Perch	159.0	144.5	-	6.54	0.031

[1]Within each factor the mean values for the presence or absence, respectively, of a perch, dust bath or nest box, are presented in the 'Yes' and 'No' columns. The 'Space' column indicates that the space reserved for the item of furniture remains available; [2]the statistical analysis for use of dust baths was restricted to cages with a perch; [3]square root transformation; [4]$\log_{10}$ transformation; [5]estimated on the basis of number of hens feeding at each hourly observation period averaged over 24 h; [6]the data have been covariate adjusted for the number of hens/cage; [7]$\log_{10}(y+1)$ transformation.

IV. DISCUSSION

The major effect of the furniture treatments was that due to the Perch treatment on bone strength. The tibia, humerus and coracoid were stronger in the Perch treatment. Increased bone strength due to a perch has previously been shown, although the bones affected vary across studies (see Barnett and Cronin, 2005). Other effects were generally

small and difficult to interpret in terms of welfare. Overall, this experiment has shown that any effects of a perch, dust bath and nest box on behavioural, physiological and body condition measurements were small. While there were some small effects of furniture on bird feather and foot condition and feather damage/cover, with dirtier and more damaged feathers but better foot condition in the Perch treatment, the overall condition remained good. Probably the most discussed issue in relation to the welfare of hens in cages is the lack of a suitable nest site and the data from the present experiment do not confirm this concern. In the presence of a nest box there was behavioural change that was affected by other items of furniture; more eggs were laid in the nest box if a perch was present. However, there were no other effects of furniture on egg laying characteristics such as interval between eggs laid. The high number of eggs laid on the wire compared to other studies (Tauson and Holm, 2002) may be due to genotype, rearing or cage modifications.

Except for one cage, the dust bath was only used if a perch was also present and this was probably a consequence of the cage design. In cages with both a perch and a dust bath, the latter was occupied for about 25% of available time. Other designs of furnished cage have a dust bath on the floor and this is likely to impact on its use. The major effects of furniture on hen condition were due to the perch, but in all cases although differences were statistically different they were small. In the Perch treatment the feathers were slightly dirtier and foot condition was slightly better at both periods and feather cover/damage was slightly worse at period 2. The slightly better foot condition may have some implications for welfare in that poor condition is likely to be associated with increased inflammation and possibly pain. The Nest Box and Dust Bath treatments had no effects on condition ($P>0.05$).

In contrast to the findings on furniture, there were significant physiological effects of group size and space allowance with some evidence that birds housed at 16 hens/cage (space allowance of 750 cm^2/bird) were stressed compared to birds housed at 8 hens/cage with the same space allowance (Barnett *et al.*, 2005). This was based on higher egg corticosterone concentrations and evidence consistent with immunosuppression at period 1. Further research is clearly warranted on this topic and on group size, space allowance and their interactions. In conclusion, in this experiment on furniture in cages, other than benefits of a perch on bone strength and possibly on foot condition, any effects of a dust bath and nest box on the welfare traits utilised were relatively small, although hens did utilise all items of furniture.

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WELFARE, PERFORMANCE AND EGG QUALITY OF HENS IN AN IMPROVED BARN SYSTEM

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Summary

The performance, egg quality and welfare of hens under a barn system at PRDC were evaluated using improved management and superior dietary treatments. Preliminary data suggests that at 50 weeks of age, production performance parameters of layer hens were satisfactory with no marked influence of dietary treatment. Although egg weight was slightly lower than expected values for cage systems this was considered beneficial for avoiding cloacal haemorrhage; as important precursor to vent trauma and cannibalism. The main problem in regards to egg quality was the number of floor eggs which were reduced but remained high (11.4 and 10.6% diet 1 and diet 2, respectively) at 50 weeks of age. This management situation needs further investigation due to egg microbial contamination. Total plumage score was substantially worsened after 40 weeks of age due to damage to the back plumage region. Mortalities were lower than in previous years with some incidence of cannibalism at 40 and 50 weeks of age in the control diet. It is concluded that appropriate birds management during the rearing period in combination with improve dietary treatments influence performance and bird welfare in barn systems.

I. INTRODUCTION

Studies in Victoria and Queensland have assessed the welfare and performance of barn systems with hens having with higher mortality (15-30%), lower body weight, better feather condition at 29 weeks old but inferior feather condition and cover at 40 and 64 weeks old. Production and egg weight were lower, with more dirty eggs, with inferior yolk colour and feed intake (FI) 7-10% higher than birds kept in cages. This overall low performance, economics, welfare and high mortality require further research. Protein and dietary amino acids, particularly sulphur amino acids, have been shown to influence cannibalism and feather pecking (Tiller, 2004). Tryptophan, a precursor for serotonin, promotes feelings of wellbeing, calm and relaxation in humans (South, 2004) and suppresses aggression in male broiler breeders (Shea *et al*., 1990), may be required at higher levels for barn birds to reduce weight loss, stress and to promote calming of birds in a stressed environment. Selenium, an essential constituent of the antioxidant enzyme glutathione peroxidase, has been shown to improve feed conversion, feather growth, egg quality and meat quality in broilers (Edens *et al.*, 2001). The evaluation of selenium in barn systems to improve bird welfare needs further investigation. Another interesting aspect of the complex issue of feather pecking is pullet body weight in which undernourished birds developed pecking and cannibalism behaviour, which may be a response to locate needed nutrients (Parkinson, 2005). The present paper reports preliminary data from a current barn production experiment at PRDC in which bird management and nutritional aspects were modified and improved in order to reduce feather peaking, cannibalism and low productivity.

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II. MATERIALS AND METHODS

Four thousand laser beak-trimmed day old chicks (Hy-Line brown) from a Bendigo commercial hatchery were floor-reared to seven weeks of age on pine shavings in a controlled environment shed (pen size 15 x 12 m). Water, temperature, husbandry management and vaccination programme (including coccidia and parasites) were provided in accordance with PRDC management. During the first 48 hours (h) continuous light was provided at 10 lux intensity (LI). From day 2 to seven weeks, light was reduced to 15 h per day to 5 LI. At 18 weeks of age, light stimulation started when the flock reached 1550 grams. Artificial lighting was used throughout to provide a 16-hour constant day-length lighting programme. Following chemical analysis, mash diets were provided following the program suggested in the Hy-Lyne management guide (2005) for adequate body weight and commercially acceptable production. After seven weeks, birds were transferred to a commercial barn system where they remained during the production cycle. The barn has been built in a naturally-ventilated clean-line poultry shed (L 40.04, W12.2, H 2.550, in meters) with a concrete floor and an opened side (north) and with shutters (south). It is operated as an open-sided barn with a fan-forced ventilation and a fogger system. The internal configuration is 4 pens of approximately 1,000 birds/pen (7 birds/m^2) with slatted areas, nest boxes and a deep-litter area. The plastic slatted areas comprise one third to one half of the total floor area. The Jansen roll-away nests are those used commercially. Automatic colony nest belts are provided with Astroturf® floor coverings. Feed and water are provided *ad libitum* from an automatic single line of pan feeders and a single line of nipple drinkers suspended over the slats for each pen. Intake in each pen was measured by individually installed silos fitted with 3 x shear beam load cells connected to a digital indicator to access and record feed delivery. Experimental diets were randomly allocated in a randomised block design to 4,000 25-weeks old pullets distributed into 4 pens within a commercial barn house. Two dietary treatments were prepared with Diet 1 (Control diet), formulated as recommended by the breeder management guide. Diet 2 was formulated as Diet 1 but with additional 30% methionine, 30% tryptophan and 0.3 mg of organic selenium added/kg of diet. The control diet was assigned to pens 1 and 3 and Diet 2 was assigned to pens 2 and 4. Eggs were collected three times daily for egg production. A sample of 10% of eggs was individually weight weekly. Nested and non-nested (floor, slats, corners) eggs were recorded including cracked and soiled eggs. FI was recorded weekly, and monthly body weights to 10% of birds in each dietary treatment. Egg quality including, shell, yolk colour, blood, meat and Haugh units were evaluated on 10 % of all eggs monthly. Temperature and humidity was continuously logged inside and outside the buildings. Litter and feather condition were assessed at intervals. At week 10, 20, 30, 40, and 50, weeks of age random samples of 5% of layer population were individually scored in each treatment (by two observers) for the condition of plumage and skin, as well as for health status of feet and comb (Tauson *et al.* 1984).

III. RESULTS AND DISCUSSION

The data in the present experiment is yet to be statistically evaluated as the experiment will continue for another 20 weeks. However, bird performance at 50 weeks of age (Table 1) indicates that production parameters of layer hens were satisfactory with not marked influence of dietary treatment. Egg production in both treatments was acceptable when compared with the 85-87% reported under cage systems (Hy-Line Guide 2005). In the present study, egg weight at 50 weeks of age was 61.4g and 61.2g for diets 1 and 2 respectively (Table 1). The expected egg

weight should be higher, at around 65.3 and 63.4 g for cage and barn system respectively (Hy-Line Guide, 2005; Thomas *et al.*, 2000). Other barn studies have also found lower egg weight (Barnet, 1999). In the present study Diet 2, which was supplemented with methionine, did not appear to influence egg weight. Linoleic acid, another dietary component influencing egg weight, was provided at 1.24% (25-32 weeks of age) and 1.18% (33-44 weeks of age), but influence egg weight. If the birds body weight, FI and production in the present study are shown to be adequate, additional levels of linoleic acid may be needed to increase egg weight. However, by increasing egg weight there is a possibility of inducing other negative aspects such as cloacal haemorrhage, an important precursor to vent trauma and cannibalism (Parkinson, *et al* 2005). Thus, the relatively low egg weight in the present study may be seen as a beneficial rather than a negative aspect, but more consideration and comparative studies with different levels of linoleic acid are needed.

Table 1. Production parameters to 25, 30, 40 and 50 weeks of age. Diet 1 (Control diet); Diet 2 has additional 30% Met, 30% Trp and 0.3 mg of organic Se added/kg of diet.

Parameter	Week 25		Week 30		Week 40		Week 50	
Diet	1	2	1	2	1	2	1	2
Hen-day out put (%)	90	93	89	91	85	85	85	84
Egg weight (g)	54.5	55	57	56.3	59.6	59.4	61.4	61.2
Egg mass (g/d)	49.1	51.2	50.7	51.2	50.7	50.5	52.2	51.4
Feed intake (g/d)	105	109	115	117	117	116	122	122
Feed efficiency (g/g)	2.14	2.13	2.27	2.29	2.31	2.30	2.34	2.37
Body weight (kg)	1.80	1.81	1.87	1.86	1.92	1.91	1.99	1.96

In the current study, the mean bird feed intake at 50 weeks of age was 122 g/b/d for both dietary treatments. This barn data is similar to the 124 g/d reported for barn systems (Thomas *et al.*, 2000) but slightly higher when compared with the 111 g/d/b in cage systems (Hy-line Guide, 2005).

Table 2. Egg quality to 25, 30, 40 and 50 weeks of age

Parameter	Week 25		Week 30		Week 40		Week 50	
Diet	1	2	1	2	1	2	1	2
Haugh units	98.5	98.0	96.7	95.9	92.7	93.0	93.2	92.2
Roche colour score	11.2	11.0	11.4	10.8	11.5	12.4	11.4	11.5
Shell thickness (mm)	0.37	0.36	0.35	0.35	0.36	0.36	0.37	0.37
Cracked eggs (%)	0.4	0.4	0.4	0.3	0.18	0.23	0.23	0.21
Dirty eggs (%)	0.6	0.6	0.7	0.6	0.25	0.25	1.7	1.7
Blood stained (%)	-	-	0.04	0.09	0.02	0.02	0.95	1.1
Floor eggs	20.2	10.3	22.4	14.2	20.4	14.5	11.4	10.6
Blood spots (%)	0.16	0.17	0.19	0.21	0.22	0.13	0.17	0.11
Meat spots (%)	0.1	0.1	0.18	0.18	0.21	0.16	0.3	0.3

The main problem with regard to egg quality in the present study is the number of floor eggs, particularly in Diet 1. Reports indicated that between 15-50% of eggs were laid on the litter under barn systems (Barnett, 1999; Lu and Dingle, 1999; Nagle *et al.,* 2004). In order to reduce floor eggs in the present study, pullets were reared in one large group in floor pens, then

transferred to the barn at a young age (7 weeks), and had limited access to the nest box by closing nests at night (Barnett 1999; Lu and Dingle, 1999, Industry staff advice). However, the number of floor eggs in our study is evident and needs further investigation particularly as it has been reported that floor eggs usually have poorer microbial quality on the shell surface with higher coliform, *E. coli* bacteria counts (Barnett, 1999). In previous barn studies at PRDC, blood stained eggs increased gradually from 0.5 to 2.5-5.9% (at 25, 41-45 weeks of age respectively). Further analysis indicated a positive correlation between egg weight and blood stained eggs (Nagle *et al*, 2004). However, in the present study the proportion of cracked, dirty or blood stained eggs were relatively low suggesting that the lower egg weight may contribute to reducing the risk for blood stained eggs. Although nearly 14% of eggs were laid on the litter, the proportion of dirty eggs contaminated with either soil or excreta was relatively low (Table 2) when compared with 8.89% from extensive systems (Glatz and Ru 2004).

Table 3. Welfare evaluation to 25, 30, 40 and 50 weeks of age

Parameter	Week 25		Week 30		Week 40		Week 50	
Diet	1	2	1	2	1	2	1	2
Back plumage score	3.76	3.78	3.73	3.78	3.24	3.08	2.07	2.07
Tail plumage score	3.98	4.0	3.98	3.99	3.7	3.7	2.49	2.63
Total plumage score	3.95	3.95	3.94	3.95	3.75	3.7	2.96	3.0
Foot score	4.0	4.0	3.94	3.94	3.94	3.97	3.96	3.98
Mortality	0.56	0.22	0.22	0.45	1.56	1.21	0.67	0.77

In general, mortality was low but the total bird feather score was reduced from 3.75 and 3.70 at week 40 to 2.96 and 3.0 for 50 weeks of age for dietary treatments 1 and 2 respectively. Damage to the back plumage is the main aspect influencing the score. These obtained values are similar to those reported elsewhere (Nagle *et al,* 2004; Barnet, 1999). Although, in our experiment birds were beak-trimmed at day old, it would appear that changes in nutrient requirements for a lower specification formula which occurred after 40 weeks of age may have negatively influenced feather cover.

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LAYER STRAINS FOR ALTERNATIVE SYSTEMS

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Summary

Public concerns for poultry welfare, and market demand for eggs produced in non-cage systems, have put pressure on the Australian poultry industry to consider alternative systems for housing layers. Two concurrent 12-month trials were conducted to compare the strengths and weaknesses of three popular brown-egg strains (ISA Brown, Hyline Brown and HiSex Brown) when housed in barn and free-range systems. Following the comparison it was found that Hyline Brown and ISA Brown were best suited to the free-range system, and ISA Brown, to the barn system. However, mortality rates were unacceptable for all three strains in both systems. Mortality decreased dramatically after beak trimming in the barn, however, it did not entirely prevent feather pecking and cannibalism, which are issues of public concern.

I. INTRODUCTION

Commercial non-cage egg production systems currently represent only a small proportion of the egg market, however the free-range and barn poultry industries in Australia are expanding as consumer demand for non-cage eggs increases. Margins are slim due to higher production costs and mortality rates, which are unacceptable to the producer, industry and public. These systems must, therefore, not only be practical and economically viable but must comply with the standards set down by organISAtions representing welfare-conscious consumers. Phasing out of older style cages will force producers to recapitalise and they may consider reinvestment in cages to be a high-risk option and settle for an alternative system (or leave the industry). The aim of this project was to provide information on the suitability of current commercial layer strains for alternative (non-cage) management systems.

II. METHODS

Equal numbers (1516) of ISA Brown, Hyline Brown and HiSex Brown day-old pullets were obtained from hatcheries on the same day, housed in floor pens and brooded and reared in accordance with the hatchery recommendations applicable to each strain. Although beak trimming may be indicated as a standard procedure for some strains, for these trials birds were not beak trimmed. All birds were housed in the experimental accommodation by approximately twelve weeks of age. The barn consisted of two 200-bird pens and one 500-bird pen of each strain in a randomised block design. The slatted areas comprised ⅓ to ½ of the total floor area. The rollaway colony nests were made of tin with Astroturf® floor coverings. The free-range paddock was divided into six fenced areas arranged in two blocks of three areas. Each block contained a shelter divided into three pens, each giving access to one of three outdoor areas, resulting in two 300-bird replicates of each strain in a randomised block design. The rollaway nests were the same as in the barn system. Some birds were kept in cages for purpose of control comparison. From 18 weeks of age, egg production and mortality were recorded daily with egg weights recorded fortnightly. Numbers of eggs laid in each nest and in various zones (floor, slats, paddock etc) were recorded, including numbers of cracked and blood stained eggs. Feed intake was measured at 4 week intervals with

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bodyweight, feather condition, foot and claw condition, egg specific gravity and yolk colour recorded at three-monthly intervals. Temperature and humidity were continuously logged inside and outside the buildings (TINYVIEW® Gemini Data Loggers) and a 16-hour lighting regime was used.

III. RESULTS

Key commercial parameters for the three strains in the free-range and barn systems are shown in Table 1.

Table 1. Effect of strain on key commercial parameters in a free-range and barn system.

	Free-range System			Barn System		
Production Parameter	ISA Brown	Hyline Brown	HiSex Brown	ISA Brown	Hyline Brown	HiSex Brown
Bird weight (g)	1937.8	1957.5	1850.0	1854.9	1901.0	1791.7
Feed per bird per day (g)	115.97	123.06	117.09	112.51	118.98	116.02
Laying percentage (%)	58.9	63.9	58.7	65.3	68.1	64.8
Egg weight (g)	63.9	62.4	61.7	63.1	61.8	61.5
Egg mass (g/day)	41.02	41.27	43.28	42.57	43.22	40.77
FCR	3.0	3.0	3.0	2.7	2.7	2.8
Eggs laid in nest boxes (%)	83	89.2	81.2	83.3	84.1	77.3
Blood stained eggs (%)	3.8	2.7	2.5	2.4	2.8	2.9
Specific gravity	1.10	1.10	1.10	1.09	1.08	1.09
Yolk colour	12.0	12.2	12.3	12.2	12.3	11.7
Mortality (%)	5.5	5.4	5.2	4.4	2.7	4.4

Results shown are average per month over a 12-month period (from 18 weeks of age). Feed conversion ratios (FCR) are for an 11-month period (from 24 weeks of age – feed conversion ratio was calculated for 11 months due to the birds coming into production during the first mo of the trial).

Mortality in the barn system was 1.4% during month one and 2.3% for month two. During month three, mortality started to approach 5% (4.9%) and the decision was made to beak trim some of the birds in the barn to evaluate the effect on various parameters (1200 trimmed versus 1500 not trimmed). Table 2 shows the key commercial parameters of the beak trim versus no beak trim birds in the barn system.

Table 2. Effect of beak trim on key commercial parameters in a barn system.

Production Parameter	No Trim	Beak Trim
Bird weight (g)	1882.8	1903.1
Feed per bird per day (g)	130.47	121.39
Laying percentage (%)	75.4	76.3
Egg weight (g)	65.6	64.8
Egg mass (g/day)	49.61	49.52
FCR	2.6	2.5
Eggs laid in nest boxes (%)	77.7	85.0
Blood stained eggs (%)	5.0	2.8
Specific gravity	1.08	1.08
Yolk colour	11.6	12.3
Mortality (%)	6.0	3.0

Results shown are average per month over a 9-mo period (from mo 3 when trimmed**).**

IV. DISCUSSION

ISA Brown birds had the lowest feed intake and FCR and the heaviest eggs in the barn and free-range systems. Hyline Brown birds had the highest feed intake and egg production in the barn and free-range systems with the highest FCR in the free-range system. HiSex Brown birds had the lowest egg production and the lightest eggs in both the barn and free-range systems, with the highest egg mass in the free-range system but the lowest in the barn and the highest FCR in the barn system. No differences were found with yolk colour and specific gravity of eggs.

Free-range had the highest mortality during the trial followed by barn and cage (Figure 1). These differences could have been influenced by various factors including body weight, diet, disease and cannibalism (Figure 2). Early in this trial barn and free-range birds had bodyweights below that of caged birds. In previous trials in commercial barn systems mortality was markedly increased in flocks that did not reach the appropriate body weight at 26 – 29 weeks of age (Parkinson and Cransberg, 2002). Within systems, Hyline Brown birds were the heaviest in the barn and free-range systems, followed by ISA Brown and HiSex Brown.

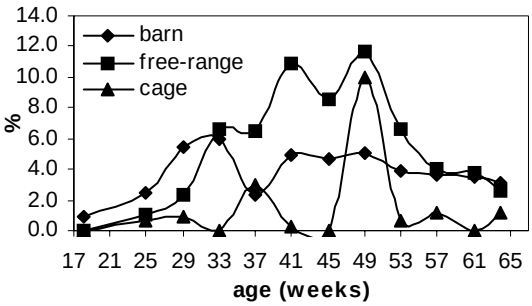

Figure 1. Effect of system on mortality.

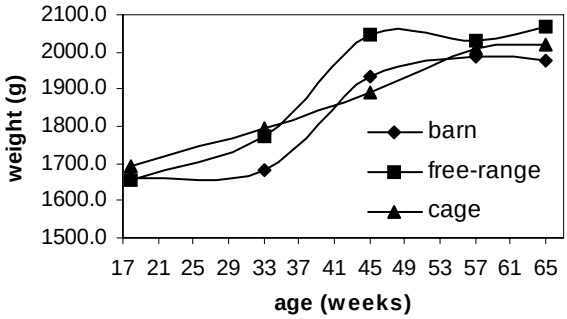

Figure 2. Effect of system on bird weight.

Changes in diet can cause a reduced or increased palatability of food and result in a decreased intake or increased competition for food, leading to stress and frustration which are reported risk factors in vent and feather pecking. A new diet may also cause diarrhoea, leading to an inflamed and reddened vent and soiled surrounding attracting pecking from flockmates. However during this trial all hens were fed the same feed therefore changes in diet were not seen as cause for the high mortality.

Previous diagnostic studies of underweight flocks have indicated problems with egg peritonitis and salpingitis (Parkinson and Cransberg, 2002), which was also observed in the present study. Salpingitis is inflammation of the oviduct and is found more frequently in birds on litter than in cages and is spread by contact between birds, or by contact between birds and faeces.

Blood stained eggs can give an indication of vent pecking in flocks and is characterised by damage to the cloaca, surrounding skin and underlying tissue by a con-specific and can progress to evisceration and death. In this trial the free-range and barn systems had a much higher incidence of blood stained eggs compared to the cage system (Figure 3).

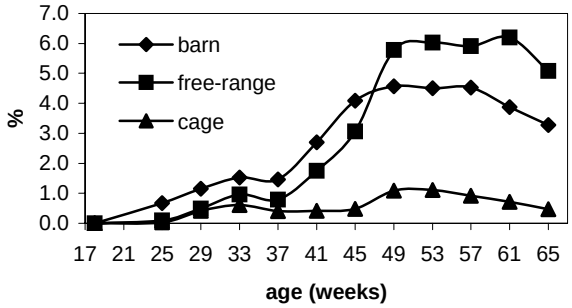

Figure 3. Effect of system on blood stained eggs.

When strains were compared within systems, ISA Brown birds had the lowest blood stained eggs in the barn and the highest in the free-range, while HiSex Brown birds had the highest in the barn and the lowest in the free-range system.

Extensive feather loss (apart from moulting) can indicate physiological or behavioural differences from natural conditions, is unattractive to the human observer and may increase the danger of exposed skin being injured. Total bird feather score was similar for all three systems with differences in the part of the body affected. In the barn and free-range systems the back and tail had lower feather scores compared to cages. Neck and wing feather scores were lower in the cages probably due to abrasion on the wire cages. The breast was most denuded in the barn and cage systems, again probably due to the wire in the cages and the slats in the barn. ISA Brown had the highest total feather score in the barn and free-range systems, with HiSex Brown the lowest. Foot and claw condition did not vary between systems or strains.

Beak trimmed birds in the barn consumed an average 9 grams less per day than non-trimmed birds and had a lower FCR compared to non-trimmed birds. Birds that were beak trimmed had a lower mortality rate than birds that had not been trimmed (Figure 4) and a lower percentage of blood stained eggs (Figure 5). This suggests an increase in vent pecking in untrimmed birds. Directly after trimming, untrimmed birds showed a dramatic reduction in the use of nest boxes in the nest boxes (Figure 6). Birds that had been beak trimmed had a higher total bird feather score throughout the trial.

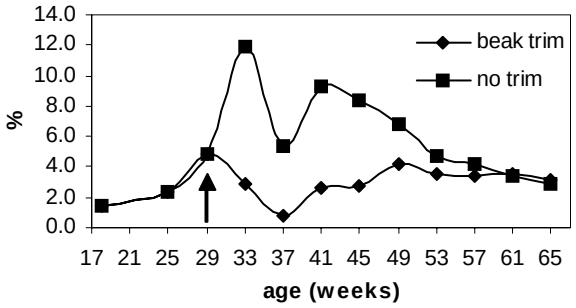

Figure 4. Effect of beak trim on mortality.

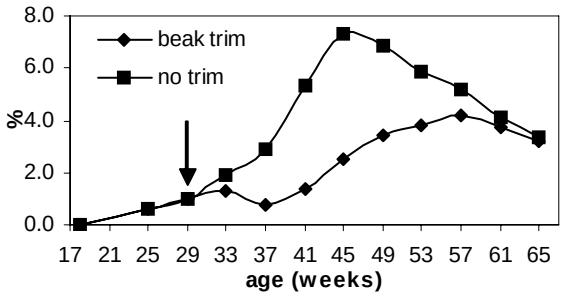

Figure 5. Effect of beak trim on blood stained eggs.

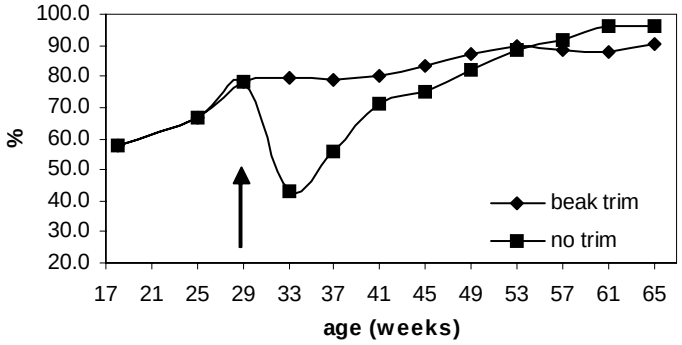

Figure 6. Effect of beak trim on use of nestboxes.

V. CONCLUSION

Following the comparison of the relative merits of the performance of ISA Brown, Hyline Brown and HiSex Brown birds in barn and free-range systems, the present trial found that Hyline Brown and ISA Brown were best suited to the free-range system and ISA Brown to the barn system. However, the mortality rates were unacceptable for all three strains in both systems. Mortality decreased dramatically after beak trimming in the barn, however,

beak trimming is not entirely effective in preventing feather pecking and cannibalism, which are issues of public concern. If we are to reduce the need for beak trimming in the Australian flock further research needs to be done to develop a specific strain for each system and improvement of the management procedures for current commercial breeds for use in alternate systems. Research on the nutrient requirements of bird strains used in the free range and barn systems is warranted to improve productivity and profitability.

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ENVIRONMENTAL FACTORS INFLUENCE THE PREVALENCE OF INFECTIOUS BRONCHITIS VIRUS

J.C. LOPEZ[1] and R. MCFARLANE[1]

Summary

Binary logistic regression analyses were conducted to assess associations between the presence of infectious bronchitis virus (IBV) in broilers and various risk factors: ambient ammonia, oxygen, carbon dioxide, humidity and litter humidity. Pairs of sheds were selected from ten large broiler farms in Canterbury, New Zealand. One shed from each of the pairs had a production or health alteration that suggested the presence of IBV and the other was a control shed. IBV was detected by RT-PCR in 50% of the farms. In 2 of the 5 positive farms where IBV was detected there were accompanying clinical signs that suggested infectious bronchitis (IB). More commonly uncomplicated infections with IBV were asymptomatic under good management. Ambient humidity was the only risk factor that showed an association (inverse) with the prevalence of IBV.

I. INTRODUCTION

Infectious bronchitis (IB) is a highly contagious respiratory viral disease of chickens characterized by respiratory and renal pathology, a drop in egg production and egg quality in layers and decreased growth and feed efficiency in broiler chickens (Cavanagh and Naqi, 2003). Infectious bronchitis virus was first isolated in New Zealand by Pohl (1967) and was subsequently shown to be serologically different from other international strains (von Bülow, 1969; Lohr, 1977). A high prevalence of IBV has been demonstrated recently in New Zealand using RT-PCR (Ramneek *et al.,* 2005); 28 genotypes differed from other international strains.

Seasonal cycles of infectious diseases have been attributed to environmental changes, pathogen appearance and disappearance and host-behavior changes (Dowell, 2001). Many diseases caused by coronavirus such as severe acute respiratory syndrome (SARS) and infectious bronchitis (IB), exhibit winter seasonality with the presence of persistent virus carriers (Dowell and Ho, 2004). The prevalence of respiratory diseases peaks during winter in poultry farms and could be caused by ineffective ventilation because of the desire to conserve heat. Reduced ventilation usually results in an increase in air pollutants, such as ammonia, carbon dioxide, dust and air-borne microorganisms (Anderson *et al.,* 1966).

Certain factors are known to reduce bird performance, exacerbate clinical disease or modulate immune response: low/high environmental temperatures (Ratanasethakul and Cumming, 1983), humidity levels (Yoder *et al.,* 1977), high ammonia levels (Anderson *et al.,* 1964), and low levels of oxygen (Olander *et al.,* 1967). Only a few of these factors have been studied in relation to an IBV infection. The aim of this study was determine the prevalence of IBV in broilers within the Canterbury province in late winter and search for associations with management or environmental factors.

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II. MATERIALS AND METHODS

a) Farm Selection

The farms eligible for the case control study were suppliers for a major broiler producer in Canterbury, New Zealand. Ten farms (the average size was 77,000 birds) were selected from a total of 29 farms that produced approximately 60-70% of the broiler chickens from Canterbury. Birds from these farms in the past have contained high levels of antibodies against IBV or exhibited productive problems. Birds within one of the sheds had some production or health signs that suggested the presence of IBV, or were known to have experienced clinical infectious bronchitis (IB) in the past. This shed was defined as the case shed and the control shed was defined as the shed believed not to contain birds that had been affected by IB.

The flock in each shed had a high degree of similarity with respect to the age of birds, number of drinkers, nature of food and litter.

b) Detection of IBV

In each shed, 6 birds were randomly selected. Each bird was examined for respiratory signs and tracheal and cloacal swabs were taken, placed in transport media (Poulvac Sterile diluent, Fort Dodge Animal Health, USA) with antibiotic (Enrofloxacin 10%, Bayer, New Zealand) and stored at -20°C. RNA was extracted by the addition of TRIzol (Life Technologies, USA) and IBV was detected using the RT-PCR assay, as described by Ramneek *et al. (*2005). The levels of ammonia, oxygen, carbon dioxide, and ambient humidity, 0.5 m above the litter surface, were measured using a Draeger multiwarn II gas detector (Dräger AG, Lübeck, German). Litter humidity was measured by manual compression (North and Bell, 1990).

c) Questionnaire and Data Analysis

A questionnaire was completed by the farm manager in order to collect information about certain farm characteristics; such as, flock size, management practices and environmental factors. A descriptive analysis was completed to provide summary statistics for all variables in the data set. Binary logistic regression analyses were conducted to assess associations between presence of IBV and various risk factors, using MINITAB Statistical Software (Minitab Inc, Pennsylvania, USA).

III. RESULTS and DISCUSSION

There is a general belief that IBV infection in broilers in New Zealand is under-diagnosed, particularly as its prevalence has been largely based on seroconversion and this may be misleading as birds are frequently tested at slaughter (35-40 days old) and have had little time to seroconvert, thus leading to false negatives. A study made by Ramneek *et al.* (2005) showed that 19% of the New Zealand broilers and layers were positive to IBV, as detected by direct RT-PCR. In this study, IBV was detected by RT-PCR in birds from 50% of the farms. In 2 of the 5 positive farms where IBV was detected there were accompanying clinical signs of IB. The lack of clinical disease in the field where IBV was present could be attributed to a number of factors, including: mildness of the IBV strains, good management practices and the absence of immunosuppressive agents such as infection bursal disease virus, chicken anemia virus, *Mycoplasma gallisepticum*, *E. coli*, or *Haemophilus paragallinarum*. These agents have been rarely found in New Zealand and the resulted pathology due to them is mild (Brooks, 2003). Ramneek (2000), studied the pathogenicity of 5 different genotypes of IBV and found that all the strains analysed induced only mild histological lesions and clinical

signs. This study was carried out under good management conditions with respect to temperature, humidity and levels of ammonia).

Environmental changes are the explanation most often used to explain the seasonality of infectious diseases (Dowell, 2001) and most avian respiratory pathogens exhibit an annual increase in incidence each winter. The temperature was higher than 24°C in only 2 farms, but otherwise was within the optimum temperature for birds at 19°C to 24°C (Yoder *et al.,* 1977). A reduction in ambient temperature (constant 16°C) has been reported to increase mortality in IBV infections (Cumming, 1969). There were no indications (postural adjustments, behavioural changes, or changes in food and water consumption) that the birds were uncomfortable with the ambient temperature.

In this trial we found a significant inverse relationship (P = 0.05; OR = 0.92) between the prevalence of IBV and ambient humidity (Table 1). Yoder *et al.* (1977) reported that at medium temperatures (19-24°C) and low humidity, flocks infected with *Mycoplasma synoviae* and IBV had a higher incidence of airsaculitis than at a high humidity. It has been proposed that drying of mucosal surfaces increases the probability of bacteria colonisation (Dowell, 2001). In humans, outbreaks by respiratory syncytial virus (RSV) have been reported to peak in seasons of lower relative humidity (Chew *et al.,* 1998). However, Ijaz *et al.* (1985) studied the survival of airborne human coronavirus and reported that a high relative humidity (80 +/- 5%) at 20 +/- 1°C, was found to be least favourable for the survival of virus aerosols.

Table 1. Logistic regression analysis of factors associated with the presence of IBV in broiler farms

Risk Factor	P Value	OR	95% CI	Mean (Range)	SEM
Temperature*	0.99	1	0.74-1.35	22.5C (20-29°C)	0.720
Humidity*	0.05	0.92	0.84-1	75.3% (50-97%)	3.490
Ammonia*	0.4	1.03	0.96-1.1	16.7 ppm (0-50 ppm)	3.100
Oxygen*	0.25	0.07	0.0-7.4	20.4 % (20.0-20.9 %)	0.054
Carbon dioxide*	0.8	0.69	0.01-60.39	0.31% (0.0-0.8%)	0.040
Litter humidity	0.1	4.6	0.74-28.47	2.6 (1.0-3.5)	0.220

OR= Odds Ratio, CI= Confidence interval, SEM= Standard error of the mean
* Measured 0.5m above litter surface.

Based on the results of previous studies (Anderson *et al*., 1964; Kling and Quarles, 1974) it was anticipated that higher levels of ammonia could increase the presence of IBV or exacerbate the clinical signs found in infected birds. Anderson *et al*. (1964) reported that 72 hours of exposure to ammonia concentrations in the range of 20 to 50 ppm significantly increased the infection rate of chickens with Newcastle disease virus when given as aerosol. However, no association was found in this study. Four of the six sheds positive for IBV had low ammonia levels (under 20 ppm).

Levels of oxygen and carbon dioxide in the poultry industry are used principally as criteria for an efficient ventilation system. Although 55% of the sheds in our studies had a

low level of oxygen (under 20.5 %) and 40% had high levels of carbon dioxide (over 0.3 %), we did not find a significant association between the levels of oxygen or carbon dioxide and the detection of IBV.

In this trial, there was no significant relationship between the litter humidity and IBV occurrence. Indeed in the majority of the of the sheds positive for IBV, levels of litter humidity were higher than recommended (North and Bell, 1990).

We can conclude within the constraints of the similar management systems described, that humidity has an influence on the presence of IBV, but there was no influence due to temperature, ammonia, carbon dioxide, oxygen and litter humidity

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PRELIMINARY STUDIES ON THE EFFECTS OF INFECTIOUS BRONCHITIS ON UNVACCINATED LAYING HENS

J.R. ROBERTS[1]

Summary

Preliminary studies were conducted on unvaccinated laying hens to investigate the effects of two strains of infectious bronchitis virus (T strain and N1/88 strain) on unprotected birds during lay. Clinical symptoms associated with the respiratory system were observed in the T-strain and N-strain groups but not the control and some birds in the T-strain group had enlarged kidneys. Feed intake tended to be depressed in the challenged birds and production was reduced in the T-strain group at 3 weeks post-challenge. However, there were relatively few effects of challenge on egg quality and no significant effects on excreta moisture. IBV antibody titres increased in response to challenge in the T-strain group but only some challenged birds produced antibodies in the N-strain group. Virus was re-isolated from the kidneys of T-strain birds at all sampling times post-challenge but only at 6-16 days for the N-strain group. Further studies are planned to document the effect of IBV challenge on unvaccinated birds.

I. INTRODUCTION

It is approximately 40 years since nephropathogenic strains of infectious bronchitis (IB) were isolated in Australia by Cumming (1963, 1965). Since that time, IB has been known to affect the respiratory system and oviduct as well as the kidneys of chickens (Jordan, 1996). The effect of infectious bronchitis virus on the oviduct of laying hens has been the subject of extensive conjecture and the effects of IB on egg quality that have been reported overseas (Jordan, 1996), have not been directly demonstrated in the Australian environment.

The current studies used White Leghorn birds that had been maintained in isolation from day-old and not vaccinated against IB, for the purposes of producing fertile eggs for other studies. Birds were maintained IB free until 65 weeks of age, at which time they were exposed to one of two strains of IB: T strain or N1/88 strain. T strain is a strongly nephropathogenic virus whereas N1/88 strain has a greater affinity for the respiratory system.

These naïve birds were used as a model for a commercial laying hen that has not been effectively vaccinated against infectious bronchitis virus. The effects of IBV on these birds would be expected to represent the most severe effect that could be expected in commercial birds.

II. MATERIALS AND METHODS

Day-old White Leghorn chicks were obtained from the Nulkaba Hatchery near Cessnock, NSW and transferred to isolation pens at the University of New England, Armidale, NSW. The birds were reared according to standard commercial practice. Birds remained in the isolation sheds and blood samples were taken at intervals to confirm that there were no antibodies to IB. The birds remained IB antibody negative to 65 weeks of age.

At 65 weeks of age, birds were divided into three groups: a control group in which birds were transferred to individual cages in small isolation sheds; a T-strain group with birds

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being transferred to individual cages in a large isolation shed and inoculated intraocularly with T-strain IBV; and an N-strain group with birds being transferred to individual cages in a separate large isolation shed and inoculated intraocularly with N1/88-strain IBV. The dose of each challenge virus was adjusted to ensure a final estimated dose of 2 x 10^5 EID_{50} per bird.

Within each treatment group, some birds were maintained throughout the experiment for the purposes of measuring feed intake, egg production, egg quality and excreta moisture. A subsample of these birds (6 per treatment group) had blood samples taken prior to challenge and three weeks following challenge for measurement of IBV antibody titre by IDEXX ELISA. The other birds were sacrificed at 3, 6, 10, 13, 16 and 21 days postchallenge for assessment of histopathological changes. Kidney tissue was stored frozen for later re-isolation of virus. For the birds that were sacrificed, blood samples were taken prior to challenge and then at the time of sacrifice for measurement of IBV antibody titre by serum neutralization and IDEXX ELISA.

Re-isolation of virus was attempted from frozen kidney tissue by injection of kidney extract into the allantoic cavity of 9-day old embryos for a total of five passages. If at least 3 of the 5 embryos were dead or virus-affected, the sample of kidney was scored as positive for re-isolation of virus.

Data presented in Figures 1-3 were analysed by ANOVA. Fisher's protected LSD was used to separate means when significant main effects were observed. Figure 3 presents percentage of samples positive for the presence of IBV.

III. RESULTS

No clinical symptoms were observed in the Control group of birds. However, rales and other symptoms of respiratory disease were observed in the N-strain group from 4 to 6 days postchallenge and in the T-strain group from 2 to 7 days postchallenge. However, the birds recovered from these symptoms.

Feed intake varied significantly over the weeks of the experiment. In the first week post challenge, feed intake was lower for the N and T groups, in comparison to the control (Figure 1).

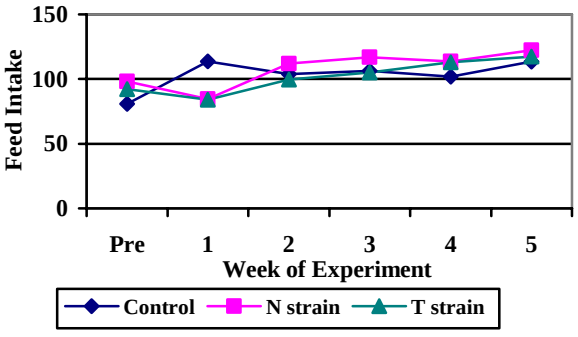

Figure 1: Feed intake (g/bird/day)

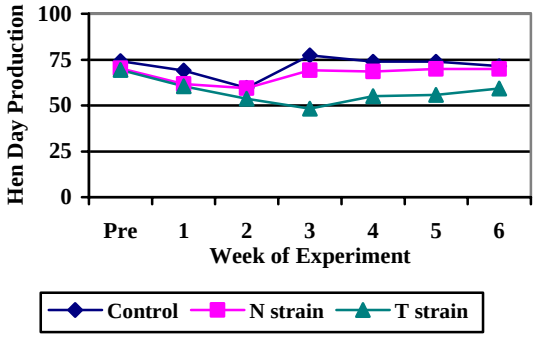

Figure 2: Hen day production (eggs/hen/100d)

Production declined in all treatment groups during weeks 1 and 2 (Figure 2). The decline in production in all groups may be attributed to the very hot weather that was experienced during those two weeks. During week 3 of the experiment, production had improved in the control and N groups but was still significantly depressed in the T group and this trend continued until the end of the experiment.

There were significant main effects of treatment group and week of experiment on measurements of egg shell quality but no statistically significant interactions between group and treatment group, indicating that challenge with either T-strain or N-strain infectious bronchitis virus had little effect on egg shell quality in this study. A similar pattern was found for egg internal quality as measured by albumen height and Haugh Units. There were significant main effects and a significant interaction for yolk colour, with yolk colour in the T group being generally lower in the post-challenge phase of the experiment.

Excreta moisture varied over the weeks of the experiment mainly as the result of changes in ambient temperature and humidity. However, there was no difference between treatment groups and no significant interaction between treatment group and week of experiment.

All birds tested negative to the presence of IBV antibodies, by both serum neutralization testing and ELISA, prior to the challenge and the control group birds all remained negative. For the N group, IBV antibody titres were negative by serum neutralization and ELISA until 21 days post-challenge in the birds that were sacrificed and were negative at 3 weeks post-challenge in the birds that were maintained throughout the experiment (Figure 3). For the T group birds that were sacrificed, all birds were positive by ELISA from 10 days post-challenge. For the T group birds that were maintained throughout the experiment, there was a large and highly statistically significant ($P<0.0001$) increase in IBV antibody titre, as measured by ELISA, at 3 weeks post-challenge (Figure 3).

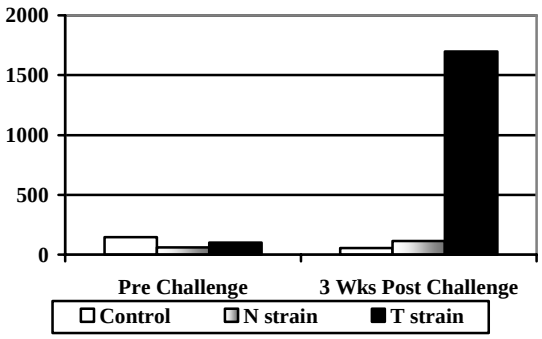

Figure 3: IBV antibody ELISA titres for birds maintained throughout the experiment

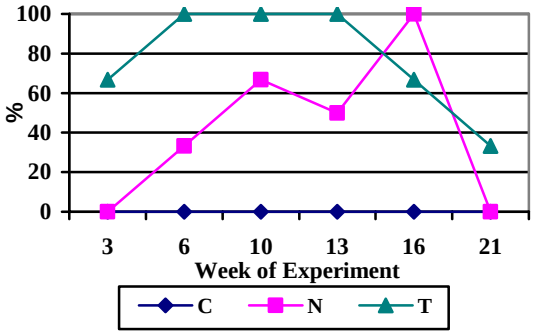

Figure 4: Percentage of kidney extracts positive for presence of virus

Virus was not re-isolated from any kidneys from the control group of birds. The percentage of birds from which virus was re-isolated in the T group increased to 100% by 6 days post challenge and was still at 33.3% 21 days post challenge (Figure 4). At the same time, it took longer (16 days post-challenge) for all birds to have virus present in the kidneys in the N group and no birds tested positive for virus in the kidneys by 21 weeks post challenge. Enlarged kidneys were not observed in the Control and N strain group but were observed in individual birds in the T strain group at 10, 13 and 21 days post challenge.

IV. DISCUSSION

The presence of clinical symptoms in birds from the N-strain and T-strain groups, as well as the large increase in IBV antibody titre in the T-strain group indicate that a significant viral challenge was delivered to the experimental groups of birds. However, the effects of challenge on feed intake and egg production were relatively mild. Feed intake tended to be lower in the N and T groups in the first week post-challenge. Egg production was

significantly lower for the T group at three weeks post challenge and was depressed for the remainder of the experiment. Excreta moisture was not statistically significantly affected by IBV challenge.

There were relatively few effects of IBV challenge on egg internal quality and egg shell quality. The lower yolk colour of eggs from the T-strain group following challenge was probably due, at least in part, to a reduction in feed intake. However, it is possible that there were also effects on other aspects of functioning.

The serum samples taken from birds that were sacrificed were negative for IBV antibody titres throughout the experiment in the control group. For the N-strain birds that were sacrificed, all serum samples were negative for IBV antibodies as measured by serum neutralization or IBV antibody ELISA titre until 21 days post-challenge. However, for the N-strain birds that were maintained throughout the experiment, all serum samples taken prior to challenge and at 3 weeks post-challenge were negative for IBV antibody titres, as measured by ELISA. For the T-strain birds that were sacrificed, most plasma samples were positive for IBV antibody by both serum neutralization and ELISA from 10 days post-challenge until the end of the experiment. For the T-strain birds maintained throughout the experiment, serum samples were negative for IBV antibodies prior to challenge and there was an increase in IBV antibody titre at 3 weeks post-challenge. These results indicate that T-strain evokes a greater antibody response, in birds that have not previously been vaccinated, than does N-strain IBV. This is emphasized in the results from the birds that were maintained throughout the experiment. The IBV antibody titres remained negative in both the Control and N-strain groups, although there was a numerical increase in the titres of the N-strain group. It appears that a single challenge by N-strain IBV in unvaccinated birds is not necessarily sufficient to induce an antibody response. However, for the T-strain group, there was a very large increase in IBV antibody titre at 3 weeks post-challenge.

Virus was not re-isolated from kidney tissue in any of the control group of birds. For the N-strain group, virus was re-isolated from 6 days post-challenge, reaching a peak of 100% of birds at 16 days post-challenge. However, virus could not be isolated at 21 days post-challenge. In the T-strain group, virus was reisolated from the kidneys of at least some birds at all days of sampling and was found in all birds at 6-13 days post-challenge. It appears that T-strain replicates in the kidneys more quickly following challenge than does N-strain. This is probably to be expected as T-strain is nephropathogenic whereas N-strain is is thought to have a greater affinity for the respiratory system.

The enlarged kidneys observed in some birds from the T-strain group are indicative of histopathological damage in these organs. The results of histopathological examination of tissue from the birds euthanased in this study are reported in Chousalkar and Roberts (this volume). Future experiments are planned to evaluate the effect of IBV challenge in unvaccinated brown egg layers that have come into lay. The results of such an experiment will assist in the identification of the impact of an intercurrent IBV infection on egg quality in commercial laying birds.

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HISTOPATHOLOGY OF TWO SEROTYPES (T & N1/88) OF AVIAN INFECTIOUS BRONCHITIS VIRUS (IBV) IN VACCINATED AND UNVACCINATED BIRDS

K.K. CHOUSALKAR[1] and J.R. ROBERTS[1]

Summary

The histopathology of Harderian gland, trachea, kidney and oviduct was studied in vaccinated and unvaccinated birds exposed to T and N1/88 strain of Infectious Bronchitis Virus (IBV). The trachea and kidney of vaccinated birds were protected to a moderate extent but the oviduct to only a small extent. The sequential histopathological changes revealed that IBV multiplies initially in the Harderian gland**,** then in the tracheal mucosa and simultaneously in the kidney and oviduct. The severity and persistence of lesions were greater in the shell gland and kidney of T infected birds whereas in trachea and Harderian gland the effects of the two IBV strains were similar. The N1/88 strain seemed to be more pathogenic for magnum of vaccinated and unvaccinated birds.

I. INTRODUCTION

Infectious bronchitis is an acute, highly contagious and primarily respiratory infection in chickens. Infectious bronchitis virus (IBV) has a great economic impact on the layer industry as it affects egg production. Besides respiratory lesions, early exposure to IBV causes extensive damage to the reproductive tract (Broadfoot *et al.,* 1956).

IBV strains vary greatly in their tissue trophism with T strain being regarded as nephropathogenic and N1/88 as respiratory. Many workers reported IBV as a respiratory syndrome (McMartin, 1993) with clinical signs being difficulty in breathing, rales, coughing, or sneezing, with or without nasal discharge. In layers, infection at an early age causes permanent damage to the oviduct (Crinion *et al*., 1971), along with some respiratory signs. In adult laying hens, respiratory signs may be in milder form and can remain unnoticed. The virus usually causes reproductive disorders with decline in egg production accompanied by soft shelled and misshapen eggs, inferior shell quality and thin and watery albumen.

The pathology of the respiratory tract and kidney has been studied by many workers (Chen and Itakura, 1996) while the pathology of the reproductive tract has been investigated on by only a few researchers (Sevoian and Levine, 1957; Crinion *et al*., 1971). The objective of this work is to describe the pathology and time frame of IBV effects in laying birds. This paper will concentrate on the Harderian gland, trachea, kidney and different parts of the oviduct.

II. MATERIALS AND METHODS

In total, 74 birds were used in this experiment with details of experimental design presented in Table 1. All the HyLine Grey birds (HL) were vaccinated with commercial vaccine at the age of day old, four weeks and twelve weeks whereas all White Leghorn (L) birds were kept unvaccinated. Leghorns at the age of 65 weeks and HyLine birds at the age of 110 weeks were challenged with two different Australian strains of virus, T and N1/88 (obtained from Dr. Jagoda Ignatovic, CSIRO, Geelong). Three unvaccinated birds (L) and

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two vaccinated birds (HL) were killed and examined on days 3, 6, 10, 13, 16, 21 post infection (p.i.). Harderian gland, trachea, kidney, magnum and shell gland were fixed in 10 % neutral buffered formalin. The tissues were processed by standard histological procedures, embedded in paraffin, and 5 μm sections cut. All the sections were stained with haematoxylin and eosin. In addition, some of the kidney and magnum sections were stained with alcian blue. All the stained slides were viewed by light microscopy.

Table 1. Experimental design.

Group	No. of birds	Vaccinated	Control	Challenged: T strain	Challenged: N1/88 strain
Leghorn (L)	44	No	7	18	19
HyLine Grey (H)	30	All	6	12	12

III. RESULTS

a) Harderian gland

In control Leghorn birds, the main features were some plasma cells in the subepithelium, intact collecting duct epithelium and acinar epithelium, occasional plasma cells and lymphocyte infiltration around the blood vessels in the glandular interstitium. In control HyLine birds, most of the findings were similar to the control Leghorn birds except for the migratory infiltration of lymphocytes in the interstitium found in the HyLine birds.

In T strain infected Leghorn birds, on 3 day p.i., there was moderate infiltration of plasma cells and plasma cells with RB, the acinar epithelium was moderately damaged but the collecting duct epithelium was severely damaged. On 6, 10, and 13 days p.i., plasma cells with RB and lymphoid cells around blood vessels were intense. On 16 and 21 days p.i. most of the ductal and acinar epithelium had regenerated, the number of RB bearing plasma cells was reduced, but the lymphocyte infiltration in the interstitium was still common. Exfoliative epithelium, along with inflammatory cells, was seen occasionally in the duct lumen at 10 days p.i. and for the remainder of experiment. Migration of lymphocytes and heterophils into the subepithelium was mild on 3, 13 16 and 21 days p.i. but moderate at 6 and 10 days p.i. In N1/88 infected Leghorn birds, most of the lesions were similar to those of T stain infection but the lesions were less severe.

In both T and N1/88 HyLine Grey birds, the lesions were severe on 3 and 6 days and moderate at 10, 13 and 21 days p.i. However there was regeneration of the collecting duct epithelium on day 10 p.i. In HyLine birds, the amount of secretion in the collecting duct lumen was increased throughout the experiment as compared to control HyLine birds.

b) Trachea

Normal tracheal epithelium, with healthy cilia and mucus glands, were seen in the control Leghorn birds (Randall and Reece, 1996).

There were no microscopic changes at three days p.i. in both T and N1/88 Leghorn groups except for lymphocytic infiltration and dilatation of blood vessels in the lamina propria. Severe pathology occurred mainly from day 6 in the form of severe loss of cilia, mucus glands and goblet cells, changes in the mucosal epithelium, oedema in the subepithelium and occasional heterophilic exudate in the tracheal lumen. Most of the above lesions persisted in moderate form in both infected groups. On day 13 p.i., most of the cilia and the epithelium had regenerated. The hypertrophied glands were normal with occasional heterophilic exudate in the lumen. Goblet cells were present in good number in T infected Leghorns but moderately absent from the N1/88 infected group. On days 16 and 21 most of

the tracheas appeared normal. However, severe thickening of the mucosa with infiltration of lymphocytes were prominent from days 13 to 21 p.i. Moderate heterophilic exudate was also present in the lumen of the trachea in some N1/88 infected Leghorn birds up to 21 days p.i. The severity of lesions in infected HyLine birds was less than infected Leghorn birds. However, in all HyLine birds including the control group, there was extensive thickening of the mucosa with lymphoid nodules throughout the experimental period.

c) Kidney

In Leghorn birds, the main kidney lesions consisted of necrosis of proximal convoluted tubules, distension of distal convoluted tubules, necrotic foci, infiltration of heterophils and lymphocytes in the interstitial space, oedema of Bowmans capsule, urate and granulocytic casts in collecting ducts and spheroids. The lesions were more apparent on the 10^{th} day p.i. in N1/88 infected birds. The pathology continued up to day 13 in both the infected groups and, at 16 to 21 days of infection, most of the tissues had regenerated, although oedema in Bowmans capsule and necrotic foci persisted. Spheroids were common only in T strain infected Leghorns. In infected HyLine birds, all the above findings were mild or moderate. Necrotic foci along with lymphoid cell infiltration persisted until the end of experiment.

d) Oviduct

In magnum and shell gland pouch of T-infected HyLine and Leghorn birds, the first feature to appear was lymphoid cell infiltration around the blood vessels in the muscular layer from the 10^{th} day p.i. However, in magnum of N1/88 infected Leghorns, prominent changes appeared from day 6 p.i. In both infected groups, on day 10 p.i., severe cilia loss and oedema in the sub epithelium were main findings. Glandular dilatation in shell gland pouch was severe in T as compared to N1/88 infected Leghorns however reverse was recorded in magnum of Leghorn birds. Alcian blue staining in magnum of infected Leghorns showed loss of mucopolysaccharides in major areas of mucosal cells. From 13 to 21 days p.i., cellular infiltration in lamina propria and muscularis layers was at a peak. Most of the tissues had regenerated at 21 day p.i. All the parts of oviduct in the control Leghorns appeared normal throughout the experiment.

All the above findings were moderate but consistent in shell gland but severe in magnum of all infected HyLine birds.

IV. DISCUSSION

In both challenged groups (T and N1/88) of Leghorn birds, the severity and time frame of lesions in the Harderian gland were almost the same which indicates that both strains are equally pathogenic for the Harderian gland although regeneration occurred more quickly in the HyLine birds as compared to the Leghorns. Our finding regarding regeneration of the ductal epithelium agrees with Toro *et al*. (1996).

Histological lesions observed in the trachea are similar to those described previously (Chen and Itakura, 1996). Lesions were similar for both IBV strains indicating similar predilection of both strains for the trachea.

The histopathological changes observed in the kidney match previous findings (Fulton *et al*., 1993). T strain was more nephropathogenic (Chong and Apostolov, 1982) as compared to N1/88 in Leghorn birds. Most of the changes in the oviduct were noticeable on the 10^{th} day p.i., a finding that is in accordance with Sevoian and Levine (1957). Glandular dilatation may be the contributory factor in albumen thinning (Butler *et al.,* 1972). The moderate inflammatory cell debris in the lumen of the oviduct may lead to the presence of

meat spots in egg albumen as reported by McDougall (1968); although pathogenesis of misshapen, soft shelled eggs and the mechanism of cessation or reduced egg production in IBV infection needs further investigation. The duration and severity of effects suggests that, in the oviduct, T strain has more affinity and pathogenicity for the shell gland where as N1/88 was more pathogenic in the magnum. The histological findings were more severe in infected Leghorns as compared to HyLine birds which indicates that the vaccine has protected the trachea to a moderate extent (Box *et al*., 1980), kidney to a large extent (Cavanagh, 2003) and oviduct to only a small extent.

In both the IBV infected Leghorn groups, histopathological lesions were more severe in Harderian gland and trachea than for infected HyLine birds, but were not devastating in kidney and oviduct as described in earlier literature. This may be due to an intrinsic factor like age influencing the pathogenesis for kidney (Albassam *et al*., 1986) and oviduct (Crinion and Hofstad, 1972). After experimental challenge with IBV, the sequential observations by histopathology suggest that virus replicates first in the Harderian gland, then tracheal mucosa and then simultaneously replicates in kidney and oviduct.

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A "COLITIS-LIKE" RESPONSE IN BROILERS AFTER A DIETARY CEREAL CHANGE

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Summary

The cereal base of a broiler starter diet was changed and the faecal excreta pH, hindgut digesta and plasma lactic acid concentrations and blood loss in the excreta were recorded. Excreta pH was altered, with or without avilamycin use. Lactic acid concentrations in the ileal digesta increased and blood loss in the faecal excreta and diarrhoea were stimulated. Avilamycin did not alter these responses. Changing the cereal base of a broiler diet results in fermentation changes associated with blood loss and diarrhoea from the bird. These data suggest that hindgut mucosae, particularly in the ileum, may be at risk of disruption and provide conditions favourable for enteric disease to occur after a dietary cereal change.

I. INTRODUCTION

In earlier work, Taylor and Jones (2004) found that inclusion of whole grain into a feed mix prior to pelleting altered physical and chemical characteristics in the broilers' gut; the pH of the ileal digesta tended to be, and excreta pH was, higher. In poultry, little work has considered changes in digesta chemistry which are associated with acidotic responses as found in ruminants, non-ruminant herbivores and monogastric animals (Clayton, 1999). In growers and layers, Taylor (2002) found changes in the hindgut digesta pH which were associated with alterations in organic acid concentrations; a substantial increase in ileal lactic acid concentration being of particular concern as was a transient decline in digesta and excreta pH. Changing the cereal base of the diet to mature, post-peak production layers (Taylor, 2003), produced a consistent decline over 48–72 h in excreta pH which was associated with increases in lactic acid concentrations. This was prominent in the distal ileum and was associated with blood loss in the excreta and histopathological results showing a 'colitis-like' irritation of the ileal mucosa. With restriction of the use of many of the in-feed anti-microbials and the concern for enteric disease, the effects of dietary cereal changes required investigation in the broiler bird. As broiler production is based in the period when the birds gut physical development, maturity of digestive enzyme systems and establishment of microbial populations are interacting, the effect of sudden and/or major changes to the cereal component of the diet upon fermentative processes is unclear. The hypothesis of previous (Taylor, 2002) and the current studies is that a sudden alteration of the dietary cereal component alters substrate flow to the birds hindgut which allows for a 'classic' lactic or fermentative acidosis to develop and which, however transient, may have adverse effects on hindgut tissue. The following experiment determined the effects of a dietary cereal change, with or without the use of avilamycin, on hindgut responses of broilers.

II. METHODS

One-d-old male broilers (Ross 308, Bartter Enterprises, Beresfield, NSW 2322) were housed in electrically-heated brooders thence large cages, in an insulated, continuously-lit room. They were offered commercial broiler starter crumbles (Bartter Enterprises) until 20 d of age. From 21 to 29 d, either the commercial starter or a wheat-based diet was fed to four

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pens of birds per treatment. The wheat diet formulation was based on a commercial feed-wheat blend but with substitution of a known "problem" wheat, HP S1. The wheat diet was made with or without the addition of avilamycin (100 mg kg^{-1}). Fresh faecal excreta were collected from the first four droppings under each cage, after the trays were cleaned, and the pH was measured (Taylor, 2002). Subsequent excreta were examined and scored for the presence or absence (1 or 0 respectively) of blood and diarrhoea. The presence of blood was confirmed by a Hemofec faecal occult blood test (Taylor (2003). After d 29, the birds were euthanased and ileal digesta were collected to measure lactic acid concentrations as detailed by Taylor (2002). Undisturbed 2 cm ileal samples were excised from 10 cm above the ileo-caecal-colic junction and fixed in Bouin's solution, coded and subjected to histopathological examination (NSW Agriculture, Menangle, NSW 2568). Data analysis (Taylor, 2002, 2003) used the MIXED Model procedure of SAS for the repeated measures (excreta pH) and the general linear model (family='binomial') of R (Ihaka and Gentleman, 1996) for binary data (blood and diarrhoea scores). The experiments were approved by the ACEC, Bartter Enterprises, and complied with the NSW Animal Research Act 1985 (as amended).

III. RESULTS

Excreta pH altered ($P<0.05$) with diet over time (Table 1), reflected in fluctuations in excreta pH in birds on the commercial diet. This was moderated on the wheat diet ± avilamycin incorporation, which maintained higher ($P<0.05$) excreta pH.

Table 1. Mean fresh excreta pH of broilers fed a commercial starter diet or an HP S1 wheat–based diet with or without avilamycin from 21 – 29 days of age (n=8).

Diet	Age (d)								
	21	22	23	24	25	26	27	28	29
Commercial	7.23^{def}	6.51^{g}	7.18^{def}	7.49^{abcd}	7.80^{abc}	7.57^{abcd}	6.93^{fg}	6.98^{efg}	6.60^{g}
HP S1 wheat	7.23^{def}	7.39^{cdef}	7.24^{def}	7.25^{def}	7.38^{cdef}	7.61^{abcd}	7.42^{bcde}	7.88^{ab}	7.49^{abcd}
HP S1 wheat + avilamycin	7.19^{def}	7.39^{cdef}	7.26^{def}	7.94^{a}	7.51^{abcd}	7.58^{abcd}	7.79^{abc}	7.61^{abcd}	7.62^{abcd}
SE	0.245								

Ileal L-lactic acid concentration (Table 2) was higher ($P<0.05$) in birds fed the wheat diet ± avilamycin than in those fed the commercial diet. Ileal D-lactic acid displayed a similar trend ($P = 0.059$). Little excreta blood loss (Table 3) was incurred on the commercial diet compared with the HP S1 wheat diets ($z = -3.213$, $P=0.001$), irrespective of avilamycin use ($P>0.889$). Diarrhoea (Table 3) was incurred on the wheat diets irrespective of avilamycin use ($P=0.103$). Examination of the ileal tissue revealed a range of cellular infiltrates, lymphoid foci, with or without lymphoid infiltrate into the coria, and/or germinal centres in the propria. The substantial differences between individual broilers resulted in no consistent treatment variation.

Table 2. Plasma and digesta concentration of L- and D-lactic acid (mMol/l) of 29 day old broilers fed a commercial or an HP S1 wheat diet with or without avilamycin from 21 – 29 days of age (n=12).

Sample	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	P	LS Mean	SE	P
Plasma	Commercial	5.17	0.423	0.78	0.03	0.121	0.11
	HP S1 wheat	5.49			0.40		
	HP S1 + avilamycin	5.09			0.17		
Ileum	Commercial	3.35 b	5.558	0.01	1.79	4.186	0.06
	HP S1 wheat	24.78 a	5.322		14.27	4.008	
	HP S1 + avilamycin	28.91 a	5.558		14.72	4.186	
Caeca	Commercial	1.99	0.315	0.11	2.26	0.348	0.10
	HP S1 wheat	1.02	0.315		1.18	0.348	
	HP S1 + avilamycin	1.51	0.329		1.55	0.363	

Table 3. Probability estimates (± SE of the probability) of blood in the excreta and diarrhoea of broilers fed a commercial diet or an HP S1 wheat diet with or without avilamycin inclusion from 21 – 29 days of age (n=8).

Feed	Age (d)									
	20	21	22	23	24	25	26	27	28	29
Commercial	0.02	0.02	0.03	0.03	0.04	0.06	0.07	0.09	0.12	0.15
	0.304	0.263	0.223	0.185	0.152	0.127	0.116	0.121	0.142	0.172
HP S1 wheat	0.06	0.08	0.11	0.15	0.21	0.28	0.36	0.45	0.54	0.64
	0.169	0.145	0.121	0.099	0.081	0.069	0.067	0.074	0.087	0.103
HP S1 + avilamycin	0.08	0.11	0.14	0.19	0.25	0.31	0.39	0.47	0.55	0.63
	0.153	0.130	0.109	0.089	0.074	0.065	0.065	0.073	0.086	0.101
Commercial	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02
	0.437	0.368	0.310	0.268	0.252	0.267	0.307	0.365	0.433	0.508
HP S1 wheat	0.21	0.30	0.42	0.54	0.66	0.76	0.84	0.89	0.93	0.96
	0.174	0.140	0.111	0.093	0.091	0.102	0.121	0.145	0172	0.201
HP S1 + avilamycin	0.16	0.26	0.38	0.53	0.67	0.78	0.87	0.92	0.95	0.97
	0.187	0.149	0.117	0.098	0.096	0.111	0.134	0.162	0.193	0.225

IV. DISCUSSION

Major changes to the dietary cereals fed to poultry, whilst avoided as far as possible, do occur in practice. Taylor (2002; 2003) considered excessive fermentation, resulting from alteration of the dietary cereal base, and found sharp, generally short-term reductions in pH and increases in lactic acid concentrations in the hindgut, particularly the distal ileum, of growers or layers. Similar changes are to be found in other monogastric animals, including humans (Cummings, 1981), pigs and dogs (Clayton, 1999), suffering a fermentative or lactic acidosis. Ileal mucosa is particularly sensitive to increased H+ concentration (Saunders and Sillery, 1982) and what may appear to be minor, transient, reductions in pH, have been associated with hindgut acidosis in several species (Clayton and Buffinton, 2000; Clayton and Jones, 2001). Subsequent increases in excreta pH are associated with the onset of colitis symptoms (Clayton and Buffinton, 2000). The major contributor to alkaline conditions in the hindgut digesta or excreta is ammonia (Newmark and Lupton, 1990) and the generally higher

pH of excreta from the birds fed the HP S1 wheat diets may have resulted from more endogenous/dietary N being lost on these diets, which, obviously, were providing for higher protein than that for which the diet was formulated. Intraluminal pooling of lactate, associated with a reduction in SCFA's, is due to a shift in bacterial metabolism caused by a lowering of pH (Vernia *et al*., 1988). A wheat-based diet fed to broilers from 1-42 d (Engberg *et al*., 2000) produced minimal lactic acid in the caeca but substantial concentrations in the ileum and rectum. Antibiotics, in combination, improved performance, increased ileal pH and reduced both ileal and rectal lactic acid concentrations. However, that lactic acid concentration *per se* was involved in depressing broiler performance was not considered. Cummings (1981) noted that D- and L-lactic acid were only found in quantity in the gut when a high glycolytic flux occurred and that in humans, D-lactic acidosis was associated with conditions like acute diarrhoea and short bowel syndrome. In the rat hindgut, accumulation of H+ and lactate restrict net water transport and cause cell sloughing with the ileal mucosa being more sensitive than the colonic (Saunders and Sillery, 1982).

The experiment supports the earlier layer work and suggests that lactic acid accumulation in the avian ileum may produce adverse effects upon hindgut mucosal integrity and create conditions favourable for subsequent bouts of enteric disease.

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PREBIOTIC PLANT EXTRACTS DO NOT STIMULATE BIFIDOBACTERIA IN BROILER CHICKENS

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Bifidobacteria were enumerated on selective MTPY agar as described by Petr and Rada (2001). Isolation and identification by fructose-6-phospahte phosphoketolase (F6PPK) activity revealed a low recovery of bifidobacteria from the plates (Table 1). The recovery percentages were used to calculate the actual number of bifidobacteria in the ileal and caecal contents of the chickens (Table 2). These results show a substantial lower number of bifidobacteria in this study than other published data in broiler chickens. The selectivity of the agar media used in some other published studies was not verified or accounted for and as pointed out by the present results, bifidobacterial numbers in broiler chickens need to be interpreted with caution due to potential methodological biases.

Table 1. Outline of identification of *Bifidobacteria* sp. among isolates from ileum and caeca of 35-d old broiler chickens fed diets with 10 g /kg plant extract[1]

Isolates	Number
In total investigated	402
Non-gas producers	51
Positive for F6PPK[2] activity	27
Distribution on treatments (ileum / caeca)	
Negative control	0 / 1
Cabbage tree shoot extract (*Cordyline australis*)	17 / 3
Wakame seaweed extract (*Undaria pinnatifida*)	3 / 3
Golden wattle extract (*Acacia pycnantha*)	0 / 0

[1]Samples were diluted and spread onto MTPY agar. Colonies appearing from the highest dilutions were picked.
[2]F6PPK = fructose-6-phosphate phosphoketolase activity.

Table 2. Number of bifidobacteria in ileal and caecal digesta of 35-d old broiler chickens fed diets with 10 g /kg plant extract[1]

Segment	Treatment[1]	No. of positive replicates	No. of bifidobacteria (CFU/ g digesta)
Ileum	Negative control	4	3.20
	Cabbage tree shoot extract	3	4.92
	Wakame seaweed extract	3	4.38
	Golden wattle extract	3	4.68
Caeca	Negative control	2	5.28
	Cabbage tree shoot extract	4	4.93
	Wakame seaweed extract	4	4.57
	Golden wattle extract	3	4.54

[1]Numbers are calculated from the proportion of bifidobacteria identified on the MTPY agar

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MANNO-OLIGOSACCHARIDES ALTER BACTERIAL POPULATIONS IN BROILERS

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The effects of different levels of manno-oligosaccharides (MOS) on the growth performance of broiler chicken were previously studied and it was shown that a dose of 2g/kg MOS in the diet was comparable to a control diet (Zn-bacitracin), especially in the first three weeks (Yang *et al*., 2004). The present experiment was conducted to examine the effects of MOS on the populations of selected bacteria (i.e., lactobacilli, *Escherichia coli*, and enterococci) in the gut lumen and mucosa of young birds.

Day-old birds (192) were randomly divided into three treatments, viz. a negative control (Control), a 2 g MOS /kg diet, and a positive control (50 ppm Zn-bacitracin), with eight replicates per treatment. The animals were offered a sorghum-wheat-based diet. At 2 weeks of age, two birds per cage were killed, and the luminal and mucosal populations of bacteria were enumerated. The results are shown in Table 1. MOS supplementation tended ($P<0.09$) to reduce the number of *E. coli* in the duodenal mucosa and the ileal contents compared to controls. Interestingly, the luminal population of lactobacilli in the ileum was significantly lower ($P<0.05$) in the MOS group in comparison with the negative control. The populations of lactobacilli in the gut did not differ between MOS treatment and the positive control. The population of enterococci was not affected by dietary treatments.

Table 1. Effects of dietary treatments on the counts (log CFU/g digesta or wet tissue) of different kinds of selected bacteria of 2-wk old chicks.

Site and microflora	Control	2g/kg MOS	Zn-bacitracin	SEM	P value
Luminal bacteria					
Ileum					
Lactobacillus	8.72[a]	8.06[b]	7.68[b]	0.14	0.04
Escherichia coli	6.25	5.78	5.83	0.22	0.06
Enterococci	5.13	5.3	5.33	0.2	0.56
Ceca					
Lactobacillus	9.41[a]	9.12[ab]	8.82[b]	0.16	0.03
Escherichia coli	8.58[a]	8.29[ab]	8.06[b]	0.11	0.02
Enterococci	6.42	6.18	5.82	0.16	0.43
Mucosa-associated bacteria					
Duodenum					
Lactobacillus	6.24	5.88	5.87	0.18	0.27
Escherichia coli	4.02	3.81	4.33	0.29	0.09
Enterococci	4.05	3.85	3.75	0.24	0.20
Ileum					
Lactobacillus	6.31	6.11	6.15	0.22	0.65
Escherichia coli	3.67	3.97	3.92	0.20	0.50
Enterococci	4.05	3.77	3.9	0.20	0.33

[a,b]Means in the same row with different superscripts differ significantly ($P<0.05$).

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SELECTED PLANT EXTRACTS MODULATE THE GUT MICROFLORA IN BROILERS

J.K. VIDANARACHCHI[1], L.L. MIKKELSEN[1], I.M. SIMS[2], P.A. IJI[1] and M. CHOCT[3]

Summary

Broiler chickens were fed diets supplemented with three different water-soluble carbohydrate extracts obtained from Cabbage tree *Cordyline australis* (*CorE*) and exudates from Golden wattle tree *Acacia pycnantha* (*AcaE*), and a seaweed, *Undaria pinnatifida* (*UndE*). Each extract was supplemented at two levels (5 g/kg and 10 g/kg) to 384 broiler chickens. The inclusion of plant extracts in feed modulated the composition of the microflora in the ileum and caeca of broilers. The ileal and caecal organic acid analysis indicated that plant extract affected microbial fermentation patterns in the ileum and caeca.

I. INTRODUCTION

It has been recognised that modulation of the natural bacterial population of the intestine in broilers through nutritional manipulation such as the selection of feed ingredients or the use of alternative feed supplements can be effective tools to control pathogens. Recent studies have shown that various oligosaccharides and polysaccharides may act as phytobiotics or prebiotics in poultry feed, exerting numerous growth promoting effects (Lan *et al.,* 2004; Vidanarachchi *et al.,* 2005). Several bioactive compounds from mushrooms and higher plants have already been identified as compounds which differentially stimulate favourable bacteria such as lactobacilli and bifidobacteria at the expense of pathogenic species in chickens (Guo *et al.,* 2004; Lan *et al.,* 2004). Stimulation of these beneficial bacteria could contribute to a balanced gut microflora, and may provide an optimal precondition for effective protection against pathogenic microorganisms and an intact immune system. Thus, this study investigated whether selected plant extracts from Australian and New Zealand plants would influence the dynamics of gut microflora in broilers.

II. MATERIALS AND METHODS

A total of 384 Cobb broilers were allocated to 8 treatments of 6 replicates (8 birds per replicates). Three different extracts were prepared from Cabbage tree *Cordyline australis* (*CorE*), Golden wattle tree *Acacia pycnantha* (*AcaE*) and a seaweed, *Undaria pinnatifida* (*UndE*), and were included at two levels (5 and 10 g/kg) in the diets. An antibiotic treatment group (45 mg Zinc-bacitracin (ZnB)/kg was used as a positive control and a non-supplemented diet (NoS) was used as a negative control.

On d 35, two birds per replicate were euthanised by cervical dislocation and digesta (about 1 g) from ileum and caeca was aseptically transferred into McCartney glass bottles containing 10 mL of a pre-reduced salt medium (Holdeman *et al.,* 1977). Total anaerobes (Wilkins-Chalgren anaerobic agar; Oxoid, CM0619), lactic acid bacteria (de Man, Rogosa, and Sharp agar-MRS; Oxoid, CM0361), coliforms, and lactose negative enterobacteria (MacConkey agar; Oxoid, CM0115), and *Clostridium perfringens* (Perfringens agar; Oxoid, CM543) were counted. For the enumeration of bifidobacteria, the diluted samples were

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spread on modified TPY agar plates according to the method described by Petr and Rada (2001). The number of colony-forming units (CFUs) was expressed as $\log_{10}$ CFUs per gram of digesta. The digesta samples were pooled within a pen and 0.2 g of ileal and caecal contents were suspended in 0.8 mL of distilled water, and the pH was measured (EcoScan 5/6 pH meter, Eutech Instruments Pte Ltd., Singapore). The concentrations of ileal and caecal organic acids were determined as described by Jensen *et al*. (1995) and quantified using a Varian CP 3400 CX gas chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA). Bacterial counts were $\log_{10}$-transformed, and molar proportions of organic acids were subjected to arcsine transformation before statistical analysis using MIXED model procedure of SAS® (SAS Institute Inc. 2000). Treatment least-squares means were compared using predetermined orthogonal contrasts and considered significant at $P<0.05$, unless otherwise stated.

III. RESULTS AND DISCUSSION

The population of lactic acid bacteria in the ileum was higher ($P<0.05$) in chickens fed the diets supplemented with plant extracts than in those fed the NoS-diet without affecting the number of total anaerobes in the ileal digesta. The lactic acid bacteria counts in the caeca were increased ($P<0.05$) by 7, 4 and 6 fold, respectively, in birds fed *CorE* (10 g/kg), *UndE* (5 g/kg), and *UndE* (10 g/kg) diets compared to the NoS group. Lower ($P<0.05$) total anaerobic bacterial counts were observed in the ileal and caecal contents of birds on the antibiotic (Zn-B) supplemented diet. However, supplementation with the antibiotic did not reduce the lactic acid bacteria counts in the ileal and caecal contents compared to the NoS group. Significantly lower ($P<0.05$) ileal coliform counts were observed in birds fed *UndE* (10 g/kg) and *AcaE* (10 g/kg) supplemented diets than counts of the NoS group. The caecal coliform counts in *UndE* (both levels) and *CorE* (10g/kg) supplemented groups were significantly lower than counts of NoS group. The number of *C. perfringens* in the ileum tended to be reduced in birds fed plant extracts compared to that in non-supplemented birds. On the other hand, supplementation with plant extracts led to a significant reduction in caecal *C. perfringens* counts. The numbers of lactose-negative enterobacteria and bifidobacteria in the ileum and caeca were unaffected by dietary inclusion of the plant extracts. Bidfidobacteria, in general, were present at very low levels.

The effects of supplementation of plant extracts on caecal organic acids (short-chain volatile fatty acids, succinate, and lactic acid) and pH values are shown in Table 2. The caecal concentration of total organic acids increased ($P<0.05$) in broilers fed diets supplemented with *UndE* (10 g/kg) and *AcaE* extracts. In addition, the molar proportions of *n*-butyrate and propionate in the caecal contents were affected by diet (Table 2), with the caecal butyrate concentration and molar proportions being markedly higher ($P<0.05$) in birds fed all plant extracts, except *AcaE* (10 g/kg) fed group, compared with the NoS group. Birds fed diets supplemented with *CorE* (both levels) and *UndE* (10 g/kg) had a higher ($P<0.05$) ileal concentration of lactate than other groups (results not shown). In general, lactate was the most abundant organic acid in the ileum whereas acetate was predominant acid in the caeca.

The ileal pH value did not differ amongst diets, but plant extracts tended to reduce it (results not shown). Supplementation of *CorE* (both levels) and *UndE* (10 g/kg) lowered ($P<0.05$) the caecal pH value compared to the NoS. This was probably attributed to increased substrate availability in the caeca for fermentation. In the present study, plant extracts increased the number of lactic acid bacteria in the ileum and caeca. Similar results have been shown in caecal contents of broiler chickens and intestine of humans fed diets supplemented with prebiotic compounds (fructans and aranbinogalactans) such as those that

are present in *CorE* (fructans) and *AcaE* (arabinogalactans) in our experiment (Cherbut *et al.,* 2003; Yusrizal and Chen 2003). The impact of lactic acid bacteria on animal health and performance is controversial. Many *Lactobacillus* spp. have been shown to act via a number of mechanisms, including competitive exclusion, to reduce the number of pathogenic bacteria in monogastric animals. Recently, an *in vivo* study by La Ragione *et al*. (2004) revealed that a single oral dose of *Lactobacillus johnsonii* is sufficient to suppress all aspects of colonisation and persistence of *C. perfringens* in chickens. However, from a nutritional point of view, an increase in number of certain *Lactobacillus* species may not be desirable since these bacteria contribute to deconjugation of bile acids by bile salt hydrolase (EC 3.5.1.24) activity, which subsequently impairs lipid digestion and absorption (Knarreborg *et al*., 2002). Although most of the groups on plant extract supplements had an increase in population of lactic acid bacteria numbers in ileal and caecal contents, the current study failed to demonstrate a growth improvement due to supplementation of plant extracts. In fact, *UndE* and *CorE* supplemented at high dose (10 g/kg) resulted in lower body weight gain (after 35 days) in broiler chickens compared to NoS group. Thus, the trade-off between benefits and costs of non-pathogenic bacterial species such as lactic acid bacteria need to be analysed with caution. On the other hand, supplementation of plant extracts was associated with a significant reduction in colonisation of *C. perfringens* in caecal contents and some plant extracts supplemented groups also had lower coliform counts in the ileal and caecal digesta. The mechanism by which supplementation of plant extracts prevents colonisation of coliforms in ileal digesta is not clear. One possible explanation for these results is that the lower pH could have an adverse effect on certain acidophobic bacteria such as coliforms. The exact modes of action of plant extracts on bird performance and gut microbial dynamics are yet to be fully understood.

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Table 1. Effect of plant extracts on bacterial counts (CFU/g digesta) in ileal and caecal digesta of 35d-old broiler chickens

Treatment	Total Anaerobes		Lactic acid bacteria		Lactose-negative enterobacteria		Coliforms		*C. perfringens*	
	Ileum	Caeca	Ileum	Caeca	Ileum	Caeca	Ileum	Caeca	Ileum	Caeca
Negative control	9.26	9.54	8.35	9.14	4.80	5.84	6.35	8.40	4.94	6.11
Positive Control	8.74**	9.22**	8.39	9.42	4.80	5.10	6.35	7.85*	4.21**	5.43*
CorE (5g/kg)	9.30	9.75	9.05***	9.38	4.76	5.18	6.28	8.22	4.83	5.25
CorE (10g/kg)	8.99	9.52	9.16***	9.97**	4.77	5.34	5.72	7.70**	4.53	5.13***
UndE (5g/kg)	9.32	9.52	9.14***	9.77*	4.76	5.35	6.08	7.69**	4.55	5.14***
UndE (10g/kg)	9.29	9.79	9.21***	9.90**	4.79	5.62	5.13***	7.94*	4.60	5.14***
AcaE (5g/kg)	9.38	9.51	8.86*	9.35	4.76	5.27	6.30	8.27	4.68	5.42*
AcaE (10g/kg)	9.47	9.54	9.13***	9.20	4.73	5.07	5.38**	8.34	4.53	5.38**
SEM	0.33	0.18	0.15	0.14	0.03	0.21	0.25	0.16	0.17	0.19

* (P<0.05), ** (P<0.01), *** (P<0.001) indicate values significantly different from the negative control.

Table 2. pH and molar proportions of various organic acids in caecal digesta of 35d-old broiler chickens fed the experimental diets.

Treatment	pH	Total organic acids (μmol/g digesta)	Molar proportions of organic acids						
			Acetate	Propionate	*n*-Butyrate	*n*-Valerate	*iso*-But+ *iso*-Vale	Lactate	Succinate
			μmole/ 100 μmole						
Negative control	7.13	91.2	71.2	11.4	10.3	2.0	1.9	ND[1]	3.2
Positive control	7.12	79.9	72.3	9.8	11.1	2.0	1.8	ND	3.1
CorE (5 g/kg)	6.78*	101.0	66.1	10.7	14.1*	2.2	2.1	ND	3.5
CorE (10 g/kg)	6.66**	102.0	69.7	10.3	14.0*	1.9	1.4	ND	2.7
UndE (5 g/kg)	6.85	101.3	66.0	7.9	15.3**	2.3	1.5	ND	3.3
UndE (10 g/kg)	6.61**	106.7*	68.3	10.6	15.9**	2.4	1.8	ND	4.7
AcaE (5 g/kg)	6.81	107.0 *	66.7	11.4	13.8*	2.1	1.5	ND	4.5
AcaE (10 g/kg)	6.84	112.1**	68.4	12.0*	13.2	1.8	1.2	ND	4.7
SEM[3]	0.12	4.15	1.87	1.08	1.13	0.19	0.27	-	0.85

* (P<0.05), ** (P<0.01) indicate values significantly different from the negative control. [1]ND = Not detected.

PRELIMINARY DEVELOPMENT OF A DIAGNOSTIC TOOL FOR DETERMINING TRUE FERTILITY IN CHICKEN EGGS

K.L. KNIGHT[1] and T.A. SCOTT[2]

Summary

Infertility and embryonic mortality are major losses to the poultry industry and classification of true fertility by identifying very early embryonic mortality is difficult, particularly when eggs have been incubated and removed after candling. Preliminary studies were carried out with the purpose of providing a definitive diagnostic tool for classifying the fertility status of incubated chicken eggs. After determining that propidium iodide staining and fluorescence microscopy was successful in determining fertility, a second trial was carried out to quantify the number of fertile eggs that were classified as "infertile" following six days of incubation. Eggs from Baiada Poultry Pty Limited (n=9600) were stored for two different periods of time (3 and 7d) prior to incubation and placed in two different positions in a standard egg trolley in an incubator (front and back). The germinal discs of 347 eggs were stained with propidium iodide and viewed under a fluorescence microscope to identify light points indicative of fertile (DNA) eggs. Overall, 11.85% of eggs initially observed to be infertile by visual inspection were found to be fertile. The effect of pre-incubation storage length, incubation position and analysis day on the ability to determine fertility was not significant. These results suggest that propidium iodide staining of the germinal disc and fluorescence microscopy is an effective tool in determining true fertility of chicken eggs.

I. INTRODUCTION

The infertile or fertile status of an unincubated egg cannot always be determined with the naked eye and Kosin (1944) reported that there would be a three percent error rate when candling eggs for 'clears'. Some eggs that are detected as infertile at candling often contain embryos that died before or soon after oviposition; yet do not display the typical characteristics of fertile eggs. Breakout analysis post candling is beneficial in that many obviously fertile eggs would be screened out whereby removing the tedium and losses associated with analysis on fresh, unincubated eggs. There are many factors that contribute to poor hatchability and embryonic mortality and much research has been carried out into determining and minimising its causes. Some research has been undertaken in defining true fertility to aid in the understanding of where along the reproductive pathway losses due to embryonic mortality occur. Liptoi *et al.* (2004) determined true fertility in duck embryos by simultaneously using outer perivitelline (OPVL) sperm counting and propidium iodide (PI) staining of the germinal disc to differentiate infertile eggs from very early dead embryos. Nonetheless, true fertility remains an ambiguous area due to the inaccurate and difficult methods currently available for detection of very early embryonic mortalities.

II. MATERIALS and METHODS

A total of 9600 eggs from Cobb 500 birds were divided evenly into two groups and placed under cold storage (18°C) at Marsden Park Hatchery. Group one was held for 3 days and group two was held for 7 days before setting in Petersime incubators. Eggs from the two

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storage times were further divided according to position in the incubator, either front or back. After incubation for six days, all eggs were candled and 1206 infertile and early dead embryos (12.56% of total eggs) were set aside for classification. Of these, 574 (47.6 %) were from the 3 day storage group and 632 (52.4 % of total) were from the 7 day storage group. Live embryos were placed back into the incubator for continued growth and hatch. Eggs were transported to the lab and stored at 4°C until classification. The four groups were those stored for 3 days and placed at the front of the incubator (3F), or the back of the incubator (3B), and those stored for 7 days and stored at the front (7F) or the back (7B) of the incubator. One tray (approximately 30 eggs) from each group was stacked in the order 3F, 7F, 3B, and then 7B and placed back in 4°C storage. Analysis was carried out on each stack at random to minimise the effects of time on the stain and on the eggs.

a) Experimental procedure

The 798 eggs identified as infertile by candling were refrigerated and stored large end up to allow movement of the germinal disc (GD) to the uppermost point on the yolk. A total of 347 of the eggs were examined from the above 798 eggs classified as infertile (and confirmed by breakout) were randomly selected. A portion of the shell at the large end was removed and the outer perivitelline membrane pulled back to expose the germinal disc. A visual assessment was then made to determine fertility according to Mauldin (1998). As described by Liptoi *et al. (*2004), the germinal discs seemingly infertile or uncertainly fertile were transferred to a microscope slide for further analysis. A scalpel was used to make a small incision through the inner perivitelline membrane on the edge of the GD and it was then removed with a small, flat-ended surgical tool. Propidium iodide (Sigma P4170, Sigma-Aldrich Co., PO Box 14508 St. Louis Missouri 63103 USA) a DNA-specific red fluorescent dye was used to stain the GD at a concentration of 5µg PI / ml 0.9% NaCl solution. For each slide, 5µl of the solution was used and a coverslip placed on top.

b) Microscope analysis

True fertility of an embryo is determined by the presence of cell nuclei (Liptoi *et al.*, 2004). If the egg is fertile, PI will stain and illuminate the nuclei but if the egg is infertile, there is no nucleus and the slide will display a dark red background without lighting points (Liptoi *et al.*, 2004). Examples of fertile eggs are illustrated in Figure 1. Propidium iodide acts by intercalating between base pairs of DNA. The cells of fertile eggs are diploid and it is estimated that at the time of oviposition, embryos are at the 30,000 to 70,000 cell stage (Emanuelsson, 1965). On the contrary, infertile egg cells remain haploid and there is no nucleus to be stained. Preliminary studies involved preparing and viewing numerous slides (n=225) under a fluorescence microscope (Olympus BX61; 400x magnification) to gain an understanding of the differences between infertile and fertile eggs. Analysis of infertile eggs was then made to identify true fertility. The remaining eggs were classified as membrane, blood ring, black eye, errors, cracked shell, shell problem or contamination according to and for the purposes of Marsden Park Hatchery.

III. RESULTS

It was hypothesised that a number of eggs that are classified as infertile by breakout analysis would be classified as fertile upon microscopic analysis. Observations of fertility and embryo viability are summarised in Figure 2. All of the eggs were candled and 1206 were set aside as infertile or embryonic mortalities. After visual assessment, 798 (8.31%) were found to be infertile, 408 (4.09%) were embryonic mortalities and there were 15 errors (fertile live embryos).

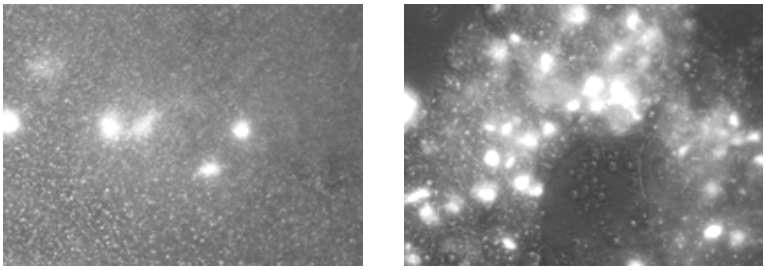

Figure 1. Examples of light points found in fertile eggs after propidium iodide staining and fluorescence microscopy (40x).

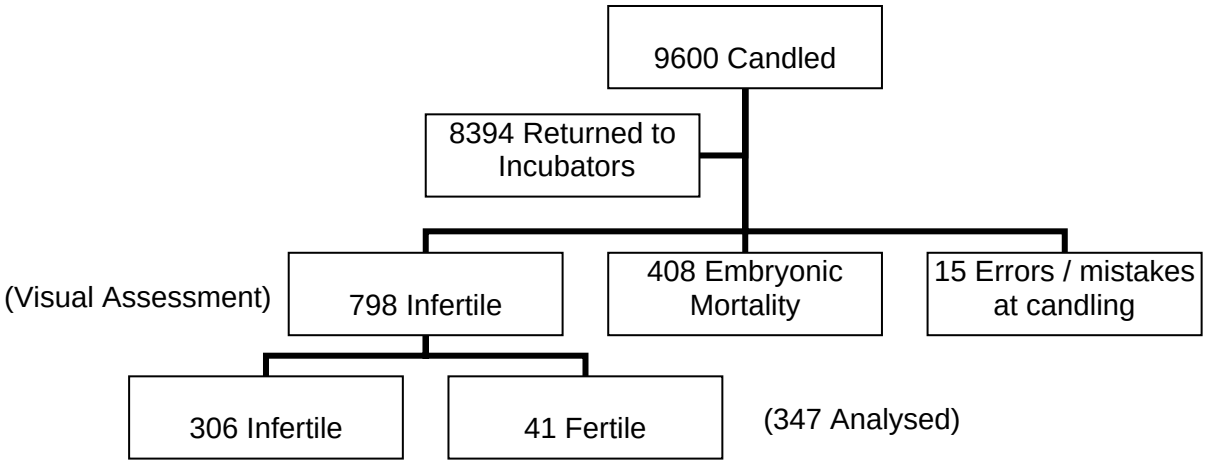

Figure 2. Observations from candling, breakout analysis and fluorescence microscopy.

Of the 347 eggs that were infertile upon visual inspection, 41 (11.85%) were found to be fertile after propidium iodide staining and fluorescence microscopy. Statistical analysis using regression of binomial proportions found that none of the factors or factor interactions had any significant effect on the number of fertile eggs (P = 0.127). The distribution of eggs identified as infertile macroscopically that were later classified as fertile but very early mortality for the two storage and egg positions is presented in Figure 3. The differences in position were not significant, but would suggest further follow up is required.

IV. CONCLUSIONS

The success of the poultry industry depends on the reproductive capacity of its birds and the health and survivability of its chicks. In particular, breeders and hatcheries aim to optimise the conditions that produce the greatest number of viable chicks. However, on average, 15% of eggs are non viable with 10% of these being infertile with the remaining 5% lost as embryonic mortality (Mauldin, 1998). The availability of a method to determine true fertility would be beneficial for isolating reproductive or management problems which lead to very early embryonic mortality. In the past, determining the fertility status of an egg has been difficult. Many claim that true fertility can be determined with candling and microscopic examination whereas others believe that the only way to be accurate is to carry out breakout analysis on an entire sample. In any case, there are likely a number of eggs that are incorrectly assessed as “infertile” when the true status is fertile.

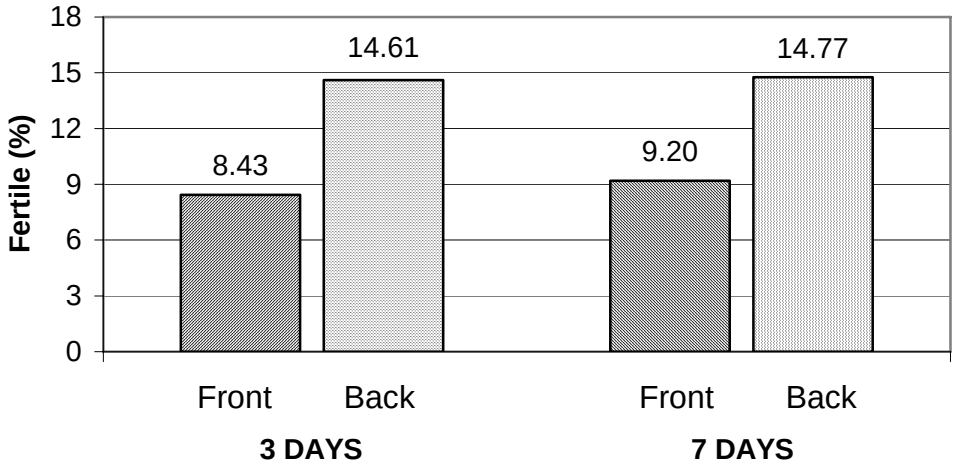

Figure 3. Comparison of fertile eggs (% of germinal discs stained with propidium iodide) for eggs stored for 3 and 7 d before incubation and incubated in front or back positions of the egg trolleys

A fluorescent dye, propidium iodide (PI) and fluorescence microscopy was used to stain germinal discs in an attempt to determine true fertility of chicken eggs. The presence of cell nuclei and hence fluorescence, is representative of a fertile egg whereas infertile eggs do not possess a nucleus and the slide will simply stain dark red.

On the basis of these preliminary trials, propidium iodide staining with the use of fluorescence microscopy technology was found to be an effective tool for determining true fertility in chicken eggs. Further analysis was then carried out to try and quantify the number of false assessments made. Almost 12% of eggs that were classified as infertile upon visual or macroscopic inspection were found to be fertile. In this study neither length of pre-incubation storage of eggs, incubation position nor the day that the analysis took place were significant in scoring the number of eggs found to be fertile. This trial has provided a definitive tool allowing further studies to be carried out into the causes of embryonic mortality and in particular, very early embryonic mortality.

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ABSOLUTE QUANTIFICATION OF MAREK'S DISEASE VIRUS SEROTYPE 2 (MDV2) USING REAL-TIME POLYMERASE CHAIN REACTION AND ITS APPLICATION TO FIELD DUST SAMPLES

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Summary

Methods for taqman real-time PCR assays to detect the three serotypes of MDV are available (Islam *et al.,* 2004), and an absolute quantification has been developed for MDV serotype 1 and serotype 3. The development of a method for absolute quantification of Marek's disease virus serotype 2 (MDV2) is described in this paper. Thus, it is now possible to perform qPCR assays for all three serotypes of MDV on a sample. Absolute quantification of MDV2 in dust samples from poultry farms across Australia in a preliminary study, revealed the presence of MDV2 in 13 of 30 samples tested.

I. INTRODUCTION

Marek's disease virus (MDV), an avian alphaherpesvirus, is one of the most potent oncogenic herpesviruses and causes a contagious, lymphoproliferative disease in chickens. MDV strains are classified into three serotypes based on their pathogenicity. Serotype 2 of MDV is a naturally occurring, infectious virus in chickens, but is nonpathogenic or only weakly pathogenic and nononcogenic in chickens (Baigent and Davison, 2004). The nononcogenic turkey herpesviruses (also referred to as HVT) are classified as serotype 3 viruses. Several strains of MDV2 are used as vaccines, either alone or combined with either strains of serotypes 1 or 3 of MDV. Immunisation with vaccinal strains of MDV does not prevent productive infection by MDV1, so multiple strains can co-exist in the host.

Since the introduction of polymerase chain reaction (PCR) in 1980, several specific assays have been developed and used to detect and quantify MDV (Zelnik, 2004). Real-time quantitative PCR (qPCR) provides a tool for the rapid detection and quantification of MDV and is increasingly preferred to conventional PCR which is labour intensive and requires post-PCR handling (Niesters, 2001). Real-time PCR assays to detect the three serotypes have previously developed by our group (Islam *et al.,* 2004), followed by methods for absolute quantification of MDV serotype 1 and serotype 3 (Islam *et al.,* 2005). In this paper we report the development and validation of a method for absolute quantification of MDV2 enabling the determination of MDV2 virus genome copy number in samples. With these methods we can now measure the viral copy number of all three serotypes in a single sample.

II. MATERIAL AND METHODS

MDV2 specific plasmid standards were developed using part of the sequence of the unique long region UL30 DNA*pol* gene containing qPCR primers described by Islam *et al. (*2004). A 283 bp fragment was amplified by standard PCR, using the reaction conditions described by Islam *et al.* (2005). Primers to produce this fragment were designed using Beacon designer 4.00 (PREMIER Biosoft International, Palo Alto, USA).

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The PCR products were purified using the Wizard® DNA purification Kit (Promega, Madison, USA) and ligated into the T-tagged site of the pGEM T-easy vector according to the manufacturer's protocol (Promega, Madison, USA). The ligation mix was transformed into competent E. *coli* (DH5α) and grown overnight on agar plates containing ampicillin/IPTG and X-gal. Positive transformants were identified using blue-white screening. DNA sequencing of the recombinant plasmid, designated pKR-DNA*pol*, was carried out by Newcastle DNA, University of Newcastle, Australia. Plasmid DNA was extracted and purified on large scale using a Wizard®plus Maxiprep DNA purification Kit (Promega, Madison, USA). The concentration of plasmid DNA was calculated on the basis of two identical agarose gel electrophoreses using twofold, fourfold and eightfold dilutions against a lambda standard containing a known amount of DNA. Purified plasmid DNA was stored at -20° C.

To establish the threshold of detection and demonstrate parallelism with the reference standard curve based on relative abundance, a series of tenfold dilutions, starting at 1x 10^6 down to 1x 10^0 plasmid molecules per reaction were made and a taqman real-time qPCR assay performed using a RotorGene 3000 real-time PCR machine (Corbett Research, Sydney, Australia). The qPCR assay, primer/probes set and reference standard curve used was set up as described by Islam *et al. (*2005). The reference standard curve comprised a 10-fold dilution series of DNA extracted from a cell-associated Maravac® vaccine (MDV2 strain MD19). To determine the reproducibility, three assays were run and intra- and inter-assay coefficients of variation calculated for both Ct value and calculated plasmid copy number. The assays were performed on separate days and all dilution series were made up new for each assay. To test the assay on a limited number of field samples, 30 dust samples from broiler farms around Australia were assayed for their MDV2 content.

III. RESULTS

a) Development of the method for absolute quantification of MDV2

The 283 bp fragment of the MDV2 specific UL30 DNA*pol* gene included the specific sequences of the qPCR primers described by Islam *et al. (*2004). These primer/probe sets do not cross-react when used in qPCR for the three serotypes (Islam *et al.,* 2004).

The fragment was cloned into the pGEM–T-easy vector (Promega Corporation) to produce the recombinant plasmid pKR-DNA*pol*. DNA sequencing of purified plasmid DNA confirmed that it contained the correct insert. Tenfold serial dilutions were made and amplification plots for the pKR-DNA*pol* plasmid standards derived from three separate assays are shown in Figure 1. The lowest dilution in the tenfold dilution series which amplified reliably was defined as the detection limit which was 10 copies of pKR-DNA*pol* plasmid. Standard curves were highly reproducible with no significant difference in slopes ($p<0.05$) between individual runs of the same assay. Based on three individual assays, the MDV2 assays showed mean intra-assay Ct- values with a CV value below 1%, while the mean inter-assay Ct- values had a CV below 3%. Calculated plasmid copy number had mean intra- and inter-assay CVs below 21.5%. Figure 2 shows a linear regression plot of pKR-DNA*pol* plasmid copy number against the previous MDV2 standard which is derived from Maravac® vaccine.

b) Absolute quantification of MDV2 genome in field dust samples

Out of the thirty samples taken from broiler farms across Australia which have been assayed previously for MDV1 and HVT, 13 samples amplified for MDV2 as shown in Table 1.

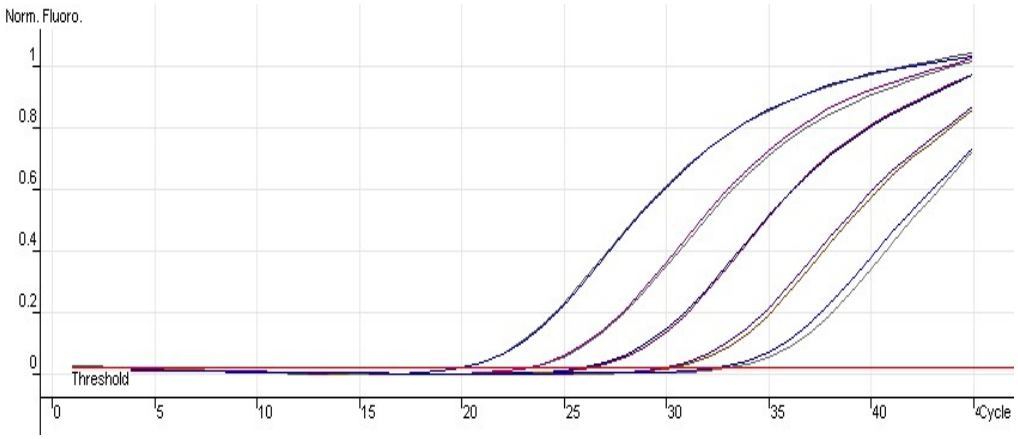

Figure 1: MDV2 assay. Amplification plot of DNA*pol* gene showing a serial tenfold dilution in duplicates of pKR-DNA*pol* plasmid copies (100000- 10), from left to right).

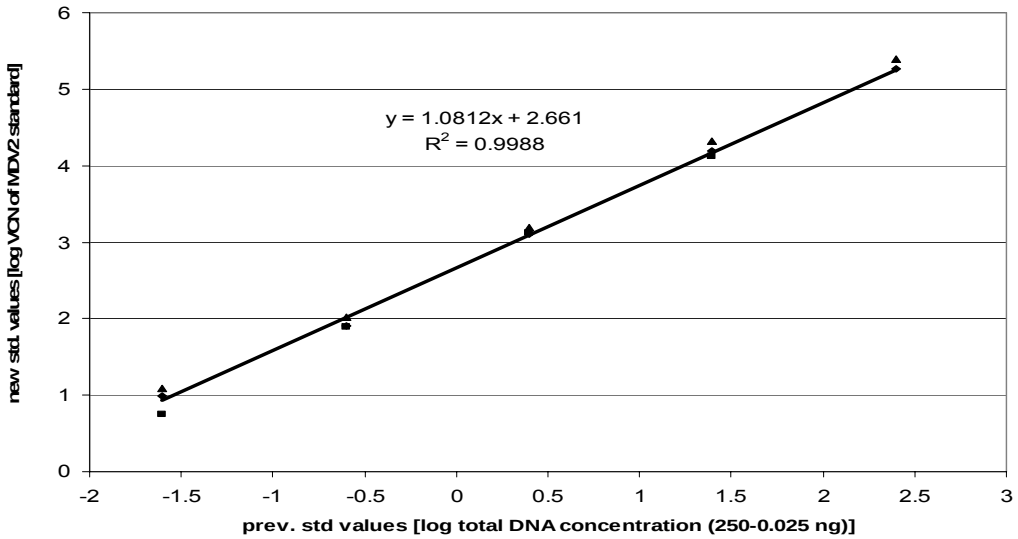

Figure 2: Conversion equation for conversion of previous standards to fully quantified standards, based on three assays.

Table 1: qPCR analysis of 30 field dust samples grouped by results for all three MDV serotypes

No. of samples	HVT vacc.	MDV1 assay	HVT assay	MDV2 assay	MDV2 VCN/mg dust [mean]
3	+	-	-	-	0
6	+	-	+	-	0
2	+	-	+	+	24054
2	+	+	+	+	8342
5	-	-	-	-	0
2	-	-	+	-	0
1	-	+	-	-	0
3	-	-	N.A.	+	22933
5	-	+	-	+	30469
1	-	+	+	+	2263
Total 30	13	9	13	13	

IV. DISCUSSION

The assay for the absolute quantification of MDV2 genome copy number in qPCR using the MDV2 specific plasmid, pKR-DNA*pol*, shows good reproducibility and the detection limit, defined to be the lowest dilution in the tenfold dilution series which amplified reliably, was 10 copies of pKR-DNA*pol* plasmid per reaction. As the lower detection limits were determined in a 10-fold dilution series, these values are regarded as conservative estimates. Therefore, the true detection limit lies in between this value and the next lower 1:10 dilution which did not amplify. The sensitivity of this MDV2 assay is greater than the sensitivity of the absolute quantification assay for HVT, reported by Islam *et al. (*2005), which amplified reliably 75 plasmid copies per reaction and is similar to that of the MDV1 assay reported by Islam *et al.* (2005) as 5 plasmid copies per reaction. One copy of the DNA*pol* gene represents one copy of the MDV genome as the DNA*pol* gene is present only in the unique long region. The generated plasmid standard curve showed parallelism with previous standards used (derived from dilutions of total DNA from 0.025–250 ng per reaction) from Maravac® vaccine indicating the same behaviour over a range from 10^3–10^5 dilutions. This data enabled us to convert the previous standard into viral copy number, thus allowing absolute quantification of qPCR assays.

From 30 field dust samples assayed to analyse the MDV2 viral load 13 samples amplified above the detection limit thus confirming its application in field samples containing a mixture of viruses. It is of interest that MDV2 was detected in 43% of dust samples from broiler farms where vaccination with MDV2 is not practiced. This suggests that wild type MDV2 is circulating in Australian broiler farms and warrants further detailed investigation.

To summarize, the application of this assay together with the MDV1 and HVT assays reported previously by Islam *et al.* (2005) enables us to absolutely quantify samples for all three serotypes of MDV. Furthermore, it is now possible to compare samples on the absolute quantification level. This should lead to improved understanding of the pathogenesis, spread, diagnosis and vaccinal control of Marek's disease.

ACKNOWLEDGEMENTS

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CONSERVING AND MONITORING SHELL EGG QUALITY

D. R. JONES[1]

I. INTRODUCTION

The goal of all producers is to make a better product. In the shell egg industry, this translates to producing a higher quality egg. Egg quality can be defined in many ways. The American consumer demands a clean and sound egg that looks appealing when cracked into a skillet. The shell egg producer could define egg quality in terms of being relatively free of defects which would result in a downgrade. There are many factors that can affect egg quality. Lambrou (1986) stated that breeding, nutrition, environment and management along with egg handling, grading, storage, packaging, and transport effect egg quality. Therefore, when attempting to enhance egg quality, a producer must consider both production and processing factors.

II. GENETICS

The poultry industry utilises genetic selection to attain desirable economic and consumer traits in products. Genetic selection can also assist in disease resistance, habitat adaptability and other factors. One of the benefits of poultry, compared to other agricultural commodities, is the short period of time between generations and the ability to produce multiple offspring in a single generation.

Several papers have examined the differences in closed, random-bred laying stocks and commercial breeds. Anderson *et al*. (2004) reported significant changes in egg shape, weight and surface area amongst the strains studied. They did not report any significant differences in percent shell weight, shell thickness or specific gravity. Another study found an increase in egg weight from the later to newer strains of laying hens compared (Tharrington *et al*., 1999). From comparing the strains with a common ancestral linkage, they concluded that genetic selection had resulted in larger size eggs with a lower percentage of yolk. Furthermore, they found egg quality was maintained or enhanced during the selection process. In an additional study (Jones *et al*., 2001), United States Department of Agriculture (USDA) egg grades were compared between closed, random-bred strains and a current commercial line. The current commercial hens produced a greater percentage of grade A eggs and lower percentage of loss eggs.

In the review document written by Hunton (1982), it was summarised that several studies selecting for low and high shell strength had been successful. Potts and Washburn (1985) found selecting for shell strength had no effect on egg weight. Johansson and colleagues (1996) selected for shell membrane attachment as a means for increasing shell strength and determined that increased shell membrane attachment resulted in thin shell eggs and vice versa. The authors felt this could be due to natural selection factors since increased shell membrane attachment could impede embryo emergence at hatch. In his review Hunton (1982), summarised that white shells are thicker than brown. Some research suggests brown eggs are more resistant to breakage, but there is great debate amongst researchers in this area. In the Anderson *et al*. (2004) study, they found that the more current genetic stocks produced eggs with greater shell strength. They did not find a difference amongst the strains for shell thickness, but as mentioned before, the more current strains produced larger sized eggs.

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III. NUTRITION

Hen nutrition can have a direct effect on egg quality. When a hen is nutritionally compromised, the body begins to shut down unnecessary processes. Reproduction is greatly diminished, and the bird becomes immunologically compromised which can lead to increased incidence of disease. If a layer diet is complete except for a lack in the appropriate level of the amino acid lysine, a laying hen will not efficiently produce eggs and the eggs laid will be of inferior quality. Dietary composition can affect egg flavor, shell quality, and yolk pigmentation (Narahari, 1980). A lesser dietary effect can be seen for albumen index, Haugh unit values, yolk index, and blood and meat spot incidence. Conversely, Blair and Lee (1972) found that increased dietary protein above a basal level of 11.5% increased Haugh units.

A lack of calcium and phosphorus in the layer diet will result in the hen leaching these minerals from the bones in order to lay sound eggs. As this condition progresses, the hen will reach a point where it will no longer remove minerals from the bones. After this, a hen will produce soft shell or even shell-less eggs. Keshavarz and Nakajima (1993) found increasing dietary calcium levels above the recommended 3.75 g calcium/hen/day did not enhance shell quality. Furthermore, phasing calcium, phosphorus or combined calcium and phosphorus concentrations in the diet did not affect shell quality. The researchers also examined the role of added cholecalciferol in the diet with increased hen age and found no affect on shell quality. Feeding oyster shell in mid- and late-lay enhanced shell quality no matter the calcium concentration of the diet. Egg production was not affected by any of the dietary treatments examined in the study. Hens laying thick shelled eggs have been found to retain more dietary calcium than thin shell layers (Clunies *et al*., 1992). There was no difference in egg production between the thick and thin shell layers. Egg weight and shell weight was increased for the thick shell eggs compared to the thin. This disagrees with the findings of Anderson *et al*. (2004) who found no difference in shell thickness for significantly different egg weights. Shell deformation was lower for thick shelled eggs (Clunies *et al*., 1992) which falls in line with the previous summary of Hunton (1982) which reported thinner shelled brown eggs to be stronger than thicker white shelled eggs.

Researchers have also examined the role of chloride in egg quality. Keshavarz and Austic (1990) determined that increased dietary levels of chloride, phosphorous or a combination of the two resulted in decreased eggshell quality and blood acid-base indicators. They speculated that the decreased shell quality could be due in part to increased calcium excretion due to the presence of high levels of chloride and phosphorous. Balnave *et al*. (1989) provided hens with 600 or 2000 mg sodium chloride per liter of drinking water. There was an increase in shell defects with no changes in egg production, weights or food or water intake associated with the treatments. The treatment group receiving 2000 mg sodium chloride per liter had an increased incidence of shell-less eggs. The shell defects persisted after the sodium chloride was removed from the drinking water. There were little to no effects on blood acid-base balance and electrolyte levels. Conversely, Hess and Britton (1989) fed laying hens low chloride diets and found virtually no effects on shell quality. There was a slight increase in specific gravity noted towards the end of the study.

A wide range of other nutritional concepts have been investigated for their role in egg quality. Hess and Britton (1989) reported that dietary protein levels did not affect egg quality or production for the diets formulated for their study. Gossypol, a component of cotton seed, has been linked to yolk mottling as has the use of some coccidiostats (Narahari, 1980). With the issue of animal waste management becoming a greater environmental concern, the use of phytase in poultry diets to reduce the mineral composition of litter is becoming more common. Scott *et al*. (2001) found egg weight to be greatest from hens fed a corn-based diet

with added phytase compared to the control diet. Furthermore, there was a decrease in the percent shell and an increase in albumen height associated with the diet. The investigators also examined a wheat-based diet with added phytase and did not achieve the same results. Distillers dried grains with added solubles have been examined for their potential in laying hen diets (Benabdeljelil and Jensen, 1989). Haugh unit values and ovomucin concentration were not increased in fresh or stored eggs produced by hens fed the ration.

IV. PHYSIOLOGICAL STRESS

Minimising physiological stress within the hen can also enhance shell egg quality. During periods of stress there is a breakdown of the reproductive tract. This can lead to reduced egg quality or production. This breakdown can also lead to an increase in the incidence of meat spots. Acute changes in the birds environment can result in a stress response. Marion *et al*. (1964) found that variations of the proportions of the parts of the egg were due to physiological changes associated with aging and the environment.

Exposure to disease can result in a decrease in shell egg quality. Many diseases can impair the reproductive tract. An example is infectious bronchitis. Initially this disease causes a respiratory response. It is then able to move through the blood stream and infect the reproductive tract. This can cause decreases in the internal and external quality of eggs produced. Both young chicks and adult hens can be infected producing these results. Infectious bronchitis is caused by a coronavirus and is a very contagious disease among chickens. Within 1-2 days, almost 100% of a flock can show signs of the disease (Trampel, 2005).

During the course of infectious bronchitis, oviduct weight and length is reduced and remains so for approximately 3 weeks. There is also a decrease in the number and height of epithelial cells in the lining of the oviduct. Another complication that can occur is the development of false layers. Soft shelled eggs or fully formed eggs are found in the abdominal cavity of a false layer. This comes about due to the egg progressing through the reproductive tract and reverse peristalsis occurring at some point forcing the egg to be deposited in the abdominal cavity.

The age of the laying flock can also have a direct effect on shell egg quality. The longer a hen is in lay, the lower the quality of the eggs produced. As the hen ages, the reproductive tract begins to decline. This results in a decrease in egg quality. Molting a laying flock can serve as a means of rejuvenating the reproductive tract. There are concerns associated with the act of molting and it should be performed in a responsible manner. After a molt, egg quality is enhanced, but declines at a greater rate than during the previous production cycle. Molting a flock multiple times also results in a greater rate of shell egg quality decline. Morris *et al*. (1985) reported a decrease in albumen quality associated with hen age which they attributed to wear on the oviduct during the laying cycle. This problem could be overcome by molting the flock, followed by a period of rest.

Silversides *et al*. (1993) reported that the percent albumen changes with hen age and egg weight. Hen age has been found to be a factor in Haugh unit (HU) values. It has been established that HU decreases as the hen ages (Cunningham *et al*., 1960; Montgomery and Stewart, 1973; Curtis *et al*., 1985; Izat *et al*., 1986). Doyon *et al*. (1986) studied the rates at which the HU and albumen height change. They stated that HU and albumen height decrease at a fairly constant rate as the hen ages. They reported that the HU decreased at a rate of 0.0458 units/day of lay (13.15 units over 287 days). The albumen height measurements decreased at a rate of 1.39 mm over the entire 287 days. Hill (1981) found that the day after the egg is laid, the HU decreased one unit for every month the hen had been in lay. Silversides (1994) reported a linear decrease in albumen height as a hen ages. The

characterisation of a linear relationship for albumen height quality and hen age is called to question when considering the report of Hill (1981) which stated that albumen quality became more variable as the hen ages. Silversides (1994) reported that strain x hen age interact significantly for Haugh unit and log albumen height, suggesting that these change at different rates for hen age and different strains. Anderson *et al*. (2004) found that as the hen ages, shell breaking strength decreased as did percent shell and specific gravity. De Ketelaere *et al*. (2002) have also reported decreased shell thickness as a hen ages.

V. ENVIRONMENT

Management practices can have a profound effect on shell egg quality and are some of the easiest to fix. Maintaining the hens on a set lighting schedule can enhance shell egg quality and egg production. Providing a clean environment aids in maintaining bird health. Proper handling of the birds reduces the incidence of body checks. A body check occurs when the egg is cracked in the reproductive tract and the bird is then able to mend the crack. These eggs are considered to be less sound than a normal shell egg. A trauma that would result in the cracking of the egg within the hen would be required. For this reason, proper employee training is imperative.

Exposure of hens to high heat results in decreased bird performance, shell thickness, and increased shell breakage (Lin *et al*., 2004). The authors reported no change in shape index. There were no changes in static stiffness, dynamic stiffness or modulus of elasticity of the shell associated with hen exposure to higher environmental temperatures. Arima and colleagues (1976) examined the role of high environmental temperature on young and old laying hens. The egg quality of the older hens was more severely affected by the increased temperature. The authors also reported that the function of the ovary and oviduct appeared to be affected by the high environmental temperature. The greatest shell strength was found in eggs produced by hens housed at 27^{o}C. The greater the environmental temperature, the lower the shell strength of the eggs produced. Additional work found that hen exposure to cyclic temperature patterns allowing for periods of cooler temperature exposure results in greater shell breaking strengths, thicker eggshells, and lower body weight changes in the hens (Deaton *et al*., 1981). When European brown egg hens were provided cool drinking water, feed intake increased as did shell thickness (Glatz, 2001). When Australian tinted layers were tested, only 5^{o}C water had an affect on feed consumption and shell thickness. The effect for this particular breed was also lost as the hens acclimatised.

VI. PROCESSING

Thompson *et al*. (1985) concluded that factors other than shell quality affect egg breakage in processing. Proper maintenance of production equipment can also improve egg quality. Collection belts which are working improperly can result in an increase in the incidence of impact checks. An impact check is a cracked egg with intact membranes that resulted from two eggs colliding. Repairing cages to prevent protruding wires decreases the risk of cracked eggs. Proper cleaning of belts, cages, and collection flats reduces the risk of shell contamination with egg contents. In the US, adhering matter results in a grade of Ainedible@. These eggs can not be sold for human consumption.

Egg processing has a definitive effect on shell egg quality. There are many steps in the processing procedure where egg quality can be enhanced. When the egg is laid it is approximately 39^{o}C. The quicker an egg is cooled, a greater level of interior quality is maintained. As the internal egg temperature increases above 7^{o}C, the protein structures of the thick albumen and vitelline membrane breakdown faster. For this reason, care should be

taken to ensure the egg is properly cooled throughout the shell egg processing procedure. One of these steps is the on-farm holding room. Collecting eggs from the laying house several times throughout the day instead of once or twice will reduce the amount of time those eggs are exposed to higher environmental conditions and they are less likely to be exposed to environmental contaminates. In the egg holding room an environment of 7–13^{o}C and relative humidity of 50–60% will help to maintain egg quality. This room should also be sanitised and the chiller system maintained in good working order to help in reducing possible microbial contamination.

Care should also be taken to process eggs as quickly as possible after being laid. For off-line farms, egg trucks should visit farms at least twice a week. Holding eggs for greater than 4 days on the farm reduces the quality of the processed shell egg. The sooner eggs can reach the market, the greater your perceived consumer quality. During transport, shaking of the eggs should be held to a minimum since shaking has been found to have a detrimental effect on egg quality (Walker *et al*., 1972).

A more ideal solution is an in-line operation where eggs move directly from the laying house to the processing line. With this process, there is no need for an on-farm holding room. There is a limit to the ability of a processor to run exclusively an on-line operation due to flocks coming in and out of production and meeting processing needs for brown and white shelled eggs. For this reason, there is a need to adjust shell egg processing to accommodate both on- and off-line eggs.

Clean flats should be utilised to move eggs from farms to the processing facility. These flats need to provide an appropriate amount of protection to prevent damage to the eggs. Either sanitisable material or disposable paper flats should be utilised. Permanent flats should be sanitised before leaving the processing facility to return to the farm. Egg trucks should also be sanitised completely before returning to any farm to increase biosecurity. If a flock has been identified as *Salmonella Enteritidis* positive, special care should be taken to prevent potential introduction of the bacteria to other contract farms and eggs in the processing facility. Current recommendations are for all known *Salmonella Enteritidis* positive eggs to be diverted to pasteurisation facilities.

When eggs enter a processing facility from an off-line farm, internal egg temperature ranges from 17–20^{o}C. In-line eggs enter a processing line with an internal egg temperature of 31 to 36^{o}C. Current USDA regulations require egg wash water to be 32^{o}C or 11^{o}C warmer than the warmest egg. When both off-line and in-line eggs are processed at the same time there can be an increase in the incidence of thermal checks. A temperature difference of greater than 22^{o}C between the egg and the wash water can cause thermal checks. A thermal check is a hairline crack in the shell surface along the vertical axis caused by abrupt changes in egg temperature. In a facility that processes both off- and in-line eggs, it would be advisable to initially process the off-line eggs then increase the wash water temperature (perhaps during a break or during lunch) to process the in-line eggs.

The internal temperature of an egg increases approximately 8^{o}C during processing. When these warm eggs are placed into paper flats, cartons, or foam cartons then packaged in wire baskets or cardboard cases, they continue to increase in temperature. A maximum temperature is reached within 4-6 hours of processing. Under the current USDA regulations eggs must be maintained at 7^{o}C ambient temperature post-processing. Research has shown that a case in the center of a 30 case pallet will require 7-14 days for the center most egg to reach an internal temperature of 7^{o}C under these conditions (Anderson *et al*., 1992). In the US, most processed shell eggs for retail sell stay in the processing facility for 1–4 days before going into distribution. Furthermore, Bell *et al*. (2001) determined in a national survey that white shell eggs in the US were on average 11 days post-processing when purchased by the consumer.

Due to the fast distribution cycle, researchers have been developing methods of rapidly cooling shell eggs at the end of processing (Curtis *et al*., 1995; Thompson *et al*., 2000). Rapid cooling of shell eggs increases the internal quality of the eggs and enhances the microbiological integrity (Jones *et al*., 2002). Haugh unit scores have been enhanced by these methods and vitelline membrane strength and elasticity have been increased compared to control eggs. Rapid cooling of shell eggs has been found to cause eggshell damage (Fajardo *et al*., 1992). The initial quality of the eggshell affects the severity of the damage. During cold storage, it has been reported that albumen height decreases while albumen pH and whipping volume increases (Silversides and Budgell, 2004). Egg and albumen weight also decreased during storage with yolk weight increasing.

Clean, appropriate packaging material should be utilised for processed shell eggs. This serves two purposes: 1) protect the egg from damage during transport and storage and 2) reduce exposure to microbial contaminants. Cartons from returned retail product should not be re-used and should be destroyed. Packaging material should not trap condensation. If a facility is located in a high humidity area, care should be taken in packaging material choices since eggs tend to sweat under these conditions. Sweating has been associated with a higher probability of microbial penetration into the egg.

Processing equipment needs to be kept in good working order. If transfer units are not maintained, eggs can be dropped and broken before processing leading to a higher organic load being introduced into the washer. High levels of organic matter can render sanitising agents in the wash water inactive. USDA regulations, in inspected plants, require wash water to be dumped every 4h in order to maintain wash water effectiveness. If eggs remain in the washer for too long of a period they can become partially cooked.

Belts should be cleaned to prevent the transfer of egg contents to other eggs. Once again, any adhering matter, including egg contents, renders an egg inedible in the US. Packer heads should be kept in careful working order to prevent damage to the eggs during packaging. Packers should also be properly calibrated to prevent crowding on the belts which can lead to an increase in impact checks.

Employee training in the processing plant is extremely important in enhancing egg quality. The movement of eggs throughout the processing plant should always be from dirty to clean areas. Employees should also understand the sanitation standard operating procedures (SSOPs) and good manufacturing practices (GMPs) utilised in the processing procedures.

VII. MONITORING EGG QUALITY

Baker and Vadehra (1970) stated that candling does not always correspond with the internal quality of an egg. Bokhari *et al*. (1995) examined the eggs pulled by candlers in commercial processing plants in California. They determined in a commercial facility with an average line speed of 240 cases/hr, 17.3 % of the pulled eggs were over-pull. Forty-three percent of the over-pulled eggs were due to the presence of cage marks. The authors concluded good employee training is necessary to prevent over-pull. Due to the subjective nature of egg candling, other methods are needed to evaluate internal egg quality. The HU measurement for interior egg quality was developed by Haugh (1937). The measurement has been modified to account for egg weight. This modification corrects for all eggs to be compared as large size eggs through mathematical manipulations, regardless of the actual weight of the egg. Therefore, it is a correlation of albumen height and egg weight. There are those who feel that this correction factor, as it is called, is unnecessary and actually causes the HU to be incorrect, thereby distorting comparisons which have been made. Researchers have suggested that a direct measurement of albumen height might be a better determinant of

interior egg quality. Silversides *et al*. (1993) suggested that the effect of egg weight on albumen height was of minor importance within flocks and inconsistent between flocks. They further stated that the statistical relationship between albumen weight and albumen height was weak. In a pooled data set, it was found that egg weight and albumen weight significantly affected HU. For this reason, the authors argued that the correction for egg weight was unnecessary. In contrast, Kidwell *et al*. (1964) reported that the correction for egg weight was probably unnecessary, but that it did not take away from the HU measurement. Baker and Vadehra (1970) contradict the complaints of accounting for egg weight by stating that the position of the thick albumen was of more importance than the actual amount when determining the HU.

Izat *et al*. (1986) and Williams (1992) stated that there was not a seasonal effect on HU. Brown egg layers have been found to produce eggs with greater HU (Curtis *et al*., 1985). Additional authors reported that HU values were more variable within the brown egg layers compared with those that lay white-shelled eggs (Hill, 1981; Williams, 1992).

Various methods have been utilised to assess egg shell quality. Both indirect and direct methods have been developed. Some of the easiest assessments to perform include: egg weight, shell weight, percent shell, shell thickness, and shell weight per unit of surface area. Another traditional laboratory measurement for shell quality is to determine egg specific gravity. There has been extensive publication of methods for its determination. With the advent of new technologies, it is now possible to assess the deformation and compression force an eggshell can withstand.

In 2002, De Ketelaere and colleagues compared a variety of methods for determining eggshell strength. They found all measurements gave different information. Sound eggs require a greater force to puncture than cracked eggs (Carnarius *et al*., 1996). Mean force was not different between cracked and leaker eggs. Shell weight, percent shell, shell thickness, specific gravity, and shell weight per unit surface area were all significantly greater for intact versus cracked eggs from a commercial processing plant (Thompson *et al*., 1986). In young and old flocks, a single specific gravity solution was able to identify eggs with low specific gravity and therefore a higher likelihood for cracking during commercial processing (Bennett, 1993). Research has shown that eggshell stiffness modulus (non-destructive shell quality measurement) was able to detect cracks when they were located in the vicinity of the testing site (Lin *et al*., 1993).

Several factors have been identified to affect eggshell quality measurements: egg temperature, time between lay and testing, sequence which indirect measurements are made, compression rate and response time of recording equipment for compression and deformation determinations, moisture content of the shell, and configuration and roughness of testing surfaces contacting the egg (Hamilton, 1982). The authors concluded that this information should be included when data is reported. The orientation of the egg can also affect detected force associated with shell breakage (Pandey *et al*., 1984). Differences exist for recorded force associated with the large and small ends as well as the equator of the egg, with the small end being the strongest. Therefore, the orientation of the egg during measurement should be presented with data.

Several correlation studies have been conducted to determine how the various measures of shell quality integrate. In 1983, Thompson and colleagues published work showing strong correlations for egg weight, shell weight, specific gravity, shell deformation, shell compression strength and shell thickness when comparing eggs from individual hens. This created an understanding that the measurements were accurate in assessing the quality of various eggs from a single hen. The report also stated that shell compression strength had a greater correlation than shell deformation between eggs. A negative correlation was found to exist between egg weight and egg specific gravity and percent shell (Pandey *et al*., 1985).

Shell thickness, shell weight per unit of surface area and percent shell were able to be predicted from specific gravity measurements. Shell weight was more accurately predicted with specific gravity and egg weight together.

Thompson and Hamilton (1986) found intact and cracked eggs were significantly different for shell weight, percent shell, specific gravity, and shell weight per unit area. Egg weight had the highest correlation with egg breakage in shipment (0.591) but this was not a strong correlation. The authors concluded that the laboratory methods examined were not adequate in predicting egg breakage during transport. Subsequently, a significant negative correlation was found between the percentage of eggs cracked during commercial processing and specific gravity and percent shell (Strong, 1989). Breaking strength, shell thickness, and shell weight were not significantly correlated with egg breakage during commercial processing. The width of the palisade layer of the shell has been found to be correlated with shell puncture force (Carnarius *et al*., 1996). Furthermore, a negative correlation has been identified between cracked eggs and percent shell, shell weight per unit surface area, specific gravity, and shell weight (Abdallah *et al*., 1993). The authors also found a positive correlation between the percentage of cracked eggs in a lot and egg weight.

Roberson *et al*. (1987) concluded specific gravity was the best method for determining the quality of intact shell eggs. They further stated that shell thickness was the preferred method for assessing broken eggs since it can easily be measured and is highly correlated with shell breaking force. Due to the errors involved with specific gravity measurements, Abdallah *et al*. (1993) recommended percent shell and shell weight per unit surface area as more accurate methods for determining shell quality. The concern over errors in assessing specific gravity in eggs is part of what prompted Bennett (1993) to analyse the potential of utilising a single solution and grouping eggs as "low" or "high" specific gravity for shell quality evaluations.

The advent of modern technology has allowed for more precise rearing and laying practices to enhance egg quality. Shell egg processing technology has also evolved to allow for the production of a safer, higher quality product. The development of more objective testing methodologies makes shell egg quality assessment much more precise. Many of the older quality determination methods are still applicable and often more appropriate in the processing environment.

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LIGHTING GROWING PULLETS – GET IT WRONG AT YOUR PERIL

P.D. LEWIS[1]

Summary

The balance between egg numbers and egg weight for egg-type hybrids and broiler breeders is mainly decided by the age and body weight of a pullet on the day it lays its first egg. The timing of this event is strongly influenced by the lighting programme applied during the rearing period. The earliest maturity for egg-type pullets reared on constant photoperiods is achieved on 10-h days. Maturity occurs about 7 d later for birds reared on 8-h days, and is marginally delayed on photoperiods > 10-h. Broiler breeders respond to constant photoperiods quite differently from egg-type pullets because they still exhibit photorefractoriness. Although the earliest maturity for constant photoperiod pullets still occurs at 10 h, first egg is delayed by about up to 3 weeks for photoperiods between 10 and 13 h, with smaller delays thereafter, and delayed by about 2 d for each 1-h reduction in photoperiod below 10 h. Egg-type pullets are not responsive to a transfer to long-days before 5 or 6 weeks, are most responsive by 9 to 10 weeks. Responses become progressively weaker thereafter, until no response 10 d before spontaneous maturation. In contrast, maturity is delayed by 3-4 weeks in broiler breeders photostimulated before 10 weeks, and complete photosensitivity does not occur in a flock until at least 18 weeks of age. From 19 weeks onwards the advance in maturity becomes steadily smaller until spontaneous maturation at about 30 weeks. A transfer from 8 h to 14 or 15 h provides maximum stimulation for both egg-type and broiler breeder pullets. Many egg-laying hybrids reach 50% egg production by 21 weeks of age without photostimulation, and so maturity cannot be retarded simply by delaying the first increase in photoperiod; some form of step-down lighting is required.

I. INTRODUCTION

Age at first egg exerts a large influence on the balance between egg numbers and egg weight, with a 7-d difference in maturity resulting in a 1-g change in mean egg weight and a 5-6 difference in egg numbers. Light is unquestionably the most potent environmental factor controlling the rate of sexual development in ad libitum fed egg-type pullets, and a major factor in control-fed broiler breeders. Precocious pullets will always lay eggs that are below breeder specification and retarded pullets rarely meet egg-number targets. Thus the main function of a lighting programme during the rearing period is to achieve optimum sexual maturation. There is no set time for sexual maturity, but there is an optimum time to achieve the balance of egg numbers and egg weight appropriate for a particular market. If an incorrect lighting programme is used, and sexual maturity occurs at the wrong time, there is little that can be done to rectify matters once egg production has started; you only get one chance to get it right.

There is a big difference in the philosophy for lighting for egg-type pullets and broiler breeders. Laying hens are no longer seasonal breeders, and so do not necessarily need to be reared on short days. In contrast, broiler breeders still are seasonal breeders, and, like turkeys, need to be given a period of short days to dissipate juvenile photorefractoriness to make them responsive to a transfer to stimulatory photoperiods.

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A further objective of a lighting programme for egg-type pullets is the encouragement of an appetite that achieves the desired growth curve. Commonly, producers have difficulty in getting modern egg-type pullets to target body weight, but it appears that generally the reverse applies in Australia, where over-weight pullets are reported to be a problem after 6 weeks of age.

In broiler breeders, growth is almost totally controlled by the feeding programme, and so lighting has little influence on body weight gain. However, lighting during the rearing phase has a huge effect on the timing of sexual development, irrespective of growth. Broiler breeders cannot simply be regarded as large chicken - they are more like small turkeys – and so lighting considerations are completely different from egg-laying stock. Getting it wrong for broiler breeders can be very costly.

II. EGG-TYPE PULLETS

a) Constant photoperiods

Constant photoperiods not only affect sexual maturation through the photosexual response, but also by modifying feed intake and growth. The earliest age at first egg (AFE) is achieved by rearing on 10-h photoperiods. AFE is delayed by about 0.3 d for each 1-h longer photoperiod, but by 4.2 d for each 1-h shorter photoperiod. This rate of change below 10 h is far steeper for modern hybrids than for early genotypes of pullet, when AFE was delayed by less than 2 d/h (Figure 1). Cumulative feed intake to 16 weeks increases by about 180 g for each 1-h extension of the photoperiod, but to mean AFE (analogous to 50% rate of lay) it is influenced by both the photoperiod and AFE, and is described by the equation: y = -12000 +221p +133A, where p = photoperiod during rearing and A = mean age at first egg (d).

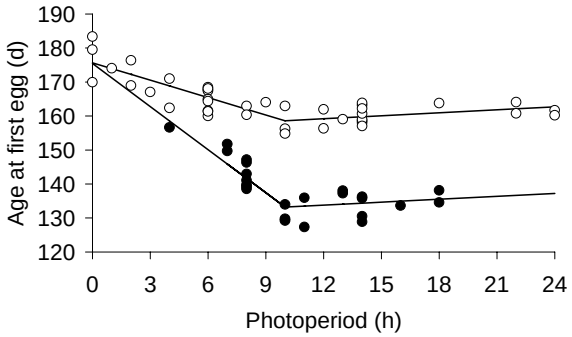

Figure 1. Mean age at first egg for ad libitum fed early (○) and modern (●) hybrids maintained on constant photoperiods

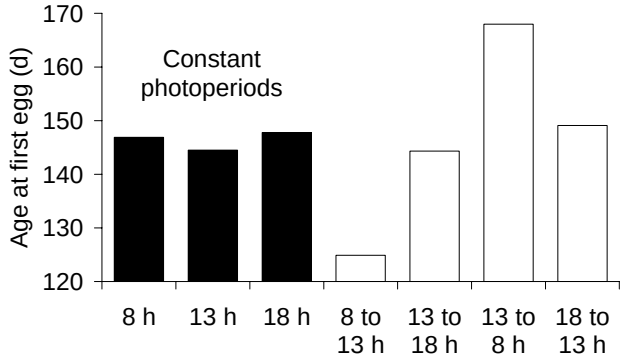

Figure 2. Mean age at first egg for modern pullets maintained on 8, 13 or 18-h photoperiods (solid bars), or given a 5-h increase or decrease (open bars) at 12 weeks.

It is common commercial practice to provide growing pullets with a short period of step-down lighting before growing them on a constant short-day to ensure the chicks have explored their environment and satisfactorily started eating and drinking. Maturity will be delayed by 1 to 2 d for each extra week taken to reach the constant short-day, depending on the stimulatoriness of the subsequent lighting regimen; the less stimulatory the programme the bigger the retarding effect of the step-down lighting (Morris, 1980). Whilst this initial phase of longer photoperiods results in higher feed intakes and faster initial growth, it does not give any improvement in total egg yield, simply fewer but larger eggs as a result of the delay in AFE (Leeson *et al*., 2005).

b) Changing photoperiods

Growing pullets respond more to a change in photoperiod than to the initial or final photoperiods themselves, irrespective of whether the change is an increase or a decrease. Data for white-egg pullets maintained on 8-, 13- or 18-h photoperiods, or transferred from 8 to 13 h, 13 to 18 h, 18 to 13 h or 13 to 8 h at 12 weeks provide a good example (Lewis *et al*., 1996). Figure 2 shows that whereas there was only a 3-d difference in AFE for pullets given constant photoperiods, more than 43 d separated the earliest treatment (an increase from 8 to 13 h) from the latest maturing group (a decrease from 13 to 8 h).

These data also demonstrate that the influences of the initial and final photoperiod are more powerful than the size of the change. Although both increments in this trial were 5 h, the transfer from 8 to 13 h advanced AFE by 22 d, whereas the transfer from 13 to 18 h advanced it by less than a day. Likewise, the decrease from 18 to 13 h delayed maturity by only 1 d, while the decrease from 13 to 8 h delayed AFE by 23 d; all maturities relative to pullets maintained on the initial photoperiod. This shows that the effect of a change in photoperiod depends on the stimulatoriness of the two photoperiods. A change between two stimulatory or between two non-stimulatory photoperiods has a lesser effect on AFE than a change between a stimulatory and a non-stimulatory photoperiod, irrespective of the direction. The rate of change in maturity for a given change in age at photostimulation can be calculated by the equation:

$$b = k_i(0.1338 + 0.1496C - 0.01884C^2 + 0.0009683C^3 - 0.00001941C^4$$
$$- 0.22396M + 0.05028M^2 - 0.00365M^3 + 0.00008216M^4) \quad (1)$$

where b = the change in AFE (d) for each 1-d delay in applying the change in photoperiod, C = difference between the initial and final photoperiod (h), M = mean of the initial and final photoperiods (h), and k_i = adjustment for the difference in responsiveness between the given genotype and ISA Brown hybrids.

The other important factor affecting the timing of sexual maturation is the age at which the changes in photoperiod are given (Lewis *et al*., 2002). Modern full-fed pullets do not respond to an increment in photoperiod until about 5 weeks of age, and not all birds within the flock are photoresponsive to an increase until about 9 weeks (Figure 3). The likely reason why increments in photoperiod at very young ages do not advance AFE, despite their ability to induce an increase in plasma luteinising hormone (LH) concentration, is that the hypothalamic-pituitary axis is insufficiently developed to stimulate the release of follicle stimulating hormone (Lewis *et al*., 1998); a consequence, in part, of suboptimal plasma concentrations of oestrogen (Lewis *et al*., 1996; Dunn *et al*., 2003). The effect of photostimulation after 9 weeks becomes progressively weaker until the time that a pullet is within about 10 d of spontaneously laying its first egg (in response to the initial photoperiod), after this time AFE will be the same as that of

non-photostimulated pullets. The steadily increasing effect between 5 and 9 weeks of a transfer to a long day for a group of birds is the result of more individuals within the group becoming photosensitive and not a change in individual responsiveness. The AFE following an increment in photoperiod can be predicted by the equation:

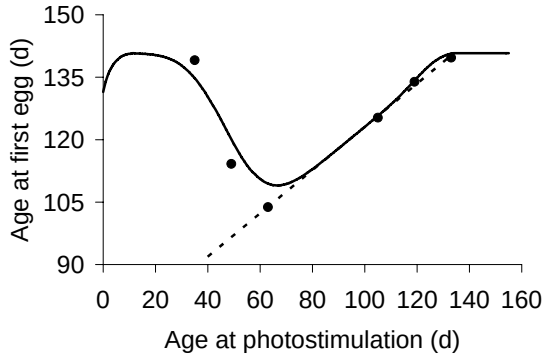

Figure 3. Mean age at first egg for pullets transferred from 8 to 16-h days at various ages and the fitted model. The broken line describes the linear regression between 9 and 19 weeks (Lewis *et al*., 2002)

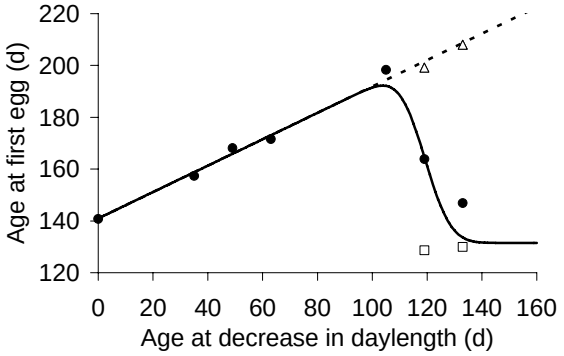

Figure 4. Mean age at first egg for pullets transferred from 16 to 8-h photoperiods at various ages (●) and the fitted model. Responders (Δ) and non-responders (□), and the dotted line describing the linear regression between 0 and 19 weeks (Lewis *et al*., 2002)

$$A_m = (1\text{-}p)A + p(1\text{-}m)[A\text{-}b(A\text{-}t)] + pmA \quad (2)$$

where A_m = predicted mean age at first egg (d), A = mean AFE (d) for pullets maintained on the initial photoperiod, p = proportion of birds sensitive to an increment in photoperiod, m = proportion of birds that have spontaneously started rapid gonadal development in response to the initial photoperiod and are within 10 d of laying their first egg, b = the rate of change in AFE (d) for a 1-d delay in transferring the pullets to the final photoperiod, and t = the age (d) at which the increase or decrease in photoperiod is given.

Lighting programmes do not include decreases in photoperiod at the end of the rearing period by design; but they can occur inadvertently. For example, when spring-hatched pullets are reared in non-lightproof or naturally ventilated rearing facilities on 'short days', transferred to a lightproof laying house, and maintained on short days for a period before the planned photostimulation, they could, in reality, receive a reduction of 4-8 h in photoperiod depending on the natural daylength at the time of transfer. Decreases in photoperiod result in a delay in AFE, but the amount of delay progressively increases as the bird gets older at a rate dependent on the initial and final photoperiod (equation 1) until a pullet is within about 10 d of spontaneously laying its first egg (in response to the initial photoperiod). The potential of a decrease in photoperiod to retard is double that of an increase to advance AFE (Figure 4). In a flock of modern egg-type pullets, the delaying effect continues uniformly until about 15 weeks, after which some pullets will mature spontaneously in response to the initial long day, whilst others will be further delayed. This can make management very difficult, especially the nutrition, with < 3-month between the early and late birds maturing. A further problem with such a flock is the suboptimal peak rate of lay (because some individuals are past their peak whilst others have yet

to mature) and large variation in egg weight caused by the wide spread of individual AFE. Mean AFE is predicted by the following equation:

$$A_m = (1\text{-}m)(A+bt)+mA \quad (3)$$

(see equation 2 for explanation of symbols)

II. BROILER BREEDERS

a) Constant photoperiods

Broiler breeders are not fed *ad libitum*, and respond differently to photoperiodic treatments when they are managed to achieve different growth profiles. However, when birds are given constant photoperiods, Figure 5 shows that the response is specific to photoperiod, and lower body weights simply delay maturity for all photoperiods (Lewis *et al*., 2004a). Broiler breeding stock exhibit photorefractoriness, and so sexual maturity in response to constant non-stimulatory photoperiods (≤ 10 h) is similar to that for egg-type birds, but it is markedly different for birds exposed to longer photoperiods (> 10 h). Broiler breeders are hatched in a state of juvenile photorefractoriness, and the rate of its dissipation is proportional to the stimulatoriness of the photoperiod and not to its length (as reported for exotic avian species). Figure 6 shows that the earliest sexual maturity is achieved by providing 10-h photoperiods and the latest when birds are maintained on constant 13 or 14 h daylengths.

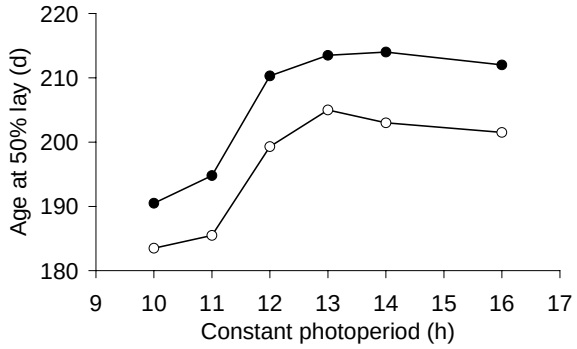

Figure 5. Mean age at 50% lay for broiler breeders grown to reach 2.1 kg body weight at 17 (○) or 21 (●) weeks of age and maintained on 10, 11, 12, 13, 14 or 16-h photoperiods (Lewis *et al*., 2004)

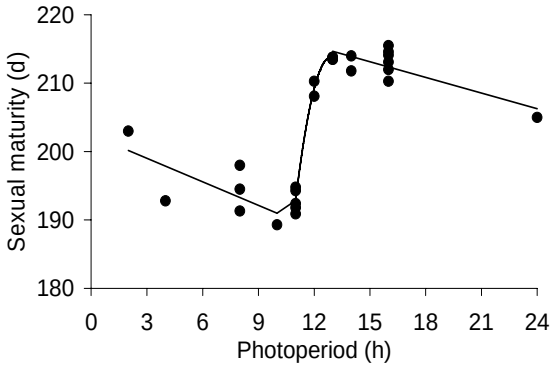

Figure 6. Mean age at sexual maturity for broiler breeders maintained on various constant photoperiods, adjusted by least squares to the response of birds reaching 2.1 kg body weight at 20 weeks (Lewis *et al*., 2004)

b) Changing photoperiods

Growth exerts its own effect on, but does not interact with, the reproductive response to constant photoperiod, but it does interact profoundly with a broiler breeder's response to an increase in daylength. One reason is that a bird cannot respond to an increase in light until it has fully dissipated juvenile photorefractoriness, and the rate at which this is achieved is dependent on the degree to which body weight is controlled. Another is that the degree of feed restriction necessary to control growth for satisfactory subsequent egg production is such that its influence is ten-fold that of the effect of the age at which a broiler breeder is photostimulated. However,

the age at which a broiler breeder is photostimulated still has a major influence on the timing of sexual maturity. When pullets are grown according to a typical primary company's body-weight

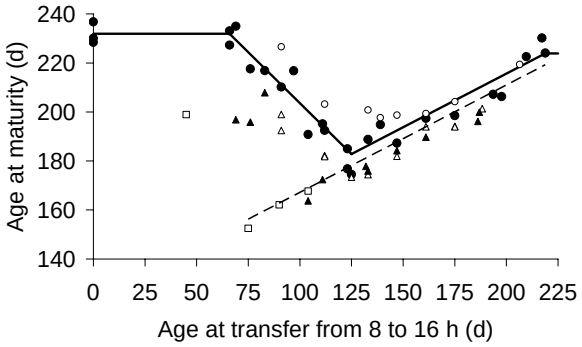

Figure 7. Mean age at sexual maturity for broiler breeders grown to achieve different body weights at 20 weeks and transferred from 8 to 16-h photoperiods at various ages. The solid line represents the mean response for pullets grown to achieve a typical 20-week body weight. The dotted line represents the common slope for all body-weight groups (unpublished from Univ. KwaZulu-Natal)

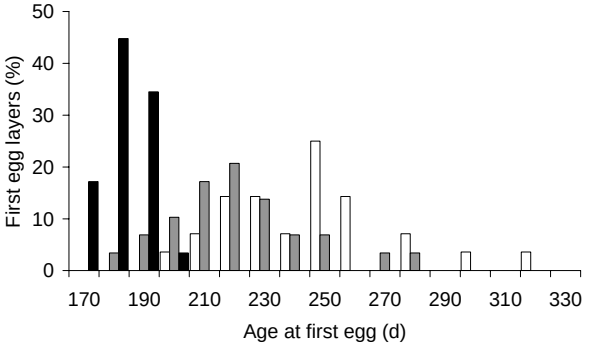

Figure 8. Percentage of broiler breeders, in 10-d classes, laying their first egg following a transfer from 8 to 16 h at 10 (white bars), at 14 weeks (grey bars) or 18 weeks (black bars) (unpublished from Univ. KwaZulu-Natal)

recommendation, the first birds are unlikely to respond to an increment in photoperiod until 10 weeks, and it will be at least 18 weeks before all the birds in a flock are photoresponsive (Figure 7). Thereafter, there is a progressive weakening of the response until, by about 30 weeks, all birds will have matured in response to the rearing photoperiod. Transfers to a stimulatory photoperiod before a pullet has dissipated photorefractoriness will delay and not advance AFE, because they mature as if reared on constant long days. Although the shape of the response profile is similar to that for egg-type pullets, events happen at different ages, and early stimulated pullets mature later and not earlier than late stimulated birds because of photorefractoriness. Between 10 and 18 weeks (for pullets grown to reach 2.1-2.2 kg at 20 weeks), there will be a bimodal distribution, as for egg-type pullets, but the consequences for broiler breeders are more severe. Figure 8 shows that photostimulation before 18 weeks results in a spread of more than 4 months in individual AFE. This will give poor peaks and a marked reduction in egg numbers to depletion age. However, unlike the response to a constant photoperiod, the age-related response to photostimulation varies with body weight. A relaxation of feed restriction facilitates a more rapid dissipation of juvenile photorefractoriness and allows the bird to respond to photostimulation at a younger age, and, as a consequence, mature earlier than normal growth broiler breeders (Figure 9).

The effect of photoperiod between 6 and 10 h during the rearing period has minimal practical effect on sexual maturity or egg production to 60 weeks when birds are photostimulated at 20 weeks, though birds reared on 8 h mature 3 to 4 d significantly earlier than those reared on 6 or 10 h (Lewis *et al*., in press).

The critical and saturation photoperiods for initiating gonadotrophin release in photosensitive normal and dwarf broiler breeders are similar, at 10.5 to 12.75 h, and 12.75 to 15.25 h respectively. This is reflected in the earliest age at sexual maturity being achieved by a transfer from 8 to 15 h in both types of stock (Figure 10).

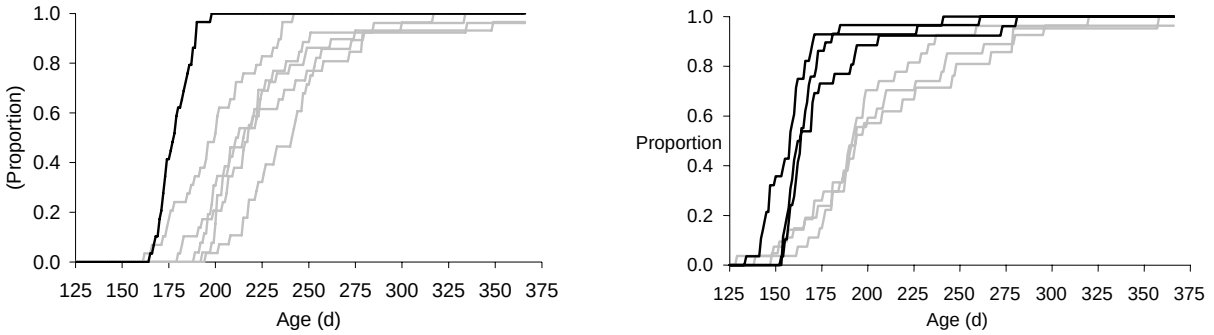

Figure 9. Effect of growing birds to 2.1 kg (left) or 2.8 kg (right) at 20 weeks on age at sexual maturity in broiler breeders transferred from 8 to 16 h at various ages between 10 and 18 weeks Black lines indicate an almost complete response and grey lines a partial or no response (unpublished data from University of KwaZulu-Natal).

A multiple regression of age at sexual maturity on body weight at 20 weeks, age and body weight at photostimulation, and final photoperiod for broiler breeders reared on 8-h photoperiods, and using data from University of KwaZulu-Natal, is described by the equation:

$$y = 549.2 - 1.9092PA + 0.00625PA^2 - 0.0445PB + 0.0000126PB^2 - 0.0342BW - 12.82p + 0.0397p^2$$

($r^2 = 0.938$, $P<0.001$, SD = 4.99)

where y = mean age at 50% lay or mean first egg (d), PA = age at photostimulation (d), PB = body weight at photostimulation (g), BW = body weight at 20 weeks (g), and p = final photoperiod (h). Using this model, Figure 11 shows that a 10% increase or decrease in body weight at 20 weeks not only alters the mean age at first maturity but also the photostimulation age that achieves the largest advance in maturity. Additionally, it shows that the optimal age for photostimulation advances linearly as growth is accelerated.

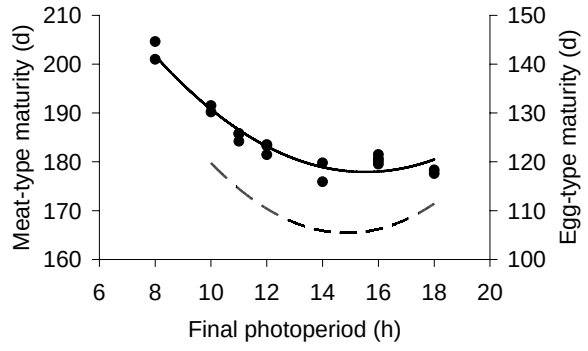

Figure 10. Sexual maturity in broiler breeder (●, solid line) and egg-type (broken line) pullets transferred from 8 h to various size photoperiods

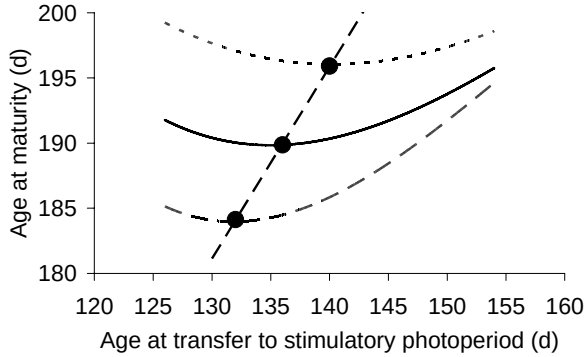

Figure 11. Effect of photostimulation age and 20-week body weight on sexual maturity (dotted line = 10% decrease, broken line = 10% increase from normal body weight at 20 weeks). The hatched line indicates the earliest age at maturity for a given body weight.

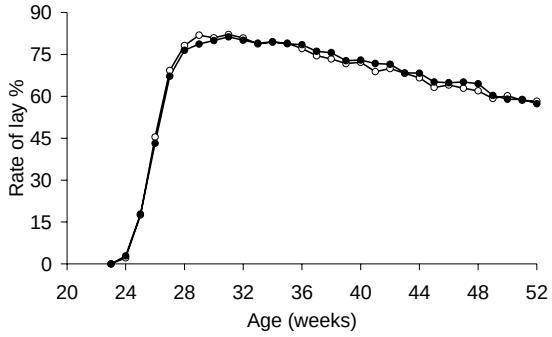

Figure 12. Rate of lay for broiler breeders transferred abruptly from 8 to 16 h at 19 weeks (○) or given an initial increase to 12 h then weekly increments of 1 h (●) (unpublished from University of KwaZulu-Natal)

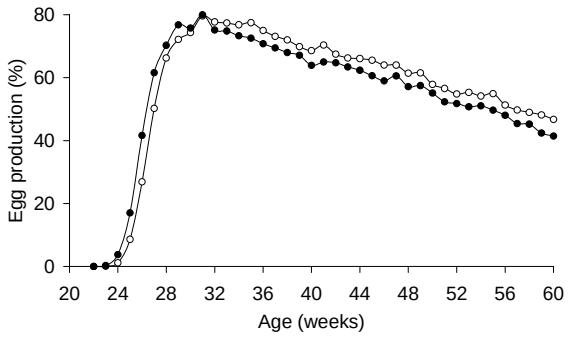

Figure 13. Rate of lay for broiler breeders transferred abruptly from 8 h to 11 (○) or 16 h (●) at 20 weeks (unpublished from University of KwaZulu-Natal)

Many producers are wary of giving an abrupt increase in photoperiod, and typically broiler breeding companies recommend a 3 to 4-h increment at about 20 weeks followed by weekly increases of 30 min to 1 h to reach a 15 or 16-h maximum. However, evidence from University of KwaZulu-Natal shows that there is minimal difference in rate of lay between an abrupt and a step-up lighting regimen (Figure 12). These findings agree with those reported by Morris *et al*. (1995) for egg-type hybrids transferred abruptly from 8 to 11 h or given a step-up programme from 18 weeks. Furthermore, evidence from three trials conducted at University of KwaZulu-Natal shows that there is nothing to be gained from increasing the photoperiod beyond 11 h, in terms of egg production, and that going to 16 h may result in inferior rates of lay after peak and fewer eggs to 60 weeks despite a 3 to 4-d earlier sexual maturity (Figure 12). However, there is a risk that hens on 11-h photoperiods could produce more floor eggs because a larger proportion of eggs will be laid before the lights come on (Lewis *et al*., 2004b)

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INCUBATION FOR UNIFORMITY

M.L.BOERJAN[1]

Summary

It is becoming increasingly apparent that for efficient broiler management the vitality and uniformity of day old chicks and poults at placement is crucial. The performance and growth of broilers to slaughter weight depend on egg quality and incubation conditions. Single-stage incubation can maximize hatchability and chick uniformity for each egg type. In this review the prerequisites for optimal single stage incubation will be discussed. Firstly, the three phases of embryonic development will be discussed: a phase of cell differentiation, growth and maturation. Secondly, tools to optimize single stage incubation for optimum chick vitality are presented. Tools are: a systematic analysis of egg shell temperature and the Pasgarscore, an objective score for chick vitality. Thirdly, the prerequisites for the design of single stage incubators for modern breeds will be discussed.

I. INTRODUCTION

It is becoming increasingly apparent that the vitality and uniformity of day old chicks and poults at placement is crucial for efficient broiler management. Generally, however, producers take vitality and uniformity of the received day old chicks for granted. The reason for this, on the one hand, is the limited exchange of information between the farmer and the hatchery manager about chick growth rates and performance data. On the other hand, it is only recently accepted that incubation related factors influence the performance and growth of broilers to slaughter weight (Decuypere *et al*., 2001; Tona *et al*., 2005).

The management of day old chick production has changed greatly during recent decades. First of all the size of hatcheries has changed such that a production of more than 2 million chicks per week is not an exception and secondly we notice a gradual transition from multistage to single stage incubation. Although the multistage incubation is still very common, the advantages of single stage incubation are being increasingly recognised. In the multi-stage incubator the climate among the eggs depends on the age of the different batches of embryos in the incubator and, therefore, fluctuates from day to day (French and Houlbrooke, 2004). Consequently, climate conditions in multi-stage incubators can not support optimal and uniform embryonic development. Single stage incubation offers the opportunity to adjust the incubation conditions to the requirements of the eggs and the embryos growing in them. Single-stage incubation can maximize hatchability and chick uniformity for each egg type. Another major advantage of single stage incubation is that after each incubation cycle the incubators can be cleaned and thereby minimize the risks of spreading microbial contamination.

To take the full advantage of single stage incubation three conditions have to be met. Firstly, knowledge must be available of those conditions needed to support embryonic development of modern breeds most optimal. Secondly, the hatchery manager must have the tools to find the appropriate incubation set points for temperature, humidity and ventilation and, thirdly, incubators must be designed to provide a homogeneous climate among all the eggs in each section of the incubator. In this review each of the prerequisites for optimal single stage incubation will be discussed as a management instrument for the incubation of uniform chicks of high vitality.

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II. THE DIFFERENT PHASES OF EMBRYONIC DEVELOPMENT

The rate of development and the vitality of the embryo depend on storage (Fasenko *et al*., 2002) and on maternal age (Applegate, 2002), while genetic selection influences important physiological systems (Christensen *et al*., 1995). The development of the avian embryo is a complex process that can roughly be divided into three phases: a phase of cell differentiation, growth and maturation. These phases are recognized through specific physiological details. From empirical data we learned that these different egg types need specific incubation protocols.

a) Embryonic differentiation

Embryonic differentiation is characterised by the formation of different tissues that will develop into the chicken's final organs in the growth phase. This first phase of cell differentiation starts in the hen, when the single-cell oocyte divides many times so that, in the un-incubated egg the embryo consists of about 30,000 cells. These 30,000 cells are organised as a plate of cells, known as the *early gastrula*, which floats on the top of the yolk.

After laying, the temperature of the egg decreases and the development of the embryo ceases or stops completely if the temperature falls below the physiological zero (25-27°C; Lundy, 1969). Embryonic differentiation continues only when the temperature of the egg rises. The differentiation phase is further characterised by a 'folding' of the early gastrula, to form a three dimensional structure in which premature organ structures of the head and heart can be recognised within 36 hours. Movement of cells mediates this folding process, whereby the cells in the early gastrula 'travel' from one side to the other, and this process is highly temperature dependent.

In the differentiation phase, it is not only the embryonic structures that develop, but also the extra-embryonic tissues - such as the amnion and chorio-allantois, both essential structures in the transport of oxygen and nutrients from the yolk to the embryo.

In this stage of development, the embryo floats to the top of the egg, where it is nearest to the eggshell, and normal, synchronised differentiation occurs only when the eggshell temperature is in the range of 37-38°C. If the temperature is in the range from 27°C to 36°C, uneven differentiation of the various tissues results and abnormal development occurs as a consequence (Romanoff, 1960). Embryonic differentiation is even less tolerant to temperatures above 38°C for longer periods, when exposed brains and eye abnormalities have been recorded. Interestingly, it has been shown that broiler embryos are even more sensitive to high temperatures during the differentiation phase than layer embryos (Decuypere and Michels, 1992).

b) Embryonic growth

During differentiation the premature organs are formed and the basic body pattern is laid down. Relatively minor changes in the size of the embryo are seen in this phase of development. Embryonic growth is characterized by an increase in mass while the development of the organs continues. The shape of the organs and, finally, the embryo is determined by the rate of growth at a specific time in the different parts of the embryonic body. Temperatures below the optimum incubation temperature of 37.5-37.8°C might result in disproportional growth: some embryonic cells and structures may grow while other do not grow. The result of a disproportional growth might be a malformed embryo (Romanoff, 1960). Temperature has a profound effect on the growth and the development of the left/right symmetry of skeletal parts and the lungs, as shown when broiler embryos were exposed to heat (39.6°C) and cold (36.9°C) for periods as short as six hours each day (Yalcin and Siegel, 2003).

The increase in mass during the growth phase is the result of a high metabolic activity and cell proliferation. The fuel for this activity is delivered by the nutrients from the egg and oxygen via the eggshell. The produced carbon dioxide and metabolic heat are by-products of embryonic metabolism.

Growth rates decline when the porosity of the eggshell becomes the limiting factor in the supply of sufficient oxygen. The growth rate and thereby the length of the incubation period depend mainly on temperature and is influenced by flock age and the length of the storage period (Lundy, 1969; Tona *et al.*, 2003).

c) Embryonic maturation

During the final phase of development the embryo undergoes a series of events that enable it to survive outside the protective environment of the shell. The rate of metabolism stabilizes and reaches the so-called plateau phase at about the 19th day of incubation in chicken and 25th day for turkey. At the plateau phase the growth rate declines because the embryo needs more oxygen than the porosity of eggshell can deliver. To be able to use yolk fat as energy source the availability of oxygen is essential. At the plateau phase the embryo suffers from anaerobic conditions. Therefore the embryo depends on carbohydrates, sugars, as energy source during the hatching period. The healthy and vital embryo is prepared for this condition because it has sufficient energy stores, in the form of glycogen, to survive the anaerobic conditions in the plateau phase. Vital tissues like heart and liver accumulate glycogen to ensure embryonic survival during the energy demanding process of maturation and hatching (Dietz *et al*., 1998). It has been suggested that lines selected for growth or egg production differ in glycogen metabolism and accumulation of glycogen stores during the maturation phase. These line specific differences might explain the different responses of the genetic lines on varying incubator climates.

A vital day old chick is an active chick that has the physiological potential to grow at the best rates with the lowest feed conversion ratios. Vitality is the result of optimum differentiation, growth and the maturation of all organs and physiological controlling circuits. The process of maturation starts shortly before hatching, the so called peri-natal period, and continues during the first week post-hatch. It has been shown that during this short timeframe, the emerging chick is equipped to cope, within certain limits, with the acute change in environmental conditions. In recent years, significant research has been undertaken on the development of the thermoregulatory system of the chick embryo and hatchling (Nichelmann and Tzschentke, 2002). This research has shown that with the development of the thermoregulatory system, the hatchling develops the capability to maintain its body temperature under changing environmental temperatures. Changes in incubation temperature at the end of embryonic development induce epigenetic adaptation, which results in a post-hatch long-lasting cold or heat adaptation. The maturation of the thermoregulatory system during the first week also includes the development of the regulation and response of heart rate baseline to changing environmental temperatures. By adapting the incubation environment throughout the last phases of incubation, and by adapting the newly hatched chick's environment during the first days post hatch, we can manage the growth development and temperature adaptation of the embryo - while it is still in the egg.

d) Metabolic heat production by modern poultry breeds

It is widely recognised that genetic improvements in poultry have resulted in an enormous diversification of breeds – all of which require specific incubation conditions (Decuypere *et al*., 2001). It is clear that embryo metabolism is changing as a result of selection for production traits. The rate of embryonic as well as post-natal growth (growth at

the farm) is determined by the rate of bio-synthesis of tissue which depends on the availability of nutrients and oxygen. A strong physiological relationship exists between the rate of bio-synthesis and metabolic heat production. In collaboration with the Humboldt of Berlin we showed that at day 18 metabolic heat production, based on oxygen consumption, is about 26% higher for Ross 308 compared to a white leghorn breed (Table 1).

Table 1. Metabolic heat production (W/1000 eggs) of a modern layer hen breed (Lohman white) and a broiler breed (Ross 308) compared to heat production (W/1000 eggs) (Janke *et al*., 2004) produced by the North Holland Blue breed (traditional) (Romijn and Lokhorst, 1960).

Days of incubation	Metabolic heat production W per 1000 eggs			
	Ross 308	Ross 508	White leghorn	Traditional
17	151.2	160.2	133.2	130
18	156.6	149.4	130.2	137
19	164.4	160.8	127.2	124
20	252	239.4	130.8	169

Metabolic heat production of the modern broiler breeds Ross 308 and the Ross 508 is about 20 % higher compared to the metabolic heat production by a North Holland blue breed a more traditional meat producing breed used in the sixties (Table 1). The higher metabolic heat production by modern broiler breeds as compared to a slower growing breed, is the result of the higher growth potential. With respect to this it is interesting to note that, at all time points measured, the Ross 508 embryo produced slightly less metabolic heat, although not significant. This might be unexpected since there is an idea that breast meat producing breeds like the Ross 508 produces more metabolic heat during incubation. From our results we conclude that a high heat production by the broiler embryo is determined by the high growth rate and not by carcass quality.

III. TOOLS FOR OPTIMIZING CHICK VITALITY AND UNIFORMITY

a) Tool: single stage incubation

Based on excellent scientific research, Lundy (1969) summarised the incubation conditions needed for optimum chick development. When he wrote his review in 1969, it was common to set eggs of several embryonic ages in one incubator: the so-called *multi-stage* incubation. In multi-stage incubators, the temperature, humidity and ventilation are set at a fixed point. The advantage of multi-stage incubation is its simplicity both with respect to the control system of the incubator as well as the management of incubation. The main disadvantage however, is that the multi-stage incubation environment cannot, by its nature, create optimum conditions for every egg set. For example, in a multistage incubator, the average eggshell temperature may vary from 37.5°C for the youngest embryos, to 39.5°C for the later embryonic stages; so it is difficult to find a temperature set point such that eggshell temperature is correct for each embryonic stage. Consequently, in multi-stage incubation, it is impossible to optimise both hatchability and chick quality, especially when dealing with variable egg quality. The single stage incubator is filled at one setting and, thus, contains eggs at one embryonic stage. To avoid overheating the set points of the different climate parameters have to be adjusted as embryonic development proceeds (Figure 1): for each day of incubation the set points are defined.

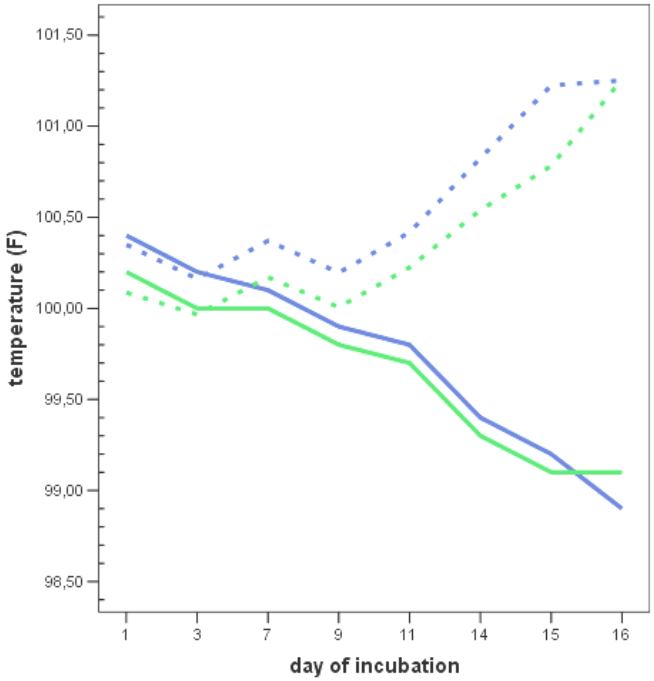

Figure 1. Two examples of temperature program for layer strains: the relation between temperature set points (lines) and average eggshell temperature (dotted lines) of at least 45 eggs is shown. Green lines: LOW incubation program; blue lines: HIGH incubation program.

For optimum chick and poult quality, fine-tuning of the incubation program might be necessary for each specific batch of eggs based on chick vitality and uniformity. These tools are respectively the Pasgarscore for objective scoring of chick vitality and a systematic analysis of egg shell temperature to find the optimum incubation temperatures.

b) Tool: Pasgarscore for chick vitality

Daily hatchery practice shows us that poor incubation conditions result low hatchabilities and poor chick vitality. For example, Pas Reform's studies, in collaboration with Wageningen University and Research centre, have shown that broiler chicks with red hocks develop significantly more leg problems at 30 to 40 days of age (De Jong *et al*., 2004).

The size of the residual yolk sac is dependent on incubation humidity and temperature, so it is clear that the vitality of an individual day old chick can be described using different aspects of the chicks' morphology. These morphological criteria have been used to develop the so-called Pasgar score (Boerjan, 2002) and, separately, researchers from the Catholic University of Leuven in Belgium (Tona *et al.*, 2003) have developed a more detailed score for chick vitality, known as the Leuven score. In both scoring systems, chicks lose points from a total of 10 (Pasgar score) or 100 (Leuven score) for abnormalities seen in navels, beaks, legs and yolk sac volume.

The Pasgar score has proven its worth in current hatchery practice. For example we applied the Pasgarscore on eggs from the same flock but stored for either 3 or 11 days before incubation. Of both lots 4.800 eggs (one trolley) were incubated in the same setter and hatcher. The Pasgarscore was determined on a sample of chicks of both groups. The mean Pasgarscore for the eggs stored for 3 days was 9.4 compared with a means value of 8.9 for eggs stored for 11 days ($P<0.05$) (Table 2). This result is in accordance with the anticipated chick quality after prolonged storage (Tona, 2003).

Because the Pasgarscore is easy to teach to hatchery personnel, is currently in widespread use to improve incubation programmes around the world. The Pasgarscore is intended to express chick quality using a number. To gain a representative quality score of a flock of chicks, a sample of at least 30 chicks must be assessed and the average Pasgar©score calculated.

Number of storage days	Mean Pasgarscore (n= number of chicks)
3	9.4 (27)*
11	8.9 (29)*

* $P<0.05$

c) Tool: Eggshell temperature as the leading parameter

We recognised the importance of temperature for optimum embryonic development and defined eggshell temperature as the leading parameter for the design of incubation programs (Boerjan, 2004). For optimum hatchability and chick quality we have found, and therefore advise that the average eggshell temperature follows a pattern within a range of 36.6-37.9°C during the first two-thirds of incubation and should not exceed 38.8 °C during the last days in the setter (Figure 1). It must be kept in mind that the larger eggs are less tolerant to higher incubation temperatures.

A way to improve the incubator's temperature program is to measure the temperature of a specific number of eggs during the different phases of the incubation process. Because embryo temperature can not be measured without destroying the egg, the temperature of the eggshell is used as a reference. The cheapest and easiest way to measure eggshell temperature is with an infrared 'fever thermometer'. The *Braun Thermoscan* is a practical instrument for such measurements, provided the instrument is used properly. To get a good idea of actual eggshell temperature in a specific incubator, a representative sample of at least 30 eggs must be measured.

IV. THE SINGLE STAGE INCUBATOR

a) Separate sections

For incubator manufacturers today, the challenge is to design incubators that can support optimum embryonic development for each egg at each stage of development. Single stage incubation requires incubators to be equipped with heating, cooling, ventilation, humidifier and turning mechanisms that are controlled accurately and independently. The uniformity and power of heat transfer from the incubator's temperature to the mass of eggs is a key aspect of incubator performance, because to achieve a uniform hatch, the eggs must be warmed rapidly and homogeneously. Homogenous temperature is best facilitated in an incubator divided into separate units, each with its own climate control.

b) Heating and cooling capacity

The heating capacity of the incubator must be sufficient to initiate embryonic development in every egg placed in one section of the incubator. Genetic selection has not only had an impact on production traits, but has also resulted in larger eggs with a decreased percentage of yolk volume. The greater volume of eggs produced by modern breeds means that a greater volume of eggs must be warmed and, therefore, the heating capacity of the modern incubator must be increased by 25% in comparison to the older, single stage incubators. In addition the preheating module facilitates a controlled and uniform preheating of eggs before setting. The cooling capacity of the incubator must also be sufficient to remove the heat produced by the older embryos, as the higher metabolic heat production of modern broiler embryos increases the risk of overheating.

In addition the temperature control system must be accurate so that unacceptably large deviations or fluctuation in temperature around the set point, or 'overshoots' are avoided. Again, the hatchery manager must have the facility to adjust the incubator temperature to keep eggshell temperatures at the desired level. In the design of the incubation program, the average eggshell temperature of a representative sample of eggs should be the leading parameter.

V. CONCLUSION

It is now understood that genetic improvements in poultry have resulted in an enormous diversification of breeds, all of which need specific incubation conditions. The embryo metabolism is changing through selection for production traits. For optimum cell differentiation and growth the embryo is dependent on a specific eggshell temperature. The importance of temperature for optimum embryonic development has been recognized and the eggshell temperature appeared to be a good reference. It is essential that the hatchery manager has the ability and facilities to control the set points of temperature, humidity and ventilation independently and as accurately as possible.

Single stage incubation facilitates optimum incubation programming, per batch and egg type. A single stage incubator should be facilitated in an incubator divided into small, separate units, each with its own climate control.

For optimum chick and poult quality, fine-tuning of the incubation program might be necessary. A tool for the objective scoring of chick vitality based on morphological criteria is the Pasgarscore. The Pasgarscore has proven its worth in current hatchery practice worldwide.

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IDENTIFYING BROILER BREEDER MANAGEMENT – NUTRITION INTERACTIONS TO OPTIMIZE CHICK PRODUCTION

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Summary

Broiler breeder hens grow more efficiently and are leaner than ever before due to positive results from broiler genetic selection strategies. Effective ovary management is an integral part of a successful breeder management program. Optimum ovarian morphology and reproductive efficiency is realized when the pre-maturational period is managed very conservatively in terms of increases in feed allocation. Feed allocation programs need to be based on a solid understanding of the reproductive physiology of these birds and defining factors influencing their growth and reproductive efficiency. By identifying 'reproductive attitudes' of individuals and their incidence in a population, more effective refinement of broiler breeder management strategies will be possible.

I. INTRODUCTION

Broiler breeders have a lot expected of them. This parent must have the genetics for rapid and efficient growth, and yet exhibit a high rate of egg production to supply the next generation of broiler chicks. While breeding programs have resulted in annual improvements in broiler growth, breast muscle yield, feed efficiency and disease resistance (McCarthy and Siegel, 1983) decades of selection for meat production traits have impaired the reproductive ability of broiler parents (Siegel and Dunnington, 1985). By the 1970's there were indications that growth selection negatively affected egg production traits. In their comparison of high and low juvenile body weight lines, Udale *et al*. (1972) showed that the high weight lines had increased rates of internal ovulation and defective egg production (36% versus 2% in high and low weight birds, respectively).

Broiler breeders are feed restricted from early in life to optimize reproductive performance. These birds have been demonstrated to be prone to multiple hierarchies – a situation that could be alleviated by feed restriction (Van Middelkoop, 1971). The use of feed restriction in modern broiler breeders has limited the expression of such negative reproductive responses to cases where birds have been overfed at a time when the ovary is especially sensitive to excess nutrient intake. These birds are changing in terms of their reaction to lighting programs as well (Joseph *et al*., 2002). Whereas specialized genetic selection has meant that egg production is not remarkably different from what it was a few years ago, hatching egg producers have had to work hard at fine tuning strain specific procedures for nutrient allocation and photoperiod management. As the modern broiler breeder continues to change due to the impact of genetic selection for improved growth efficiency and meat yield, there is value in understanding how our management priorities have changed along with the bird.

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II. MANAGING OVARIAN FOLLICULAR DYNAMICS

The ovary is the core of a successful broiler breeder. This is the point where the internal balance between growth and reproduction will interface with external management methods. In general, a hen with a well coordinated reproductive system will have an ideal state of physical maturity and number of large ovarian follicles (ovarian, yolky follicles greater than 10 mm diameter) at sexual maturity to support a strong, sustainable reproductive effort.

However, many external factors can affect egg production. Specific feed ingredients, bird age, and flock management decisions can directly affect semen quality, the oviduct environment, and the egg environment. Furthermore, even small degrees of over or under feeding have been shown to negatively impact egg and chick production (Katanbaf *et al*., 1989a; 1989b: Robinson *et al*., 1998a; 1998b). Something as simple as not adjusting feed allocation for temperature changes can affect nutrients available for reproduction and storage due to altered metabolic requirements. Understanding the ovarian function of the chicken and its interaction with nutritional status, age, and strain is essential to the effective production of fertile eggs with a high probability of hatching.

Both bird age and feeding level can influence how the ovary develops. Extra feed can highly stimulate large yellow follicle development, although this stimulation is greater at younger photostimulation ages (Renema *et al*., 2000). Broiler breeders are thought to be most responsive (or 'estrogenic') in the weeks immediately following photostimulation. During this period, the estrogen output by the ovarian follicles is increasing, and will not decrease to its mature baseline concentration until egg production is underway (Bacon *et al*., 1980). Allowing excess nutrients at this time may potentially be the most detrimental for normal ovary development. The most critical period for avoiding sudden increases or excessive feed allocation appears to be 2 to 4 wk after photostimulation. This period is a time of flux in the management of nutrients, as the bird switches from primarily growth to a reproductive state. The reproductive and metabolic hormone pathways do not appear to be mature enough to withstand the challenge of a sudden increase in nutrient intake.

The *ad libitum* feeding of broiler breeder females from photostimulation results in an 80 to 100% greater increase in Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) than in feed restricted birds by 3-d after photostimulation resulting in an accelerated sexual maturation process (Renema *et al*., 1999). This feed-driven accelerated sexual maturation process is typically also associated with elevated ovarian large yellow follicle numbers. The primary influence on how many large, yolky follicles form on the ovary is body weight. However, when you compare birds of the same size, the one consuming more feed will have more large follicles (Hocking, 1993).

A flock with poor ovarian control will have a normal peak production, but undergo more rapid decline in egg production. Unfortunately this may be indicative of either too few or too many large yellow follicles. Having too few large follicles can result in gaps in laying sequences and hence shorter than normal sequences. This is a problem seen in hens undergoing follicular atresia (follicle dissolution), photostimulated too early, or being underfed. Having too many large ovarian follicles is a problem associated with obesity or overfeeding (Yu *et al*., 1992). The hen can grow even more overweight as the rate of egg production remains low or goes into early decline due to excess feed intake.

III. BIRD VARIABILITY AND REPRODUCTIVE EFFICIENCY

If consumers could dictate how the poultry industry functioned, variation in growth, conformation and efficiency would not exist. The food industry is striving to achieve an increased level of product consistency and bird-to-bird variation is not wanted. However, this same variability is essential for the continued movement in genetic selection programs towards more desirable bird-types. Without natural variation, genetic progress would be stalled at the current bird-types. The 'ideal hen' with the perfect balance between growth and egg production traits, is more of an exception to the rule rather than the common occurrence in the broiler breeder barn.

Commercially, body weight, egg production, fertility and hatchability values are collected on a barn or farm basis. However, variation among individual hens affects how they do or do not respond to environmental or treatment conditions. For example, a high proportion of the unsettable eggs produced by a flock are from a small number of birds (Renema *et al*., 2001). Individual response to environmental cues can also vary. Proudman and Siopes (2002) reported no change, moderate sensitivity, or extreme sensitivity in turkey hens given a short-term reduction in light period. A range of variability in egg weight exists within individual hens. Egg weight-based differences in hatchability have been reported to be more closely related to deviations in egg weight from the individual hen mean egg weight than from the population mean egg weight (Wilson, 1991). University of Alberta egg weight data shows a 15 g range in breeder flock egg weight at a given age, while variation within individual hens ranges from 5 to 15 g (Renema, unpublished observations).

There are good reasons to work towards achieving a uniform flock – particularly at the end of the pullet phase, when a high proportion of the birds will ideally respond similarly to the photostimulatory cue. Whereas delaying photostimulation to allow the smaller pullets more time to become physically mature may appear counterproductive for the productivity of the larger birds, most hens will compensate with a greater rate of lay that will typically result in similar overall production (Robinson *et al*., 1996).

IV. EARLY PULLET GROWTH AFFECTS BODY WEIGHT UNIFORMITY

Early feed management practices are believed to have a long-term impact on frame size, fleshing, and body weight uniformity. Falling short of 4 week weight or protein intake targets is believed to adversely affect frame size and hen weight management. To test the impact of early feeding on frame size and fleshing, a study was performed comparing the effects of full feeding broiler breeder pullets until 1 or 3 weeks of age on frame size, fatness and fleshing at 4, 8, 12, and 16 weeks of age (Renema *et al*., 2005). The transition to feed restriction was smoothed by starting it already at 1 week of age. Ross 308 pullets (720) were placed at day of hatch (8 pens) and provided *ad libitum* access to feed for 1 (1WK) or 3 weeks (3WK) of age. Body weight was recorded twice/week to allow the growth profiles to be gradually converged (target of 8-10 weeks of age). At 4, 8, 12, and 16 weeks, external carcass and fleshing scores were recorded for all birds, and 14 birds/pens were dissected for assessment of muscle mass, fatness, and reproductive development.

Table 1. Body weight (g), shank length (mm), chest width (mm) and relative breast muscle weight (%) of broiler breeder pullets fed *ad libitum* for 1 (1WK) or 3 (3WK) of age and dissected at 4, 8, 12, or 16 wk of age.

	Body weight		Shank length		Chest width		Breast muscle	
Age	1WK	3WK	1WK	3WK	1WK	3WK	1WK	3WK
4 wk	451[b]	586[a]	61.5[b]	66.9[a]	34.5[b]	38.7[a]	12.7[b]	13.8[a]
8 wk	893[b]	963[a]	81.0[b]	84.1[a]	52.7	53.3	13.0[b]	13.6[a]
12 wk	1,318	1,248	98.0	98.4	55.1	56.5	15.9	15.9
16 wk	1,634	1,613	103.1	102.3	58.7	57.3	17.2	17.2

By 3 weeks of age, the daily gain of the 3WK pullets was double that of the 1WK pullets, which resulted in significantly more weight and fleshing at 4 weeks of age (Table 1). The 3WK birds weighed 30% more, had a larger shank and keel length, and carried a higher proportion of breast muscle (12.6% compared to 11.5%) than the 1WK birds. The groups still differed in some traits at 8 weeks of age, but were similar in comparisons after 10 weeks of age, when the body weight profiles met. The body weight uniformity of the 1WK birds was better than that of the 3WK birds from 14 weeks of age (CV of 13.0% compared to 16.7%). At 16 weeks of age, the frame size and fleshing of the birds were indistinguishable, and body weight uniformity was worse in the 3WK birds. In sister pullets from this study that continued on to sexual maturity, there were no differences in how these birds entered lay, or in carcass or reproductive traits at that time (Renema, unpublished observations). Maintaining very tight control body weight profiles through more frequent feed allocation decisions may contribute more to the effective management of broiler breeder productivity than simply following feeding guides.

V. INFLUENCE OF GROWTH HISTORY ON FEED UTILIZATITION

In many ways, the post-peak feeding period is not as critical as the pullet phase, or the sexual maturation and early production period. The condition of the reproductive system at the onset of production has long-term effects on the potential reproduction of the hen (Robinson *et al*., 1998a, 1998b). While the post-peak period (from approximately 32 to 60 weeks of age) is the most financially important, production problems at this time are generally the result of damage done earlier in the life of the breeder. For example, overfeeding of hens for as little as 2 weeks between 23 and 31 weeks of age has been found to reduce fertility and hatchability throughout production (Ingram and Wilson, 1987).

In a recent study, we grew broiler breeders on rapid or slow BW profiles between 5 and 12 weeks of age, and merged the BW targets by 32 weeks of age. By the time birds were dissected at 58 weeks of age there was a clear, long-term effect of pre-peak nutrient allocation on muscle, fat and the ovary (Table 2). The hens fed the early aggressive, HIGH profile carried a slightly increased proportion of breast muscle than those of most of the less aggressive treatments by sexual maturity (data not presented). At 58 weeks of age, it was striking how the HIGH profile hens still had more breast muscle, but also had a smaller ovary and less abdominal fat. These hens entering lay with extra fleshing maintained this extra muscle mass at the expense of the ovary. Birds of all treatments were maintaining very little fat. With reduced nutrient stores to rely on to cover shortfalls in nutrient requirements and the easy diversion of nutrients into fleshing, this shows how effective management continues to demand a higher degree of attention to detail.

Table 2. Breast muscle weight (g), abdominal fatpad weight (g) ovary weight (g) and total egg production (#) at 58 weeks of age in broiler breeders grown on growth profiles varying in target body weight between 5 and 32 weeks of age

Growth profile	Breast muscle	Abdominal fatpad	Ovary	Total eggs
High	451[b]	586[a]	61.5[b]	66.9[a]
Moderate	893[b]	963[a]	81.0[b]	84.1[a]
Standard	1,318	1,248	98.0	98.4
Low	1,634	1,613	103.1	102.3

It can be difficult to formulate diets to optimize egg production, fertility, and hatchability as little is known about the nutritional requirements of the embryo. Growth-selected stocks have low immuno-responsiveness (Siegel *et al*., 1984) due to either inadvertent negative selection pressure combined with growth efficiency selection. The developing embryo is especially sensitive to vitamin deficiency, which will result in death, malformation or some other atypical response (Leeson and Summers, 2001).

The importance of the macro-minerals and electrolytes for the maintenance of hen productivity is well established. This has meant that study of the carry-over of minerals from hen to chick for the enhancement of early growth and immunity is being done with the trace minerals (Kidd, 2003). Manganese, selenium, zinc, and vitamin E in the maternal diet have been identifies as important for the improved immunity of the progeny and ability of the embryo to survive incubation.

VI. LINKING GROWTH AND REPRODUCTIVE EFFICIENCY

Examination of individual growth and egg production profiles can reveal breeder hens that do not fit the classic balance between birds that lay well at the expense of growth, or grow very well while producing fewer eggs. Following a recent study, an individual 'snapshot' of production was compiled using feed intake, actual and target BW profiles, BW gains and losses, egg production, egg weight, and final carcass fat and protein concentrations. Each hen appears to have a different balance between the pull to lay eggs or to grow. However, some hens were present that were able to lay eggs very well and gain body weight relative to the flock average. Conversely, some birds on the same feed allocation grew very poorly and did not produce many eggs. To create an objective score of these various 'reproductive attitudes' scoring the balance between the pull to lay eggs or to grow, hens were also scored for overall efficiency (Figure 1).

Variation in feed utilization among hens exists that cannot be explained by metabolic body weight, body weight gain, and egg mass output (Van Eerden *et al*., 2004; Luiting and Urff, 1991a). This variation can be reflected in hen Residual Feed Intake (RFI). The RFI is the difference between observed and predicted feed intake and is a measure of feed efficiency that estimates the remaining part of the variation in feed consumption that cannot be accounted for by changes in growth, maintenance or egg production (Schulman *et al.,* 1994). Birds that consumed less feed than we calculated they needed had a negative residual feed intake. These are great birds because they are more efficient than we calculate they should be. Birds that consume more feed than we calculate they need for their activities end up with a positive residual feed intake – meaning that they are consuming more feed than we calculate they should. This remaining variation can be the result of differences in: 1) maintenance requirements, 2) partial efficiency in energy utilization, 3) energy demanding processes not accounted for, and 4) measuring errors (Luiting and Erff, 1991b).

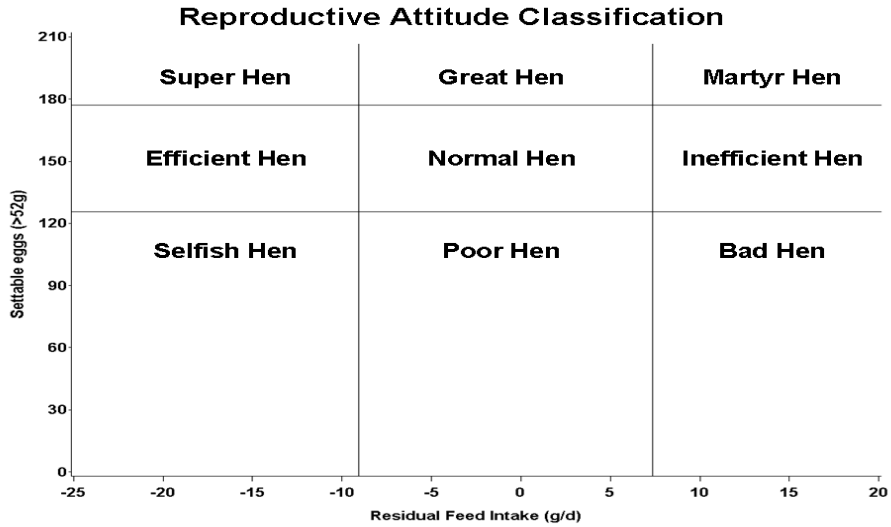

Figure 1. Template of 'reproductive attitude' classification. Hen growth and egg production efficiency was scored on residual feed intake and settable egg production.

If the reproductive attitude of the hen demonstrates good overall efficiency, will this carry forward to the broiler offspring? Defining the relationship between maternal efficiency and the quality of the broiler offspring is an essential step in providing support to future decisions on future breeder management methods and the provision of high quality broilers with desirable meat and growth traits.

VII. CAUSES OF VARIATION IN EFFICIENCY

One of the major sources of variation of RFI in laying hens is a difference in maintenance requirements among birds. Gabarrou *et al*. (1998) reported that a great part of variation in maintenance requirements in laying hens may be due to feeding activity, with less efficient hens demonstrating a higher regulatory thermogenesis resulting in dissipation of excess energy as heat. The liver, gut, and reproductive tract of broiler breeders represent 26 and 30% of the total energy expenditure in fed and fasted hens (Spratt *et al*., 1990). Differences in size and/or metabolic rate of these organs may have a considerably effect maintenance requirements. Interestingly, fasting increases liver and reproductive tract tissue metabolism in broiler breeders indicating the major role that liver plays in energy metabolism in fasten hens; and a compensatory response of magnum to an absence of dietary substrates for egg synthesis (Spratt *et al*., 1990).

Reports on causes of variation in RFI and heat production in laying hens were examined by Luiting (1990). Major sources of variation were attributed to physical activity, feathering density, basal metabolic rate, area of nude skin, body temperature, and body composition. However, further studies using divergent selection have shown a great importance of body composition and lipid metabolism to explain RFI variation Gabarrou *et al*., 1998). While basal metabolic rate was found to be similar in high and low RFI lines, differences in feeding activity and regulatory thermogenesis were found (Gabarrou *et al*., 1998). Variation in maintenance requirement may be attributed to differences in body composition. This can be affected by bird genetics, behavior and management, and may affect fat and protein deposition in body tissues, lipid metabolism, egg composition and the size and metabolic rate of liver, gut and reproductive tract.

Heritability of RFI has been estimated in laying hens from 0.30 to 0.60 (Hagger and Abplanalp, 1978; Schulman *et al*., 1994; Luiting and Erff, 1991b). Schulman *et al*. (1994) looked for the genetic correlations of RFI and economically important traits in laying hens,

and only found a genetic correlation with feed consumption. Interestingly, when broiler stocks are provided a choice of protein and energy compared to a single, complete diet, they do not maximize their growth (Siegel *et al*., 1997). Instead, they will grow more slowly with a reduced feed efficiency, and ultimately be more fat while also having an enhanced immune response. In the continuing push to grow broilers more efficiency, sight of what is 'normal' for the bird must not be lost in commercial stocks.

VIII. CONCLUSIONS

The newer broiler breeder genetic strains are becoming more specialized and appear to have more specific management methods associated with them. Effective ovary management is an integral part of a successful breeder management program. Managing the broiler breeder female for optimal chick production requires an understanding of reproductive physiology, nutrition, and their interaction. With new analytical and descriptive tools to apply to daily broiler breeder management, the modern manager will be able to cope with the increasingly specific needs of the modern heavy breeder. By identifying 'reproductive attitudes' of individuals and their incidence in a population, more effective refinement of broiler breeder management strategies will be possible.

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SELENIUM IN POULTRY NUTRITION: FROM IMPROVEMENT OF REPRODUCTIVE PERFORMANCE TO FUNCTIONAL FOOD

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Summary

Selenium (Se) plays an important role in avian reproduction. It is transferred from the feed to the egg and further to the developing embryo. As a part of various selenoproteins expressed in the chicken embryo, Se participates in regulation of various metabolic processes and is responsible for effective antioxidant defence. Our data indicate that organic selenium in the form of Se-enriched yeast is an effective means for increasing Se concentration in the egg yolk, albumin and eggshell. Chicks hatched from Se-enriched eggs were characterised by increased Se concentrations in their tissues and this increase was observed not only in newly hatched chicks but also persisted up to 4 weeks posthatch. Indeed, maternal diet was shown to affect chicks in postnatal development. Organic selenium in the form of Sel-Plex was successfully used to produce Se-enriched eggs in more than 25 countries worldwide. It was proven that consumption of such eggs could increase Se concentration in blood of volunteers. Therefore, Se-enriched eggs are considered as an effective delivery system to improve Se status of humans.

I. SELENIUM FOR BREEDERS

The most important opportunity to regulate antioxidant system of the newly hatched chick is by using organic selenium in breeder diets. Data presented by Paton *et al*. (2002) indicate that inclusion of sodium selenite in the maternal diet has a limited potential in increasing Se concentration in eggs. Indeed, there was no difference in Se content of the egg when sodium selenite was used at doses 0.1, 0.2 or 0.3 ppm. In contrast, when organic Se was used in the form of Sel-Plex a gradual increase in Se content in the egg (from 7.1 μg up to 11.2 μg/egg) was observed (Paton *et al*., 2002). Similarly, increasing organic selenium content in the diet of laying hens (from 0.1 up to 0.5 ppm) was associated with increased Se concentration in the egg yolk (by 2.6-fold) and albumen (by 5.2-fold; Pappas et al, 2005e; Table 1). Two groups of quail (3 families in each group consisting of 4 females and 1 male) were formed at the beginning of the reproductive period. They were fed a commercial maize-based diet containing 0.1 ppm feed-derived Se, supplemented with 0.2 ppm selenite Se (control group) or 0.5 ppm organic selenium (Sel-Plex^{TM}) for 6 months after which eggs were analysed and incubated in standard conditions (Karadas *et al*., 2004a). Selenium concentration in egg yolk and egg white significantly increased as a result of organic selenium supplementation. The highest increase (8.8-fold) was observed in egg albumin, while Se concentration in egg yolk increased only 2-fold. It seems likely that inclusion of fish oil in the parental diet can affect ability of the embryos to assimilate selenium. Indeed, dietary fish oil did not change Se content of the egg, however, Se concentration in the liver of the newly hatched chick increased by 42% in the liver and by 36% in the breast muscle. However, at high dietary Se supplementation, this effect of fish oil was not seen (Pappas *et al*., 2004ab). In general, due to organic Se supplementation (0.4 ppm) of the maternal diet the Se concentration in the liver and breast muscle of the newly hatched chicks increased by 3.6 and 4.1-fold respectively. Se enrichment of quail eggs was

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associated with a significant increase in the Se concentration in the liver, muscle and brain of the newly hatched quail (Karadas *et al*., 2004a). In comparison to the control birds the highest increase in selenium level was observed for the liver (3.5-fold) with a smaller (about 2-fold) increase in other tissues (Karadas *et al*., 2004ab). Our data indicate that selenium in maternal diet affects Se concentration in tissues of postnatal quail. Indeed, when newly hatched quail from Se-enriched eggs and normal quail eggs were placed on low Se-diet (0.1 ppm) the selenium concentration in tissues dropped dramatically for the first 2 weeks posthatch. This finding suggests that Se accumulated in the liver of newly hatched quail is actively used during the first days post-hatch. It is possible to suggest that Se absorption from the diet is not sufficient during the first few days of life and the chick must rely on reserves of the element accumulated during embryogenesis. However, the difference in selenium concentration between control and experimental groups was significant at 2 weeks posthatch. These results clearly indicated that the maternal diet affects not only newly hatched quails, but also chicks in postnatal development. When a similar experiment was conducted with chicken breeders, it was shown that the maternal effect of Se was also significant at 14 days posthatch (Pappas *et al*., 2005a). Indeed, pre-hatch maternal nutrition affected the progeny's Se status leading to significantly increased Se concentration in the chicken liver two weeks posthatch and potentially could affect antioxidant defences of the chickens.

Table 1. Effect of Se supplementation of the maternal diet on the Se concentration (ng/g) of hatching eggs and day-old chicks (Adapted from Pappas *et al*., 2005a)

Tissue	0.1 Se	0.1 Se + FO	0.5 Se	0.5 Se + FO
Yolk	183	194	505	496
Albumen	40	42	209	205
Breast	58	79	235	217
Liver	199	282	684	698

*/ The diet contained 0.1 or 0.5 ppm organic selenium and also supplemented with fish oil (FO)

Recently a new experiment was conducted at SAC to address this question (Pappas et al., 2005a). Maternal diet was supplemented with 0.4 ppm organic selenium in the form of Sel-Plex and comparison was made with a basic diet containing 0.1 ppm feed-derived selenium. As a result of dietary Se supplementation the Se concentration in the egg yolk, albumin, shell, shell membrane and perivitelline membrane was significantly increased (Karadas *et al*., 2005). The newly hatched chicks were placed on basal diet (0.1 ppm) without Se supplementation for the next 4 weeks posthatch. After hatching, chickens fed diets low in Se (0.1 ppm) but originating from parents fed diets high in Se (0.5 ppm) had, up to 4 weeks post-hatch, significantly higher blood Se levels than those originated from parents fed diets low in Se (0.1 ppm; Pappas *et al*., 2005b). Furthermore, our results indicate that maternal diet affected selenium concentration in the liver up to 3 weeks posthatch and in the breast muscle up to 4 weeks posthatch. GSH-Px activity in the liver and muscles was also elevated. Taking into account recent data presented by Koutsos *et al. (*2003) showing similar effect of carotenoids in the maternal diet on the carotenoid concentration in 4-week-old chickens, it is possible to suggest that Se and probably carotenoids could affect gene expression during the embryonic development. As a result, Se/carotenoid assimilation in postnatal development could be affected. Alternatively, antioxidant system could be affected and less Se/carotenoids being used for metabolic needs and higher concentrations of these compounds were observed in tissues. Indeed, this hypothesis needs further clarification, however, it is clear that maternal effect is seen beyond newly hatched chicks.

The benefit of organic selenium in breeder diets lies in its efficient absorption, transport and accumulation in egg and embryonic tissues. This results in improved antioxidant status of

the newly hatched chick. As the levels of major natural antioxidants (vitamin E and carotenoids) in chicken tissues progressively declined after hatch, the antioxidant enzymes become a critical arm of antioxidant defence. It would also appear that the embryo absorbs greater amounts of Se during days 10 to 15 of incubation than during other periods. Improving the transfer of Se from the hen's diet by using a Se-yeast instead of inorganic sodium selenite is a useful strategy to improve the nutritional status of the embryo as well as that of the newly hatched chick (Cantor *et al*., 2003).

II. SELENIUM REQUIREMENT FOR BREEDERS

The Se requirement of poultry in physiological conditions is thought to be quite low, varying from 0.06 ppm (laying hen) up to 0.2 ppm (turkey, duck; NRC, 1994). However in commercial conditions associated with various stresses the Se requirement increases substantially. Although there was no significant difference between 0.2 and 0.4 mg/kg Se supplementation of the maternal diet in GSH-Px activity in the liver of day old chicks in our experiment, 0.4 mg/kg Se gave more protection against peroxidation due to higher levels of vitamin E and glutathione in the liver of day old and 5 day-old chicks (Surai, 2000a). Indeed, 0.4 ppm Se supplementation in the breeder's diet significantly increased the Se concentration in all examined tissues. The age of the breeders had a significant effect on the accumulation of Se in the tissues. For example, at peak production when breeders where 27 wk old, those that were supplemented with NRC Se levels in their diet exhibited lower Se concentration in 3 out of 5 examined tissues compared to the levels that were noted when breeders were younger (Pappas *et al*., 2005c). Therefore, there was an indication of depletion over time for the breeder hens that were fed NRC recommended Se levels. On the other hand, hens fed the high Se diets (0.5 ppm total Se) were supplied with enough Se to maintain their requirements as well as build Se reserves in the tissues.

Since the process of Se transfer from feed to egg yolk, and subsequently to embryonic tissues, has received limited attention (Cantor, 1997; Paton et al., 2002), there is no clear answer as to which level of Se supplementation is optimal for broiler breeders. However, an analysis of published research and commercial data indicates that 0.3 ppm organic selenium in the form of Sel-Plex would be a recommended dose of Se dietary supplementation for breeders. For example, at 21 weeks of age Hubbard Ultra-Yield broiler breeders (11,600 pullets and 1530 roosters) were placed in each of two floor barns on two separate farms (Sefton and Edens, 2004a). They were reared on Selenite until placement and after that fed on Selenite or Sel-Plex (0.3 ppm) for 33 weeks of production. Sel-Plex supplementation was associated with improvement of fertility (by 0.4-4.5%), hatchability of fertile eggs (by 1-6%). As a result, the number of settable eggs were greater in Sel-Plex group showing an advantage of over 67,000 on one farm and over 8,500 on the second farm at 27 and 30 weeks of production respectively. An average 4.5 extra chicks per hen capitalised on Sel-Plex treatment was realised during the field trial. When data were combined for the whole production period (41 weeks on Farm 1 and 43 weeks on Farm 2) it was concluded that Sel-Plex in broiler breeder diets was very beneficial from both production and economic viewpoints (Edens and Sefton, 2003; Sefton and Edens, 2004b). Indeed, 5.63 extra chicks per hen housed were obtained as a result of Sel-Plex supplementation and it was calculated that the improved performance resulted in an increased potential revenue of +$1.17 per hen housed.

From photostimulation (22 wk) Ross 508 pullets were fed a Se-free laying ration (-Se), a standard Selenite-supplemented ration (0.3 ppm, Selenite) or Sel-Plex-supplemented (0.3 ppm) ration (Renema and Sefton, 2004). The egg production was similar, with 175, 173 and 178 eggs produced by 58 wk of age. However, the rate of lay was affected after 48 wk of age, when hen-housed production was 68% in Sel-Plex group and 60 and 61% in –Se and Selenite groups

respectively. In the Sel-Plex supplemented group settable egg production from 40 weeks (87.4) was higher than in –Se (80.6) or Selenite (83.7) birds. Prior to 34 wk, hatchability averaged 88% in Sel-Plex eggs compared to 80% in Selenite group and 77% in –Se group. Sel-Plex also reduced shell defects (Renema and Sefton, 2004; Renema. 2003).

Table 2. The effect of dietary selenium level and source on reproduction traits of broiler breeders (Adapted from Ranema, 2004)

	Embryo mortality and culls		Reproduction parameters			
	Day 1-14, %	Day 15-hatch, %	Fertility, %	Hatchability, %	Hatch of fertile, %	Chick production, No
No Se	5.33	3.66	86.9	77.9	88.6	131.3
Selenite	3.72	3.85	90.1	82.5	91.5	139.1
Sel-Plex	3.52	3.14	90.1	83.5	92.5	145.3

Two experiments with broiler breeders have been recently conducted in Brazil. In the first study, conducted at an integrated farm in the state Sao Paolo 42,000 Cobb breeders were divided in two groups and allocated in four farms (Rutz *et al*., 2003). The control group was fed on a corn-soybean meal basal diet containing 0.5 ppm Se as sodium selenite, while in the experimental diet sodium selenite was replaced by 0.3 ppm Se from Sel-Plex and birds were on these diets throughout the laying period. In the second trial, conducted in the state of Parana 15270 Cobb breeders were used. The control diet was supplemented with 0.2 ppm Se in the form of sodium selenite and in the experimental diet sodium selenite was replaced by 0.3 ppm selenium as Sel-Plex (Rutz *et al*., 2003). The results indicate that replacement of sodium selenite by Sel-Plex gave 1-2 extra chicks per hen housed. Similar results were reported by Renema (2004) showing increased egg production at 49-58 weeks and chick produced per hen housed (Table 2) as a result of replacement of 0.3 ppm sodium selenite by the same amount of Sel-Plex. Recently, it has been shown that embryonic mortality in eggs laid by 23 week old broiler breeders was higher in the first and last week of incubation and significantly reduced as the age of the flock increased. In the 27 week old breeders mortality in week 3 of egg incubation was 3.5%, and 10.6% in the soya oil and fish oils supplemented breeders (Pappas *et al*., 2005d). Inclusion of Sel-Plex (0.4 ppm) in the diets decreased mortality to 3.1 and 6.2% respectively.

Unfortunately there is no data available on Se requirement of egg-type breeders in commercial conditions. Indeed, higher rate of egg production and lower feed consumption in laying type of breeders indicate that their Se requirement could be similar or even higher than that in broiler breeders. It seems likely that 0.3 ppm Se in the form of Sel-Plex could also be effective in the egg-type breeder diet. However, more research and commercial trials are needed to address this question.

III. SELENIUM-ENRICHED EGGS

Before the advent of commercially available organic selenium for food animal diets, the main problem as regards the enrichment of eggs with selenium was the low efficiency of transfer of inorganic selenium (selenite or selenate) to the egg. In fact, even high doses of selenite in the diet of laying hens were not able to substantially enrich eggs with this trace element (for review see Surai, 2002).

Studies at the Scottish Agricultural College showed that egg selenium content can be easily increased when Sel-Plex is included in the diet at a level to provide 0.4 ppm Se (Surai, 2000abc, Table 3). In fact Se content in the egg was increased from 7.1 μg up to 30.7 μg as a

result of dietary supplementation with organic selenium. This finding is in agreement with data of Paton *et al. (*2002) indicating that whole egg Se is directly affected by the level of the organic selenium in the diet of the laying hen. As a result, the technology for production of eggs delivering ~50% of selenium RDA was developed and successfully tested (Surai *et al*., 2000a).

Table 3. Selenium in the egg (adapted from Surai, 2000b).

Organic Se added to the feed, ppm	Se in egg yolk, ng/g	Se in egg white, ng/g	Se per egg, μg	RDA from one egg
0	298.3	50.7	7.10	11.4
0.2	605.3	193.7	18.04	28.9
0.4	854.0	403.7	30.67	49.1
0.8	1087.3	621.7	43.35	69.4

Our investigation of the commercial characteristics of Se enriched eggs (Yaroshenko *et al*., 2003ab; Dvorska *et al*., 2003) indicate that:

a) Inclusion of increased levels of organic selenium or their combination with vitamin E for 12 weeks did not affect egg production, egg weight, ratio of yolk/white, feed consumption, FCR or body weight of laying hens
b) There was no significant difference in carotenoid and vitamin A levels in the egg yolk
c) Increasing diet Se increased vitamin E in the egg yolk and total Se in a single egg reached 35-40 μg, providing 55-73% RDA
d) During egg storage at 20°C for 7-14 days lipid peroxidation occurred in the egg yolk and MDA concentration significantly increased
e) Egg yolk enrichment with selenium was associated with a significant decrease in MDA accumulation, which was related to increased GSH-Px activity. When yolk was incubated at 37°C, lipid peroxidation was enhanced and the protective effect of increased diet Se was significant
f) These data clearly indicate that enrichment of eggs with Se and vitamin E is beneficial not only from the point of view of nutritional value of the eggs but also as an important technological solution to decrease lipid peroxidation in eggs during storage.

It seems that Se in eggs is highly available. For example, a recent clinical trial conducted in the Ukraine showed that consumption of two Se-enriched eggs per day for eight weeks significantly increased the Se level of the plasma of volunteers (Surai *et al*., 2004). In fact sixty volunteers (30 in control and 30 in experimental group) successfully finished the trial. Eggs consumed in the control group contained 7-9 μg Se/egg and experimental eggs were enriched with selenium (28-32 μg Se/egg). Blood was collected before the beginning and at the end of experimental period and Se was determined in plasma by hydride generation atomic absorption spectrometry with fluorometric detection. The level of selenium in plasma of volunteers living in the Kiev area of Ukraine (0.055-0.081 μg /ml) was on the low side of the physiological range and was somehow lower than we reported earlier in volunteers in Scotland (Surai *et al*., 2000). Consumption of commercially available eggs for eight weeks only slightly increased Se in plasma, which reached physiological level (0.075-0.085 μg/ml). In contrast, consumption of two Se-enriched eggs daily, which together delivered the daily requirement of 55-65 μg Se, for eight weeks was associated with a significant increase in Se concentration in plasma. Plasma Se reached 0.09- 0.14 μg/ml, indicating improving Se status of volunteers (Surai *et al*., 2004). This is the first clinical trial to prove that selenium-enriched eggs could be used as an important

vector to improve selenium status in countries with low Se consumption like Scotland or Ukraine. Se availability from eggs for human needs further elucidation and effect of different dietary sources of Se on its concentration in plasma probably depends on the Se status of the human. For example, in our previous clinical study in Scotland a consumption of one Se-enriched egg per day for 8 weeks did not change Se concentration in blood which was in a physiological range (Surai *et al*., 2000). In contrast, in the case of low selenium status, the consumption of eggs enriched with Se during 3 months significantly increased serum and hair Se level (Yu *et al*, 1996). Similarly, in Chinese children from the area endemic for Keshan disease, Se content in hair increased due to consumption of Se-enriched eggs during 3 years (Yu *et al*, 1998).

After the successful clinical trial with Se-enriched eggs in Ukraine, the production of such eggs, enriched with Se and vitamin E, started commercially and now the eggs called "Basket of life" can be found in the supermarkets in Ukraine. This development is very important for this region. From the one hand Se deficiency was documented in people working in Chernobyl area (Tutelian *et al*., 2002; Golubkina *et al*., 2002). On the other hand selenium and other antioxidants can be especially beneficial for people living in radionuclide-contaminated areas of the Ukraine. Currently various companies all over the world market Se-enriched eggs including Mega-Eggs in Ireland, NutriPlus eggs in Malaysia, as well as selenium enriched eggs in Russia, Thailand, Australia and the US. Prices for those eggs varied from country to country and are similar to those for free-range eggs.

In the UK the only designer egg available through the major supermarkets is the 'Columbus' egg produced by the Belgium company Belovo. These eggs, enriched in n-3 fatty acids and vitamin E, first appeared in Belgium in 1997, and since then they have been sold in the UK (1998), Netherlands (1999) and India, Japan and South Africa (2000). Currently production of the Columbus egg exceeds 50 million eggs per year in Europe. These eggs are characterised by a balanced nutritional lipid composition (C18, n-6:n-3=1:1) and a favourable structural lipid ratio (long-chain PUFA, n-6:n-3 = 1:3). When fed to selected groups of people, Columbus eggs have been shown to improve the circulating cell membrane fatty acid composition by favourably altering the n-6:n-3 ratio (De Meester *et al*., 1998). Recently, Columbus eggs were also enriched with selenium, delivering with a single egg more than 50% RDA (35 μg) of this trace element as well as 10 mg vitamin E (66% RDA) and 75 μg iodine.

Since these types of designer eggs find their way to supermarket shelves in other countries the selenium status in those countries can also be improved. This could be a result of successful knowledge transfer from developer and producer of the feed additive (Alltech, Inc., USA) via scientific evaluation and development of the effective technology of designer egg production (Surai, 2002) to the egg producer and food retailers to consumers. To satisfy consumer demand in the UK, free range Columbus eggs enriched with n-3 PUFAs, vitamin E and selenium are also on the supermarket shelves.

It is important to mention that in many countries there are several different competitive companies producing Se-enriched eggs. For example in Ukraine Kievskaya Poultry farm produces 1.2 million Se-enriched eggs per day, while in Russia Seimovskaya poultry farm (Nizni Novgorod region) produces about 65,000 Se-enriched eggs per day, Magnitogorskaya (Magnitogorsk city), Aksaiskaya (Rostov region), Voktinskaya (Izhevsk region) and Volzhaninskaya (Yaroslavl region) poultry farms produce 40,000; 30,000; 15, 000 and 10,000 Se-enriched eggs daily. In Belarus, Soligorskaya (Minsk region) poultry farm produces about 40,000 Se-enriched eggs daily. Se concentration in those eggs in Russia, Ukraiune and Belarus varies in a range of 20-30 μg/egg. Similar range of Se in Se-enriched eggs can be found in other countries with a target to achieve a 50% RDA in Se with a single egg. There is also a possibility to produce eggs with much higher Se content. For example, Combs (2000) reported that using

selenium in the form of high-quality Se-enriched yeast in the chicken diet at the level 1.2 ppm it is possible to achieve Se content in the egg up to 200 μg.

If egg selenium content is enhanced along with increased levels of omega-3 PUFAs and vitamin E (Columbus) or vitamin E and specific carotenoids (Super eggs, Surai, 2001) in levels comparable with RDA, this could further widen opportunities for producers and consumers to meet specific nutrient demands that aid in maintaining a healthy life style. There is also an opportunity to produce Se-enriched quail eggs which can find their way into niche market.

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THE IMPACT OF SHELL EGG PROCESSING ON FOOD SAFETY

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Summary

The microbial quality of shell eggs is affected by many factors such as: hen health, production environment, nutrition, storage conditions, processing conditions, processing facilities, etc. Most of the regulations and guidelines utilised for the processing of shell eggs in the U.S. are based on research conducted before the early 1970s. This research focused on egg quality more than food safety. There has been an absence of current research examining the role of processing technologies, procedures and equipment on product safety and quality. For this reason, our laboratories began to examine individual aspects of shell egg processing to determine what, if any, changes should be made in order to enhance shell egg food safety in the U.S. We started with a regional survey of the effectiveness of sanitation practices utilised by shell egg processors in the southeastern U.S. We then progressed to examining the changes in microbial quality of shell eggs during prolonged refrigerated storage. Most recently, we have monitored the changes in microbial quality of the shell egg as it progressed through the processing line. The results of the studies have shown that there is room for improvement, but the microbial quality of the eggs produced is high.

I. INTRODUCTION

The microbial quality of shell eggs is affected by many factors such as: hen health, production environment, nutrition, storage conditions, processing conditions, processing facilities, etc. In the United States, the washing of shell eggs for retail sale is a requirement for all product marketed under the United States Department of Agriculture (USDA) grade shield (USDA, 2005). These guidelines state that wash water temperature must be at least 32^0C or 11^0C warmer than the warmest egg. Wash water pH must be maintained at pH 10 or greater. Furthermore, a post-wash sanitising rinse of 100-200 ppm chlorine or its equivalent must be applied. All shell eggs packaged in containers ultimately destined for consumers are required to be maintained (during storage and shipping) at 7^0C (USDA, 1999). Most of the previously mentioned regulations and guidelines are based on research conducted before the early 1970s and this research focused on egg quality more than food safety.

Currently, two federal agencies in the U.S. are drafting and publishing proposed rules focused on ensuring the microbial safety of shell eggs and egg products. There has been an absence of current research examining the role of processing technologies, procedures and equipment on product safety and quality. For this reason, our laboratories began to examine individual aspects of shell egg processing to determine what, if any, changes should be made in order to enhance shell egg food safety in the U.S. We started with a regional survey of the effectiveness of sanitation practices utilised by shell egg processors in the southeastern U.S. We then progressed to examining the changes in microbial quality of shell eggs during prolonged refrigerated storage. Most recently, we have monitored the changes in microbial quality of the shell egg as it progressed through the processing line. An overview of each of these studies will be presented in this talk.

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II. SHELL EGG PROCESSING FACILITY SANITATION

The cleanliness of a processing facility has often been associated with the cleanliness of the final product. Moats (1981) stated that bacterial counts on the surface of washed eggs correlated with counts on equipment surfaces and in wash water. In addition, the major source of contamination for the wash water was found to be the eggs, not the equipment. Bartlett *et al*. (1993) found that merely maintaining wash water at the recommended temperatures and pH was not enough to keep bacterial levels on the equipment $<10^5$ CFU/ml. Unfortunately, many processors feel that if they meet the minimum wash water guidelines during processing, the need for thorough post-processing cleaning is negated.

The principle contamination sources in a processing facility have been identified as: direct and indirect contact surfaces, water, air and personnel (Slade, 2002). Drains, solid waste handling, transportation equipment within a plant, and maintenance personnel and equipment have also been identified as possible sources of contamination (Shapton and Shapton, 1991; Evancho *et al*., 2001; Kornacki and Johnson, 2001; Carsberg, 2003; Davies and Breslin, 2003) Cleaning processes may not be as effective due to lack of employee knowledge. Plant personnel frequently do not read the labels of the detergents utilised during cleaning (Powitz, 2002). This can contribute to ineffective cleaning. Furthermore, employees may not be aware what effect their actions can have on product quality or plant sanitation.

In 2002, our laboratory coordinated, in conjunction with research personnel from Auburn University, the University of Georgia and North Carolina State University, a survey to determine the effectiveness of sanitation practices in nine shell egg processing plants throughout the southeastern U.S. A summary of the results are presented in Tables 1 and 2. Plants were sampled immediately after processing ended (POST) and again just before the start of the following processing day (PRE). Table 1 shows the results from the direct contact surface portion of the study (Jones *et al*., 2003). Non-contact surface results are presented in Table 2 (Musgrove *et a*l., 2004). No significant differences were found between POST and PRE operational sampling of the same locations. While conducting this study, investigators did not inquire about sanitation practices utilised in each facility so as not to influence the normal procedures utilised each day.

These results not only indicate the need for more effective plant sanitation practices, but several locations in the process have been identified as areas of important concern. The high levels of aerobic bacteria and *Enterobacteriaceae* found at the check detector are of particular concern, since all washed eggs must come in contact with the check detector. Currently, this portion of most processing lines is not able to be completely cleaned due to design limitations of the equipment. The same situation exists for the packer head brushes. All eggs must come into contact with a packer head brush before being placed into a carton. With new HACCP-based (Hazard Analysis and Critical Control Points) regulations impending from USDA Food Safety Inspection Service, sanitation issues need to be addressed for effective prerequisite programs to be in place. There is a great need for more focused work in this area to aid both regulatory groups and industry to make a successful transition to HACCP-based processing.

Table 1. Effect of sanitation practices on aerobic plate and Enterobacteriaceae counts for direct contact surfaces.

Sample Site	Post APC (log CFU/ml)	Pre APC (log CFU/ml)	Post VRBG (log CFU/ml)	Pre VRBG (log CFU/ml)
Farm Belt (n=5)	5.55 ± 0.45	5.02 ± 0.56	0.77 ± 0.44	0.72 ± 0.45
Guide bar for farm belt (n=5)	5.48 ± 0.79	4.42 ± 1.02	1.10 ± 0.51	0.75 ± 0.55
Vacuum loaders (n=7)	6.16 ± 039	4.79 ± 0.93	1.97 ± 0.55	1.05 ± 0.58
1st Washer brushes (n=9)	3.71 ± 0.75	3.11 ± 0.73	0.39 ± 0.25	0.43 ± 0.30
2nd Washer brushes (n=6)	2.98 ± 0.99	3.34 ± 0.76	0.37 ± 0.37	0.18 ± 0.18
Oiler flaps (n=2)	4.04 ± 0.65	4.64 ± 0.79	ND	0.60 ± 042
Spools (n=9)	2.96 ± 0.31	3.52 ± 0.41	0.29 ± 0.18	0.31 ± 0.19
Check detector (n=9)	4.03 ± 0.58	3.87 ± 0.39	1.48 ± 0.64	0.68 ± 0.27
Re-wash belt (n=9)	6.23 ± 0.57	5.84 ± 0.50	1.41 ± 0.75	1.66 ± 1.02
Guide bar for re-wash belt (n=3)	4.83 ± 0.69	5.64 ± 0.78	0.66 ± 0.37	1.57 ± 0.33
Packer head brush (n=9)	2.65 ± 0.34	3.33 ± 0.65	ND	0.58 ± 0.37
Packer head belt (n=9)	3.13 ± 0.66	3.56 ± 0.59	0.46 ± 0.30	0.41 ± 0.41

P>0.05 In table 1. VRBG = violet red bile glucose agar; utilised for the determination of *Enterobacteriaceae*
ND = none detected

Table 2. Effect of sanitation practices on aerobic plate and Enterobacteriaceae counts for non-contact surfaces.

Sample Site	Post APC (log CFU/ml)	Pre APC (log CFU/ml)	Post VRBG (log CFU/ml)	Pre VRBG (log CFU/ml)
Floor under farm belt	6.5 ± 0.6	6.2 ± 0.5	2.7 ± 0.6	1.8 ± 0.8
Floor under nest-run loader	5.9 ± 0.3	5.7 ± 0.6	2.7 ± 0.7	1.6 ± 0.5
Floor under washers	5.7 ± 0.9	4.8 ± 0.6	2.3 ± 0.5	1.2 ± 0.5
Floor under packer heads	5.2 ± 0.4	5.2 ± 0.8	2.7 ± 0.6	2.4 ± 0.6
Drain near washers	5.7 ± 0.9	5.2 ± 0.8	2.7 ± 0.6	2.4 ± 0.6
Wall near washers	3.8 ± 0.7	3.8 ± 0.7	0.4 ± 0.3	0.2 ± 0.2
Outside of inedible bin	4.8 ± 0.3	4.7 ± 0.2	1.3 ± 0.3	0.9 ± 0.3
Post-processing cooler floor	2.4 ± 0.2	2.7 ± 0.4	0.1 ± 0.1	0.1 ± 0.1
Nest-run cooler wall	4.0 ± 0.3	4.0 ± 0.6	ND	ND
Nest-run egg cart shelf	6.7 ± 0.3	6.7 ± 0.2	2.9 ± 0.6	2.4 ± 0.2
Nest-run cart wheel	6.1 ± 0.1	5.7 ± 0.2	2.1 ± 0.3	1.4 ± 0.4
Loading dock floor	4.9 ± 0.3	4.8 ± 0.3	1.0 ± 0.3	0.8 ± 0.3
Store delivery crate	2.7 ± 0.4	2.6 ± 0.6	0.2 ± 0.2	0.1 ± 0.1

P>0.05
ND = none detected

III. SHELL EGG MICROBIAL QUALITY DURING EXTENDED STORAGE

Shell eggs are a unique agricultural commodity because when they reach the consumer, they are still in the original packaging as when they left the hen. In historical egg research, it has been determined that many of the natural defences present in the egg degrade over time. Board (1966) summarised a collection of previous research and determined there was a 20 day lag between shell penetration and the contamination of the egg contents. Furthermore, it has been found that stored or aged eggs are more easily infected when inoculated (Elliott, 1954). In another study, the infection rate during inoculation was greater

for eggs with a moderate weight loss, which is common during egg storage (Kraft *et al*., 1958).

The washing of shell eggs for retail sale has not always been a common industry practice in the U.S. Currently, all eggs are washed before retail sale in the U.S., Canada, Japan, Sweden and a significant proportion are also washed in Australia. A previous report has stated that visibly clean eggs usually have fewer microorganisms on the surface than dirty (Board *et al*., 1964). Garibaldi and Bayne (1960) described a trend towards washing eggs because it took less time to wash them than sort clean from dirty. This study was undertaken to determine what effect current U.S. shell egg processing technologies had on the microbial quality of eggs during prolonged storage.

While the work of Jones and colleagues (2003) found a lack of efficacy in current egg processing facility sanitation practices on reducing the number of microorganisms present on direct egg contact surfaces. A recent study illustrated that the washed eggs were contaminated with microorganisms at lower frequency and with fewer cells than were found in unwashed eggs. Furthermore, no differences were found in aerobic bacteria, *Enterobacteriaceae* and pseudomonads cultured in the pooled contents of unwashed or washed eggs (Jones *et al*., 2004).

Eggs are a difficult product to accurately sample for microbial contamination, especially when attempting to evaluate the growth of organisms in specific segments of the egg (Mayes and Takeballi, 1983). Due to the nature of the egg, completely separating the components without crossover contamination is almost impossible. Furthermore, research (Mayes and Takeballi, 1983) has shown the multiplication of organisms in the contents can be slowed by the viscosity of the egg white, pH, lysozyme and conalbumin. Characteristics of the shell have also been implicated in limiting the ability of organisms to enter the egg contents. Garibaldi and Stokes (1958) reported a complete cessation of bacterial penetration *in vitro* when the shell and both membranes were present. Others have found a linear response between shell porosity and microbial infection of the contents (Kraft *et al.,* 1958). Orel (1959) demonstrated a greater resistance to microbial penetration and egg spoilage at room temperature when shell specific gravity was greater than 1.080. Some researchers have considered changes during the storage of eggs and found eggs of three different shell permeabilities had similar spoilage levels until 15 days of storage when the most permeable began to spoil at a greater rate (Fromm and Monroe, 1960).

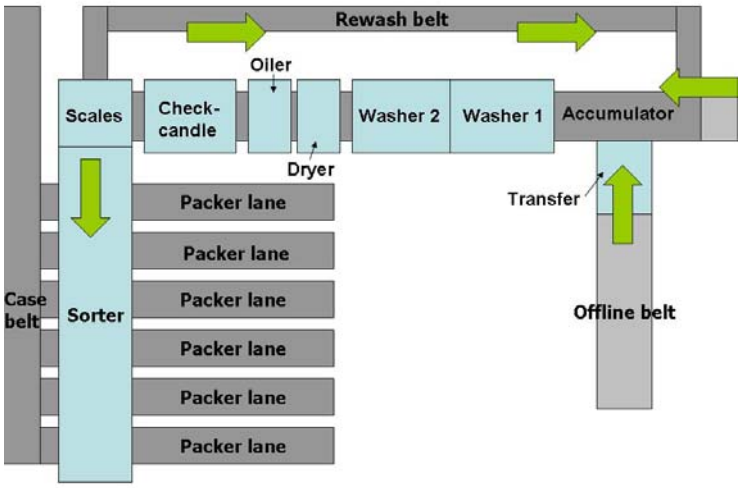
In a recent study conducted in our laboratory (Jones *et al*., 2004), we avoided some of these issues by utilising eggs from an in-line processing facility. Under these circumstances, eggs were always less than 24 hours old when processed. The birds from which the eggs were derived were in a multi-aged facility including young, old and post-moult flocks. Therefore, the eggs sampled represented the spectrum of physical and microbial characteristics the consumer would be exposed to in the retail market.

Additional researchers have found the shell membrane to lose its effectiveness as a microbial barrier as a challenge increased (Hartung and Stadelman, 1962). The yeast and mould results for the shell surface and egg contents at week eight of storage further support this finding. Miller and Crawford (1953) reported spoilage organisms to be virtually 100% absent from the contents of fresh eggs. In the current study, fewer than 10 CFU/ml of pseudomonads in pooled egg contents were detected in samples and only after three weeks of storage. Fluorescence, from *Pseudomonas fluorescens*, in the past had been utilised to determine when an egg had spoiled. Visible fluorescence in the egg is not detected until 5.0 log CFU/ml (Imai, 1976). Board and colleagues (1964) had difficulty finding fluorescent pseudomonads in shell eggs. In the current study, a single pool of eggs reached 4.2 log CFU/ml at nine weeks of storage.

Recent research has indicated that commercial sanitation operations did not significantly reduce aerobes and *Enterobacteriaceae* on the direct contact surfaces in shell egg processing facilities (Jones *et al*., 2003). However, the present study demonstrates that bacterial populations were greatly diminished by current processing techniques. This reduction in shell contamination is further continued throughout storage and in the contents of the eggs. The storage time in the current study was much greater than the 30 day sell by date and 19 days post-processing when most eggs are purchased (Bell *et al*., 2001; Patterson *et al*., 2001). Therefore, current federal guidelines for the production and processing of shell eggs appear to have a beneficial effect on the microbial quality of the eggs being produced, even during long-term storage.

IV. CHANGES IN MICROBIAL POPULATIONS DURING SHELL EGG PROCESSING

A further study (Musgrove *et al*., 2005) was conducted to determine microbial populations on the surface of shell eggs as they progress through the processing line. Samples were collected from three in-line egg processing facilities. Each plant was visited three times. A typical schematic of a U.S. shell egg processing facility is shown in Figure 1. The sampling sites are labeled on the figure.



Rinses from shells of eggs collected at the accumulator indicate that populations of yeasts and molds were not significantly different for any of the three plants. For all other populations analysed from eggs collected at the accumulator, eggs from the second plant were the least contaminated. For aerobic microorganisms and *E. coli*, eggs from the first and third plants were equivalent while eggs from the second plant were contaminated to a significantly lower level of *Enterobacteriaceae*. All the populations surveyed decreased throughout processing in every plant.

For aerobic microorganisms, yeasts and moulds, *Enterobacteriaceae*, and *E. coli*, greatest numbers of organisms were recovered from shell rinses of eggs collected at the accumulator or the re-wash belt. Pre-wash counts were higher than those obtained from eggs at most other sample collection sites (in-process and post-process). Stages of processing were grouped as pre-processing (accumulator, pre-wash, re-wash belts), in-processing (washers, sanitiser rinse, dryer, oiler), or post-processing (scales, packer lanes). From the pre-processing to post-processing stages, average prevalence of aerobic mesophilic

microorganisms, yeasts and moulds, *Enterobacteriaceae*, and *E. coli* decreased from 100 to 80.6%, 80 to 62%, 60 to 10% and 35 to 2%, respectively.

There were some differences in microbial levels recovered from egg shells collected at different plants on different visits (replications). Each plant was visited within two weeks of each other in sequential fashion to prevent a seasonal bias. Prior to processing, aerobic microorganisms, *E. coli*, and yeasts and moulds were determined to be less than a log CFU/ml rinse different among the plants. Despite differences in plant age, processing capacity, and water quality, all three plants were contaminated at similar levels for yeasts and moulds, *Enterobacteriaceae* and *E. coli* at the end of processing.

Despite plant differences, the way shell eggs were washed, graded, and sorted was similar. Regardless of plant or microbial population, highest bacterial and fungal counts were observed at the accumulator or the re-wash belts. Eggs at these points along the processing chain are visibly dirty or unwashed eggs. In fact, wash water at one plant was harsh enough that all populations were decreased by greater than a log CFU/ml at the pre-wash rinse. The second plant achieved the same result for all populations except for aerobic microorganisms, which were reduced in washer 1. The third plant achieved a log reduction in washer 1 for the four directly plated populations only after eggs reached the first washer.

Data for each population were averaged for the three plants and separated by sample site. Aerobes reached the lowest levels by the dryer while *Enterobacteriaceae* and *E. coli* were reduced to the lowest levels by washer 1. Yeasts and moulds were reduced at pre-wash rinse but increased again at oiling. Oiling follows drying, accomplished by forcing warm air over the eggs as they emerge from the sanitiser rinse. A survey of air quality in shell egg processing plants indicated poorest yeast/mould air quality near the dryers and washers (Northcutt *et al*., 2004). De Reu *et al.* (2003) compared aerobic shell populations on eggs collected from production through retail from cage and organic production systems. Their results indicated that air quality affected shell counts regardless of production system. However, by the end of the processing chain, all microbial populations determined in our study were significantly reduced compared to pre-processing levels.

Sanitising rinse application is just one of the hurdles designed to diminish microbial egg shell contaminants. In a 1979 study, Moats (1979) visited commercial facilities in Maryland and Pennsylvania that used different combinations of washing compounds and sanitising or water rinses. Microbial populations on shell eggs in plants using sanitiser rinses were very low (<50 cells or CFU's /shell), and significantly lower than one plant using an unsupplemented water rinse. However, when sanitiser rinse was temporarily cut off in plants that employed this type of rinse, populations on the shell did not change. Moats (1979) concluded that a lack of significant change in egg shell bacterial numbers indicated that sanitiser rinse was at most an indirect effect. In a separate study, Moats (1980) obtained population data from equipment surfaces, wash water, and eggs. Based on correlations, he concluded that the sanitiser rinse was of no use. Our data offers no sound argument against his conclusion. United States Department of Agriculture (2005), Agricultural Marketing Service American Microbioloical Societyguidelines specify that sanitiser rinses must be compatible with detergents and of a strength equivalent to100-200 ppm chlorine. Chlorine compounds perform optimally between pH 6.5-7.5 (Curtis and Johnston, 1998), much lower than that measured for wash water in this study. Other compounds have been analysed to replace chlorine but none has been as effective (Kuo *et al*., 1997).

Prevalence data for individual plants and an average of the three plants were organised by stage of processing. Once eggs were introduced into the washer, microbial populations were reduced and biologically relevant increases were not observed through the remainder of the processing chain. Sanitation affects microbial populations during shell egg processing (Moats, 1981). Certain sections of the equipment are not water proof (scales), are

difficult to reach (re-wash belt), or are difficult to remove and clean regularly (packer head brushes). However, contact with these surfaces did not result in significant increases in counts.

These data indicate that commercial egg processing significantly reduced levels of aerobic, yeasts and molds, *Enterobacteriaceae* and *E. coli* populations recovered by shell egg rinses. Populations decrease once eggs reach the first washer and remained low through packaging. Therefore, while current sanitation practices utilised by shell egg processors in the U.S. could be more effective, the product has a high microbial quality post-processing which remains during extended storage.

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PRELIMINARY RESULTS OF *IN OVO* STRATEGIES TO INCREASE BREAST MEAT YIELD IN BROILER CHICKENS

S.J. WILKINSON[1], and T.A. SCOTT[1]

Summary

Emerging strategies to enhance and support high-value breast meat development of broiler chickens include *in ovo* injections to supply nutrients to the developing embryo as well as early nutrition. In order to minimise losses associated with hypertrophy of muscle cell fibres, *in ovo* feeding may provide an opportunity to stimulate muscle fibre numbers and supportive vascularisation of these tissues; thereby, avoiding myopathy of large muscle fibre size. This paper provides a summary of the preliminary work conducted to determine effective *in ovo* strategies to increase breast meat development of young broiler chickens. The results of this preliminary work suggest that *in ovo* strategies may be useful in increasing chick body weight as measured at day-0 and day-7 post hatch. Further work is required to determine more suitable compounds for use *in ovo* as well as their effects on body weight.

I. INTRODUCTION

Breast meat yield of broiler chickens accounts for approximately 60% of the value of the bird at processing. Typically, breast meat yield as a proportion of total body weight varies from 17 – 25% (Havenstein *et al.,* 2003), thereby significantly affecting the profitability of chicken meat production. Muscle size and mass is determined by factors such as genetics, nutrition, health as well as environmental conditions. Physiologically, chickens develop muscle fibres (hyperplasia) during embryo development, and for approximately 7 days post hatch, after which muscle fibre numbers do not alter (Halevy *et al.,* 2003). Growth of muscle after 7 days post hatch is by hypertrophy (muscle fibres increase in size). Excessive hypertrophy such as that found in fast growing strains of broilers may lead to the muscle exceeding the available blood supply resulting in deep pectoral myopathy (Maltby *et al*., 2004). Therefore it is preferable to increase muscle fibre number, and presumably supply of supportive vasculature, in the pursuit of greater breast meat yield. Additionally, work conducted in beef by Crouse *et al. (*1991) linked smaller muscle fibres to improved meat quality, quantified as tenderness. The objective of the work described in this paper is to investigate strategies to increase breast meat yield in broiler chickens by stimulating hyperplasia, focussing on delivering nutrients to the embryo *in ovo* to increase body weight at hatch.

II. MATERIALS AND METHODS

a) Egg incubation

A series of trials was conducted to determine the effect of *in ovo* injection of various substrates on body weight of chickens. For all experiments, eggs (Cobb) were obtained from a commercial hatchery (Baiada Pty Limited, Marsden Park, NSW) and transported to the Poultry Research Unit located at the University of Sydney, Camden. Eggs were weighed and randomly allocated to setting trays forming treatment groups. Eggs were placed in force draught research incubators (Aussieset 100, Bellsouth, Narre Warren, VIC) set at 37.2 ºC and 65% relative humidity (RH), turning 90^{o} automatically every 60 minutes. At day 7 of

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incubation, eggs containing infertile and early dead embryos were identified by candling and removed. Eggs were transferred to numbered hatching trays at day 18 of incubation and the temperature reduced to 35.3 ºC and 65% RH. At day 22 of incubation, hatched chicks were weighed and tagged. Unhatched eggs were removed and later classified for probable failure to hatch or produce viable chicks.

b) *In-ovo* injections

At day 16 of incubation, eggs were removed from the incubator for *in ovo* injection using a modified method from Noor *et al. (*1995). Treatment solutions were made to volume using physiological saline (0.9% NaCl). Eggs were sprayed with 70% ethanol and a hole punctured into the broad end of the egg using a dissection needle. The full length of a 32mm, 23-gauge needle was inserted to inject treatment solutions. A control group that received no injection and a sham group (0.9% NaCl) were also used. A further control group that had an empty needle inserted into the hole was also added to determine the effects of injection on hatchability and viability. Holes were sealed with melted paraffin wax and the eggs were returned to the incubator. Approximately thirty individual and combinations of, substrates and 3000 eggs were tested over the five experiments. Generally, a 1% solution was administered although in some instances, a high, medium and low concentration was administered to ascertain dose responses.

c) Analysis

Data were recorded and analysed for ANOVA using the GLM procedure of SAS 9.1. Differences between treatment groups were deemed significant if the P-value was ≤ 0.05.

III. RESULTS AND DISCUSSION

Day-0 (incubation) egg weights increased with paternal flock age. However, for each treatment group within each experiment there were no statistical differences. Table 1 lists the correlations observed over the five experiments for egg weight, egg length, day-0 (hatch) body weight and day-7 body weight. As expected, a high correlation between egg weight and body weight (day-0) was observed. The correlation between day-0 and day-7 body weight was low and as such, hatch body weight would not be a good indicator for market weight prediction.

Table 1. Correlations of egg weight, egg length and body weight (day 0 and 7).

	EW00D	EL00D	BW00D	BW07D
EW00D	1.00	0.69**	0.82**	0.11*
EL00D		1.00	0.65**	NS
BW00D			1.00	0.25**

EW00D – egg weight day-0; BW00D – body weight day-0; EL00D – egg length day-0; BW07D – body weight day-7

*, **, NS indicate levels of significance P<0.05, P<0.01, Not Significant, respectively.

The data in Figure 1 depict the treatment effects of *in ovo* administration of test substrates (proprietary information) on day-0 body weight, relative to the control group. The bars in Figure 2 illustrate the same information for day-7 body weight. As the two figures demonstrate, there are generally positive effects of *in ovo* injections on hatch and 7-d body weight. Eighteen treatment groups have been identified to increase body weight at day 0 relative to the control group (Figure 1). The majority of these treatment groups continued to outweigh the control group at day 7 (Figure 2) with 12 treatment groups representing gains in

body weight greater than 10% and 7 treatment groups gaining greater than 20% of the control group. Some treatment groups did not perform like this and resulted in negative values at day-7 compared to their day-0 status.

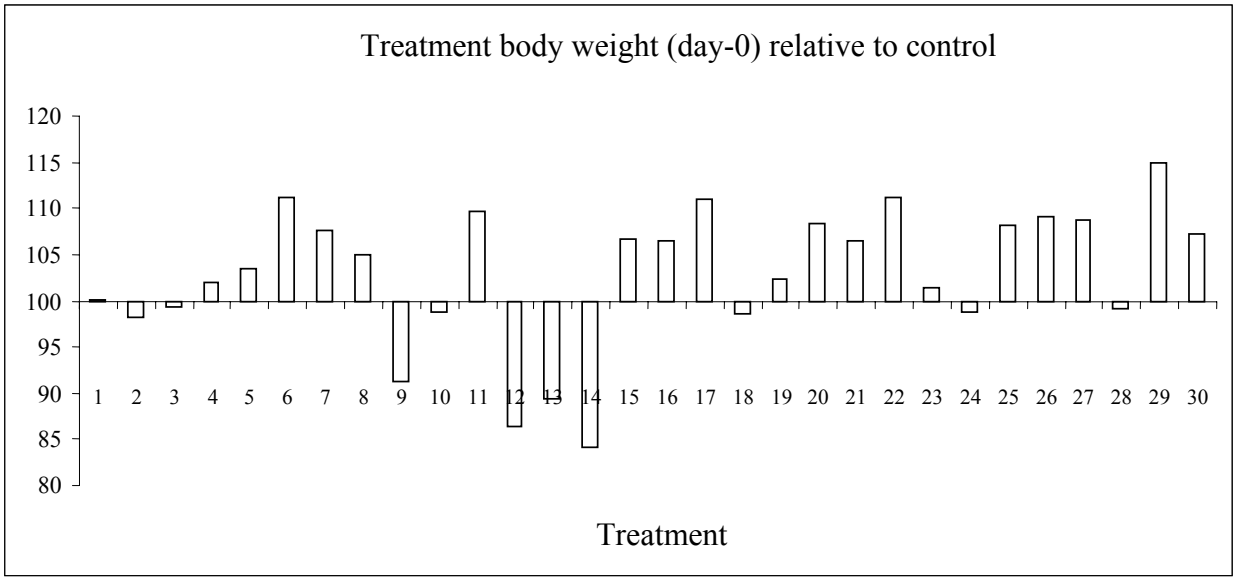

Figure 1. Effect of treatment group relative to control on day-0 body weight.

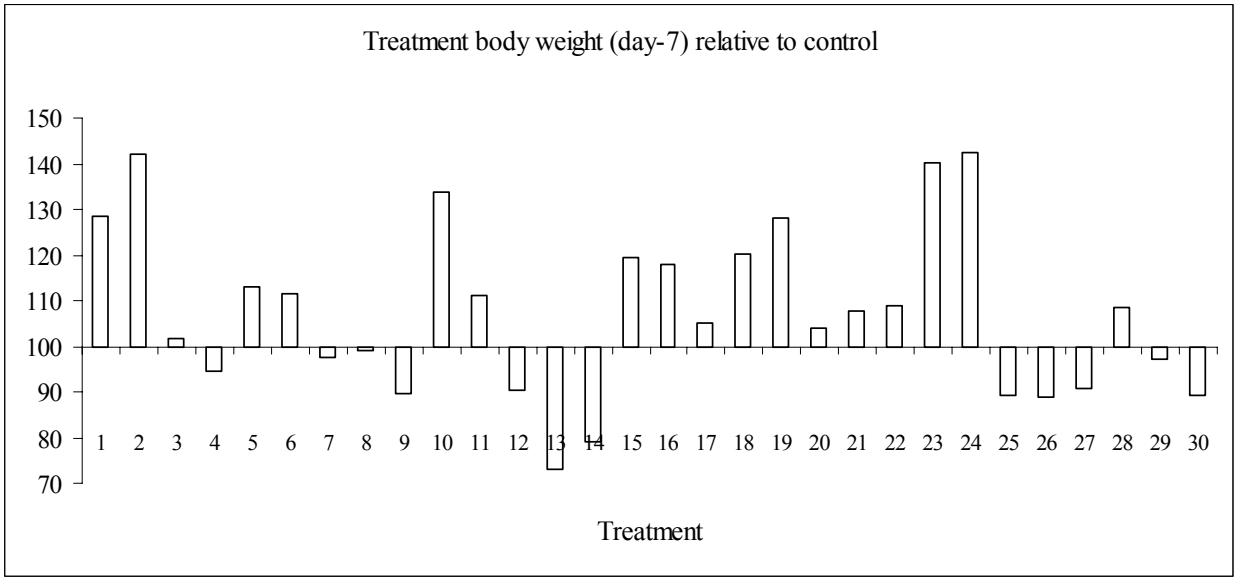

Figure 2. Effect of treatment group relative to control on day-7 body weight.

The results of this preliminary work concur with studies of previous authors who have reported varied results utilising different in-ovo strategies as tested here (Coles *et al*., 1999; Ipek *et al*., 2004). Some treatment groups (eg. 1, 2, 23 and 24) provided minor, if any, stimulus (Figure 1) *in ovo.* However, stimulus was provided during the post-hatch period of continued hyperplasia, as depicted in Figure 2.

Work conducted by Uni and Ferket, (2004) suggests that approximately 2-5% of chicks do not survive the adjustment period post hatch, whilst many that do display signs of stunted growth, inefficient feed utilisation, reduced disease resistance, or poor meat yield. Furthermore, these authors continued to suggest that many of these problems may be

overcome with *in ovo* feeding to the late term embryo as well as early nutrition post hatch. Further studies by Uni *et al*. (2005) reported positive effects of *in ovo* feeding to late term embryos that lasted through to 25 days post hatch. Further work is being conducted to elucidate beneficial substrates for use as *in ovo* feeding supplements and their effect on targeted tissue i.e. breast muscle and/or gastrointestinal tissue.

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DEVELOPMENT OF A COMPETITIVE EXCLUSION PRODUCT FOR POULTRY MEETING THE REGULATORY REQUIREMENTS IN THE EUROPEAN UNION

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Summary

Newly hatched broiler chickens of the modern poultry husbandry do not come into contact with the mother hens. This lack of contact is believed to result in a delayed development of the intestinal micro-flora, and as a consequence broilers at very young age are particularly susceptible to pathogen colonisation. To date, the animal production in the European Union faces a very difficult situation due to the ban of antibiotic growth promoters. Competitive exclusion (CE) treatment in animal nutrition may contribute to a successful replacement of in feed-antibiotics. In particular the CE concept is able to increase the colonisation resistance of day old chicks by stabilising the indigenous micro-flora against *Salmonella*, one of the main bacterial contaminants transmitted by poultry. Within the frame of an EU-funded CRAFT project, our work was aimed towards research and development of a safe microbial feed additive for chicken. The CE product, in contrast to existing products represents an effective combination of well-studied intestinal organisms which meet the regulatory requirements in the EU. For this purpose, a stepwise risk assessment of potential probiotic strains had to be established. Insights obtained in this study are presented and discussed in context of the main safety aspects.

I. INTRODUCTION

For many decades antibiotic growth promoters (AGPs) have been used in the feed for farm animals. Because of the general problem of increased resistance of bacteria and the decreasing acceptance of consumers for this type of additive, antibiotics have been banned step by step throughout the European Union. Due to the problems anticipated in association with the total ban of antibiotic growth promoters (e. g. performance losses in animal husbandry, food borne disease in humans, increased use of therapeutic antibiotics), the animal production in Europe now faces a critical situation. Especially the poultry industry deals with the problem of transfer of pathogens (*Salmonella, Campylobacter, E. coli*) from chickens to humans via the food chain.

Probiotics derived from the animals gut are of current interest because they offer alternatives which should find acceptance by both the producers and consumers. Within an EU-funded CRAFT project titled "Development of a competitive exclusion (CE) product for poultry meeting the regulatory requirements in the EU" a step-wise risk analysis was established to carefully investigate the presence of the main risk factors related to the use of micro-organisms as feed additive (e. g. spread of antibiotic resistances, virulence activity).

The project focussed on the development of a defined CE product, which consists of a series of beneficial bacteria that conform with the requirements for registration in the European Union. The CE product is intended to be applied to chicks during their first days of life offering protection from infections with pathogenic bacteria by stabilizing the natural gut micro-flora and by increasing their colonisation resistance against potential pathogens.

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II. MATERIAL AND METHODS

a) Isolation and characterization of intestinal bacteria

Isolation of strains was performed from fresh intestinal contents of healthy chickens of various ages. Two 14-day-old broilers (C1, C2) and two 28-day-old broilers (C3, C4) from a local commercial poultry farm and one adult, home-grown chicken of 12 weeks (C5) were the sources of the bacterial isolates. Standard cultivation techniques were performed for isolation of intestinal bacteria under aerobic, facultative anaerobic and strict anaerobic conditions.

Characterisation of bacterial isolates was conducted using a polyphasic approach combining morphological, physiological and genotypic methods. Protein patterns were compared by SDS-PAGE using a discontinuous Laemmli system (SE600 Amersham Biosciences). Isolates with more than 90 % similarity were clustered and their assignment to the same species was considered. Acid profiles were recorded for representative bacterial strains by HPLC analysis using a Hewlett Packard 1047 station.

16S rDNA sequence analysis was performed for 5 of the most relevant strains that were further deposited in an official culture collection (DSMZ). For determination of the sequence, purified amplification products were sequenced on a LI-COR 4000 L (LI-CORE Inc., NE., USA). 16S rRNA gene sequence similarity studies were carried out by using FASTA3.

b) Challenge feeding trial

Broiler chicks were challenged three days after hatching with 0.1 ml of a suspension of *Salmonella enteritidis* containing 1.0 x 10^6 CFU/mL. Birds were sacrificed in groups: group 1 two days after infection; group 2 four days after infection; group 3 seven days after infection. Colony counts of *Salmonella enteritidis* were determined in cecal contents of sacrificed birds.

III. RESULTS AND DISCUSSION

a) Strain collection and composition of the competitive exclusion product

Classification of bacterial isolates resulted in a well-defined culture collection of intestinal strains. Taxonomic grouping by comparison of whole cell protein patterns of isolates enabled the selection of 121 representative strains for further analyses in order to identify potential product strains. Differentiation among probiotic strains with regard to the type of fermentative metabolism revealed three major groups of lactic acid producers: 1) strict homofermentative LAB generating lactate as the sole metabolite, 2) strict heterofermentative LAB producing a mixture of ethanol, acetate and lactate and 3) heterofermentative bifidobacteria fermenting hexose through the "bifid shunt" producing acetate and lactate. The clusters of aerobically and facultative anaerobically isolated strains from the 14-day- and 28-day-old industrial broilers C2 and C4 were dominated by members of the *Enterobacteriaceae* representing a percentage of 56% and 75%, respectively. As shown in Figure 1(A), clusters of anaerobically isolated strains contained more than 92% lactic acid bacteria (LAB), mainly bacilli of the genus *Lactobacillus* sp. (85%), cocco-bacilli of uncertain identity (8%) and cocci of the *genera Enterococcus* and *Pediococcus* (7%). About 47% of the *Lactobacillus*-assigned isolates were clustered, but remained unidentified on species-level. Three species of *Lactobacillus* isolates representing clusters encompassing many members were identified as *Lactobacillus salivarius*, *L. johnsonii* and *L. reuteri*, collected in a percentage of 17%, 11% and 10%. Protein similarity clusters deriving from the home-grown chicken C5 encompassing the mostly facultative anaerobes which were found to

be present in various parts of the chicken intestine. A percentage of around 60% was dominated by several genera of lactic acid bacteria, mainly by *Lactobacillus* sp. (~ 45%) and *Enterococcus* sp. (~ 15%). LAB clusters consisted of *L. reuteri*- and *L. salivarius*-assigned clusters, genus-assigned *Lactobacillus*-clusters (accounting for ~ 9%) and non-identified clusters (~ 4%), respectively. One main cluster was formed by strict anaerobic bifidobacterial strains which represented a percentage of around 15%.

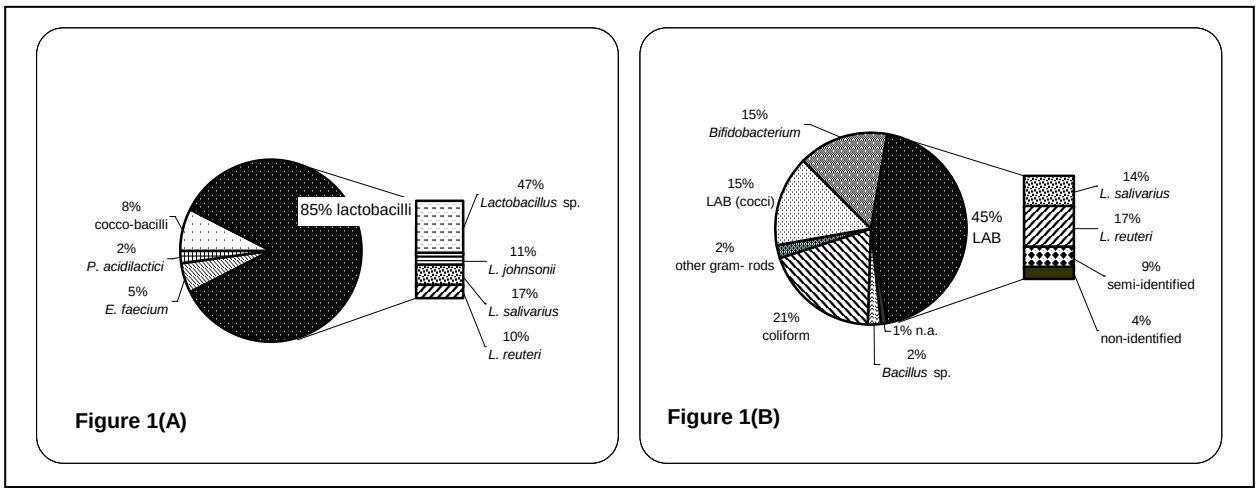

Figure 1. Composition of intestinal strain collection of anaerobically isolated bacteria deriving from broilers C2 and C4 (A) and from the home-grown chicken C5 (B)

16S rDNA sequencing revealed that the most suitable probiotic strains are affiliated to different species of well-known lactic acid producers: *Enterococcus faecium*, *Pediococcus acidilactici, Lactobacillus salivarius* ssp. *salivarius*, *L. reuteri*, and *Bifidobacterium animalis* ssp. *animalis* (Table 1).

Table 1. Percent similarity of 16S rDNA sequences of potential probiotic strains to sequences of their closest bacterial relatives available in the EMBL nucleotide sequence database

Sequence [nt]	Similarity [%]	Phylogenetic assignment	Intestinal origin
1094	99.6	*Pediococcus acidilactici*	caecum
1033	100.0	*Enterococcus faecium*	jejunum
1139	98.9	*Bifidobacterium animalis* ssp. *animalis*	ileum
1083	99.7	*Lactobacillus reuteri*	crop
1066	99.5	*Lactobacillus salivarius* ssp. *salivarius*	caecum

Abbreviations: nt (nucleotides)

Additional investigations performed by DGGE analysis of chicken intestinal tract (data not shown) confirmed the presence of these strains throughout the chicken intestine.

It can therefore be assumed that a feed additive comprising such a multi-species composition will be well tolerated by newly hatched chicken, well suited for propagation within the digestive tract to perform its competitive exclusion activities.

b) Challenge feeding trial

The challenge trial revealed significant differences of the treatment groups (1-3) in comparison with the control group (4). A positive trend was deducted for application of the

CE product (groups 2 and 3; PoultryStar) compared to an acidifier product without bacterial supplementation (group 1).

Table 2. Medium values of viable counts of Salmonella enteritidis (SE) in cecal contents of sacrificed birds.

Group	treatment	log $_{10}$ of SE per g cecal content
1	acidifier product	2,43 (N-3,68)
2	acidifier + CE product (PoultryStar)	< 2
3	CE product (PoultryStar)	<2
4	control	3,62 (N-5,53)

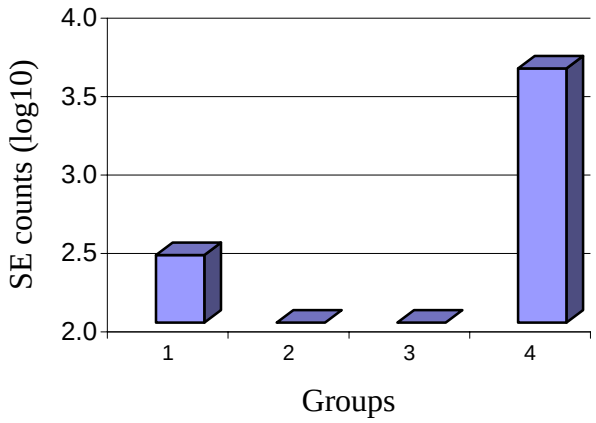

Figure 2. *Salmonella enteritidis* counts within caecal contents of challenged birds (log_{10})

IV. CONCLUSION

Feed additives for replacement of antibiotic growth promoters have to be designed with respect to safety and efficacy criteria in order to obtain registration. Competitive exclusion is a powerful principle to prevent newly hatched chicks from potential pathogenic infections (e.g. *Salmonella enteritidis*) and to positively support gut health of young birds.

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NUTRITIONAL VALUE OF BARLEY CULTIVARS FOR BROILER CHICKENS AS INFLUENCED BY β-GLUCANASE SUPPLEMENTATION

Z.V. TILMAN[1], V. RAVINDRAN[1], P.C.H. MOREL[1], G.D. COLES[2] and M.E. CUNDY[3]

Summary

The influence of β-glucanase supplementation on the apparent metabolisable energy (AME) and apparent ileal digestibility coefficient (AID) of protein and amino acids in a normal, hulled barley cultivar and three hulless barley cultivars was investigated. The three hulless cultivars differed in starch characteristics (waxiness) and β-glucan contents. The AME of barley was influenced ($P<0.001$) by the cultivar type. The AME of the (unsupplemented) normal, hulled barley was determined to be 12.68 MJ/kg DM, while the values for the three hulless cultivars were 12.92, 10.87 and 10.20 MJ/kg DM. These data suggest that starch characteristics and β-glucan contents are additional factors that may influence the available energy in barley. β-glucanase supplementation improved ($P<0.001$) the AME of all barley cultivars, with improvements ranging from 5.4 to 21.9%. The cultivar type had no influence ($P>0.05$) on the AID of protein and average AID of amino acids. Enzyme supplementation improved ($P<0.001$) the AID of protein and amino acids in all barley cultivars.

I. INTRODUCTION

The nutritional value of barley for poultry is limited by the presence of mixed-link (1-3) (1-4) β-glucans that increase digesta viscosity and lower the digestion and absorption of nutrients, but their adverse effects can be largely overcome by supplementation with exogenous β-glucanases (Bedford, 1995). Reports on the influence of supplemental β-glucanase on the feeding value of barley, however, have been inconsistent, which may be partly related to differences in cultivars. Barley cultivars differ not only in the fibre content (hulls), but also in β-glucans and starch amylose: amylopectin ratios (waxiness). It is generally thought that the available energy and nutrient digestibility are lower in conventional, hulled barley compared to hulless cultivars due to the presence of fibrous hulls, but the type of starch may also be of nutritional significance (Bergh *et al.*, 1999). In the present study, the influence of exogenous β-glucanase supplementation on the apparent metabolisable energy (AME) and apparent ileal digestibility coefficient (AID) of amino acids in a conventional, hulled barley cultivar and three hulless barley cultivars was investigated.

II. MATERIALS AND METHODS

The three hulless cultivars differed in starch characteristics and β-glucan contents (Table 1). The assay diets contained 943 g/kg barley, and were fortified with minerals and vitamins. Titanium oxide (3 g/kg) was included as an inert marker for the estimation of amino acid digestibility. The enzyme (Allzyme BG; Alltech, Inc., Nicholasville, KY) was incorporated at the rate of 1000 g per tonne diet. Each of the eight dietary treatments (four barley cultivars, without and with enzyme) was assigned to four pens of five 28-day old male broilers each. The assay diets, in mash form, were offered for 7 days and the total excreta

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collection was carried out during the last four days for the determination of AME. At the end of the AME assay, the birds were euthanased and digesta from the lower half of the ileum was collected at the end of the trial and the digestibility coefficients of protein and amino acids were calculated using marker ratios in the diet and digesta. Two-way analysis of variance was employed to determine the main effects (barley cultivars and enzyme) and their interactions by using the General Linear Model procedure of SAS (1997)

Table 1. Composition of the barley cultivars (dry matter basis)[1]

	C-NS	H-NS	H-W	H-W-BG
Crude protein, g/100 g	11.6	10.4	10.5	13.7
Gross energy (MJ/kg)	18.0	18.1	18.2	18.3
Neutral detergent fibre, g/100 g	15.8	11.6	9.8	10.3
Acid detergent fibre, g/100 g	4.70	1.81	2.17	2.26
Starch, g/100 g	59.7	65.5	64.2	58.6
Amylose, g/ 100 g starch	47	38	13	5
Amylopectin, g/ 100 g starch	53	72	87	95
Total NSP[2], g/100 g	18.87	12.34	15.33	16.51
Total β-glucan, g/100 g	5.02	4.00	5.46	7.03

[1] C-NS, conventional (hulled) normal starch; H-NS, hulless, normal starch; H-W, hulless, waxy; H-W-BG, hulless, waxy, high β-glucan.
[2] Non-starch polysaccharides.

III. RESULTS AND DISCUSSION

The results are summarised in Table 2. The AME of barley was influenced ($P < 0.001$) by the cultivar type. The AME of the (unsupplemented) normal, hulled barley was determined to be 12.68 MJ/kg DM, while the corresponding values for the three hulless cultivars were 12.92, 10.87 and 10.20 MJ/kg DM. These data suggest that starch characteristics and β-glucan contents may be more important than fibre (hulls) in determining the available energy content in barley for poultry. Cultivars with waxy starch, which contain high levels of amylopectin, had the lowest AME values. This finding is in contrast to the general belief that amylopectin is more susceptible to amylolytic degradation than amylose and that waxy starch may be more digestible than the normal starch type (French, 1984). Interestingly, our data is consistent with those of Newman and Newman (1987) who reported that broiler chickens fed diets based on barley with normal starch performed better than those fed waxy barley.

β-glucanase supplementation improved ($P<0.001$) the AME of all barley cultivars, with improvements ranging from 5.4 to 21.9%. The AME response tended to be greater when the initial AME of the cereal was lower, as indicated by the cultivar x enzyme interaction ($P=0.07$).

Table 2. Apparent metabolisable energy (AME) and apparent ileal digestibility (AID) of protein and selected amino acids of four barley cultivars for broilers as influenced by β-glucanase supplementation.

Treatment		AME, MJ/kg DM	AID of protein	Average AID of amino acids[2]	AID of threonine	AID of lysine
Barley type[1]	Enzyme					
C-NS	-	12.68	0.63	0.66	0.52	0.62
C-NS	+	13.58	0.74	0.75	0.61	0.72
H-NS	-	12.92	0.64	0.66	0.57	0.61
H-NS	+	13.82	0.76	0.77	0.68	0.73
H-W	-	10.87	0.61	0.62	0.53	0.56
H-W	+	12.83	0.74	0.73	0.62	0.68
H-W-BG	-	10.20	0.68	0.70	0.60	0.70
H-W-BG	+	12.43	0.76	0.75	0.69	0.75
Pooled SEM		0.26	0.03	0.02	0.02	0.02
Main effects						
Barley type	C-NS	13.13	0.69	0.71	0.57	0.67
	H-NS	13.37	0.70	0.72	0.63	0.67
	H-W	11.85	0.68	0.68	0.58	0.62
	H-W-BG	11.31	0.71	0.73	0.65	0.73
Enzyme	-	11.67	0.64	0.66	0.56	0.62
	+	13.16	0.74	0.75	0.65	0.72
Probability, P ≤						
Barley type		0.001	NS	NS	0.07	NS
Enzyme		0.001	0.001	0.001	0.001	0.001
Barley type x enzyme		0.07	NS	NS	NS	NS

[1] See Table 1.
[1] Average of 17 amino acids.

The cultivar type had no influence (P > 0.05) on the AID of protein and average AID of amino acids. The average AID of 17 amino acids in the hulled barley and the three hulless cultivars, in the absence of supplemental enzyme, were 0.66, 0.66, 0.62 and 0.70, respectively. However, significant differences (P<0.05 to 0.10) in the AID of threonine, phenylalanine and arginine were observed between the four cultivars, with the hulless, waxy barley (H-W-BG) generally having the highest and the hulless, normal barley (H-NS) having the lowest digestibility (data not shown). Enzyme supplementation improved (P<0.001) the AID of protein and average AID of amino acids by 15.6 and 13.6%, respectively. The AID of all individual amino acids in the four barley cultivars were improved by the added enzyme and there were no significant (P>0.05) cultivar x enzyme interaction for these parameters.

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THE OPTIMAL DOSE OF PECTINASE IN LUPIN-BASED DIETS FOR LAYING HENS

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Summary

The hypotheses tested were that egg layers should benefit from pectinase supplementation of lupin-based diets and that pectinase might allow the inclusion of up to 20% whole or dehulled lupins in diets without compromising production performance or causing wet droppings. A 2x2x4 complete factorial experiment (whole and dehulled lupins, 10 and 20% lupin inclusion and 0, 0.6, 0.8 and 1.0g pectinase/kg diet) was conducted for 10 weeks. The lowest dose (0.6g/kg diet) of pectinase was the most effective for reducing water intake, wet droppings, viscosity of the digesta, soiled eggs, and food conversion ratio. This dose was also the most effective for increasing the digestibility, metabolisable energy, and egg yield and shell thickness. There were no interactions between lupin inclusion rate, whole and dehulled lupins and pectinase. The higher doses, 0.8 and 1.0g/kg diet, had no beneficial effects over the lower dose and may be detrimental. Hens performed slightly better when the lupins were dehulled. Increasing lupins to 20% in the diet slightly increased wet droppings and soiled eggs and reduced metabolisable energy with/without pectinase. A dose of 0.6g/kg pectinase improved the nutritive value of whole and dehulled lupins for laying hens and should allow feed manufacturers to include 20% lupins in diets while controlling wet droppings to a manageable level.

I. INTRODUCTION

Despite lupins being a rich source of protein and energy and locally available throughout the year, feed manufacturers in Australia are unable to incorporate more than about 10% of whole or dehulled lupins in layer diets. This is because lupins contain considerable amounts of indigestible and complex cell-wall carbohydrates known as non-starch polysaccharides (Evans *et al*., 1993; Chesson, 1993). These polysaccharides mainly consist of pectic substances that cannot be digested because poultry lack the specific enzymes to hydrolyse them into simple sugars. Furthermore, pectins increase the viscosity of the digesta in the intestine and this interferes with digestion and absorption of nutrients. Pectins also increase water-holding capacity of the digesta and increase water intake by the bird. This leads to low metabolisable energy of the diet and poor weight gain, wet droppings and dirty eggs. These effects might be reversed if pectins could be broken down. There is some evidence that pectinases improve the nutritional value of feed for layers but the optimal dose has yet to be determined (Burnett, 1965; Patel and McGinnis, 1980). We have established the optimal dose of pectinase for broilers in lupin-based diets to be 0.8g/kg diet (Ali, 1997) but, because layers have a more-developed digestive tract than broilers, we suggest that layers may respond to a lower dose.

The hypotheses tested were 1) egg layers are expected to benefit from a dietary pectinase but respond at a lower dose than broilers but and, 2) pectinase should allow feed manufacturers to use 20% lupins in layer diets without compromising production performance or the dry-litter condition of layers.

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II. MATERIALS AND METHODS

Whole and dehulled lupins were included at 10 and 20% in a commercial layer diet based on wheat (63%) as a main ingredient. Each of the lupin inclusions was treated with four doses of pectinase, polygalacturonase, (pectinase at 0, 0.6, 0.8 and 1.0g/kg diet). The diets were formulated to be isocaloric and isonitrogenous to meet all nutrient requirements of laying hens. Two hundred and forty hens (20-weeks old) of brown Hy-Line were housed in metabolism cages in a layer shed. The hens were fed to experimental diets in mash form for 11 weeks. Feed and water were supplied *ad libitum*. The powdered pectinase (polygalacturonase Peclyve CP) had a pectinase activity of 2200 unit/g with traces of pectin methyl esterase and pectin lyase. Body weight, feed and water intake, egg and egg-shell weights, shell thickness, Haugh unit and number of soiled eggs were measured weekly and egg production was recorded daily. Total excreta were collected twice daily for determination of moisture content and metabolisable energy. At end of the experiment, the birds were killed and the ileal digesta were extracted for measurement of viscosity. The experiment consisted of a 2x2x4 complete factorial of 10 and 20% lupins, whole and dehulled lupins and four doses of pectinase each with 15 replicates. Data were analysed by Analysis of Variance and, if significant, differences between two means were tested using Tukey's Honestly Significant Difference.

III. RESULTS

The lowest dose of pectinase (0.6 g/kg diet) increased ($P<0.05$) food conversion efficiency by 4%, digestibility of dry matter by 7%, metabolisable energy by 4%, egg production by 3% and egg-shell weight by 2%. It reduced viscosity ($P<0.05$) by 11%, water intake by 7%, wet droppings by 6% and soiled eggs by 8% (Table 1). *Ad libitum* food intake was 118g/bird/d and was not altered by the addition of pectinase. Several other parameters (egg weight, Haugh unit, yolk colour and shell thickness) were also not altered by pectinase. There were no interactions between pectinase and whole or dehulled lupins or between pectinase and the level of lupins in the diet. There was no advantage of adding higher doses of pectinase (0.8 and 1.0 g/kg diet) on any of the parameters although wet droppings and soiled eggs seemed to increase ($P>0.05$) slightly.

IV. DISCUSSION

The first hypothesis was accepted because layers clearly responded to pectinase treatment of lupin-based diets. Furthermore, we obtained maximum response at the lowest dose (0.6g/kg diet) used which, as anticipated, was lower than the level (0.8g/kg) we found best for broilers. The second hypothesis was also accepted because 0.6g/pectinase/kg diet allowed layers to handle 20% lupins without compromising production performance or increasing wet droppings. Pectinase reduced wet droppings of layers fed 20% lupins to levels normally seen on diets containing 10% lupins. By breaking down pectins of the lupins, pectinase reduced the viscosity of digesta and released nutrients confined within the cell-wall lattices, which allowed them to be digested and absorbed. As a consequence, digestibility of dry matter and metabolisable energy of the diet were increased and food conversion ratio decreased. The increase in egg production and egg-shell weight were most likely due to the increase in digestibility of dry matter and metabolisable energy of the diet. The decrease in percentage of soiled eggs was likely due to the decrease in water intake and wet droppings.

Dose level is particularly important and there is likely to be a compromise between sufficient breakdown of pectin to release nutrients but not too much to release large numbers

Table 1. Responses of hens to lupin-based diets supplemented with pectinase (PG)

PG dose	Whole lupins		Dehulled lupins		Mean
g/kg diet	10%	20%	10%	20%	
Food conversion ratio (g food : g egg)					
0	2.02 ± 0.03	2.04 ± 0.03	1.89 ± 0.03	1.95 ± 0.03	1.97[a]
0.6	1.94 ± 0.03	2.01 ± 0.03	1.82 ± 0.02	1.92 ± 0.02	1.92[b]
0.8	1.96 ± 0.03	2.00 ± 0.02	1.85 ± 0.02	1.89 ± 0.02	1.93[ab]
1.0	1.96 ± 0.02	1.99 ± 0.02	1.87 ± 0.02	1.90 ± 0.03	1.93[ab]
Digestibility of dry matter (%)					
0	57.4 ± 1.9	55.4 ± 1.9	59.6 ± 1.9	57.7 ± 1.0	57.5[a]
0.6	61.2 ± 1.7	59.5 ± 1.8	63.8 ± 1.8	60.5 ± 1.6	61.3[b]
0.8	60.7 ± 1.8	57.6 ± 1.9	60.8 ± 2.4	59.3 ± 1.4	59.6[ab]
1.0	60.0 ± 1.6	56.8 ± 1.5	59.4 ± 2.6	59.0 ± 1.7	58.8[ab]
Apparent metabolisable energy (MJ/kg DM)					
0	10.4 ± 0.1	9.7 ± 0.1	10.8 ± 0.1	10.1 ± 0.1	10.4[a]
0.6	11.0 ± 0.1	10.1 ± 0.1	11.4 ± 0.1	10.5 ± 0.1	10.8[b]
0.8	10.8 ± 0.1	10.0 ± 0.1	11.2 ± 0.1	10.3 ± 0.1	10.6[ab]
1.0	10.8 ± 0.1	10.0 ± 0.1	11.1 ± 0.1	10.3 ± 0.1	10.5[a]
Viscosity (m.Pas/sec.)					
0	4.48 ± 0.15	5.91 ± 0.21	4.21 ± 0.14	5.88 ± 0.21	5.12[a]
0.6	4.02 ± 0.14	5.28 ± 0.22	3.77 ± 0.16	5.29 ± 0.23	4.59[b]
0.8	3.98 ± 0.17	5.23 ± 0.17	3.72 ± 0.15	5.26 ± 0.21	4.55[b]
1.0	3.87 ± 0.13	5.23 ± 0.16	3.72 ± 0.15	5.27 ± 0.23	4.52[b]
Water intake (ml/hen/day)					
0	206 ± 4	218 ± 7	184 ± 6	226 ± 7	209[a]
0.6	188 ± 4	203 ± 7	167 ± 5	209 ± 7	193[b]
0.8	189 ± 3	210 ± 7	174 ± 6	209 ± 5	195[b]
1.0	192 ± 4	211 ± 6	169 ± 7	211 ± 6	195[b]
Faecal moisture (%)					
0	60.9 ± 1.8	63.6 ± 1.9	57.5 ± 2.0	61.6 ± 1.0	60.9[a]
0.6	56.7 ± 1.6	59.1 ± 1.9	51.6 ± 1.8	57.9 ± 1.0	56.3[b]
0.8	57.4 ± 1.8	60.9 ± 1.9	54.6 ± 2.1	58.7 ± 1.2	57.9[b]
1.0	58.0 ± 1.9	61.3 ± 1.6	55.9 ± 2.1	59.2 ± 1.1	58.6[ab]
Soiled eggs (%)					
0	5.53 ± 0.19	5.80 ± 0.20	5.11 ± 0.20	5.53 ± 0.10	5.49[a]
0.6	5.07 ± 0.16	5.35 ± 0.20	4.59 ± 0.19	5.21 ± 0.11	5.05[b]
0.8	5.10 ± 0.19	5.45 ± 0.21	4.82 ± 0.23	5.33 ± 0.13	5.18[ab]
1.0	5.21 ± 0.19	5.54 ± 0.16	5.02 ± 0.24	5.25 ± 0.13	5.26[ab]
Egg production (egg/100 hens/day)					
0	89.2 ± 0.9	89.0 ± 1.0	89.2 ± 0.8	87.5 ± 0.9	88.7[a]
0.6	91.8 ± 0.9	91.5 ± 1.0	91.8 ± 0.9	89.7 ± 0.9	91.2[b]
0.8	90.8 ± 0.9	89.9 ± 0.8	91.0 ± 0.9	89.2 ± 0.9	90.2[ab]
1.0	90.5 ± 0.9	88.7 ± 0.8	91.0 ± 0.8	89.2 ± 1.1	89.8[ab]
Shell weight (g)					
0	6.07 ± 0.04	6.09 ± 0.05	6.11 ± 0.05	6.02 ± 0.06	6.07[a]
0.6	6.22 ± 0.05	6.14 ± 0.07	6.25 ± 0.04	6.08 ± 0.07	6.18[b]
0.8	6.22 ± 0.05	6.15 ± 0.06	6.15 ± 0.04	6.11 ± 0.07	6.16[ab]
1.0	6.20 ± 0.05	6.14 ± 0.05	6.12 ± 0.05	6.08 ± 0.07	6.14[ab]

Means within columns with different superscripts differ significantly ($P<0.05$).

of small, indigestible molecules, mono-galacturonic acid units. For example, 0.6g/kg pectinase diet significantly reduced faecal moisture from 60.9 to 56.3% but a higher dose of pectinase reduced it less (58.6%). If too many small units of mono-galacturonic acid are released, they are not only poorly metabolised by poultry (Longstaff *et al*., 1988; Longstaff and McNab, 1986; Yule and Fuller, 1992) but they are also hydrophilic which increases their water-holding capacity (Kertesz, 1951; McCready, 1970; Schejter and Marcus, 1988). Excessive release of galacturonic acid units will increase the osmotic pressure in the gut. This would increase water in the digestive tract and lead to an increase in wet droppings. There is evidence from several studies that low levels of enzyme outperform higher levels in both layers and broilers (Petersen and Sauter, 1968; Patel and McGinnis, 1985; Boling *et al*., 2000; Lazaro *et al*., 2003) but some results are equivocal (Francesch *et al*., 1995; Scott *et al*., 1999).

As anticipated layers performed slightly better when the lupins were dehulled but the difference was not great. This suggests that lupin hulls do not act simply as a diluent because they are responsible for increases of viscosity, water intake, faecal moisture and soiled eggs. So the dehulling process may improve the nutritive value of lupins for layers. In conclusion, pectinase improved the nutritive value of whole and dehulled lupins for laying hens and the most appropriate dose was 0.6/kg. Higher doses might be detrimental. A pectinase level of 0.6g/kg of diet should allow feed manufacturers to include 20% lupins in layer diets while allowing the wet dropping problem to be managed successfully.

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BROILER CHICKENS COULD BENEFIT FROM ORGANICALLY-COMPLEXED COPPER, IRON, MANGENESE AND ZINC

Y.M. BAO[1], M. CHOCT[2], P.A. IJI[1] and K. BRUERTON[3]

Summary

Trace minerals are essential for broiler growth but supplemental inorganic trace minerals usually result in a high level of mineral excretion. Organically-complexed Cu, Fe, Mn and Zn may be alternative supplements in broiler diets due to a low rate of excretion. However, the requirements of these organically-complexed trace minerals for broilers are not known. Therefore, the current experiment was conducted to examine the effect of three levels of Cu (2, 4, 8 mg/kg), Fe (20, 40, 80 mg/kg), Mn (20, 40, 80 mg/kg) and Zn (20, 40, 80 mg/kg) fed as proteinates (Organic 1, 2, 3) on broiler performance and tissue trace mineral contents in comparison to a negative control (low-mineral basal diet) and a positive control (supplemented with sulphates). Both inorganic and organic minerals improved ($P<0.05$) broiler growth. The diets organically supplemented with 4 mg Cu, 40 mg Fe, 40 mg Mn and 40 mgZn per kg diet (Organic 2) achieved ($P<0.05$) a superior FCR and lower mineral excretion than the inorganic control ($P<0.05$). The Cu, Mn and Zn contents in the tibia increased with the level of supplementation but there was no significant difference in plasma Cu, Fe, Mn and Zn contents among treatments. So organically-complexed Cu, Fe, Mn and Zn appeared to meet broiler requirements at lower levels than inorganic supplements and did not compromise broiler growth.

I. INTRODUCTION

In the past 20 years, the body weight of the broilers at 42-49 days of age has increased by 25-50 g per year (Leeson, 2003) but the requirements for trace minerals such as Cu, Fe, Mn and Zn have still been thought to be the same as those recommended by the National Research Council for Animal Nutrition (NRC, 1994). Furthermore, these values are largely based on research information obtained 20-40 years ago. Obviously these levels relate only to inorganic mineral salts including sulphates, oxides and carbonates. These inorganic minerals are believed to prevent clinical deficiencies and/or allow the bird to reach its genetic potential for growth. However, due to the concern of build-up of heavy metals through application of poultry litter to cropland, the level of trace minerals in poultry manure is regulated in Europe, Japan and the U.S.A. (Bruerton, 2004). It has been proposed that the total amount of dietary zinc and copper for broilers should not exceed 40 and 8 mg/kg (including natural ingredients), respectively (Dozier *et al.*, 2003). Thus, organically-complexed trace minerals, which are considered to be beneficial in reducing the excretion of minerals (Scott *et al.*, 1982; Leeson, 2003), would provide an alternative source of trace minerals in broiler diets. However, the requirements of these organically-complexed trace minerals for broiler are not known. Most studies on organically-complexed trace minerals for broilers have used conventional diets, which usually exceed requirements of trace minerals for broilers, the supplement responses were not observed. The present study used a semi-

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conventional basal diet, providing lower levels of Cu, Fe, Mn and Zn than NRC levels to evaluate possible response of broilers to organically-complexed minerals.

II. MATERIALS AND METHODS

A total of 164 one-day old Cobb broilers were randomly assigned to 5 treatments, each with 8 replicates of 4 birds per replicate. The pelleted basal diet was formulated with sorghum and isolated soy (negative control, ME 13.4 MJ/kg; CP 22.5%;and a Cu, Fe Mn and Zn free premix). Three levels of Cu (2, 4, 8 mg/kg), Fe (20, 40, 80 mg/kg), Mn (20, 40, 80 mg/kg) and Zn (20, 40, 80 mg/kg) were fed as proteinates (Organic 1, 2, 3), compared with an inorganic diet with minerals provided as feed-grade sulphates (Cu 5, Fe 70, Mn 80, Zn 50 mg/kg; positive control).

Body weight and feed intake were recorded weekly. Body weight gain and FCR were determined weekly and corrected for mortality. On day 14, groups of four chicks were individually weighed and transferred to metabolism cages. After a four-day adapting period, total excreta were collected for 4 days. On day 29, all birds were killed and blood samples were collected in heparinised tubes individually. Liver, bile, spleen and right tibia from each bird were removed, weighed and pooled per cage to analyse for mineral contents.

The data were analysed statistically using one-way ANOVA with SPSS software. The mineral contents in tissue and blood were analysed using a linear and quadratic regression model.

III. RESULTS AND DISCUSSION

a) Broiler Performance

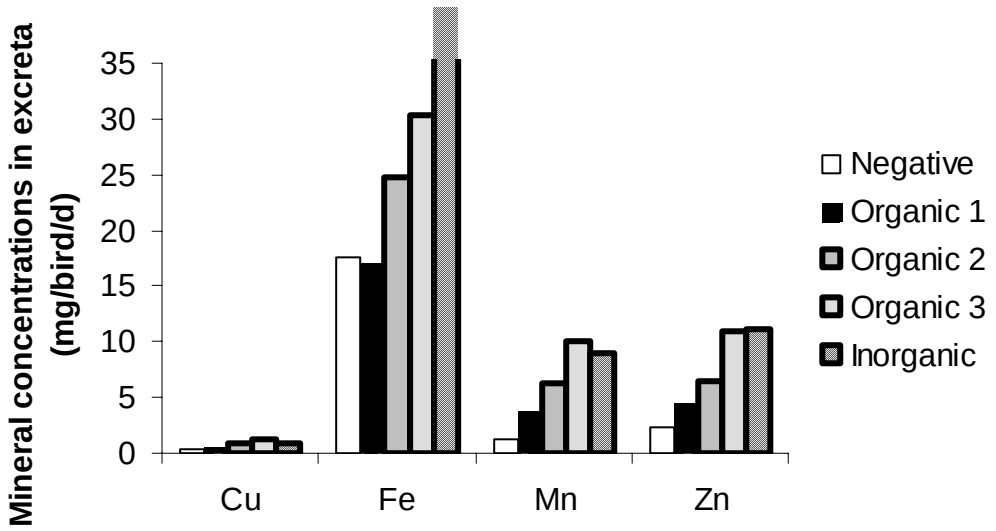
During the first week, there was no significant difference in feed intake between basal diet and experimental diets but the growth of broilers over the 4-week period on the basal diet was compromised by a deficiency of trace minerals (Table 1). Supplemental Cu, Fe, Mn and Zn, regardless of their source, improved ($P<0.05$) broiler performance. Organically-complexed Cu, Fe, Mn and Zn showed positive effects on live weight gain and FCR in a dose-response manner. The diet supplemented with organic Cu (4 mg/kg), 40 mg Fe/kg, 40 mg Mn/kg and 40 mg Zn/kg (Organic 2), achieved ($P<0.05$) a superior FCR than the inorganic positive control. However, The FCR of the diet supplemented with the highest levels of organic proteinates tended to decline.

Table 1. Effect of different diets on live performance for broilers

Treatment	0-7 d	0-29	0-29	0-29
	Feed intake (g/bird)	BWG (g/bird)	Intake (g/bird)	FCR (g/g)
Basal	147.61[a]	980[c]	1568[b]	1.590[a]
Basal+Organic 1	146.9[a]	1381[b]	2077[a]	1.510[b]
Basal+Organic 2	145.6[a]	1499[a]	2100[a]	1.403[c]
Basal+Organic 3	151.0[a]	1495[a]	2137[a]	1.432[c]
Basal+Inorganic	154.6[a]	1482[a]	2210[a]	1.493[b]
Pooled SEM	2.16	42	44	0.016

[a,b,c] Means within the same column with no common superscript differ significantly ($P<0.05$)

From these results it is evident that during the first week, the intake of birds on the basal diet and supplemental diets was similar and therefore eliminated differences in response to supplementation. Thereafter, differences in growth rate and intake were only due to different sources and concentration of trace minerals. These results suggest that organically-complexed minerals might meet the requirements of the birds more efficiently than their inorganic counterparts. It may not be necessary to supplement these organically-complexed minerals at levels as high as those with inorganic elements. This conclusion was confirmed by the results of excretion (Figure 1).



Figue 1. Effect of diet on mineral excretion

From Figure 1, it can be seen except that the diet supplemented with the highest levels of organic Mn and Cu excreted relative more minerals than those on the inorganic diets, other two elements in organic groups excreted less as expected. The excretion of Mn and Zn in organic 2 group was significantly less than organic 3 and inorganic group ($P<0.05$). It is probably due to the highest level of organic Mn exceeding broiler's requirement. This result is in agreement with the effect of organic Zn in conventional diets (Collins and Moran, 1999). It showed that Zn sulfate had a lower rate of excretion than an organic Zn supplement because they used much higher supplemental level of Zn from different sources. So it seemed the organically-complexed mineral absorption was also determined by the mineral levels in the diet. If the organically-complexed minerals more efficiently met the requirement of broilers, more minerals would excrete when the diet supplemented with higher levels of organic minerals.

b) Mineral concentration in tibia

The mineral concentrations of tibia bone are shown in Table 2 and Figure 2. Table 2 and Figure 2 clearly show that Cu, Mn and Zn concentrations in tibia increased linearly with these mineral intakes but Fe concentration in tibia did not show this effect. It probably means that the bone is the exchangeable pool for Mn, Zn and Cu. The current basal diet showed a deficiency of Mn, Zn and Cu. When these minerals are deficient in the diet, the bird would mobilize them from this exchangeable pool. In contrast to previous results, supplemental organic zinc and Mn did not lead to a higher concentration in tibia than the inorganic positive control but reach a plateau at a lower supplemental level. This is probably due to organic minerals having the smaller exchangeable pool, being more readily available for the bird to

use the minerals. Therefore, it is possible and practical to use a lower level of organically-complexed Cu, Fe, Mn and Zn in broiler diets due to better performance and less excretion.

Table 2. Concentration of trace minerals in tibia of chicks fed on different diets

Diet	Cu µg/g bone	Fe µg/g bone	Mn µg/g bone	Zn µg/g bone
basal	2.96	54.66	2.64	61.15
basal +organic 1	4.82	69.38	3.61	99.03
Basal+organic 2	5.90	67.46	3.56	139.73
Basal +organic 3	5.95	65.68	4.07	148.91
Basal + inorganic	6.37	66.62	4.47	160.16
R2	0.23	0.05	0.52	0.78
P	<0.05	NS	<0.01	<0.01

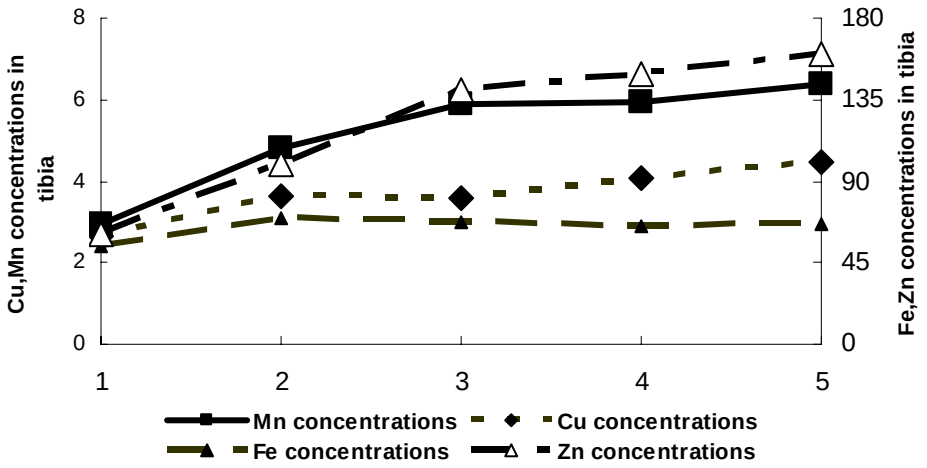


Figure 2. Tibia mineral concentration among different diets

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A "COLITIS-LIKE" RESPONSE IN GROWERS AFTER A DIETARY CEREAL CHANGE

R.D. TAYLOR[1]

Summary

The commercial wheat blend of a grower diet was changed, with or without the use of an exogenous feed enzyme. Over 48 h the faecal excreta pH and instances of diarrhoea and excreta blood loss were recorded. At 48 h the hindgut digesta pH, plasma and hindgut digesta lactic acid and digesta short-chain fatty concentrations were recorded. Changing the wheat decreased excreta pH, irrespective of enzyme use. Lactic acid concentrations in the caecal digesta increased and blood loss in the faecal excreta and diarrhoea occurred. The feed enzyme moderated some responses. Changing the wheat blend of a grower diet results in short-term hindgut fermentation changes, diarrhoea and blood loss. This suggests that the integrity of hindgut mucosa is vulnerable to damage when modest changes to a diet are made.

I. INTRODUCTION

Taylor (2002; 2003ab) showed that alterations to the dietary cereals fed to growers or layers reduced excreta pH over 48-72 h and modified the concentrations and relative proportions of short-chain fatty acids. Substantial increases in lactic acid concentrations could be produced in the ileum, caeca or colon. Lower pH and increased lactic acid concentrations were associated with fresh blood loss in the faecal excreta and histopathology indicating damage to ileal or colo-rectal mucosae. Promotion of exogenous feed enzyme use in layer diets has been aimed at the apparent benefit of improved feed conversion efficiency (Bird, 1996). Enzyme activity is influenced by pH (Marquardt and Bedford, 1996), and so a cereal change altering digesta characteristics may affect the exogenous enzyme performance. The interaction of responses to cereal change and enzyme use may alter digesta characteristics with unforeseen consequences for the bird. Previous work entailed change to the type of cereal. The following experiment examined a more subtle change; variation of the source of a single cereal type. Digesta characteristics and responses in immature birds were examined upon substitution of a commercial wheat-based diet with an alternative wheat blend, with or without application of an exogenous feed enzyme.

II. METHODS

AZTEC 101/007 growers (Bartter Enterprises, Griffith, NSW) were reared from day-old and fed commercial starter (11.94 MJ ME/kg, 198.7 g CP/kg + enzyme (Ronozyme WX)) to 8 weeks, and grower (10.99 MJ ME/kg, 153.9 g CP/kg) rations (Ridley Agriproducts, Tamworth, NSW). At 15 weeks of age, the commercial diet, or one based on an alternative feed-wheat blend (Taylor, 2002; 2003ab), with or without the enzyme, was fed for 48 h. Each 12 h, excreta trays were cleaned and the pH of fresh faecal excreta was measured (Taylor, 2002). Subsequent excreta were scored for the presence or absence (1 or 0 respectively) of blood and diarrhoea (Taylor, 2003ab). At 48 h, the birds were bled, euthanased and digesta were collected to measure short-chain fatty, and lactic acid concentrations. Methods and data analysis were described by Taylor (2002; 2003ab). The

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work (Authority No. ACEC 0004, ACEC, Bartter Enterprises) complied with the NSW Animal Research Act 1985.

III. RESULTS

Wheat diets reduced (P<0.05) mean excreta pH irrespective of enzyme application (7.64[a], 7.26 [b] and 7.21 [b] ± 0.069 for commercial, wheat and wheat + enzyme (E) feeds respectively) but did not change (P>0.05) pH of digesta in different gut sections (Table 1). Diet did not alter (P>0.05) plasma nor ileal or colonic digesta L- and D-lactic acid concentrations (Table 2). Caecal concentrations were greater (P<0.05) when the wheat-blend was fed; moderated by enzyme-supplementation.

Table 1. Excreta and digesta pH (least squares mean ± SE) of growers fed a commercial or cold-pelleted alternative wheat-blend diet, with and without enzyme (E), for 48 h at 15 weeks of age (n=12).

Feed	Excreta pH over time (h)					Digesta pH		
	0	12	24	36	48	Ileum	Caeca	Colon
Commercial	7.44	7.76	7.29	7.86	7.87	8.91	6.04	8.90
Wheat	7.48	7.52	6.72	7.61	6.97	8.78	6.07	8.77
Wheat + E	7.32	7.12	6.85	7.51	7.26	8.76	5.90	8.84
SE			0.158			0.069	0.182	0.058

Table 2. L- and D-lactic acid concentrations (mMol/l) of growers fed a commercial or alternative wheat-blend diet, with and without enzyme (E), for 48 h (n=12).

Sample	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	P	LS Mean	SE	P
Plasma	Commercial	5.30	0.369	0.52	0.03	0.008	0.16
	Wheat	5.84			0.03		
	Wheat + E	5.35			0.01		
Ileum	Commercial	6.08	2.744	0.64	0.75	1.166	0.38
	Wheat	9.70			3.02		
	Wheat + E	7.28			1.41		
Caeca	Commercial	3.20 [b]	2.372	0.03	3.15 [b]	2.340	0.03
	Wheat	13.70 [a]	2.835		13.38 [a]	2.797	
	Wheat + E	9.23 [ab]	2.652		8.99 [ab]	2.617	
Colon	Commercial	8.55	1.886	0.23	1.76	0.579	0.79
	Wheat	3.82	1.886		1.17	0.647	
	Wheat + E	5.46	1.722		1.48	0.610	

Use of the wheat-based diet, without enzyme, resulted in greater (P<0.05) propionic acid concentration in the caecal digesta (Table 3). Birds fed the wheat-blend diet (irrespective of enzyme inclusion), had higher mean blood scores (P=0.002) than birds maintained on the commercial diet and blood scores over time (Table 4) were lower (z = -1.724, P=0.085) from birds on the commercial diet. Diet change caused diarrhoea in most birds (z=1.976, P=0.048 and z=2.059, P=0.040; wheat and wheat + E diets respectively).

Table 3. Ileal and caecal digesta VFA concentrations (mMol) of growers fed a commercial or cold-pelleted alternative wheat-blend diet, with and without enzyme (E), for 48 h at 15 weeks of age.

Feed	Ileal VFA (mMol)				Caecal VFA (mMol)			
	C2	C3	C4:0	Tot C2-C7	C2	C3	C4:0	Tot C2-C7
Commercial	6.94	0.12	0.03	7.33	87.53	6.52 [b]	11.10	106.24
Wheat	6.90	0.16	0.04	7.41	102.14	10.18 [a]	9.16	122.98
Wheat + E	7.05	0.15	0.06	7.54	105.25	5.82 [b]	17.93	130.14
SE	0.749	0.061	0.031	0.729	9.140	1.415	3.273	10.453

Table 4. Probability estimates (± SE of the probability) of blood in the excreta and diarrhoea of growers fed a commercial or cold-pelleted alternative wheat-blend diet, with and without enzyme (E), for 48 h at 15 weeks of age (n=12).

Factor	Feed	Time (h)				
		0	12	24	36	48
Blood	Commercial	0.02	0.02	0.02	0.02	0.02
		0.437	0.309	0.252	0.309	0.437
	Wheat	<0.01	<0.01	<0.01	<0.01	0.25
		>1.000	>1.000	>1.000	>1.000	0.164
	Wheat + E	0.05	0.09	0.15	0.25	0.38
		0.212	0.150	0.101	0.089	0.121
Diarrhoea	Commercial	0.20	0.20	0.20	0.20	0.20
		0.138	0.098	0.080	0.098	0.138
	Wheat	0.14	0.23	0.36	0.52	0.67
		0.144	0.099	0.071	0.077	0.107
	Wheat + E	0.15	0.26	0.40	0.56	0.71
		0.139	0.096	0.070	0.078	0.108

IV. DISCUSSION

Altering the cereal fed to growers and layers can produce rapid reductions in digesta and faecal excreta pH and increases in lactic acid concentrations in the hindgut (Taylor, 2002; 2003ab). Similar responses are associated with a fermentative or lactic acidosis found in ruminants (Allison *et al*., 1975), non-ruminant herbivores (Garner *et al*., 1975) and monogastric species (Cummings, 1981; Clayton, 1999). The current results support earlier work (Taylor 2002; 2003ab) in that a change in the source of the same cereal type used in the commercial diet can have deleterious effects. A change of diet increased total organic acid production in the caeca but the lactic acid increase was moderated when the feed enzyme was used. Bustos *et al*. (1994) suggested that D-lactic acidosis associated with short-bowel syndrome in humans was of concern for gut integrity and that the overproduction of D-lactate in the gut was the problem. A reducing environment favours lactate formation, lactate is not readily absorbed and does not elicit bicarbonate exchange (Newmark and Lupton, 1990). This contributes to a lower gut lumen pH. As indicated earlier (Taylor, 2002, 2003ab), the reduction in hindgut digesta pH may be transitory. The diarrhoea noted in the current work may be equated with the reduced net water transport and increased cell sloughing of rat ileum and colon found with increased H+ and lactate concentrations observed by Saunders and Sillery (1982). Hindgut mucosal damage is indicated by rapid and significant increases in blood loss (Vernia *et al*., 1988). Blood loss and diarrhoea are markers used in animal models

of the aetiology of inflammatory bowel disease (Okayasu *et al*., 1990; Clayton and Buffinton, 2000). This work adds to earlier work with growers and layers and suggests that any change in the cereal mix fed to poultry may have an immediate, adverse impact upon hindgut tissues and which may predispose the bird to enteric disease.

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PRELIMINARY ASSESSMENT OF THE VARIABILITY IN BROILER LITTER COMPOSITION AND ITS IMPLICATION FOR VERMICULTURE

J. R. TURNELL[1], G. HINCH[2] and R. D. FAULKNER[1]

Summary

The success of value adding operations like vermiculture for the broiler industry is to some extent influenced by variation in the animal wastes resource. Broiler litter samples were taken from sheds under different management systems using a sampling technique designed to identify possible sources of variation in broiler litter composition. Analysis of broiler litter samples focused on chemical attributes that would impact on vermiculture, and these included; macro-nutrients, trace-elements, dry weight, pH, EC and gross energy. The results show low variability in the major parameters that could influence the conversion of broiler litter to a profitable resource in a vermiculture system.

I. INTRODUCTION

Broiler litter or litter is normally comprised of cellulose bedding material, manure and feathers. Once scraped from shed floors and transported it forms a free flowing granular material, including a small proportion of caked pieces (Gilmour *et al.*, 2004; Marshall *et al.*, 2000). Approximately 738,000 tonnes of litter is produced each year in Australia which equates to 1.72 kg of litter/bird/7 weeks for a single batch cleanout system. This litter resource is seen by producers as a waste product that can be difficult to dispose of.

Regional availability of dry organic materials dictates which bedding material broiler growers will use to establish a litter base. The most commonly used bedding materials are rice hulls, wood shavings, sawdust and paper products (Gilmour *et al.*, 2004) and the typical shed will require approximately 40 m^3 of material to house 29,000 birds. These birds will in turn produce 100 m^3 of litter which is equivalent to 50 tonnes. The bedding material is normally spread approximately 5cm deep and can serve several flocks per year, however single flock cleanouts are common in Australia and increasing because of biosecurity issues.

Chemically, litter is comprised of proteins, carbohydrates and lipids or fats, with carbohydrates comprising the majority of biodegradable materials in the form of cellulose, starch, and sugars. Litter also has significant gross-energy value, which is comparable with wood (Kelleher *et al.*, 2002). Previous studies have shown that the chemical and physical composition of litter is highly variable as a result of different diets, litter retention times and other management practices (Nahm, 2003; Patterson *et al.*, 1998). However, it appears that no studies have been conducted in Australia to evaluate variation in litter chemical composition between sheds, growers or integrators.

The purpose of the present study was to determine if litter chemical composition varied significantly between growers of two integrators that operate in different regions of Australia. These results would then be evaluated in terms of the potential impact that variability of composition may have on a vermiculture system.

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II. MATERIALS AND METHODS

Litter samples were collected from broiler sheds/farms that belonged to two integrators that were located in Queensland and NSW, respectively. Two growers belonging to integrator 1 (I1) were used and five sheds with three replicate samples were taken in the winter of 2004 at the time of cleanout. A 15kg sample was taken after the litter had been scraped from the shed and bucketed to the truck. One grower (I1-G1) used wood shaving the second (I1-G2) used rice hulls as litter. Both growers cleaned out sheds after every batch of birds and had the same batch retention time.

The second integrator (I2) was represented by three growers (I2-G1, I2-G2 and I2-G3) which ran 2, 3 and 4 batches of broilers before cleanout, respectively. Samples were taken in the winter of 2005. All three growers used a mixture of pine sawdust and shavings as bedding and had the same batch retention time. Six sheds with three replicates were sampled at the time of cleanout, with 1kg cores taken randomly in three zones of the modern evaporative-cooled shed. These zones included areas near cool pads, mid-shed positions and near the fans.

Samples were refrigerated until they were homogenised and sub-samples taken using a soil splitter. Dry weight was determined after samples were oven dried for 48 h at 80°C and then ground. An inductively coupled plasma (ICP) spectrometer was used to determine the concentration of macro (N, P, Ca, K, Mg and S) and micro-nutrients (Na, Fe, Al, Zn, Mn, Cu, B and Mo). Total nitrogen was determined using kjeldahl nitrogen digests (TKN) and spectrum absorption. The pH and EC was determined using a 1:5 (litter:water) suspension after agitation for 1 h.

Data were analysed using analysis of variance (LSD, $\alpha = 0.05$) and excessive variation was defined as being that greater than 0.25 coefficient of variation. This definition was based on the variability deemed acceptable by a vermiculture expert.

III. RESULTS

The two growers I1-G1 and I2-G2 were operating with the same number of batches, bedding and batch retention times. The results show significant differences for N, S and EC for these samples. Three growers for I2 were operating with different numbers of batches before cleanout, while bedding and batch retention times were the same. There were significant differences for N, Ca, K, Na, Fe, Al, B, dry weight, pH and EC (Figure 1 and Table 1).

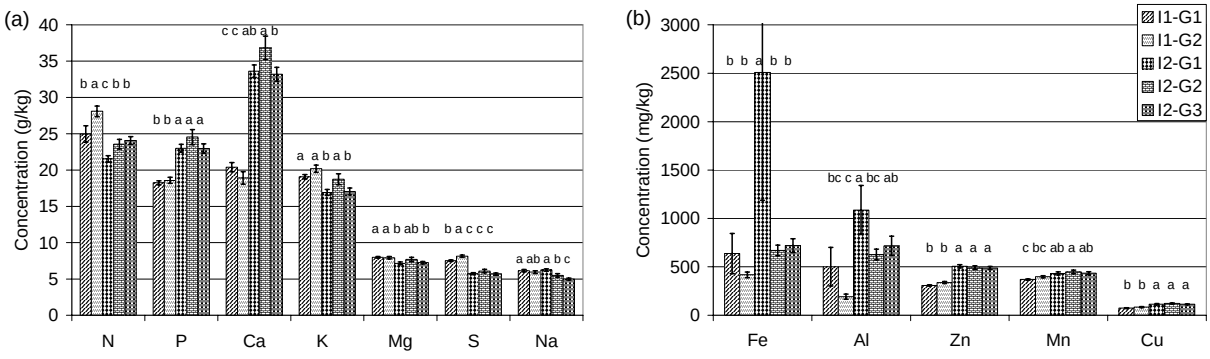

Figure 1. Concentrations of macro-nutrients ((a) not including Na)) and trace-elements (b), in the litter from five growers (G), belonging to 2 integrators (I).

Only four trace-elements exceed a 0.25 cv including Fe and Al for both integrators and Cu and Mo for I1 (Table 2). The composition of all litter samples are deemed acceptable for vermiculture possibly with the exception of EC, N, and Na.

Table 1. Mean and SE for gross-energy, pH, EC, B and Mo for each grower.

Integrator- I Grower- G	Gross energy (MJ/kg)		Dry weight (%)		pH		EC (mS/cm)		B (mg/kg)		Mo (mg/kg)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
I1-G1	16.7	0.2	85.9 ^{a}	0.7	6.9 ^{b}	0.1	7.6 ^{c}	0.3	37.2 ^{a}	1.7	4.1 ^{b}	0.3
I1-G2	16.8	0.1	84.1 ^{ab}	0.7	6.8 ^{bc}	0.0	9.0 ^{b}	0.2	40.0 ^{a}	1.5	3.4 ^{b}	0.2
I2-G1	-	-	83.2 ^{b}	0.4	7.2 ^{a}	0.0	9.4 ^{b}	0.3	21.6 ^{b}	1.2	9.9 ^{a}	0.5
I2-G2	-	-	79.2 ^{c}	1.4	6.7 ^{c}	0.1	11.6 ^{a}	0.6	22.7 ^{b}	1.1	9.9 ^{a}	0.4
I2-G3	-	-	84.2 ^{ab}	0.4	7.1 ^{a}	0.0	9.4 ^{b}	0.3	17.7 ^{c}	0.7	9.4 ^{a}	0.3

Table 2. Coefficient of variation (cv) in litter for each grower, expressed as a percent.

Grower	N	P	Ca	K	Mg	S	Na	Fe	Al	Zn	Mn	Cu	B	Mo	Dry weight	pH	EC	Gross energy
									-------- % --------									
I1-G1	18	6	13	6	5	6	11	126	154	8	7	22	18	32	3	3	18	6
I1-G2	10	8	18	9	7	7	12	27	52	12	11	28	15	28	3	1	10	2
I2-G1	9	10	11	10	11	10	11	224	98	14	15	22	24	21	2	2	12	NA
I2-G2	12	18	18	17	16	16	18	35	37	16	18	21	21	16	7	6	21	NA
I2-G3	9	12	12	12	11	10	12	42	59	14	15	21	17	16	2	2	15	NA

IV. DISCUSSION

The purpose of this study was to compare chemical composition of litter from several growing operations associated with two Australian broiler integrators, in an attempt to define potential problems that might exist for vermiculture or other systems that might use litter. The mean values for all parameters measured suggest broiler litter on average is a good a potential food source for worms. The concentrations of salts (EC) especially N and Na in litter are a challenge for the vermiculturalists and would require specific management if litter is the sole food source for worms (Fauser, 2005).

Some macro-nutrient and trace-element concentrations are significantly different between growers belonging to the same integrator and between growers belonging to different integrators (Figure 1 and Table 1). The significant difference in EC between I1-G1 and I2-G2 is interesting since salts could affect the commercial value of the litter resource, making I1-G1 litter more attractive. This would also be the case for litter from I2-G2 and confirms that any successful value-adding operation must be able to deal with the variability in litter composition that exists between growers (Figure 1).

If within farm variation is high this would be of great concern to vermiculturalists as cast analysis can be expensive if it has to be done often to guarantee quality. High variation can also impede management of the vermiculture system potentially leading to worm deaths; especially when N is involved. Apart from Fe and Al, Cu and Mo, variability was low, and therefore improves litters value for vermiculture (Table 2). One sample contributed to the majority of variation of Fe and Al for I1-G1, suggesting that sampling methodology might be at fault, for example road base from the shed floor may have been included in the sample. Even at these concentrations Fe and Al is unlikely to affect the vermiculture system other than possibly reducing the value of casts. Also, the slightly higher variation in Cu and Mo for I1 is not excessive for vermiculture and increasing the number of samples analysed could see this variation reduce.

V. CONCLUSION

Poultry litter is renowned for being highly variable in composition and there is a perception that this variation also exists within growers of our major broiler integrators. The general lack of variation in the parameters investigated in this preliminary study suggests that this may not be the case in Australia. If this is true a vermiculturalists could be reasonably well assured that the litter could provide a reliable food source for worms providing they accommodate for salts.

Sampling is needed throughout the year to provide information on temporal variation in litter composition along with further analysis of metals.

ACKNOWLEDGEMENTS

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AN APPROACH TO MODELLING AVIAN INFLUENZA IN THE AUSTRALIAN POULTRY INDUSTRY

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There have been five outbreaks of Highly Pathogenic Avian Influenza (HPAI) in Australia during the last three decades, all of which have been contained by the implementation of aggressive slaughtering-out policies (Westbury 1997; Selleck *et al*., 2003). In each case, secondary spread was minimal from the index property, perhaps aided by the low poultry population density in each region (Westbury 1997, Turner 2005). However, an outbreak of HPAI in an area with higher poultry density may have much more severe consequences, as indicated by the magnitude of the Newcastle disease outbreak at Mangrove Mountain, NSW in 1998 (Turner, 2005).

Development of effective contingency plans to contain future outbreaks of HPAI in the Australian poultry industry is a priority for government and industry. In the absence of detailed understanding of avian influenza epidemiology under Australian conditions, simulation modelling may be a useful tool to gain insights into the spread of disease within and between different sectors of the poultry industry and regions of the country. In this paper we describe the development of a disease simulation model to investigate the potential impact of HPAI on the Australian poultry industry.

The proposed poultry industry model will investigate the spatial and temporal spread of disease between farms using a stochastic state transition approach, similar to those used to model the potential spread of Foot-and-Mouth Disease in Australia and overseas. The basis of this model will be a dataset of commercial poultry producers including chicken meat, table egg, turkey, duck, quail, emu, ostrich and smaller independent chicken farms identified by a telephone survey conducted in 2005, which was commissioned by the federal Department of Agriculture, Fisheries and Forestry and undertaken by independent contractors. The survey recorded the location and size of farms together with their routine horizontal and vertical contacts and biosecurity procedures.

The model will simulate the spread of disease through time and space, taking into account the potential for spread of infection between farms. Contacts between infectious and susceptible farms will include pathways such as contaminated personnel, vehicles or equipment, the movement of infectious birds and local transmission, which are widely recognised as mechanisms for the secondary spread of HPAI.

Several mitigation strategies will be included in the model to represent movement controls, the culling of infected and dangerous contact farms, vaccination and disease surveillance. Modules will also assess the economic cost of disease outbreaks and their control.

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MICROARRAYS: CHIPPING AWAY AT THE MYSTERIES OF CHICKEN GENOMICS

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Summary

Our knowledge of avian genomics has rapidly increased over the past few years, culminating in the recent publication of the chicken genome sequence and the development of several microarray platforms to study gene expression. Microarrays have enabled chicken biologists to investigate the expression of thousands of genes across many conditions, including genetic control of development, cellular differentiation, and adaptation to biological challenges. Thus, it is easy to see why microarrays have such a vast potential in not only chicken biology but the entire agricultural sector. It is anticipated that the continued development of microarray technology will be paralleled with improvements in the health and productivity of chickens.

I. INTRODUCTION

From their origins in Southwest Asia, chickens have spread to all corners of the world due in great part to their usefulness to man. There is an ever increasing demand for chicken meat. The ability of producers to meet this demand has been assisted by an approximate halving in typical production time to marketable weight in the past 50 years. This huge time reduction is due to both traditional breeding methods and improved management strategies. The rate of improvement is still being maintained and with the advent of new molecular based technologies it is sure to continue.

Microarrays are tools that allow exploration and discovery at a genomic level. Essentially, microarrays are assays that can be used to concurrently measure the expression of thousands of genes. Microarrays are often used to capture a "molecular portrait" of the living cell or tissue at the moment of sampling. By parallel comparison of the portrait among samples of different physiological or pathological origin, a molecular signature can emerge which is characteristic of the different state. This information then facilitates a higher-level understanding of the physiology or pathology state of the organism thus offering insights into fundamental aspects of cell biology. These microscopic arrays of large sets of DNA sequences, together with the recently obtained knowledge of the chicken genome, have the potential to revolutionise chicken biology.

II. THE CHICKEN GENOME

The chicken is the first bird and first production animal to have its genome sequenced (Hillier *et al*., 2004). It is an important non-mammalian model organism particularly for embryological studies. While knowledge of the chicken genome will not provide immediate solutions to current poultry problems, such as avian influenza, it has opened the door to a range of promising research that is sure to impact the industry in the coming years. So far, the chicken genome has expanded the number of known or predicted genes from approximately 13,000 previously identified to over 18,000 (Burt, 2004). Included in the most recent set are a large number of immune related genes not previously described in chickens (Burt, 2004). These genes are likely to play a role in many of the infectious diseases that

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threaten poultry production. Currently many diseases are controlled with vaccinations and antibiotics. These current treatments often have limitations and new methods of control need to be investigated. The chicken genome has the potential to play a key role in unravelling ways to improve current disease control methods such as vaccines (Dhiman *et al*., 2002) and also to identify new ones, by allowing a more comprehensive understanding of how the chicken responds to pathogenic challenges. In addition, this wealth of knowledge together with microarray technology will allow chicken biologists to gain an enhanced understanding of the biology of a chicken at a gene specific level. Regardless of whether the aim is to enhance current vaccines, or develop new disease control strategies, knowledge of the chicken genome is undoubtedly a powerful tool to study immune competence in birds.

III. BASIC PRINICPLES OF MICROARRAYS

The majority of the cells in the chicken contain the same set of chromosomes and identical genes. The phenotypic differences among cells of different types are determined largely by the level of expression of the genes. Some genes may be switched on, 'expressed' in one cell type and switched off 'not expressed' in a different cell type. Essentially microarrays measure this gene expression, which is a term used to describe the transcription of information contained in the DNA into messenger RNA (mRNA) molecules that are translated into proteins, which in turn perform the most critical functions of cells. The study of mRNA produced by a cell, including the various types and their amounts, allows insight into what genes are being expressed. The assumption is made that in general mRNA levels give a good overall indication of the amount of the equivalent encoded protein. Gene expression is a dynamic and highly regulated process that enables a cell to respond to environmental stimuli and to its own changing needs. Normal cell conditions see a harmonious expression of a range of different genes that are critical to normal growth, development and function. Disruptions or changes to this gene expression equilibrium may result in disease.

Microarrays typically consist of a small glass slide onto which hundreds to thousands of DNA templates are attached (spotted) to known locations. This 'spotting' can be done by a contact pin printing robot, ink-jet printing, or by photo-lithographic synthesis (similar to production of computer chips) and other in-situ synthesis methods (Dhiman *et al*., 2002). At present there are a few different types of chicken arrays available including a cDNA (Figure 1) and oligonucleotide array produced by the International Chicken Consortium and a commercial Affymetrix GeneChip® array.

Microarray technology works by exploiting the ability of an mRNA to specifically bind to the template DNA from which it originated. However, the real power of microarrays lies in their ability to screen thousands of DNA templates (spots) in a single experiment and thus gain a snapshot of the gene expression within a cell. There are two main types of microarray experiments, dual colour and single colour. For a dual colour experiment RNA from cells from two different conditions are reverse transcribed to produce complimentary DNA (cDNA). The cDNA is then labelled with two different fluorescent dyes: for example a red dye (Cy5) for the first condition and a green dye (Cy3) for the second condition. The labelled cDNA from both conditions are then hybridised to the microarray, allowing labelled gene products to bind to their complementary sequence (spot) present on the microarray (Figure 2). The attached fluorescent dyes enable the amount of bound cDNA to be measured: for example if cDNA from condition one is in abundance then the spot will appear red, or if the cDNA from the second condition is in abundance the spot will be green and if the cDNA from both conditions is present in equal amounts then the spot will appear yellow (a mixture of red and green) (Xiang and Chen, 2000). Single colour microarrays are in principle the

same as dual colour microarrays, however, they use RNA from a single condition and compare results across slides.

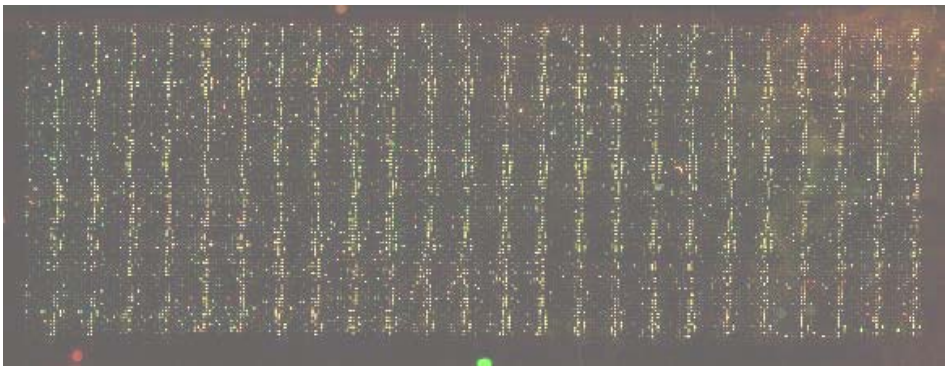

Figure 1. A 13,000K cDNA array (Cogburn *et al*., 2003) hybridised with a control and an infected sample labelled with Cy5 and Cy3 respectively (Our laboratory). Physical dimensions of this array are 54 mm x 18 mm.

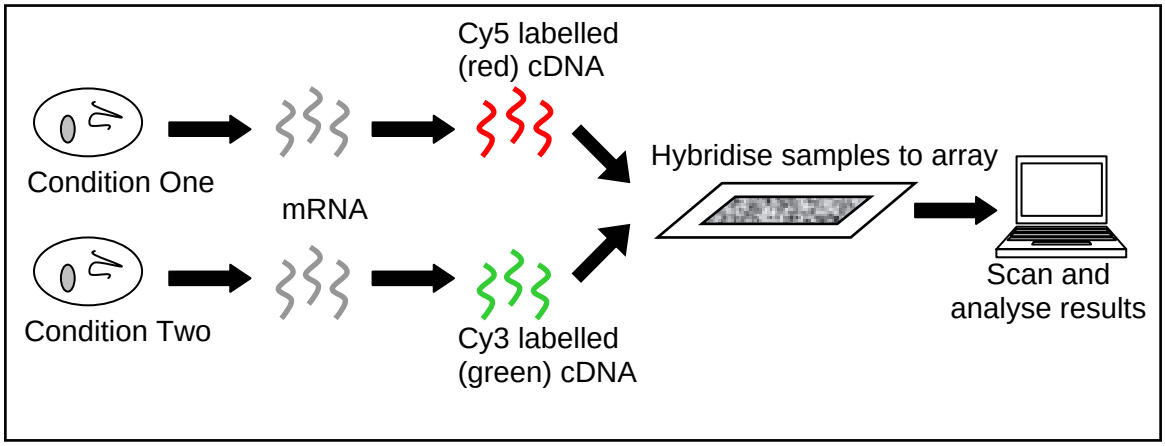

Figure 2. Overview of the experimental steps in a dual colour microarray experiment.

The measurement of the fluorescent intensities (colours) from each spot is obtained using an image scanner (Xiang and Chen, 2000). The raw output microarray data from the image scanner is used to obtain the relative expression levels of the genes in both samples. Each spot on the array is identified and quantified using image analysis software that compares the spot intensity to the background. Following this the data is normalised and a host of statistical tests are applied to determine what genes are being significantly expressed in both conditions. At present there are many programs available to perform the statistical analysis of microarray data, each with different analyses. The rapid development of microarray technology has resulted in the absence of established standards for detection of differentially expressed genes or even a standard unit for what determines gene expression levels. It is for this reason that microarrays in their current form are sometimes regarded as not a stand alone experiment and results are often confirmed using other methods such as quantitative PCR (Q-PCR; Beckman *et al*., 2004).

Microarrays are not exclusively used to measure gene expression. Another application is the detection of single nucleotide polymorphisms (SNPs), in a gene sequence.

SNP microarrays are spotted with known target sequences usually from a single gene, with each spot differing by only one or a few specific nucleotides (Jalving *et al*., 2004). A further application of microarrays is comparative genomic hybridisation (CGH) (Muller, 2001). Each of the spots on a CGH microarray contain large pieces of genomic DNA which have a known chromosomal location and the hybridisation mixture has fluorescently labelled DNA harvested from two different conditions: for example DNA from normal and diseased tissue. These microarrays detect changes in the number of copies of a particular gene involved in a disease state: for example growth of a tumour. Single nucleotide polymorphisms can have powerful uses in genetic mapping experiments aimed at defining the genetic causes of superior trait characteristics. The adaptation of microarrays to analyse SNP's plus the vast number of new SNP's identified in an adjunct project to the genome sequencing effort, gives poultry breeders very powerful new capabilities to use in the improvement of chicken strains.

IV.CURRENT POULTRY MICROARRAY APPLICATIONS

Microarrays are increasingly being used in the world of poultry research. To date this technology has been used for a handful of applications, including response of cultured chicken cells to Marek's disease virus (MDV) (Morgan *et al*., 2001), where they found that MDV infection was linked to expression of TSA-1, a gene important for T-cell differentiation and activation. In addition, differential gene expression between hypothyroid and hyperthyroid chickens and the developing liver has been detected using specific cDNA arrays (Cogburn *et al*., 2003). A recent microarray study focusing the developing thymus tissue (Cui *et al*., 2004), has provided an initial profile of the developmental patterns of expression of genes important in the chicken immune system. Microarrays have also been utilised to explore gene expression differences between a range of Broiler chickens including malabsorption syndrome resistant and susceptible lines (van Hemert *et al*., 2004) and to investigate responses to chemical treatments on the expression of activated T-cells (Kampa and Burnside, 2002). Microarrays have also been utilised in a number of different bird species including turkey (Munir and Kapur, 2003; Karaca *et al*., 2004) and quail (Mott and Ivarie, 2004). Our laboratory is currently investigating chicken gene expression in response to a number of infectious agents including chicken anaemia virus, Marek's disease virus, Mycoplasma and several gut related pathogens. Through these studies we hope to gain a superior understanding of the chicken's response to pathogenic challenges, in particular the innate immune response and begin to enhance this response to a range of infectious diseases.

V. THE FUTURE OF MICROARRAYS

While microarrays are still in their infancy it is easy to extrapolate ideas on the future uses of this technology. In addition to the enhancement of treatment and control of poultry diseases this technology could potentially aid diagnosis. It is plausible that in the future poultry farmers may be able to use a simple hand held device to quickly diagnose diseases. Microarrays may also play a prospective role in management and breeding for specific traits or gene profiles. Regardless of what the crystal ball holds for microarrays it is evident that over the coming years this area of technology is sure to be challenging, exciting and fruitful.

VI. CONCLUSION

For many years biologists have had the ability to investigate expression of a small number of genes in a small number of conditions, however, with the advent of microarray technology it is now possible to explore thousands of genes across many conditions. Thus, it

is easy to see why microarrays have such a vast potential in not only chicken biology but the entire agricultural sector. In the case of poultry this potential has been accelerated by the recent sequencing of the chicken genome. This information will complement the information obtained from microarrays and allow chicken biologists to gain a greater understanding of genetic control of development, cellular differentiation, and adaptation to biological challenges. It is anticipated that the continued development of microarray technology will be paralleled with improvements in the health and productivity of chickens.

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CHICKEN PRODUCTION IN EUROPE – WHERE DO WE GO FROM HERE

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Summary

Chicken production in Europe continues to grow, but the growth is coming from new EU member states and Eastern Europe, not the established producers like France. Imports from Asia and Brazil are also taking an increasing share of the growing further processed chicken products, encouraged by the dominant European retailers. Food scares and food safety remain high on the agenda of the media. Animal welfare is seen by governments and retailers as the most important factor in meat production, to address this EU are bringing in new welfare regulations for chicken meat production, this seeks to place controls and checks on all parameters within chicken meat production. Running parallel to this is the control of food pathogens in poultry production in the EU, namely *Salmonella, Campylobacter*. All these controls place an increasing financial burden on the producers, so improving productivity of the breeder and broiler is therefore of increasing importance. Effects of breeder flocks age on chick quality and viability and vitamin interventions that may assist progeny growth are discussed. Achieving good 7 day broiler weights are important as they are correlated to finished weight. This is accomplished by increasing amino acids in starter feed, but higher nutrient density in subsequent diets can be important for economic growth, but not for all breeds. In all diets correct essential amino acid ratios are critical, but the next limiting amino acids will vary depending on the raw materials used, particularly those required in all vegetable diets, these are considered.

I. INTRODUCTION

World chicken production has changed dramatically over the last 50 years. America still dominates global production with South America and Asia growing year on year, Avian Influenza not withstanding. Europe essentially is now divided into two camps, those inside and those outside the EU. Current predictions suggest that some of the CIS countries are on the threshold of unprecedented annual production growth (Russia, Ukraine etc.). Within the EU New Member States (NMS) like Poland are growing, whilst established producers like France, UK and Italy are showing negative growth. It is the loss of traditional export markets to the Middle East and NMS and imports from Asia, Brazil and NMS, along with a slowing in chicken consumption that has been the root cause of this change.

Just as chicken is a global business so is retailing, the top ten retailers are dominated by European companies, with three French companies led by Carrefour with Wal-Mart USA ranking third, UK's Tesco ranking fifth and Ito-Yokado Japan ranking sixth. Retailers are an important outlet for chicken products, in the UK 77.6% of retail poultry sales go through the multiples. This shows that within the UK, retailers have achieved total dominance over sales and as a consequence have demanded and obtained, with the acquiescence of the industry, control over the production process. This is done through the Assured Chicken Production (ACP) which uses a red and blue tractor logo to identify it is being produced to "British Farm Standard".

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II. MEDIA LINKS CHICKEN PRODUCTION AND HUMAN HEALTH

Despite the hard work of the industry to meet the rigorous standards the codes of practice demand, there remains a high degree of mistrust within the media, welfare groups and public. Recent food scares such as the discovery of 'Sudan 1', a potentially carcinogenic red dye found in chilli powder and in the food colourant 'Sunset Yellow', both of which are added to a wide variety of food products. According to a recent UK survey (Partos, 2005) this has left one in four shoppers claiming that food is now more risky than twelve months ago, particularly the presence of additives in food.

The welfare of commercial chicken production still seems to captivate the media, a recent spate of TV programmes in the UK have now focused on chicken production and relationship to human health. The Channel 4's programme 'Dispatches' 28th August 2005 showed modern poultry production and ascribing this to issues such as 'hock burns' as reported by Broom and Redmann (2005) and linking fatness of modern chicken to human obesity. The interviewee Dr M. Crawford. (2005) stated that whole modern chickens were fattier, levels of Omega 3 were lower and Omega 6 were higher as compared to chickens produced in 1970. He then implied that this adverse ratio was the cause of the rise of mental health illness. The claim of the adverse ratio however is probably true, due to EU and retailers policies, where fishmeal, animal proteins and animal fats in many EU countries are banned, high Omega 6 vegetable oils have replaced the oil contribution from these banned materials. But clearly linking chicken consumption to obesity and brain issues are unsubstantiated, however these health issues do remain a dilemma within certain western societies (Noble 2001). Another TV programme; BBC 'Real Story' August 2005 had tested a number of chickens and claimed they contained "Superbugs" and this was as result of antibiotic use on farm and thus was a major concern to human health. The data that showed that 50% were contaminated with multi-resistant *Escherichia coli* and that 8% had antibiotic resistant *Campylobacter* and 4% had *Vancomycin Resistant Enterocci* (VRE). In defence the 'Health Protection Agency' said that there were no new areas for concern. They stated that *E.coli* resistance is due to the high level of use of antibiotics in humans, although VRE in chickens can lead to VRE in the human gut, it only affects people already ill in hospital and inadequate hygiene and poor cooking can lead to food poisoning from bacteria like *Campylobacter*. The ban in the EU of all prophylactic AGP's (antibiotic growth promoters) from 1st January 2006 is the final act made in an effort to reduce resistant bacteria entering the food chain, since Avoparcin was first banned in 1997 (an analogue of Vancomycin).

The issue of food borne pathogens entering the food chain remains a world wide problem. Within the EU, Directive EU-92/117 laid down the principals for monitoring/eradication of pathogenic Zoonoses this was followed by subsequent amendments including directive 2003/99/EC and regulation 2160/2003/EC, these are committed to reducing all serotypes of *Salmonella* in all poultry and *Campylobacter jejuni* in broilers. The UK's Food Standards Agency has targeted to reduce all food borne diseases by 20% by April 2006, reduce *Salmonella* contamination of UK produced retail chicken by at least 50% by April 2005 and a 50% reduction in incidence of UK produced chickens which test positive for *Campylobacter* by 2010. National surveys are being or are about to be conducted, however methodology for eradication differs with each pathogen, a top down approach worked for *Salmonella* but is less effect for others (Ducatelle *et al.,* 2001), but improved hygiene measures for *Salmonella* also have beneficial effect on *Campylobacter* (Brendtson *et al.*, 1996).

III. IMPACT OF RESEARCH AND MEDIA EXPOSURE

Normally these programmes have little long term impact on production practices or poultry meat consumption, but it would seem that consumer purchase habits may be changing, possibly the combined effects of food scares, newspapers and TV celebratory chefs telling viewers to cook from fresh ingredients that have improved UK fresh meat sales. Perhaps this change away from ready cooked meals is a part of the natural sales cycle that products have. It is mainly imported chicken meat that dominates this ready cook sector, where as fresh meat is mostly produced locally and it is this fresh market that the European industry feels it needs to protect, until packaging technology permits importation of "fresh" meat from South America. This could be good news for the UK and European producers, however the impact in Europe may be less, as the ready meal market was less significant, as cooking from fresh is more traditional. The only impact of the programmes to-date appears to have been that some UK retailers have decided that the level of hock burns (HB) is to be zero. A number of European countries like France cut off the hock; in this case measurement with CCTV at the slaughter house may become the norm. Broom and Redmann (2005) reported a HB survey; they found 82 % HB in dressed carcasses of fresh whole birds from 11 supermarkets. They surveyed both conventional and organic produced birds, measuring a wide range of hock and breast marking, leg bruising and other carcass blemishes. They showed a clear link with bird weight and the severity of hock marking, 384 conventional birds were examined, with a weight range of 1.6 to 3.4kg. By comparison, the small sample size of 26 organic carcasses were examined with a weight range of 1.2 to 1.6kg these showed lower levels of hock marks about 60% versus 80% in conventionally reared. Total hock burn (small/medium/large) within same weight range, found 42% in organic and 82% in conventionally produced birds. In summary the report indicates that too high a percentage of birds reared under current UK (ACP) standards have HB levels above the 15% allowed, this and other carcass marks are indicative of poor welfare and the industry must take steps to reduce this.

IV. NEW WELFARE REGULATION IN THE EU

It is coincidental that HB along with Foot Pad Dermititis (FPD), as in the Scandinavian chicken production model, has become the measurement associated not only with bird welfare but also with stocking density (SD). The draft 2005/0099 Proposal for a Council Directive "laying down rules for the protection of chickens kept for meat production" has been circulated to member states for comment. There are a number of options that have to be considered by negotiating teams from each country. The likely outcome could be a two tier system, linking a lower stocking density 30kg/mt^2 to poultry units that meet required standards of management and housing, including; drinkers feeders, litter, ventilation and heating, noise, light, inspection, cleaning, training, record keeping and surgical intervention. Those farms that wish to stock at higher densities, 38kg/mt^2, must meet with enhanced standards that involve; site assessment, better training and monitoring of welfare parameters at the slaughter house. This monitoring includes recording of all mortality and FPD. Failure to comply with these criteria will trigger an official inspection and could eventually result in enforced reductions in SD. The commercial ramifications of SD reductions could be significant for those production companies/sites that are unable to meet the rules. In the UK the current ACP standard is 34kg/mt^2 and 80% of UK production fall within 30-38kg/mt^2, some European countries tend to be a higher; 40kg/mt^2+. According to DEFRA the UK's ministry responsible for implementation say that it could cost the average grower with a unit of 650,000 birds £15,000 a year and the whole broiler industry

£15-20 million, based upon a 2% increase in average production costs. The Dutch industry already with a SD at about 42kg/m^2, has had some calculations done by LEI - Wageningen University (2005), they suggest average farm income would fall by €11,000 and €31,000 if SD was 38 or 30kg/m^2 respectively

A recent paper by Jones *et al.* (2005) confirms earlier findings by (Dawkins *et al.,* 2004) who showed clearly that stocking density should not be considered in isolation and other environmental factors; like temperature, humidity, air and litter quality are critical to the welfare of the broiler. Their survey of 10 major broiler producers in UK and Denmark found that the more accurately you can control temperature and relative humidity (RH) throughout the life of the flock the better the health and lower the mortality of the broiler bird. Prolonged periods of high temperature and RH negatively affected FPD, impaired gait, produced leg angulation abnormalities, increased mortality and stress. The management, environmental and bio-security factors (Jones *et al.,* 2005; Stamp Dawkins *et al.,* 2004; Martrenchar *et al.,* 2001) that will be required to meet the higher stocking density standards are out of the scope of this paper. But undoubtedly all these factors have an impact on bird productivity, but clearly feed and feed components impact on the levels of FPD found (Harms and Simpson, 1982; Harms *et al.,* 1977; Ekstrand *et al.,* 1998; Martrenchar *et al.,* 2001).

The EU poultry industry must now await the final conclusions of the commission in response to the member states inputs, it is how the regulations will be implemented in each member state that remains unpredictable and as often stated the 'devil is in the detail'. Many major UK producers don't feel that the achieving the higher SD required will be a problem, only the details on FPD, lighting and mortality need clarity. But with such diverse SD's such as Sweden at 30kg/m^2 and Poland at 42kg/m^2 achieving the targets may prove more damaging for some EU countries. But assuming that the principals of the regulation remain, then achieving a more level playing field should be good for the industry and the welfare of the birds.

V. PRODUCER OPTIONS

What options do producers have under the new regulations? If stocking density is to be reduced then a number of actions will be required to minimise the impact on the current productivity, output and economics.

Maximising total live weight produced per square metre is a valid option if processing plants and finished products fit the market outlet that producers work within, as product weights could range from 1.5kg to 3.00kg+. This can only be achieved by flock thinning, the lower the maximum SD then potentially the greater the number of thinning operations are needed. Under today's requirements then *Campylobacter spp.* may then become a problem, as the stress of feed withdrawal and thinning has been associated with a high incidence of *Campylobacter spp.* (Hald *et al.,* 2001). Humphrey (2004) in a pilot survey found that reductions in colonisation of flocks with *Campylobacter spp.* could be reduced with good bio-security. Van de Gissen *et al.* (1998) found the same in broiler flocks in Holland and in further research done by the University of Bristol (unpublished) showed that it was possible for 'good farmers' to get negative flocks and 'bad farmers' to get positive flocks, but infection during thinning could be associated with poor hygiene of crates and catching crews.

Improving broiler productivity and efficiency is a clear objective of producers. As far as nutrition is concerned this can be approached in two ways, firstly via the broiler breeder to improve chick numbers, chick quality and viability, and secondly via the growing broiler to improve productivity and efficiency.

VI. BROILER BREEDER OPTIONS

In a review of broiler breeders, Kemp *et al.* (2001) highlighted that the nutrient transfer to the hatching egg was complex, and the effect that the feed and nutrient intake may have on hatchlings. They suggested only small changes to hatchery and broiler performance were required to pay for a 10% investment in breeder feed cost. In countries where white (maize free) diets are standard an inclusion of 40% maize could improve productivity, but indicated research may be required to understand the maize effect. Clearly the antioxidant role that is provided by carotenoids was shown by Surai *et al.* (1995) also it's sparing effect of vitamin E in egg yolks in maize based verses wheat based diets was also demonstrated by Surai and Sparks (2001). Hossain *et al.* (1998) in maize based diets showed that increasing dietary vitamin E from 25 to 100mg/kg in 25mg steps linearly improved the antibody log titre of the broiler, but this does not explain all the benefits shown by Kemp.

The major issue with chick quality tends to be in period up to post peak, smaller egg size in early lay effects day old (DO) body weight and 7 day weights, as does early access to feed (Noy and Sklan, 1998). Leeson (2004) found the increasing peak breeder feed allowance could positively affect the DO chick weights. Work with a UK customer (unpublished) using Ross 308 progeny from breeders at 25, 34, 46 weeks of age showed that breeder flock age effects DO weights and their potential to achieve good 7 day weights. 7 day weights achieved were 131g, 151g and 57g from 25, 34 and 46 wks respectively. Also within the same flock age you get variation in DO weights these were averaged into 35g, 37g and 40g groups; lower the weight, lower the 7 day weights (142g, 147g and 154g respectively). Differences occur even within the same day old weight (37g), but at different flock ages 25, 34 and 46; younger the flock the lower the 7 day weight; 137g 147g and 158g, respectively. Clearly there are differences between chicks from young and older breeder flocks, Hussein *et al.* (1993), found better yolk : albumen ratios in broiler and layers eggs from older flocks. Noble *et al.* (1986) suggested that there was malfunction of yolk lipid assimilation and mobilisation from yolk content in embryos from young parent flocks and this may limit utilisation of vital nutrients at a critical time. Vieira *et al.* (1998) measured amino acid and mineral contents of eggs from 27wk and 62 wks old flocks in order to identify differences but found little difference. It is normal for egg size to increase as the breeder ages and commensurate increases in yolk size, however work (unpublished) looking at eggs from 37 week old Ross breeders found that within one flock at 37 week of age, yolk as a % of egg size fell as egg size increased. What was also interesting, that not only is does egg weight vary but yolk % varied within a single egg weight, for a 59 gram egg the range was between 25–34%, this may be normal due to genetic variance or flock uniformity or differing bird sexual maturity but it could affect DO size and viability. DSM customer trials conducted in Portugal have shown that feeding the vitamin D_3 metabolite 25-OH-D_3 to breeding birds increased egg weight, egg yolk size and improved hatchability. Other studies conducted by Isogen (1997) with laying birds also showed that feeding 25-OH-D_3 can increase egg and yolk size, but also appears to shift the yolk : albumen ratio in favour of more yolk. Currently we have no data linking this effect on yolk size with chick size or viability. It is believed that increasing the amount of 25-OH-D_3 deposited into the egg rather than cholecalciferol may have benefits for the embryo in assisting skeletal development. Work carried out by Gonzales *et al.* (2003) with in OVO inoculation of various levels of 25-OH-D_3 into hatching eggs at 17 days found it stimulated embryo metabolism and lowered to hatching time by 2 to 4 hours. There were no negative effects in performance of progeny up to 10 days of age. Weytejens *et al.* (1999) suggested that there is less well developed thermoregulation ability in chicks from young broiler breeders. So ensuring that DO chicks from different flock ages are not mixed on farm, will allow growers to make management /

temperature adjustments for chicks from young flocks and in an ideal world grading eggs may improve flock uniformity, however this may not be a practical option.

Commercial experience has shown that feeding the young breeder flocks with a vitamin booster has reduced the number of chick quality problems and improved chick viability. Rebel *et al.* (2004) demonstrated that feeding high levels of vitamins and minerals gave about five fold improvements in lymphocyte, basophil and macrocyte. Kemp *et al.* (2001) suggested that although no hard data existed a generous supply of vitamins in the peak to peak production may be worthwhile. Trials subsequently conducted by Aviagen Ltd in the UK (unpublished) comparing vitamin boosted breeder of 31 and 42 weeks of age with non boosted flock of 31 and 45 weeks demonstrated growth improvements of the progeny over 22 to 49 day period, modest improvements in FCR, BMY and between 2.5% and 1.5% reductions in mortality for the 31 and 42 week respectively. Results from the same trial however demonstrated that additional improvements could be made by raising the amino acid by 20% above the Ross standards for all parameters except mortality.

VII. BROILER OPTIONS

Maximising productivity of the broiler is normally a function of optimising nutrient density. This has proved more a challenge in Europe where in most countries as the feeding of animal protein has been banned under the Council Decision (2000/766/EC) of 4 December 2000 as part of the protection measures to prevent BSE. High nutrient density is more difficult to achieve when only vegetable proteins are available, so materials like potato protein and maize gluten meal are more commonly used particularly in starting feeds. The feed industry had to strike a balance between performance, levels of dietary ingredients and nutrient density. Clearly reducing crude protein has economic benefits on feed cost and ammonia excretion; work by Kidd *et al.* (2001) has shown that each % decrease in crude protein decreases nitrogen excretion by about 8%. Miles *et al.* (2004) showed that birds exposed to levels of ammonia greater than 25ppm (a parameter applied in the EU welfare regulations) would negatively effect growth and breast meat yield. However, we currently seem unable to reduce proteins by increased addition of amino acids as that achieved in the pig industry. If we go too low and reduce performance we must be undersupplying the next limiting amino acid/s. For example nonessential amino acids like serine and glycine may become marginal, especially glycine in all vegetable diets; trials by Corzo *et al.* (2004) showed dietary glycine + serine needed to be 1.76% and 1.80% for optimal growth and FCR, somewhat higher than the NRC (1994) level of 1.24%. Under normal dietary formulation conditions the levels of addition of the three essential limiting amino acids; lysine, methionine and threonine will be limited by setting minima for the less limiting dietary amino acids (e.g. isoluecine, valine, arginine and tryptophan). However the data is less clear as to the order and dietary levels of these amino acids. Work by Kidd (2005) looked at the three main cereals plus soybean (SB) in combination with animal proteins, poultry meat (SB+P) and meat blend (SB+M) using two amino acid densities both at standard amino acid ratios. For all sorghum based diets, arginine was limiting, in wheat based diets it was valine in SB diets and isoleucine in SB+P and SB+M diets respectively. In corn based diets it was valine, isolecine and tryptophan in SB, SB+P and SB+M respectively. Breeding companies make dietary recommendations for their birds, Aviagen in particular has encouraged growers to maximise 7 day weights a) because as the time from hatch to death continues to decline and b) there is a good correlation to 35 and 42 weights. Using the Ross dietary specifications as a base the Avagen has demonstrated the negative effects of undersupplying nutrients and positive effects and increasing amino acid density, both in growth, feed efficiency, breast meat, uniformity and economics. They have recently fine tuned recommendations to suit the

optimal biological response and revenues of poultry products. A recent report by Kidd *et al.* (2005) backs up the higher nutrient specifications advocated for the Ross bird. The trial varied dietary amino acid density, designated high (H) and medium (M) so that each treatment was fed different combinations of H and M at each of the 6 time periods from DO to 55days. The data clearly demonstrated that feeding a high nutrient density feed throughout the growing period to 'as hatched' Ross 708's improved all the productive parameters at both 35 and 55 days including income over feed costs.

This requirement for high nutrient density however may not be required for all broiler strains. Recent experience of feeding the Cobb 500 under European feed and management conditions has highlighted these strain differences and feeding for high growth rates suitable for the Ross bird does not work. A different approach has to be taken if the breed differences are to be exploited; the advisory data provided by the Cobb Breeding Company should be followed, which essentially is a combination of lower nutrient density and lighting programmes, giving claimed economic benefits using cheaper diet costs.

VIII. CONCLUSION

In conclusion, it is apparent that chicken production in Europe is at a crossroads, with the new member states seeing a need to increase production to meet local and export demands, in line with their growing economies. Meantime the more affluent states within the EU are struggling to balance the economic demands of the retailers, the welfare demands of the EU commission and the muddled perception in the consumer's minds of factory farming, additives, welfare etc.

The industry needs to respond to the concerns of the consumer, but dealing with the media and welfare groups remains problematic, as some appear to have entrenched views. Clearly the more that can be done to improve food safety and welfare can only be a good thing; unfortunately the EU probably leans too much to "The Precautionary Principal" rather than a pragmatic principal. As with many regulations within the EU the interpretation and implementation can be capricious, there is no reason to expect the EU broiler welfare regulations to be any different, perhaps with some new member states struggling to satisfy all the requirements. Perhaps it is ironic that the view of the regulators was that meat birds were growing too fast and yet the industry's only response in the current economic climate is to maximise the genetic and economic potential of both breeders and broilers. If European fresh meat chicken production is going to survive in the future in today's climate of minimal use of antibiotics and improving food safety then it is only achievable by improving nutrition, housing and management and rigorous bio-security.

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GUIDELINES FOR THE DEVELOPMENT AND REGISTRATION OF ANTICOCCIDIAL VACCINES FOR POULTRY

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Summary

Guidelines have recently been published in Avian Pathology (Chapman *et al*., 2005b) that are intended as an aid in the design, implementation and interpretation of laboratory, floor-pen and field studies for the assessment of the efficacy and safety of live anticoccidial vaccines for immunisation of chickens and turkeys against *Eimeria* species. In addition to efficacy and safety requirements, manufacture, quality control, and licensing considerations are discussed. The guidelines do not address sub-unit vaccines, but many of the principles described will be relevant to such vaccines if they are developed in the future. Guidelines are available in some countries for avian vaccines of bacterial or viral origin but specific standards for anticoccidial vaccines in poultry have not, as far as we know, been produced. Information is provided on general requirements of registration authorities (based upon regulations applicable in the European Union and the USA) for obtaining marketing authorisations for vaccines. These guidelines may assist poultry specialists in providing specific information for administrators involved in the decision-making process leading to registration of new vaccines, and are intended to facilitate the worldwide adoption of consistent, standard procedures.

I. INTRODUCTION

It is increasingly recognized that live vaccines, based upon attenuated or non-attenuated strains of important species of *Eimeria*, provide a valuable alternative to chemotherapy for the control of coccidiosis in poultry (Chapman, 2000; Chapman *et al*., 2002; Williams, 2002a,b). Anticoccidial drugs are still widely used to control coccidiosis, particularly in broiler chickens and meat type turkeys. However in some countries, such as those of the European Union, the desirability of including chemicals in animal feeds has been questioned; this has led to the withdrawal of several anticoccidial drugs from the marketplace. Other problems for prophylactic chemotherapy include reductions in the efficacy of drugs due to the acquistion of resistance by the parasites and a decline in investment in the discovery of new active agents that could serve as their eventual replacements.

The need therefore for alternative methods of coccidiosis control is urgent. For many years vaccines comprising oocysts of non-attenuated strains of pathogenic species, such as *E. acervulina*, *E. maxima*, and *E. tenella* in chickens, and *E. adenoeides*, *E. gallopavonis*, and *E. meleagrimitis* in turkeys have been available for use by the poultry industry. The former have principally been employed during the rearing phase of broiler breeders and to a lesser extent replacement layers, and the latter occasionally in meat type turkeys. The development of new methods of application, in particular methods that allow administration at the hatchery, has improved the practicability of vaccinating broiler chickens, and the

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development of vaccines based upon non-pathogenic (attenuated) strains of *Eimeria* has helped allay concerns of possible adverse reactions following vaccination.

The improved prospects for coccidiosis vaccines have encouraged researchers in many countries to develop vaccines for local use and in the foreseeable future several new vaccines are likely to be introduced. Companies that are experienced in the provision of high quality vaccines for the poultry industry will produce some of these vaccines but many other companies lacking such experience are also likely to be involved. It is important that all commercial vaccines, whatever their source, should be evaluated using recognized procedures and that they should be produced to the same high standards. The purpose of the guidelines is to provide a framework upon which the design, implementation and interpretation of laboratory, floor-pen and field studies for the assessment of the efficacy and safety of live anticoccidial vaccines can be based. Although the guidelines do not specifically address non-viable subunit vaccines many of the basic principles described will apply when these are developed.

II. IDEAL CHARACTERISTICS

Several ideal characteristics for any live anticoccidial vaccine can be identified. A vaccine should:

1. Induce protective immunity against economically important species of *Eimeria*.
2. Be safe for the target host, non-target animals and humans.
3. Not represent an environmental hazard.
4. Comprise parasites of normal or low virulence.
5. Comprise parasites that remain viable during storage for a reasonable period of time.
6. Protect against field strains from those geographical areas where the vaccine is used.
7. Be administered by a commercially practical method to ensure that as many birds as possible receive an immunising dose.
8. Have no adverse effects upon final performance or other production criteria.
9. Be compatible with other poultry vaccines.
10. Be free from viral, bacterial, mycoplasmal, fungal, and chemical contaminants.
11. Be cost effective compared with other methods of coccidiosis control.
12. Include drug sensitive lines that may reduce drug resistance in field populations.
13. Raise no problems with residues or impose a need for mandatory withdrawal periods.

III. EXPERIMENTAL PROCEDURES

Birds should be vaccinated under conditions that duplicate as far as possible the manner in which vaccination will be carried out in the field; subsequently they should be intentionally challenged with the parasites to see whether they have acquired protective immunity. Since immunity is species specific, the ability of a vaccine to protect against homologous parental strains of different species included in the vaccine and heterologous strains of recent field origin from different geographical locations will be necessary. Currently live oocyst vaccines are administered on a single occasion (often in the hatchery). It has been demonstrated that even for highly immunogenic species (such as *E. maxima*), reinfection, whether by vaccinal oocysts or oocysts present in the environment, is necessary for the establishment of protective immunity (Chapman *et al*., 2005a). Therefore, an important aspect of experimental design is that following vaccination birds must be reared in floor-pens to allow adequate exposure to oocysts; the challenge phase of experiments can be carried out in wire floored cages or pens and single-species challenges should be used (Williams and Catchpole, 2000). In the field natural challenge most frequently occurs when

birds are three to five weeks of age. Acquisition of immunity following vaccination should therefore be demonstrated by challenging birds at four weeks or earlier.

Once satisfactory results have been obtained from experimental studies then large-scale tests can be carried out in the field; this is important in order to establish that a vaccine is safe to use under field conditions. Ideally, such trials should be carried out in all geographical regions where a vaccine is intended for use.

IV. CRITERIA FOR EFFICACY

The criteria conventionally used to evaluate drug efficacy, such as weight gain, mortality, feed conversion and the presence of lesions in the intestines may similarly be used to determine the extent of immunity development following vaccination and subsequent challenge. Advantages and disadvantages of these criteria have been reviewed (Williams and Catchpole, 2000). The most useful criterion is the measurement of weight gain of vaccinated birds during the acute phase of infection following challenge with a titrated dose of oocysts that causes a decrease in weight gain without mortality. Assessment of efficacy requires comparison with the weight gain of challenged birds reared in the absence of infection (susceptible controls). In the opinion of the authors, the determination of lesion scores, using the method described by Johnson and Reid (1970), is of questionable value. Lesion scoring requires considerable expertise, is inherently subjective and, unfortunately, does not necessarily correlate with protection because lesions may be present in the gut of partially or completely immune birds even though their weight gain is not depressed.

V. REGISTRATION

In some countries registration authorities have produced guidelines for avian vaccines but specific standards for anticoccidial vaccines in poultry have not apparently so far been published. A detailed knowledge of any local requirements is essential if a product is to obtain approval. In the USA, approval to manufacture and sell veterinary anticoccidial vaccines must be sought from the USDA and requires extensive documentation to support an application for a US Veterinary Biologics Product and Establishment License. A license is also required to import vaccines from overseas. However, vaccines may be produced and used without USDA approval provided that the product was manufactured under a veterinarian-client-animal relationship, for sole use by a company in their own animals (Duquette, 2005). It is unclear whether such vaccines will be produced to the same safety and efficacy standards as those required for formal approval In the EU procedures for registration are complex and this is dealt with in detail in the guidelines. Some of the topics covered include efficacy requirements, safety and environmental considerations, quality control in terms of purity, sterility, potency, quantification and stability etc., manufacturing practice, all to pharmacopoeial standards where specified, and last but not least necessary documentation. A monograph titled “Coccidiosis vaccine (live) for chickens” is currently under preparation and will be published in the European Pharmacopeia.

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A COMPARISON OF GENETIC, NUTRITIONAL AND ENVIRONMENTAL EFFECTS ON BONE CHARACTERISTICS AND OSTEOPOROSIS IN LAYING HENS

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Summary

Skeletal quality and strength in hens at end of lay are affected by genetic, nutritional and environmental factors. This study investigated the relative contributions of these factors to maximising overall bone quality, and the mechanisms by which these factors operate. White Leghorn-type hens from lines selected over 9 generations for high (H) or low (L) bone strength were reared together, then housed in individual cages or an aviary. Hens in the cages were fed diets containing limestone in particulate or powdered form, those in the aviary were fed only the powdered limestone diet. Egg production and shell characteristics were recorded and groups of hens were killed at different ages up to 56 weeks for measurement of morphological and mechanical characteristics of tibia and humerus. Data at 56 weeks showed that the genetic effect was greatest, with tibia and humerus strengths being 83 and 35% respectively higher in the H line. Aviary birds showed improvements in tibia and humerus strength of 32 and 35% respectively compared to caged birds. The improvement with the particulate limestone diet was about 20% for tibia strength, with little effect on humerus strength. The genetic and environmental effects were found to be related to decreased rates of cortical bone resorption, associated with lower osteoclast numbers. There were no interactions between the factors, with effects of genetics and nutrition and genetics and environment being additive. Thus genetic improvements in bone strength would be expected to reduce fracture incidences in all types of housing.

I. INTRODUCTION

Bone fractures and other forms of skeletal damage are a severe welfare problem in laying hens. Surveys have suggested that 30% of laying hens experience one or more fractures during their lifetimes (Gregory and Wilkins, 1989; Gregory *et al*., 1990). Osteoporosis is an important contributory cause because it results in a progressive weakening of bones over the lives of the hens. We have previously shown that there is a strong genetic influence on osteoporosis and have bred lines divergently selected for high (H) or low (L) bone strength at end of lay (Bishop *et al*., 2000). Nutritional factors, particularly feed in a particulate source of calcium, and allowing birds to exercise can also improve bone strength (Fleming *et al*., 1994: Fleming *et al*., 1998; Fleming *et al*., 2003). The purpose of the present study was to determine the relative effectiveness of, and any interactions between, genetics, nutrition and environment in alleviating osteoporosis. Caged hens of the H and L lines were fed on diets containing particulate or powdered limestone. Hens of both lines were also housed in an aviary-type system but it was only possible to feed one diet, chosen to be the powdered limestone diet. This design gave answers to two questions. Firstly, it enabled genetic, nutritional and environmental effects and interactions to be studied. Secondly, by monitoring bone characteristics in caged and aviary hens at different ages, it provided an opportunity to gain insight into the mechanism by which exercise increases bone strength. A previous study (Newman and Leeson, 1998) has suggested that transferring birds from cages to an aviary stimulated structural bone growth, but this conclusion is at variance with our own theory that little or no structural bone formation occurs in hens during lay.

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II. MATERIALS AND METHODS

Two hundred-eighty day–old female White Leghorn LSL-type chicks from each of the high (H) and low (L) bone strength lines from the 9th generation of selection were reared together in a room of 60 m^2, deep-littered with wood shavings and fitted with bell drinkers and tube feeders. They were fed on standard starter and grower diets until 15 weeks of age. The birds were weighed at 8 and 14 weeks. Birds were ranked according to weight at 14 weeks and a subsample of 25 H and 25 L pullets were identified for baseline biomechanical and histomorphometric assessment at 15 weeks. At that age, a total of 260 birds were allocated to individual layer cages in a randomised block design consisting of 2 lines x 2 dietary treatments and a further 210 were placed in an aviary environment which consisted of a single room of 60 m^2, deep littered with wood shavings and equipped with perches, straw bales and nest boxes. Lighting pattern (14 hours light: 10 hours dark) and intensity (10-20 Lux) were the same for both locations. At 56 weeks, there were 62 hens of each of the H and L lines in cages and 38 H and 52 L hens in the aviary.

The dietary treatments consisted of a standard layer ration with the limestone portion of the diet fed either entirely as flour or as particulates of 2.5-4 mm. Both forms of limestone originated from the same quarry. All birds in the aviary system were fed the diet containing limestone flour. At four weekly intervals, eggs were taken for measurement of biomechanical properties from both cage and aviary birds. At 21 weeks of age, 20 birds of the L line from the cage environment and fed the diet containing limestone flour and 20 L line birds from the aviary environment were culled for biomechanical and histomorphometric assessment. Culls were also made at 25 weeks, when 24 birds of each line from each of the two diets in the cage environment and 24 birds of each line from the aviary environment were culled, and at 56 weeks, when the remaining birds were culled. At each cull, birds were weighed and killed by an overdose of barbiturate. The right tibia was dissected out and used for biomechanical assessment. A 5mm cross sectional sample from the mid shaft of the left tibia was taken for histological assessment.

Methods for bone analyses are described in Fleming *et al*. (2003). Data were analysed by factorial analysis of variance (ANOVA) using the GENSTAT statistical package. (*Genstat for Windows*^{TM}, Lawes Agricultural Trust, Rothamsted Experimental Station, England.). Data for the aviary cage comparisons were analysed on an individual bird basis because of the lack of replication in the aviary.

III. RESULTS

a) Line and diet comparisons in caged birds

Bodyweight of the H and L line birds at 15 weeks did not differ between the lines. Tibia dimensions showed little difference between the lines, though width was almost significantly ($P<0.06$) higher in the L line. Histomorphometric measurements indicated that the external x-sectional area of the tibia (another measure of bone width) was again almost significantly ($P=0.06$) higher in the L line and internal area (the area of the marrow cavity) was significantly ($P<0.05$) greater in the L line. However, the cortical area of bone and its bone content was very similar in both lines so that the true cortical area (the area of mineralised bone in the tibia x-section) did not differ between the lines. Nevertheless, the tibia breaking strength was very much greater in the H line ($P<0.01$) and the breaking stress (breaking strength in relation to the true cortical area) was also significantly ($P<0.01$) higher in the H line. Humerus characteristics at 15 weeks did not show any important differences.

Egg production traits over the production period up to 56 weeks are shown in Table 1. Egg number was significantly ($P<0.01$) higher in the H line but was unaffected by diet.

Mean egg weight was significantly lower in the H line ($P<0.01$) but there was no effect of diet. Both shell thickness and egg shell breaking strength were unaffected by line but were significantly ($P<0.01$) higher with the particulate calcium diet.

Bone data for birds killed at 56 weeks are summarised in Table 1 and represent group sizes of 24. Data were excluded from a few birds in each line that had poor egg production towards the end of the experiment or whose bone pathology showed abnormalities or signs of new structural bone formation indicative of the birds having gone out of lay. Body weights did not show any significant effects of line or diet ($P>0.05$). Tibia length was unaffected by line or diet, tibia weight was significantly ($P<0.01$) higher in the H line but width was lower ($P<0.01$) in this line. Width was also significantly lower ($P<0.05$) in birds fed particulate limestone. Tibia RD was significantly ($P<0.01$) higher in H line birds and in those fed particulate limestone. Histomorphometric measurements confirmed smaller ($P<0.01$) total external area of the tibia x-section in the H line and internal area was also smaller ($P<0.01$). However, area of the cortical ring was significantly ($P<0.01$) higher in the H line and, with no line effect on the proportion of mineralised bone in the cortex, the true cortical bone area was also significantly ($P<0.01$) greater in the H line. Tibia breaking strength and stiffness and bending stress were all significantly higher in birds of the H line ($P<0.01$) and in birds fed particulate limestone ($P<0.01$). There were no line x diet interactions for any of the histomorphometric or biomechanical traits.

Mean number of osteoclasts per microscope field were significantly lower in the tibias of H line birds ($P<0.05$) and much lower in birds fed particulate limestone ($P<0.01$). Osteoclast sizes were unaffected by line or diet. Medullary bone content was significantly higher ($P<0.01$) in birds of the H line but was unaffected by diet. Numbers of osteoclasts and osteoclast surface areas as proportions of amount of medullary bone area were smaller in H line birds ($P<0.01$) and in those fed particulate limestone ($P<0.01$). There were no interactions between line and diet in any of these traits.

Humerus weight and width were unaffected by line but length was shorter ($P<0.01$) in the H line. Humerus radiographic density (RD) was higher ($P<0.01$) in the H line. Breaking strength and stiffness were both significantly ($P<0.01$) higher in the H line. Diet did not affect any of these traits at this age but there was a significant line x diet interactions for humerus stiffness ($P<0.05$).

b) Environmental comparisons

The hens adapted well to the aviary, moving around the floor and flying up on to the bales and perches readily. Aviary birds had egg production comparable to caged birds. Egg weight and shell thickness and strength were all significantly higher in the caged birds, but the differences were numerically small.

Bird weight and biomechanical, dimensional and radiographic characteristics of the tibia in H and L line birds in the aviary at 56 weeks are given in Table 2. The same line effects were seen as in the diet comparisons (Table 1), with the exception that difference in the proportion of mineralised bone in the cortex now attained statistical significance ($P<0.05$), with a higher proportion in the H line. Comparisons of location effects showed that live weight was significantly ($P<0.01$) lower in the aviary birds. Tibia weight was significantly ($P<0.05$) higher in aviary birds but tibia length and width did not differ between locations. Tibia external area, determined histomorphometrically, was significantly ($P<0.01$) smaller in the aviary birds, as was the internal area of the marrow cavity ($P<0.01$). Tibial cortical area was significantly ($P<0.01$) higher in the aviary birds but there was no location effect on proportion of mineralised bone. True cortical area, whole bone RD, breaking strength and stiffness were significantly ($P<0.01$) higher in the aviary birds and bending stress was also higher ($P<0.01$).

Osteoclast numbers and medullary bone areas in the tibia at 56 weeks (Table 2) showed line effects and significances that were the same as those seen in the cage comparisons in Table 1. There were no significant effects of location on the osteoclast or medullary bone traits.

Line effects on the humerus (Table 2) were similar to those seen in the cage comparisons (Table 1). Location comparisons showed that humerus length and medullary bone score were unaffected by location, that humerus weight was significantly ($P<0.01$) lower in the aviary birds and that RD, breaking strength and stiffness were all significantly ($P<0.01$) higher in the aviary birds.

Mean values over the H and L line hens in cages and aviary at 25 and 56 weeks for some of the most meaningful traits are compared in Table 3. The data for the tibia show significant ($P<0.01$) declines between the ages in both true cortical area and osteoclast number and an increase ($P<0.01$) in medullary bone. Differences between aviary and caged birds were greatest at 25 weeks for all these traits except medullary bone. There was a significant age x location interaction in true cortical area, with the difference between aviary and caged birds narrowing over the period. Neither tibia nor humerus breaking strength showed any significant ($P>0.05$) change or interaction over the ages, though the numerical difference between the husbandry systems was less for both bones at 56 weeks.

IV. DISCUSSION

The results from the experiment confirmed the major effects of genetic selection over nine generations and feeding particulate limestone in improving osteoporosis resistance in caged laying hens, with the genetic effect by far the greater. Thus the genetic advantage in tibia strength of the H line over the L line at the end of the laying period was approximately 65% whereas the improvement with the particulate limestone diet was about 20%, with no interaction between the factors. The two effects were additive, with hens of both the H and L lines showing similar responses to the particulate limestone diet. There was also a pronounced genetic improvement in humerus strength (30%) but negligible overall nutritional effect. It is thus apparent that although good nutrition can help bone strength, genetic selection is a far superior approach.

The results also confirmed that selection for bone characteristics had not affected rate of egg production or bodyweight of hens (there is a factor in the selection equation designed to prevent weight change). However, a surprise finding was that eggshell thickness did not differ between the lines, though there was the expected nutritional effect. Results from a previous large study of birds from generation 3 had suggested superior shell quality in the L line but the present results, on a smaller number of birds, appear to indicate that the earlier findings were not representative and that prolonged selection for better bone quality has little or no effect on shell quality. A further large study is currently being carried out on the breeding populations of the lines to check these findings.

Measurements of bone characteristics suggested the mechanisms underlying the resistance to osteoporosis of the H line. Bones were stronger at end of lay as a result of (a) slightly more structural bone formation during growth (b) much less resorption of structural bone during the laying period and (c) greater accumulation of medullary bone during the laying period. The first effect was probably due to increased osteoblast function, though direct measurements on these cells were not made. However, the measurements made on osteoclast numbers provided the explanation for effects (b) and (c). Osteoclasts are situated mainly on medullary bone in laying hens but some also attach to structural bone and will resorb both types of bone. Osteoclast numbers were very much lower in hens of the H line and this would be expected to result in a lower overall rate of bone resorption. This would

explain both the lower rate of structural bone loss and the greater accumulation of medullary bone in these birds. Medullary bone has less intrinsic strength than structural bone but can nevertheless contribute to overall bone strength.

Keeping some of the birds in an aviary also allowed a study of environmental effects. Unfortunately, only one aviary could be constructed so it was not possible to study nutritional interactions but the experiment allowed a study of genetic effects within the aviary. Hens were observed to fly frequently in the aviary, spending considerable time on perches or other elevated positions on straw bales or in tiered nest boxes. Egg production in the aviary was very similar to that in cages but aviary hens tended to be lighter, as might be expected from their greater degree of exercise. Bone analyses showed effects of both genetics and environment. The genetic effect was very similar to that seen in caged birds, with tibia and humerus strengths being 83 and 35% respectively higher in the H line at the end of lay. Aviary birds showed improvements in tibia and humerus strength of 32 and 35% respectively compared to caged birds. This improvement in the aviary birds occurred during the early period in the aviary and was not enhanced later in the laying period. There were no genetic x environmental interactions in any bone characteristics. The aviary/cage comparisons confirm earlier findings that exercise can have a proportionately greater beneficial effect on humerus than tibia bone strength, particularly when birds have the opportunity to fly. However, the magnitude of the genetic effect on the humerus was similar to that of the environmental and the genetic effect on the tibia was relatively much larger.

The environmental comparison also shed light into the mechanism by which exercise resulted in stronger bones. Appositional growth of long bones takes place by bone formation on the periosteal (outer) surface and is accompanied by resorption on the endosteal (inner) surface of cortical bone. In the present study, external dimensions of bones did not differ between caged and aviary birds throughout the laying period, suggesting that there was no additional cortical bone formation in aviary birds after the initial growing period. However, the thickness of the cortical bone was greater in the aviary birds, suggesting that exercise decreased cortical bone resorption, at least during the early laying period. In confirmation of this, osteoclast numbers were found to be lower in the bones of the H line at 25 weeks. These results contradict the conclusions of Newman and Leeson (1998) that exercise resulted in increased bone formation and suggest that the mechanisms of genetics and exercise in improving hen bone strength show some similarities related to lower osteoclast numbers resulting in less structural bone resorption. However, there is a difference between the mechanisms in effects on medullary bone. In the genetic model, reduction in osteoclast numbers is associated with a greater increase in medullary bone amount whereas exercise-induced reduction in osteoclast numbers does not accelerate medullary bone accumulation.

Some of the differences in the environmental effects were greater at 25 weeks than at 56 weeks. By 56 weeks, there had been a narrowing in the environmental effects on tibia true cortical area and tibia and humerus breaking strength and a marked decline in osteoclast number. This decline was greatest in the caged birds with the result that differences between the two environments were no longer detectable by 56 weeks. It is thus apparent that the effects of exercise occur during the early laying period when the depression in osteoclast numbers suppresses structural bone resorption. Later in lay, continued exercise fails to maintain this relative suppression of osteoclasts and structural bone resorption and there is no further benefit to bone quality.

The additivity of genetic and environmental effects on bone strength and the similarities of the genetic effects in both husbandry systems have important implications for future strategies for reducing skeletal damage in laying hens. The increasing use of alternative systems for laying hens is likely to result in improvements in bone strength. However, evidence to date suggests that this is not necessarily accompanied by decreases in

skeletal damage (Gregory *et al*., 1990); birds have greater opportunities for more severe accidents that can still fracture stronger bones. Thus genetic improvements in bone strength that are independent of husbandry system would be expected to reduce fracture incidences in all types of housing.

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Table 1. Bird weight, egg production and tibia traits of birds of the H and L lines at 56 weeks housed in cages and fed diets containing limestone in flour or particulate form.

	Line	Limestone		*SED*	Significance of effect		
		Flour	Particulate		*Line*	*Diet*	*Diet x Line*
Liveweight (g)	H	1822	1771	46.1	P=0.1	NS	NS
	L	1854	1852				
Egg number to 56 weeks	H	220	222	7	**	NS	NS
	L	208	207				
Shell strength (N)	H	33.72	34.69	0.287	NS	***	NS
	L	33.39	34.87				
Tibia weight (g)	H	10.629	10.624	0.2208	***	NS	NS
	L	9.927	10.219				
Tibia length (mm)	H	113.6	111.6	0.751	NS	NS	*
	L	112.8	113.5				
Tibia width (mm)	H	6.185	6.023	0.0635	***	*	NS
	L	6.368	6.315				
Tibia breaking strength (N)	H	361.5	432.0	24.4	***	**	NS
	L	217.2	262.3				
Tibial true cortical area (mm^2)	H	6.192	6.402	0.253	***	NS	NS
	L	5.406	5.329				
Tibia bending stress (N/mm^2)	H	58.6	68.3	4.88	***	**	NS
	L	39.8	50.9				
No of osteoclasts /microscope field	H	9.23	5.41	1.05	*	***	NS
	L	10.15	8.17				
Total MB area per field (μm^2)	H	17323	19121	1627	***	NS	NS
	L	11243	11333				
Humerus weight (g)	H	4.93	4.59	0.302	NS	NS	NS
	L	4.62	4.59				
Humerus length (mm)	H	75.5	74.6	0.499	***	NS	NS
	L	76.4	76.2				
Humerus width (mm)	H	6.44	6.39	0.075	NS	NS	NS
	L	6.55	6.44				
Hum. breaking strength (N)	H	239.9	220.5	13.84	***	NS	P=0.067
	L	167.4	184.3				
Humerus RD (mm Al eq.)	H	1.489	1.432	0.0686	**	NS	NS
	L	1.313	1.334				

Table 2. Bird weight and tibia traits of birds of the H and L lines at 56 weeks housed in either cages or an aviary system and fed diets containing powdered limestone.

	Line	Location		Av. SED	Significance of effect		
		Aviary	Cage		*Line*	*Location*	*Location x Line*
Liveweight (g)	H	1722	1821	32.4	NS	***	NS
	L	1729	1854				
Tibia weight (g)	H	11.13	10.63	0.18	*	***	NS
	L	10.27	9.93				
Tibia length (mm)	H	112.9	113.6	0.662	NS	NS	NS
	L	113.0	112.8				
Tibia width (mm)	H	6.14	6.19	0.053	**	P=0.07	NS
	L	6.29	6.37				
Tibia breaking strength (N)	H	492.2	361.5	19.4	***	***	*
	L	269.7	217.2				
Tibial true cortical area (mm^2)	H	6.816	6.192	0.213	***	**	NS
	L	5.870	5.406				
No of osteoclasts /microscope field	H	8.04	9.23	1.05	*	NS	NS
	L	10.75	10.15				
Total MB area per field (μm^2)	H	21395	17323	1703	***	P=0.08	NS
	L	11058	11243				
Humerus weight (g)	H	5.47	4.93	0.22	P: 0.07	**	NS
	L	5.19	4.59				
Humerus length (mm)	H	74.8	74.6	0.39	***	NS	NS
	L	76.4	76.2				
Humerus width (mm)	H	6.35	6.39	0.054	NS	***	NS
	L	6.29	6.44				
Humerus breaking strength (N)	H	308.1	239.9	10.9	***	***	NS
	L	240.9	167.4				
Humerus RD (mm Al eq.)	H	1.684	1.489	0.048	***	***	NS
	L	1.467	1.313				

Table 3. Age comparisons for cage and aviary birds (H and L lines combined).

	Age wks	Location		*Av. SED*	Significance of effect		
		Aviary	Cage		*Location*	*Age*	*Location x Age*
No of osteoclasts /microscope field	25	10.56	12.59	0.68	*	***	P=0.087
	56	9.40	9.78				
Total MB area /field (μm^2)	25	10930	12104	1314	NS	***	P=0.085
	56	16227	14180				
Tibial true cortical area (mm^2)	25	7.574	6.395	0.176	***	***	**
	56	6.329	5.807				
Tibia breaking strength (N)	25	371.6	292.7	23.0	***	NS	NS
	56	380.1	305.7				
Hum. breaking strength (N)	25	292.6	204.9	11.6	***	NS	NS
	56	273.8	209.6				

EUROPEAN CONSUMER PERSPECTIVES ON EGG QUALITY

J M HERNANDEZ[1]

Summary

A number of consumers in Spain and Europe were interviewed with the objective of validating and ranking in eggs some food quality attributes proposed by the European Consumer Association (BEUC). Results showed how "Safety"and "Freshness" were the most important quality factors. "Nutritional value" and "Sensory characteristics" being also key elements according to consumers' opinions. Since 1997, additional surveys have been conducted in France, Germany, Italy, UK, Spain, Poland, and Greece to find out how consumers perceive egg quality from a sensory point of view. Consumers defined egg quality as related to shell strength, albumen consistency, and intense yolk colour. When offered egg samples with different yolk colours (8, 10, 12 and 14 in the DSM-Fan) a majority of surveyed persons in all countries preferred colour 14, the deepest one. Carotenoids have been historically used by the feed industry to provide the homogenous yolk colour demanded by consumers although recently researchers have started to work on the potential biological benefits of some carotenoids for animals and humans.

Levels of most vitamins in eggs are directly linked to the levels in the diet of the hen. Layer feed conversion rates have improved dramatically (approx. 40%) during last 30 years so that it is essential to re-evaluate vitamin levels in feed accordingly. Recent research has compared the effects of two vitamin levels in laying hen diets (average industry levels versus optimum vitamin nutrition (OVN) levels) on the vitamin deposition and quality of eggs. Results indicate that almost all vitamin levels (A, E, B1, B2, B12, pantothenic acid, folic acid, biotin) found in whole egg were statistically increased when hens were fed OVN premix (although not enough to be considered as vitamin enriched eggs), thus improving the nutritive value compared to control eggs. Nutraceuticals such as vitamin enriched eggs are another way to increase their added value which is of particular interest for some specific vitamins like folic acid and most recently vitamin D. Long-term lack of vitamin D increases the risk of osteoporosis and fractures in older age groups; now an important proportion of European consumers. Recent experiences in laying hens fed a combination of vitamin D and its active metabolite, 25-hydroxyvitamin D added in the diet as HyD®, show the possibility of enriching eggs with vitamin D under EU conditions, where a maximum vitamin D level of 3,000 IU is set for layers.

I. INTRODUCTION

In November 1999, Beate Kettlitz, Food Officer at the European Consumer Organization (BEUC), pointed out some of the most relevant factors for consumers when judging food quality (Kettlitz, 1999). The proposed list was as follows with no special order of importance:

- Appearance and organoleptic perception: flavour, taste, texture, etc
- Safety
- Nutritional value
- Clear labelling
- Brand's reputation

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- Convenience
- Product consistency
- Suitability for specific occasions
- Ingredients
- Price
- Packaging
- Origin
- Ethical aspects
- Environmental considerations

In order to obtain sound consumer feedback about the "food quality package" suggested by BEUC, several consumer studies were run by professional consumer research companies on a national basis. The first objective was to validate with consumers in those markets whether those attributes covered most of characteristics, factors, items, etc on food quality mentioned spontaneously by consumers from different geographical, social and economic conditions. The second objective was to rank, after a quantitative phase, the final food quality factors obtained previously and, therefore, define the priorities which should be taken into account when trying to fulfil food quality and the consumers' needs.

a) Egg quality surveys: BEUC attributes

Germany (Hernandez and Seehawer, 2001) and Spain (Hernandez, 2000) were the markets initially selected to undertake this consumer research with the objective of validating and ranking those attributes proposed by BEUC in eggs. These countries were chosen for this research due to their different social, economical, cultural and geographical characteristics, accounting at the same time for a good portion of the European consumer population (around 120 million persons).

T.I.P. Biehl and Wagener conducted the survey in Germany and Centro Informático de Estadísticas y Sondeos S.A. did the same in Spain. Both studies were run independently in 2000/01 and structured in a similar way through two phases (Figure 1).

Universe: persons older than 18; in Spain living in towns/villages with more than 1,000 inhabitants (99% of the Spanish population). In Germany, national representation was guaranteed by calculating the sample in this study from the eight Nielsen geographical areas commonly used in national consumer research.

Sample size in Spain: 3,100 telephone interviews distributed geographically by Comunidad Autónoma (17 Regions in Spain) and matching quite closely the average Spanish consumer profile (sex, age, studies/incomes). Sample error of +/- 1.76%, for p=q=50 and 95% interval of confidence.

Sample size in Germany: 2,000 telephone interviews distributed geographically by Nielsen Area (8 Areas in Germany). Sample error of +/- 2.01%, 95% interval of confidence.

As indicated in Figure 1 and 2, results show how "Safety", "Hygiene" and "Freshness" were the most important quality factors, "Nutritional value" and "Sensory characteristics" being also key elements according to consumers' opinions.

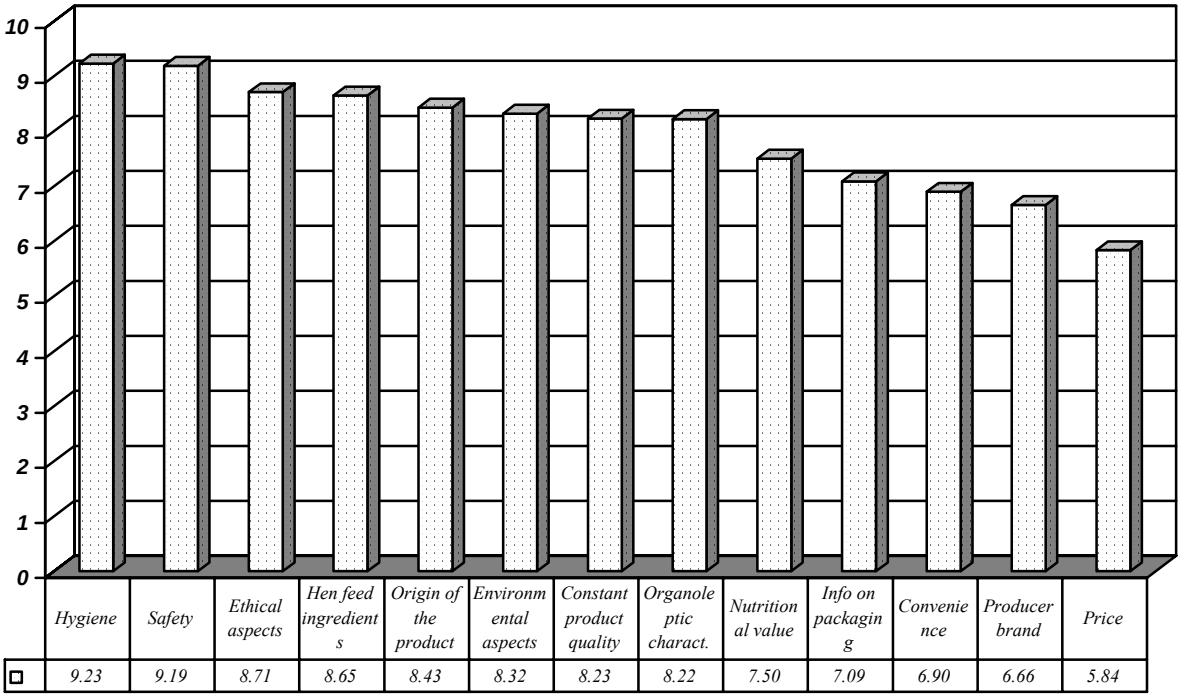

Figure 1. Ranking (0-10) of "Food Quality Parameters" (BEUC) for Eggs in Germany, 2000 (2.000 consumers).

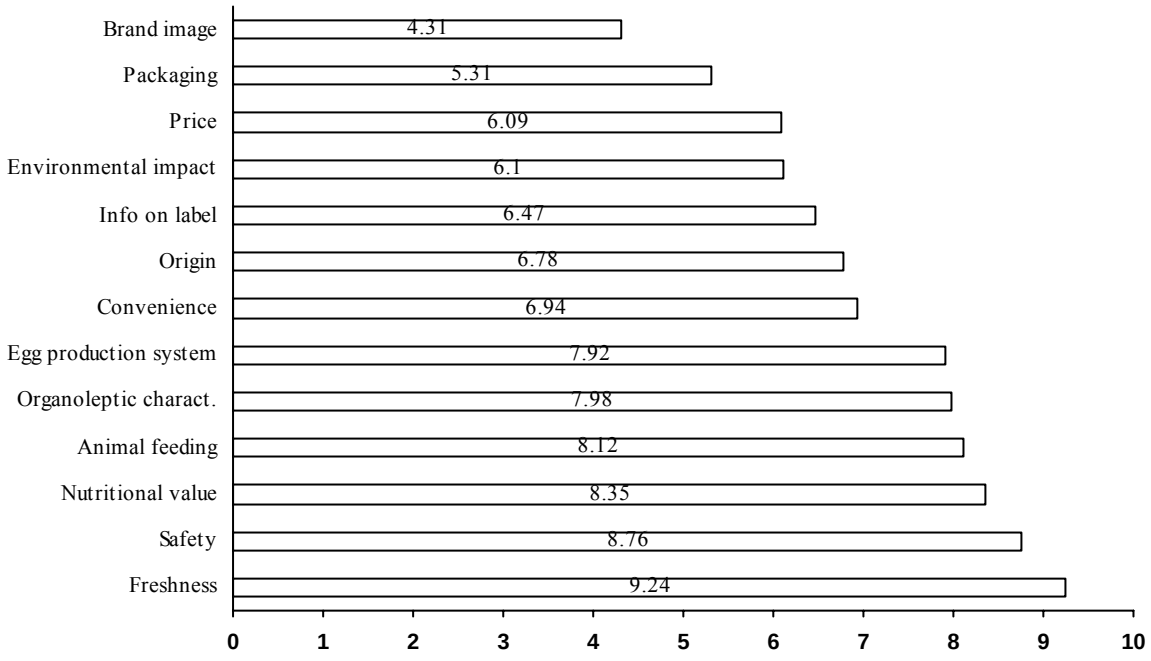

Figure 2. Ranking (0-10) of "Food Quality Parameters" (BEUC) for Eggs in Spain, 2001 (3,085 consumers)

b) Consumer surveys on the sensory aspects of egg quality

Since 1997, additional studies with more than 2,000 consumers have been conducted in European countries such as France, Germany, Italy, the UK, Spain, Poland, and Greece to find out how consumers perceive egg quality from a sensory point of view.

Consumers defined egg quality through the tangible characteristics of the egg, most especially shell strength, albumen consistency, and intense yolk colour. When offered egg samples with different yolk colours (8, 10, 12 and 14 in the DSM Fan) a majority of surveyed persons in all countries preferred colour 14 (Hernandez *et al.,* 2000). Figures 3, 4, 5 and 6 show this data in more detail as well as its relative importance in the main countries evaluated.

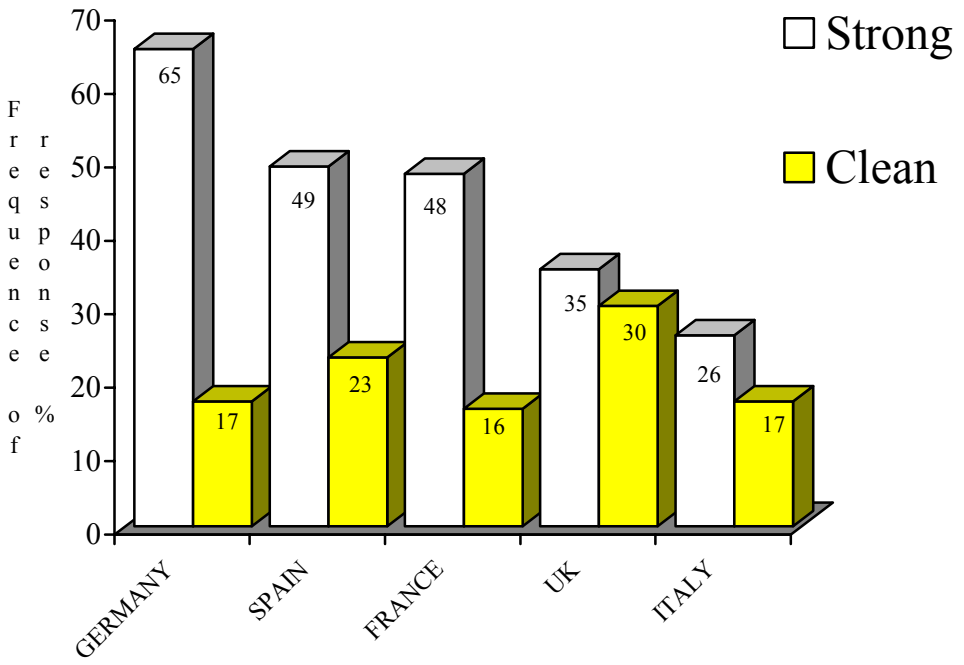

Figure 3. Criteria for a Quality Egg Shell in Europe. "What would you expect the shell of a quality egg to be like?" n= 2,122 consumers (multiple response)

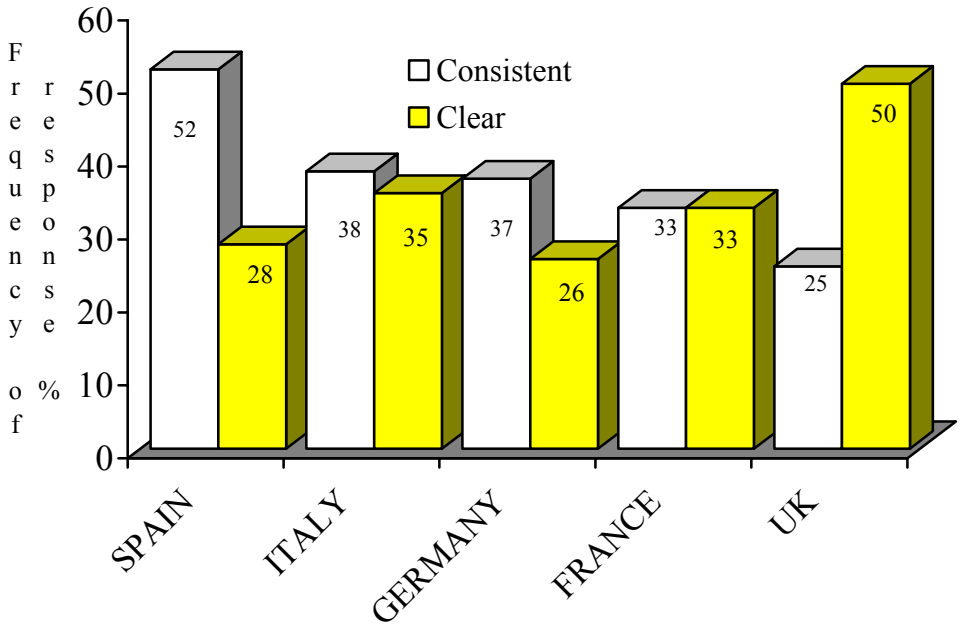

Figure 4. Criteria for a Quality Egg Albumen in Europe. "What would you expect the white of a quality egg to be like?" n= 2,122 consumers (multiple response)

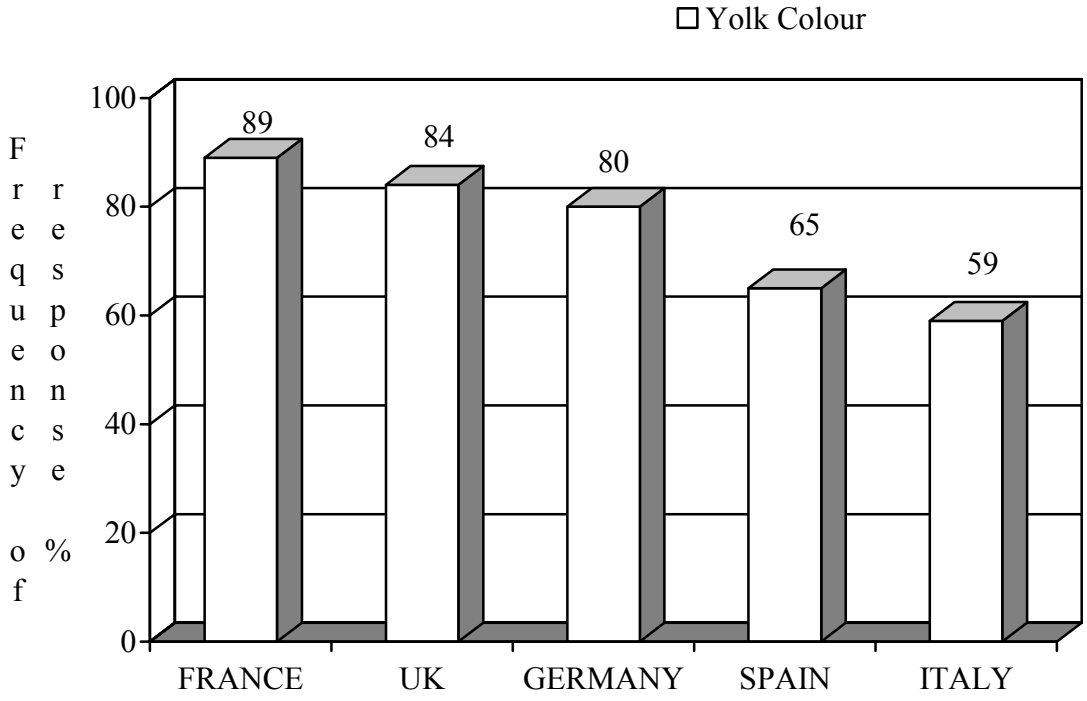

Figure 5. Criteria for a Quality Egg Yolk in Europe. n= 2,122 consumers (multiple response)

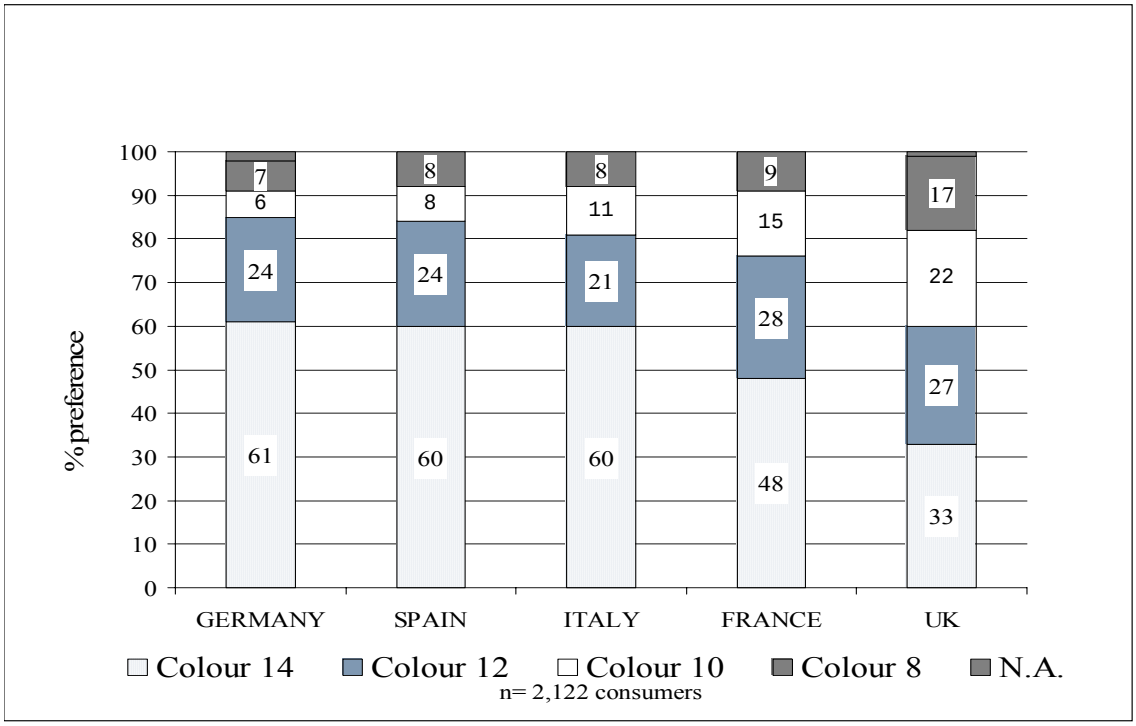

Figure 6. Criteria for a quality egg yolk. "And what would you expect the yolk of a quality egg to be like" – Preferred yolk colour, (DSM, Yolk Colour Fan)

Carotenoids have been historically used by the egg industry to provide the homogenous yolk colour demanded by consumers. Recently researchers have started to work on the potential biological benefits of some carotenoids for animals and humans. Recent data support the hypothesis that dietary canthaxanthin in breeders can modulate antioxidant systems in the developing chick as well as contribute to decreasing embryo mortality during hatching (Surai *et al*., 2003). On the other hand a large number of epidemiological studies in humans indicate that dietary intake of lutein and zeaxanthin is associated with a reduced risk of cataracts and age-related macular degeneration.

c) Improving the egg nutritional value

With regards to the nutritional value of the eggs it is important to remark that eggs are an excellent source of high quality and easily digestible protein as well as a good source of certain vitamins and minerals (Table 1). Levels of most vitamins in eggs are directly linked to the levels in the diet of the hen. Layer feed conversion rates have improved dramatically (approx. 40%) during the last 30 years so that it is essential to re-evaluate vitamin levels in feed accordingly if we want to offer eggs to consumers with, at least, the same nutritional value than in the past.

Recent research (Pérez-Vendrell *et al.,* 2004) has compared the effects of two vitamin levels in laying hen diets (average levels used by the Spanish egg industry versus Optimum Vitamin Nutrition (OVN) levels) on the vitamin deposition and quality of eggs. Results indicate that almost all vitamin levels (A, E, B1, B2, B12, pantothenic acid, folic acid, biotin) found in whole egg were statistically increased when hens were fed OVN premix (but not enough to be considered as vitamin enriched eggs), thus improving in that way the nutritive value compared to control eggs.

Table 1. Vitamin enriched eggs: current levels, R.D.A. (Recommended Daily Allowances in humans, Spain).

Vitamin	Amount / egg	Amount / 2 eggs (100 g)	RDA	% of RDA in 100 g of egg
Vitamin A	96 µg	192 µg	800 µg	24
Vitamin D	1.05 µg	2.1 µg	5 µg	42
Vitamin E	0.96 mg	1.92 mg	10 mg	19
Vitamin B1			1.4 mg	0
Vitamin B2	0.20 mg	0.40 mg	1.6 mg	25
Vitamin B6			2 mg	0
Vitamin B12	1.02 µg	2.04 µg	1 µg	204
Folic acid	15 µg	30 µg	200 µg	15
Niacin	2.04 mg	4.08 mg	18 mg	23
Biotin	12.12 µg	24.24 µg	150 µg	16
Calpan			6 mg	0
Vitamin C			60 mg	0

Most eggs produced nowadays contain a lower level of some vitamins compared to those levels published for eggs in the official nutritional tables. This fact is probably related to the improvement of layer feed conversion mentioned previously due mainly to better animal genetics and management compared to the past and the non-corresponding adaptation of vitamin levels in feed: the lower the vitamin intake by the hen, the lower the vitamin deposition in the egg. On the other hand, OVN eggs contained a higher level of those vitamins which allowed eggs to have a nutritional value similar or even better than the ones published in official nutritional tables.

Nutraceuticals such as vitamin enriched eggs are another way to increase their added value. Currently there are several brands in the market offering eggs enriched with vitamin A, E, Folic Acid, Biotin, etc as a way to balance the diet of certain segments of the human population such as pregnant women, children or elderly people. Egg producers must guarantee in these eggs a higher content of the enriched vitamin (min. +15% RDA, Recommended Daily Allowance for humans) compared to the average level of such vitamin in a standard egg.

Recent experiences have been run in France and the UK with laying hens fed a combination of vitamin D3 and its active metabolite, 25-hydroxyvitamin D3, added in the diet as HyD®. First results have shown the possibility of enriching eggs with vitamin D under EU conditions where there is a maximum vitamin D3 level of 3,000 IU in layer feed. By doing that the egg industry might contribute to the solution of an important need in human nutrition: the lack of enough vitamin D which is associated to an increase on the risk of osteoporotic fractures in older age groups and which is affecting a large proportion of Europeans (Ovesen, 2005).

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