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Syd Wilkins received his tertiary education at Hawkesbury Agricultural College and developed a career as a Poultry Officer in the NSW Department of Agriculture, becoming its Senior Poultry Officer by the late 1950's. In the mid to late 1960's he transferred to Allied Feeds, where he remained as a Senior Executive of the Allied Mills Group until his untimely death in 1982. During all this time he was very active in the affairs of the WPSA Australian Branch, including a period as President. He was also elected as one of the Vice Presidents of WPSA world body in the 1970's, a position he still held on this death. He was actively involved in the conduct of the 1974 and 1978 World's Poultry Congresses in New Orleans and Rio de Janeiro.

Syd Wilkins was also involved with the PHRF virtually from its beginning, and served many years as its Vice-President. He was the recipient of the Australian Poultry Award in 1974. Syd's career was contemporary with the application of the 20th century poultry technology revolution in Australia. In his own low-key and unassuming way he contributed very significantly to its progress.

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CONTENTS

DEVELOPMENT OF DIGESTIVE FUNCTION IN NEONATAL POULTRY: PHYSIOLOGICAL LIMITATIONS AND POTENTIAL

V. RAVINDRAN

Summary

The intestine is the primary nutrient supply organ. Early development of digestive function in newly hatched chick will therefore enable it to better utilise nutrients, grow efficiently and achieve its genetic potential. Published data on the growth and digestive function of the gastrointestinal tract in neonatal poultry are reviewed. Some potential strategies to improve gut growth and function are also presented.

I. INTRODUCTION

The first week after hatch is the most critical period in the life of a broiler chicken. When the chick emerges from the egg, its digestive and immune systems are still immature and it is not well prepared to face environmental challenges. First, there is the transition from yolk to oral nutrition. Associated with this are the substantial physical and functional development of the digestive tract and organs and the development of active immunity. The capacity to digest food and, absorb and transport nutrients appears to be limiting during the early life of broilers. To achieve their genetic potential, the neonate must quickly adapt to efficiently digesting and utilising nutrients from relatively complex exogenous dietary sources in which energy is supplied predominantly by carbohydrates.

As the growing period of broilers continues to shorten, the early nutritional management of the chick becomes increasingly important. In Australasia, with the average age to reach a slaughter weight of 2 kg averaging 33-35 days, the first week represents a large proportion of the total production period. The relative daily growth rate of broilers is also high during the early growth phase. On the first day body weight increases by 14 % per day, reaching a peak of 22 % per day on Day 11 and declining thereafter to approximately 9 % per day by Day 17 (Nitsan *et al*., 1991a). Changes that accompany post-hatch growth include phenomenal growth of the gastrointestinal tract (GIT), increased secretion of digestive enzymes, increases in overall gut surface area for absorption, improved nutrient transport systems and development of the immune system. An understanding of these changes may, therefore, allow better utilisation of the full capability of the immature GIT at hatch. This paper aims to briefly review the current literature on intestinal growth and function during the first 7 to 10 days of life and examine potential early nutrition strategies that may support maximum efficiency during the latter stages. The immune system of the bird is only partly developed at hatch (Schut and Myers, 1991), but the development of this system has been shown to respond to early nutrition and dietary nutrients. In particular, the development of gut-associated immune system is critical for protection against exogenous organisms during early growth. Excellent reviews of the ontogeny of the immune system in neonatal poultry are available (Dibner *et al*., 1998; Dibner, 2001) and will not be covered herein.

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II. GROWTH OF GASTROINTESTINAL TRACT

The post-hatch changes in the absolute and relatives size of GIT and organs have been studied by several researchers (Nir *et al*., 1993; Nitsan *et al*., 1991 a, b; Dunnington and Siegel, 1995) and an exhaustive review of the topic is available (Sklan, 2001). All studies point to dramatic growth of GIT during the first week, although there is disagreement on the exact point of maturation (see reviews by Dibner *et al*., 1996; Sell, 1996; Noy and Sklan, 1997, 1998).

The bird places a high priority on early intestinal growth to ensure the development of nutrient supply functions, which are necessary for subsequent growth of demand tissues, such as muscle (Croom *et al*., 1999). It has been reported that avian species with high growth rate are characterised by rapid early development of GIT and digestive organs (Lilja, 1983). The implication of this finding may be that potential inefficiencies in early GIT growth could limit maximum phenotypic expression in birds with superior genetic potential. Feed intake during early life appears to be limited by the size of GIT (Brake, 2001; Morel *et al*., 2001) and this may have effects on subsequent growth and efficiency, particularly in the fast-growing modern broiler.

(a) Gastrointestinal tract

In common with all organs, the GIT follows an allometric relationship with body weight. In the days following hatching, the weights of proventriculus, gizzard and small intestine increase more rapidly in relation to body weight than other organs and tissues. This enhanced growth is maximal in chicks at 4 to 8 days of age and thereafter there is a relative decline. The mass of the small intestine increases almost 600% within the first 7 days (Noy *et al*., 2001). The length of the small intestine and its individual component regions also increase with age. However, the relative proportion of jejunum plus ileum remain similar at hatch and at 21 days of age, accounting for about 82 and 84% of the length of the small intestine, respectively (Iji *et al*., 2001; Nir *et al*, 1993).

The early development of the small intestine is stimulated by the ingestion of feed. Changes occurring in the GIT seem to match the many fold increase in feed intake during the post-hatch period. Pinchasov (1995) reported that birds with immediate access to feed and water had significantly higher relative GIT weight at 30 hours post-hatch than those held without food for the first 24 hours. They also found that oral administration of nutrients into the crop immediately after hatching increased the GIT weight in a dose-dependent manner.

(b) Associated digestive organs

Nitsan *et al.* (1991a) showed that allometric growth of the pancreas reached a maximum of approximately 4 times that of body growth at 8 days of age and thereafter declined to approximately 2.5 times at day 23. Nir *et al*. (1993) reported that for the first two weeks after hatch the relative liver weight increased faster than bodyweight.

III. CHANGES IN GUT MORPHOLOGY

The function of the GIT is strictly related to its microscopic structure. The post-hatch microscopic changes in gut morphology have been reviewed (Dibner *et al*., 1996; Noy and Sklan, 1997, 1998). The dramatic post-hatch increases observed in the weight and length of the small intestine are small relative to the growth of gut mucosa. The villus height and area

increase rapidly at different rates in different intestinal segments, reaching a plateau at 6-8 days in the duodenum and ten days in the jejunum and ileum. Crypt depth, which reflects enterocyte maturation rate, increased linearly in both duodenum and jejunum until 10-12 days (Uni *et al*., 1995).

Iji *et al*. (2001) showed an increase in crypt depth, villus height and villus surface area in chicks between hatch and 21 days of age. They found a highly developed gut mucosa structural development at hatch, with gross changes occurring in the mucosa structure over the 21 days, which was attributed to exposure to dietary nutrients. Cell proliferation was rapid, and cells also migrated rapidly up the villus, suggesting a rapid response to short periods of exposure to dietary factors. In very young chicks, enterocytes approached the extrusion zone within 96 hours of formation, but were only three quarters of the distance in chicks older than 14 days. This may be associated with a shorter enterocyte life span, with a greater need for cell replenishment, which would increase nutrient demand for gut mucosal maintenance. As enzyme activity is expressed over a large proportion of the villus, the increase in total villus area will improve the total digestive capability of the chick.

IV. DEVELOPMENT OF DIGESTIVE ENZYMES

The pancreas and intestine are functionally immature at hatch, but undergo rapid maturation thereafter. The secretion of digestive enzymes by pancreas and the brush border of the small intestine are low at the time of hatch (Sell, 1996), but increase after hatch although the rate of increase was different for different enzymes (Tarvid, 1995; Noy and Sklan, 1997).

Activities of lipase, amylase and proteases all increase during the first week of life. Pancreatic amylase activity has been shown to increase three-fold between 1 and 10 days post hatching, whereas trypsin and lipase activities increased five to six-fold (Nitsan *et al*., 1991 a, b; Nir *et al*., 1993). Similarly the total activity per gram of intestine increased steadily for maltase and sucrase, which are important enzymes in carbohydrate digestion (Uni *et al*., 1998).

It is, however, not clear whether the availability of enzymes limits early growth. Nir *et al.* (1993) observed that, during the first week of life, broiler-type chicks consumed almost twice as much feed as egg-type chicks. This large feed intake was reflected in the intestinal contents, but not compensated for by either an increase in the relative sizes of the pancreas and small intestine, or by the activity of the digestive enzymes. It was concluded that in the broiler-type chicks a similar secretion of digestive enzymes had to cope with a higher amount of chyme than in the egg-type chicks. This suggests that the development of the pancreatic enzyme system may be a factor limiting broiler chick performance in early life. On the other hand, differences in digestive enzyme levels between lines selected for light and heavy 8-week body weights could be observed at 3-4 days after hatching but disappeared quickly (Nitsan *et al*., 1991b; Dunnington and Siegel, 1995; Uni *et al*., 1995).

V. DIGESTION OF NUTRIENTS

Information on the digestion or utilisation of nutrients by the newly hatched chick is scanty, but it is generally accepted that digestibility of nutrients increases over the first week of life. Starch digestion appears not limiting in young chicks (Moran, 1982) and digestibility of starch in maize-soybean diets, measured at the excreta level, is reported to reach values of over 95% by 4-10 days of age (Brake, 2001; Noy and Sklan, 1997; Uni *et al*., 1995). This aspect, however, may have to be re-evaluated in light of current evidence that ileal

digestibility of starch from wheat can be as low as 51% even in 3-week-old broilers (Svihus and Hetland, 2001).

Several studies have shown that fat digestion may be low in young chicks and increases with age (Carew *et al*., 1972; Whitehead and Fisher, 1975; Polin and Hussein, 1982; Krogdahl and Sell, 1985). One report, however, indicates that the capacity to digest fats is fully developed by 4 days of age (Smulikowska, 1998). The poor digestibility in young chicks has been attributed to low lipase activity or to insufficient bile secretion. Polin and Hussein (1982) reported that supplementation with bile salts enhanced fat digestion in 7-day-old chicks. It should be, however, noted that the type of diet and fats used varied between the above reports.

Digestibility of both unsaturated and saturated fats is low in young chicks (Carew *et al*., 1972; Polin and Hussein, 1982), but evidence clearly indicates that fats with high proportions of saturated fatty acids are poorly digested compared with unsaturated fatty acids. Carew *et al*. (1972) reported that digestibility of maize oil was 84% from 2 to 7 days of age compared with 40% for beef tallow. Insufficient bile secretion is thought to be the major contributing factor to this difference. The solubilisation and absorption of saturated fatty acids are more negatively affected in the absence of bile salts than that of unsaturated fatty acids (Garret and Young, 1975). According to Smulikowska (1998), the low initial bile level limits the formation of micelles necessary for the digestion of tallow, which contains high levels of the non-polar palmitic and stearic fatty acids. These cannot form mixed micelles spontaneously, but only in the presence of micelles formed from unsaturated fatty acids and conjugated bile salts. As a result, fat sources such as soybean oil are more readily digested than tallow by young chicks.

Perhaps the poor digestion of protein, indicating limited proteolysis in young chicks is one ~~intriquing~~intriguing aspect of digestion in the immediate post hatch period.. Protein digestibility continues to increase from 70-80% on day 4 to 90% on day 10-14 (Noy and Skan, 1997; Uni *et al*., 1995). Noy and Sklan (1995) found that ileal protein digestibility of a maize-soybean diet was 78-80% in 4 to 7-day-old chicks. The digestibility increased to 90% at 21 days. This observation is surprising since several researchers have reported that pancreatic enzymes are not a limiting factor during week 1 and perhaps points to our lack of knowledge on other related processes such as the efficiency of absorption and amino acid transport systems.

The poor digestibility of fats and proteins are reflected in poor energy utilisation efficiency during the first week of life. Zelenka (1968) found that the metabolisable energy content of the diet was 11.29 MJ/kg at Day 7 and this increased to 12.59 MJ/kg ay Day 14. Polin and Hussein (1982) similarly found that the metabolisable energy content increased from 12.43 MJ/kg at Day 7 to 13.60 MJ/kg at Day 14.

VI. CONCLUDING REMARKS AND FUTURE RESEARCH NEEDS

The digestive system is relatively underdeveloped at hatch and appropriate dietary strategies during the first week of life are therefore relevant to achieve the maximum genetic potential of the modern broiler. A shift from blood-borne to oral nutrition, essential to provide substrates for the rapid growth of demand organs (muscle, bone etc), requires rapid growth and maturation of the supply organs, including the gastrointestinal system. Available evidence conclusively demonstrate that short- and long-term increases in body weight (Noy and Sklan, 1998) and development of the immune system (Dibner *et al*., 1998; Dibner, 2001) can be obtained by early access to feeding. These growth responses have been attributed to the stimulation of GIT function and the resultant improvements nutritional maturity.

Nevertheless, some data also suggest that intestinal growth (Noy and Sklan, 1997) and function (Croom *et al*., 1999) of modern broiler chicks may not be adequate to support efficient growth. Thus scope exists for the modern bird to attain its genetic potential though nutritional manipulation of digestive capacity during the critical first few days after hatch. Even an enhancement of early GIT growth by a day or two will go a long way towards improving the efficiency of the bird over its grow-out period. Some future areas for research are suggested below.

(a) Nutrient requirements of young chicks

Little precise data is available on the nutritional requirement of the broiler chick during the first 7 days of life. Available data is based on experiments that start at 7 days or include the first 7 days within the global 3- or 4-week "starter" period. There is clear evidence that the nutritional requirement of young chicks are different (e.g. glycine + serine, Schutte *et al*. 1997; sodium, Brake, 2001) A re-evaluation of this approach is urgently needed in modern broilers since the first 7 days now represent 20% of their productive lives. These studies must also include the possible differences between male and female chicks. Gender effects are often overlooked, but can cause significant differences in GIT growth.

(b) Manipulation of GIT growth prior to hatching

Opportunities to improve GIT growth in the embryo may be possible through breeder nutrition and this should be explored. *In ovo* administration, into the amnion fluid of the late term embryo, of selected amino acids and sugars also represent a potential area for future research. Another avenue would be *in ovo* administration of certain bioactive factors during incubation which have been shown to cause dramatic increases in growth and feed efficiency as a result of increased post hatch skeletal muscle growth (Croom *et al*., 1999).

(c) Enhanced functional development

Studies are needed on the role of specific nutrients on the maturation of the gastrointestinal system and immune system development; these may include vitamins, minerals, polyunsaturated fatty acids, amino acids and peptides. A feed supplement based on this concept is commercially available and has been shown to improve early growth of GIT and the bird (Brake, 2001).

(d) Influence of carbohydrate sources

Our current knowledge of ontogentic allomtery of GIT and nutrient digestion is based on chicks fed maize-soybean diets. Studies are needed to define these aspects in chicks fed wheat- and sorghum-based diets and the influence of exogenous enzymes will also be relevant in this context. As noted by Lilburn (2002), the choice of ingredients for pre-starter diets needs to be addressed from the perspective of formulating to maximise availability of nutrients, especially carbohydrates, as opposed to formulating to a published set of energy or amino acid levels.

(e) Enhanced nutrient absorption.

Biotechnology may provide opportunities for improving nutrient absorption. e.g. pro-absorptive agents such as epidermal growth factor (EGF) and peptide YY (YYY). These gastrointestinal factors have been shown to enhance the intestinal absorption of carbohydrates, fatty acids and amino acids (Bird *et al*., 1996).

(f) Role of microflora

The gut microflora play a key role in preventing colonisation of enteropathogens and the development of the gut immune system. Interactions between feed composition and intestinal microflora with reference to gut growth and function are well known (Drasar and Barrow, 1985; Tannock, 1999). It is also known that feed additives designed to modify the intestinal flora, such as exogenous enzymes and antibiotics, may also influence growth and microscopic structure of the intestine. With the likely ban on the use of in-feed antibiotics and possibly ionophore coocidiostats in the future, an understanding of the interrelationship between gut microbiology and gut health and function becomes increasingly relevant. In this regard, the recent advances in molecular-based techniques to characterise gut flora profile are particularly exciting.

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FEEDING PATTERNS IN CHICKENS: EFFECTS ON ENDOCRINE AND METABOLIC STATUS

J. BUYSE and E. DECUYPERE

Summary

Domestic fowls are continuous feeders and the daily feed intake pattern is mainly determined by the imposed lighting regimen. Turning broiler chickens from having a nibbling feeding pattern into periodic or meal eaters can improve feed conversion, providing there are a sufficient number of meals per day of high quality feed. The use of intermittent lighting readily imposes a meal-feeding pattern on chickens that were previously continuous feeders. These lighting schedules assure an improved feed conversion, nitrogen retention efficiency and more uniform slaughter weight. The underlying causal physiological mechanism is the more concave growth trajectory. Feeding only a single meal per day or alternatively, skip-a-day feeding, have profound effects on endocrine status and intermediary metabolic processes. These changes can be explained in terms of maintaining metabolic homeostasis. Refeeding gradually restores these processes, and the time course of these changes in plasma metabolite levels precede those of hormones. Dietary macronutrients and especially protein also affect endocrine and metabolic functioning.

I. INTRODUCTION

Domestic fowls are photoperiodic nibblers by nature, meaning that they consume feed continuously throughout the entire daylight period. Commercial broiler chickens are reared under continuous lighting because it is believed that feed intake is then maximal and hence growth rate would also be maximal. In contrast, laying hens are kept under shorter photoperiods e.g. 14 or 16 h light per day because such lighting programs are necessary for the entrainment of the ovulatory cycle and hence egg production rate. When feed is provided *ad libitum*, feed intake behavior of continuously lit broiler chickens consists of short though very frequent and regular feeding bouts ('nibbling'), giving rise to a rather constant daily feed intake pattern. Laying hens show an increase in feed ingestion at the onset of lighting and an anticipatory increase towards the end of the photoperiod (Savory, 1980). Broiler chickens reared under a day: night lighting schedule also develop such an anticipatory feed intake behavior (Savory, 1980; Buyse *et al*., 1993). The changes in feeding pattern, whether compulsorily imposed (e.g. through meal feeding; fasting: refeeding cycles or skip-a-day feeding), or induced by the lighting program, have pronounced effects on the physiology of birds and hence on their performance.

The present paper focuses on the physiological consequences of alterations in feed intake pattern (quantitative factor) as well as diet composition (qualitative factor) for domestic fowl, mainly broiler chickens.

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II. CHANGING THE FEEDING PATTERN

a) Shorter photoperiods (day: night schedules)

Broiler chickens have a voracious appetite and nibble feed throughout the photoperiod. By providing nearly continuous light (CL: 23 h light (L): 1 h darkness (D)), it is assumed that feed consumption is then maximal, a condition that is believed to be necessary for maximal body weight gain. Indeed, reducing the daily photoperiod from 23L to 18L or less has a negative effect on growth rate due to a lower feed consumption or lower feed efficiency (Robbins *et al*., 1984). If daylength is not too short (>12 – 14 h L), feed consumption during the scotoperiod is insignificant (Buyse *et al*., 1993). Broiler chickens however can learn to eat during darkness, and older work showed that light is not really essential for feeding to occur properly (Cherry and Barwick, 1962; Squibb and Collier, 1979). On the other hand, broiler chickens learn to develop strategies in order to try to overcome the long nocturnal period without feed intake. This includes increased anticipatory feed intake towards the end of the photoperiod, mechanical storage of ingesta in the gastrointestinal tract and its gradual release during the night (longer nocturnal feed transit time) (Buyse *et al*., 1993) and reduced gastric motility (Duke and Evanson, 1976). During the nocturnal fast, heat production declines by more than 40 % (Buyse *et al*., 1993) and heart rate and rectal temperature drop as well (Klandorf *et al*., 1978; Decuypere and Kühn, 1984). This reduction in metabolic rate is attributable to lower nocturnal levels of the metabolism stimulating thyroid hormone 3,3',5-triiodothyronine (T_3) (Klandorf *et al*., 1978; Buyse *et al*., 1987). The anticipatory feed intake behavior towards the end of the photoperiod needs time to develop (Squibb and Collier, 1979) and this learning process can be speeded up by simulated dusk (Savory, 1976).

The practical relevance of such day: night lighting schedules for broiler chickens will necessarily receive renewed interest as in the framework of EU welfare legislation for broiler chickens, continuous lighting will be forbidden. It must be recognized that nearly continuous lighting has detrimental effects on sleep, eye conditions, physical activity and leg health, immune-competence, incidence of ascites and Sudden Death syndrome (for reviews, see Gordon, 1994; Buyse *et al*., 1996a,b).

b) Meal feeding schedules

The obligatory conversion of broiler chickens from being 'nibblers' to 'periodic feeders' may improve growth rate and feed efficiency, at least when a sufficient number of meals are provided on a daily basis and with a diet of a high energy content (Conard and Kuenzel, 1978). Furthermore, Reece *et al*.,(1986) concluded that certain meal feeding programs might improve feed conversion during periods of both hot and cold temperature stress but not if grow-out temperature is normal. Meal feeding also reduces the incidence of leg abnormalities, independently of changes in body weight (Su *et al.*, 1999).

c) Intermittent lighting schedules

A behavioral pattern of meal feeding is easily achieved when intermittent lighting (IL) schedules consisting of repeated short L: D cycles such as 1L:2D or 1L:3D are used. The alternating short light: dark cycles drastically change the daily feed intake pattern. Feed intake is principally limited to the L period of each L: D cycle, though some feeding does occur during the D period and more so as the D period increases in duration (Buyse and Decuypere, 1988). The plasma triglyceride (TG) concentrations clearly follow the feed intake

pattern as increased levels are observed during the L period and beginning of the D period, and decrease towards the end of the D period. The increase is explained by the digestion of dietary lipids and their incorporation in portomicrons and Very Low Density Lipoproteins (VLDL) on the one hand, and the *de novo* lipogenesis and subsequent secretion of VLDL on the other hand. Plasma glucose levels remain unchanged across the L: D cycle, illustrating again the strict glucose homeostasis in chickens (Simon, 1989). This periodic feeding pattern is also associated with marked changes in energy expenditure rhythmicity (Buyse *et al*., 1994) compared to the more or less constant heat production of CL chickens throughout 24 h each day. When lights are turned on, heat production increases rapidly due to increased heat increment associated with feeding and physical activity. This may even lead to a higher heat production during this period of illumination compared to CL chickens (Ohtani and Leeson, 2000). When lights are turned off, heat production declines although at a slower rate (Buyse *et al*., 1994; Ohtani and Leeson, 2000). IL does not affect feed transit time (Buyse *et al*., 1994) but the crop alternatively is filled and emptied with the pattern of intermittent lighting (Hooppaw and Goodman, 1976).

There are many reports available comparing the impact of intermittent lighting programs introduced at young age on broiler performance compared to the widely used CL schedule 23L:1D (for reviews, see Buyse *et al*., 1996a,b). In summary, broiler chickens reared under IL attain similar or even higher commercial slaughter weights as compared to their CL counterparts, though endogenous (sex, genotype) as well as exogenous (diet composition, feeder space, etc) interact with the imposed lighting schedule on body weight. In contrast, feed conversion is consistently improved by using IL. The effect of IL on fat deposition is again variable as literature reports indicate reduced (in most cases), equivalent or even higher fat accretion with IL compared to CL. The major causal mechanism for the improved biological performance (and in this case also the financial returns) with IL is undoubtedly the changed growth trajectory. Imposing IL at young age (after a few days of CL) is associated with a temporary reduction in feed intake and hence growth rate. However, when the chicks are accustomed to the new lighting environment, compensatory growth is manifested. Due to this concave growth trajectory cumulative maintenance needs are reduced, which contributes to the improved feed conversion. IL schedules do not affect apparent metabolizability (Buyse *et al*., 1994; Ketelaars *et al*., 1986).

During the period of compensatory growth, endocrine changes occur as well. Indeed, IL chickens manifesting compensatory growth are characterized by significantly elevated plasma growth hormone (GH) levels, which is the mechanistic result of a higher GH mass secretion per burst, hence higher GH amplitude (Buyse *et al*., 1997). It is hypothesized that the enhanced GH secretion is a causative factor for the improved protein retention (Buyse and Decuypere, 1994) that occurs during catch-up growth (Buyse *et al*., 1996c). In addition, during the period of compensatory growth male broilers have significantly higher plasma testosterone levels (Kühn *et al*., 1996), suggesting a positive role of this androgen in catch-up growth and probably also in GH secretion.

d) Daily fasting and refeeding cycles

In intensive livestock production, it is important to provide the animals with sufficient feed of high quality in order to obtain the best performance. However, there is only one branch of animal husbandry that requires a rigorous feed restriction: broiler breeder management. Indeed, if broiler breeder pullets are fed *ad libitum*, the hens become too heavy and obese, many animals have to be culled and above all, reproductive capacity is very low. . Therefore, pullets have to be severely restricted in their daily feed allowance in order to reach

an acceptable production of fertile eggs of sufficient quality. Such a single daily fasting and subsequent refeeding cycle has profound effects on metabolic and endocrine functioning.

We have recently investigated the effects of daily meal feeding (one daily meal of 40 to 45 g/chicken/day, consumed in about 0.5 h) as practiced during breeder rearing on the somatotrophic and thyrotrophic axes of 4-week-old broiler chickens (Buyse *et al*., 2000; Buyse *et al*., 2002). Compared to their *ad libitum* (AL) fed counterparts, feed-restricted (FR) chickens showed a much more pronounced pulsatile GH release as reflected in a higher amplitude and mass of GH secreted per burst and pulse frequency. Free and total hepatic GH receptor numbers of FR chickens were significantly lower than those of AL chickens, indicating that high circulating GH levels down regulate their own hepatic receptors. The reduced hepatic GH receptor capacity is also assumed to be the reason, at least in part, for the lower plasma Insulin-like growth factor-I (IGF-I) levels in FR chickens. This uncoupling of IGF-I from GH – which is also recognized in other animal species and in humans – represents a mechanism to reduce cell growth and proliferation in favor of substrate (catabolism) and energy (lipolysis) mobilization in order to maintain homeostasis. It was also demonstrated that feed restriction of broiler breeder pullets resulted in a progressive increase in plasma IGFBP-28kDa and IGFBP-34kDa but not in IGFBP-40kDa levels (Bruggeman *et al*., 1997). In this way, there is few free IGF-I available for its anabolic actions. During fasting conditions, energy expenditure decreases due to lower plasma T_3 levels as a means to preserve body homeostasis (Decuypere and Kühn, 1984). All studies have also clearly shown that plasma thyroxine (T_4) levels are increased with fasting (e.g. Buyse *et al*., 2000; Reyns *et al*., 2002). Simultaneously, hepatic T_3 and T_4 concentrations are decreased and increased, respectively (Reyns *et al*., 2002), illustrating that the T_4 availability in the liver is not diminished during fasting. All relevant studies reported a marked augmentation of the hepatic IRD-III activity during fasting whereas for ORD-I activity, a decrease or no changes were observed.

Feed deprivation is also associated with reduced plasma TG, uric acid, and lactate levels, whereas plasma free fatty acid (FFA) levels and ketone bodies are increased. It is evident that dietary sources of lipids are absent in feed-deprived animals and that the *in vivo* hepatic fatty acid synthesis activity is strongly impaired (Muiruri *et al*., 1975), which explains the low plasma TG levels in these animals. As fasting is associated with low circulating insulin (lipogenic activity) and elevated glucagon (lipolytic and antilipogenic activity) levels in chickens (Simon, 1989), this may be the endocrine basis of the reduced hepatic lipogenic activity in feed-deprived chickens. The elevated lipolytic activity in feed-deprived animals is very likely to be induced by glucagon, a very potent lipolytic hormone in avian species (Langslow and Hales, 1969) and possibly also by GH for which the lipolytic properties have been clearly demonstrated *in vivo* (Vasilatos-Younken *et al*., 1988) and *in vitro* (Buyse *et al*., 1992a). The high plasma FFA levels could also be directly responsible for the inhibition of the hepatic lipogenesis (Leveille *et al*., 1975). Plasma lactate levels are significantly depressed in feed-deprived chickens compared to the levels of their *ad libitum* fed counterparts. In view of the reduced metabolic rate of feed-deprived animals, there is no need for anaerobic energy production; hence lactate production from glucose is limited. The reduced plasma uric acid levels result from the absence of dietary protein degradation and low protein turnover. Wilson and Miles (1988) reported that plasma uric acid levels of meal-fed broiler breeder males were highest at 2 h and lowest at 24 h postfeeding. However, prolonged fasting causes again an increase in plasma uric acid levels due to degradation of endogenous protein sources (Buyse *et al.*, 1995). At first sight, the effects of fasting on plasma glucose levels of fowl seem to be inconsistent as some studies report a decline in plasma glucose concentrations whereas others found no differences in glycemia. However,

the duration of feed deprivation is of utmost importance in this respect, as plasma glucose levels may initially decline but are then restored after prolonged fasting due to increased gluconeogenesis (Decuypere and Kühn, 1984; Dewil *et al.,* 1999).

Refeeding gradually reverses the fasting-induced alterations in plasma hormone levels. Plasma GH and T_4 levels decrease whereas plasma T_3 and IGF-I levels increase after the introduction of feed (Buyse *et al*., 2000; Buyse *et al*., 2002). The activities of intermediary metabolic processes are restored as well; though typically show an initial 'overshoot' phenomenon (relative to control *ad libitum* fed counterparts) as demonstrated for plasma glucose (Buyse *et al*., 2002), and insulin (Rosebrough *et al*., 1984) levels, hepatic glycogen storage ((Rosebrough *et al*., 1984) and *in vivo* lipogenesis (Muiruri *et al*., 1975; Leveille *et al*., 1975).

It is important to stress that the time course of postprandial changes differs between metabolites, which clearly precede those of endocrine factors. The order in responsiveness after feed was reintroduced was glucose>uric acid≥FFA>lactate>TG for the plasma metabolites and GH>T_3>T_4>IGF-I for hormones (Buyse *et al.,* 2002). Based on these different postprandial time courses of changes, several functional relationships can be proposed. Glucose is believed to be the primary trigger for the normalization of the effects of fasting on these plasma parameters by restoring hepatic GH receptor capacity as well as decreasing IRD-III activity.

The magnitude of the postprandial hormone and metabolite responses is also dependent on the composition of the diet provided. Indeed, Collado and Tasaki (1981) clearly showed that the postprandial increase in plasma TG levels was more pronounced when the introduced diet contained a low % of protein (12 %) compared to a high protein diet (30 %). The sharp postprandial increase in plasma glucose levels is also more pronounced when carbohydrate is included in the provided meal, though without concurrent changes in plasma insulin concentrations (Aman Yaman *et al*., 2000). These authors also showed that dietary protein intake is more important for the stimulation of protein fractional synthesis rate in muscle and liver of previously fasted chickens compared to their carbohydrate or protein consumption. It was also demonstrated that the rapid increase (within 1 h) in liver and muscle protein synthesis in refed chickens was not correlated with circulating plasma IGF-I concentrations, of which the postprandial increase lags considerably behind (Buyse *et al*., 2002).

e) Skip-a-day feeding

Feeding on alternate days or skip-a-day feeding programs exert even a more drastic effect on bird's physiology. This management practice used to be applied in broiler breeder rearing as flock uniformity would be improved (Costa, 1981). However, such feeding programs lead to a higher feed conversion as reflected in the lower body weights of skip-a day fed pullets compared to their daily fed counterparts receiving the same feed allotment (e.g. Bennett *et al*., 1990). Indeed, on the feeding day, all skip-a-day fed birds can consume enough feed, and the excess energy is stored mainly as fat. On the feed-off days, this energy is mobilized and the FFA are oxidized to yield metabolizable energy. The diurnal variations in heat production (HP) have been clearly demonstrated by Bennett *et al*. (1990). On the feeding days, skip-a-day broiler breeder pullets had a much higher HP than their daily fed counterparts whereas the opposite was true on the feed-off days. On a daily basis, the total HP was significantly higher for the skip-a-day fed pullets, which were hence less efficient. These repeated cycles of energy storage and energy expenditure are energetically less efficient and are also reflected in the Respiratory Quotient, averaging 1.05 on the feeding

days and decreasing to 0.67 on the feed-off days (daily fed hens had a RQ ranging from 0.8 to 0.9). These RQ values reflect carbohydrate oxidation (and fatty acid synthesis) and fatty acid oxidation, respectively. During the repletion days, the *in vivo* synthesis of proteins in various tissues was also much higher than during the fasting days, illustrating the anabolic status when feed is available (Pinchasov *et al*., 1988). Alternate-day feeding does however improve immunocompetence (Praharaj *et al*., 1996) and reduces the prevalence of leg deformities (Boa-Amponsem *et al.*, 1991).

III. DIET COMPOSITION

Not only do the quantity of food consumed and the feeding frequency have marked effects on chicken performance and on endocrine and metabolic status, but also the diet quality, and in particular its macronutrient content affect performance (Buyse *et al*., 2001). The effects of dietary crude protein (CP) levels on these physiological processes are best documented. In summary, a reduction in dietary CP content results in increased plasma GH concentrations, as a consequence of the higher amplitude, baseline and pulse frequency values compared to broilers provided an isoenergetic diet with a normal protein (20 – 22 % CP) content (Buyse *et al*., 1992b). Again, it is believed that the enhanced pulsatile GH secretion is a causal mechanism for the improved protein conversion efficiency of moderately protein-restricted animals (Buyse and Decuypere, 1994). Chickens fed on a low CP diet also have reduced plasma uric acid levels, indicative of a low protein degradation rate, besides their low protein ingestion (Malheiros *et al*., unpublished results). Plasma IGF-I concentrations are lowered by feeding low CP diets whereas plasma IGF-II levels remain unchanged (Leili and Scanes, 1998). In addition, circulating IGFBP levels are also influenced by protein malnutrition, although dependent on their molecular mass and severity and duration of the protein restriction (Leili and Scanes, 1998). However, in contrast to feed-deprivation, there is no evidence that protein restriction is associated with a down regulation of hepatic GH receptors, but rather the contrary has been found. Protein-restricted chickens are characterized by reduced plasma T_4 and increased plasma T_3 levels (see Buyse *et al*., 2001). This is very likely to be a consequence of the high circulating GH levels, as this peptide is known to inhibit the IRD-III activity (Darras *et al*., 1995). The elevated plasma T_3 levels are thought to be the causal mechanism for the increased heat production of chickens fed a low CP diet, which is a way to deal with their 'luxus' energy consumption, besides a higher fat deposition (Buyse *et al*., 1992b). The higher fat deposition in protein-restricted chickens is also reflected in elevated *in vitro* and *in vivo* lipogenesis and hepatic lipogenic enzyme activities (e.g. Rosebrough and Steele, 1985).

We recently observed that an isocaloric substitution of fat by carbohydrate calories in isocaloric and isonitrogenous diets has no clear effects on plasma hormone and metabolite levels, except for a temporary elevation in plasma T_3 and FFA levels (Malheiros *et al*., in press and unpublished results). These results again demonstrate that the protein content *per se* has greater influence on these parameters and hence on chicken performance.

Although there is a wealth of information on the effects of dietary energy and macronutrient content on body weight gain and composition and on the amount of feed consumed, there is little information on how these dietary variables affect the feed intake pattern or the voluntary regulation of feed intake in terms of meal size and frequency in the domestic fowl. Taher *et al*. (1985) reported that when SCWL roosters were abruptly changed to a diet with a higher energetic density, they decreased the amount of feed consumed by decreasing meal size and duration but increasing the number of meals. Roosters changed to a low energy diet ate more feed, increased their meal size and duration as well as number of meals. Other factors that affect feeding patterns are physical form of the diet (mash *versus*

pellets: Nir *et al*., 1994; Aerni *et al*., 2000; protein concentrate *versus* ground, cracked or whole grain corn: Yo *et al*., 1997) and environmental temperature (Taher *et al*., 1985).

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PHYSIOLOGICAL AND CHEMICAL CONSTRAINTS TO STARCH DIGESTION AND GLUCOSE ABSORPTION IN BROILERS

B. SVIHUS

Summary

It is now clear that starch digestibility may be sub-optimal in broilers when certain sources of starch are fed. This is probably caused by an incomplete digestion that is either due to a too low enzyme secretion, and/or an over-consumption of feeds and thus a too short time available for digestion. Glucose absorption and metabolism does not seem to be a limiting factor. Structural features of starch that may affect digestibility are discussed, and these may include amylose/amylopectin ratio, starch granule size, and quality and quantity of non-starch components on the surface of the starch granule.

I. INTRODUCTION

The high ability of the chicken to digest starch and absorb glucose, as an adaptation to a diet with large amounts of starch-containing seeds, is well known. To achieve such a high utilization rate of starch, the chicken has a large pancreas that secretes a juice with a high concentration of enzymes (Hulan and Bird, 1972). Thus, starch is effectively and rapidly broken down in the duodenum and jejunum. Through work with humans, evidence arose (Anderson *et al.*, 1981; Englyst and Cummings, 1985) that a fraction of starch was not digested in the small intestine. This fraction was later termed resistant starch (Berry, 1986; Englyst *et al.*, 1996). In some Australian studies with broilers, faecal starch digestibilities of wheat diets lower than 0.82 were observed (Mollah *et al.*, 1983; Rogel *et al.*, 1987; Choct *et al.*, 1995), suggesting that starch resistant to digestion can be a significant factor also for these types of animals. Low starch digestibilities have recently been confirmed on a faecal (Wiseman *et al.*, 2000; Maisonnier *et al.*, 2001; Marron *et al.*, 2001; Svihus and Hetland, 2001) as well as an ileal level (Marron *et al.*, 2001; Svihus, 2001; Svihus and Hetland, 2001; Weurding *et al.*, 2001; Hetland *et al.*, 2002). In several of the reports, a considerable variation among cereal species, and also among varieties within species, has been observed. This indicates that some factors intrinsic to the cereals may reduce digestibility of starch. One such factor may be soluble fibre, and it has been shown that starch digestibility may improve as a result of addition of xylanase to wheat diets (Choct *et al.*, 1995, 1999; Svihus, 2001). However, recent results indicate that starch is not fully digested even when enzymes are added (Svihus, 2001). A lack of correlation between starch digestibility and viscosity may also indicate that other factors are causes for low digestibility of starch (Carré *et al.*, 2002). In this review, an attempt will be made to investigate potential causes for an incomplete starch utilisation by broilers.

II. STARCH DIGESTION

Starch digestion generally occurs in the small intestine by the action of α-amylase, dextrinase and glucoamylase. A limited extent of swelling of starch granules may take place

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in segments of the alimentary canal anterior to the small intestine, but it is reasonable to believe that starch to a large extent will be present as intact starch granules in the small

intestine. Starch in extruded feeds, where the granules have disintegrated during processing, represents an exception from this general rule. Lynn and Cochrane (1997) observed that digestion occurred along channels in the plane of the central disc of the lenticular wheat starch granule. It was also observed that these channels gave rise to extensive digestion in the interior of the starch granule, resulting in disintegration of the centre of the granule prior to the periphery. Planchot *et al.* (1997) also concluded that amylolysis occurred first randomly along furrows and then through pores that increased in size. Hydrolysis mainly occurred along amorphous zones.

It is uncertain whether enzyme secretion may be a limiting factor for starch digestion, since literature seems to be scarce on this topic. However, the numerous publications showing a reduced starch digestion rate under certain circumstances indicate that enzyme secretions may be a limiting factor. It is possible that for broilers, the very short time available for digestion may result in enzyme production becoming a limiting factor. Results from our laboratory indicate that digestibility of a diet with a low digestibility increases when feed intake is reduced due to changes from pellets to mash (Svihus and Hetland, 2001), and this indirectly indicates that feed intake is correlated to starch digestibility. Further studies at our laboratory have indicated that there is a slight negative correlation between feed intake of individual birds on identical diets and the apparent metabolisable energy (AME) value determined. Such negative relationships are illustrated in Figures 1 and 2.

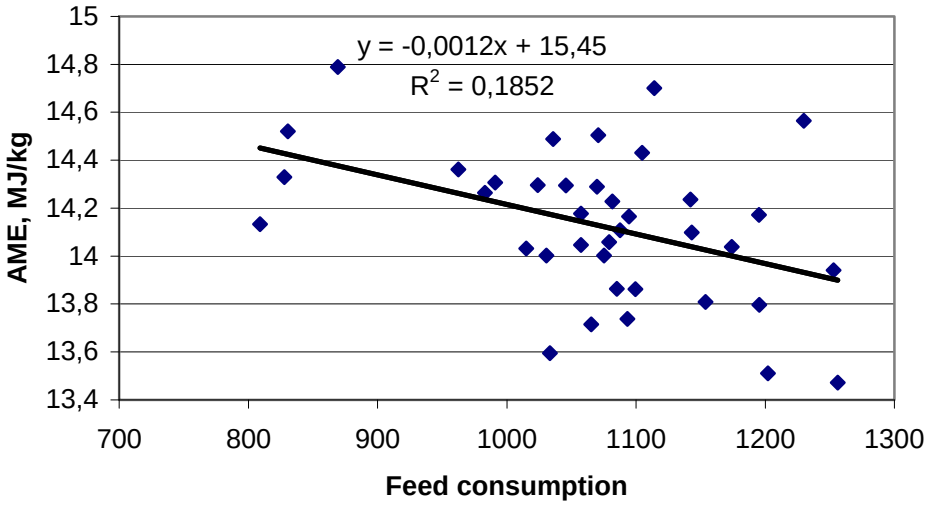

Figure 1. Relationship between feed consumption and AME determined on individual birds fed on a ground maize-based diet.

These data also show that feed intake varies among birds. It is thus possible that over-consumption among individual birds in a flock results in impaired starch digestion due to an increased passage rate. This is consistent with many observations of a reduced variation among individuals concurrent with an increased starch digestibility (e.g. Svihus and Hetland, 2001). The question may then arise on the causes for over-consumption among individual birds. Peter Siegel was among the first to indicate that intensive breeding for weight gain had resulted in broilers that were capable of over-consuming feed due to disturbance of the appetite control centre in the brain (Siegel and Dunnington, 1987). Lacy *et al.* (1985) showed that broilers, as opposed to layers, did not respond to intra-hepatic glucose infusions with reduced feed intake. This indicates also that peripheral appetite control centres have been disturbed in broilers. It can thus be postulated that modern breeds of broilers may over-consume feeds, with a resulting impairment in starch digestibility for some diets. Results from

our laboratory indicate that there may be an interaction between gizzard function and over-consumption of feeds. When feeds have more structure, either through the use of whole cereals or through the addition of large fibre particles (oat hulls or wood shavings) to the diet, an improvement in starch digestibility and a reduced variation between birds is commonly observed (Rogel *et al.*, 1987; Svihus and Hetland, 2001; Hetland *et al.*, 2002). Our hypothesis is that an active and well-developed gizzard will function as a food intake regulator that will assure that the bird does not over-consume feeds. This is due to the active retention of large particles until they are broken down, which results in a larger filling of the gizzard and thus less room for more feed to be consumed, as well as a stimulative effect of gizzard activity on gastro-duodenal refluxes. Unpublished results from our laboratory indicate that the use of whole wheat or insoluble fibres stimulate gastro-duodenal refluxes.

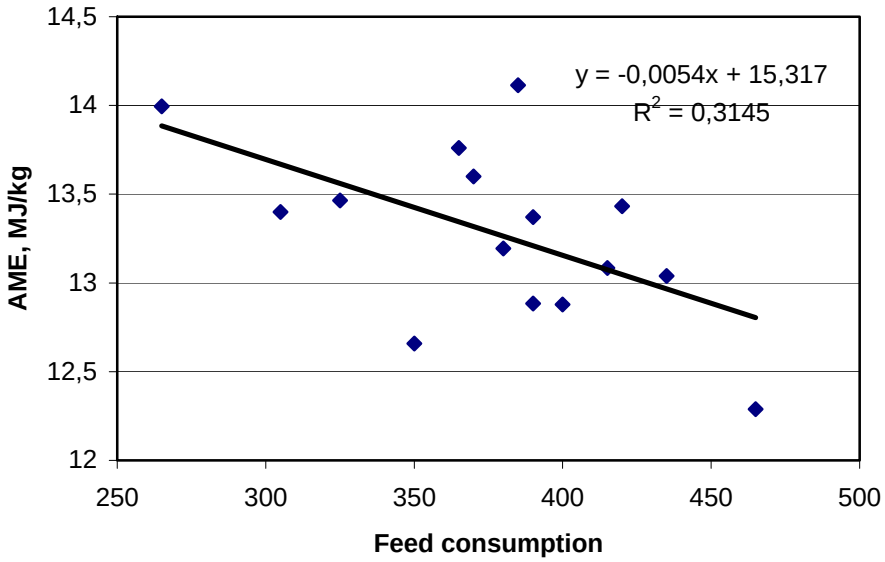

Figure 2. Relationship between feed consumption and AME determined on individual birds fed a ground wheat-based diet.

III. GLUCOSE ABSORPTION AND METABOLISM

Glucose is absorbed into the bloodstream mainly by active absorption. It can be speculated whether glucose absorption could be a limiting factor and thus be the cause for low starch digestibility. However, experiments have indicated that glucose absorption is not a limiting factor for starch utilisation (Riesenfeld *et al.*, 1980; Noy and Sklan, 1996). This is also consistent with unpublished results at our lab, where ileal samples from birds that had a low starch digestion showed that starch content in the ileum could be as high as 30 percent, while free glucose content was low and never higher than 2 %. This indicates that digestion of starch, and not the absorption rate of glucose, is the limiting factor. Birds have a glucose metabolism that is quite different from other warm-blooded animals. The blood glucose level is about three times higher and the regulation mechanisms are more focused on keeping the blood glucose levels in the blood high via the action of glucagon (6 to 8 times higher concentration in blood than mammals) than on down-regulating the level (Hazelwood, 2000). This is also illustrated by the fact that depancreatized birds rather die of hypoglycemia than of hyperglycemia. The turnover rate of glucose is also twice that of mammals (Brady *et al.*,

1978). It is reasonable to believe that this regulation mechanism ensures a very high ability to absorb glucose from the small intestine. It has been shown that the enterocytes play a role in glucose homeostasis by converting up to 35 % of the absorbed glucose into lactate (Riesenfeld *et al.*, 1982; Raheja *et al.*, 1975). This may facilitate a rapid transferral of glucose from the intestinal lumen to the blood stream. The other main regulator of blood glucose levels is the liver, which turns excess glucose and lactate from the portal blood into fatty acids that can subsequently be transported to lipid cells for storage. The high capacity of the liver to turn glucose into fatty acids provides another regulatory mechanism that ensures a stable blood glucose level. At periods of fasting, the liver is responsible for producing glucose via glycogenolysis, gluconeogenesis, glucose 6 phosphatase systems and lipolytic pathways (Hazelwood, 2000). Due to this mechanism, chickens have been shown to be rather resistant to hypoglycemia or hyperglycemia during discontinuous feeding (Belo *et al.*, 1976; Brady *et al.*, 1978) and when fed diets with varying carbohydrate contents (Raheja *et al.*, 1975). A high ability of the liver to regulate the blood glucose level may thus be necessary for a steady and high glucose absorption.

IV. STRUCTURAL FEATURES OF STARCH

It is clear that properties of the starch ingested will affect digestibility, since numerous experiments have reported that glucose response and digestibility of starch varies with source. However, there is still lack of knowledge on the exact causes for these variations in starch digestibility.

Two distinct populations of starch exist. Amylopectin consists of α1-4 glucose chains with frequent branches due to α1-6 bonds, while amylose is characterised by very few branches. Indeed, no structural continuum is observed between these two types of α-glucans (Buléon *et al.*, 1998). Usually, less than half the amylose will be branched and the amount of branch points will be less than 20 per molecule, while for amylopectin the average amount of branch points will be approximately once per 20 glucose units (Hizukuri *et al.*, 1997). Most starches contain between 20 – 25% w/w amylose although some waxy starches contain very little, and other starches, such as amylomaize, may contain 65-70% amylose (Parker and Ring, 2001). Several studies have shown that the amylose/amylopectin ratio is negatively correlated with starch digestion (Xue *et al.*, 1996; Ankrah *et al.*, 1999; Abdel-Aal *et al.*, 2002; Åkerberg *et al.*, 1998; Topping *et al.*, 1997; Bornet *et al.*, 1990; Zhou and Kaplan, 1997; Bednar *et al.*, 2001; Ito *et al.*, 1999; Saito *et al.*, 2001). However, the nature of these studies does not allow a conclusion on whether this effect is due to the primary structure itself, or if it is an effect of differences in starch granule structure that vary in concert with amylose/amylopectin ratio. The effect of amylose/amylopectin ratio on starch digestibility could also be mediated through interactions with starch granule size and organization, although the nature of this interaction does not appear to be fully revealed.

Starch is accumulated in granules in the endosperm. The granules may vary in size from 1 to 50 μm. Cornell *et al.* (1994) found that the main peak for starch granule size was around 22 μm for wheat starch, 8 μm for rice starch and 38 μm for potato starch. Cornell *et al.* (1994) found a trimodal distribution curve with peaks at 0.8, 4, and 22 μm in starch isolated from wheat, with the majority of the starch volume distributed around the 22 μm peak. Raeker *et al.* (1998) found the volume percentage distribution to be 9.7 – 15.2 % for the small granules, 13.4 – 27.9 % for medium size granules and 57.9 – 76.9 % for large granules. Tang *et al.* (2001) separated starch granules from barley into three classes with an average size of 18.4, 12.3 and 2.2 μm. The granule size distribution and shape are considered important for the functional properties of the starch. As less than 5 % by weight of starch

granules are physically damaged in roller-milled wheat flour (Sahlström *et al.*, 1998), it can be expected that starch granules to a very high extent will be present as intact granules in cereals ground for use in feed. Thus, the organisation of the starch granule if ungelatinised is likely to be important for the extent of digestion in animals. It has been speculated that size of the starch granule may affect digestibility, as the relationship between surface area and starch volume, and thus contact between substrate and enzyme, decreases as size of the granule increases. A higher starch digestibility of cereals with small granules (oats and rice) compared to wheat and potato with larger starch granules has also been observed (Manelius and Bertoft, 1996; Bednar *et al.*, 2001). Such comparisons are associated with uncertainty since other differences may also come into effect. Franco *et al.* (1992), however, separated starch granules from cassava and corn into different sizes and studied breakdown in the presence of amylase and amyloglucosidase. Small starch granules had a higher percentage of hydrolysed starch after 36 hours incubation than large starch granules. Chiotelli and Le Meste (2002) observed a higher water affinity of small starch granules than large starch granules due to a lower extent of crystallinity, and this could result in increased availability for amylolytic enzymes. The observation that starch digestion takes place not only on the surface of the starch granule but also in the interior of the granule through channels and amorphous regions (Lynn and Cochrane, 1997), however, may reduce the dependency of a large surface on rate of starch digestion.

Several non-starch components have been found associated with the starch granule. These components may also represent a challenge during digestion. One of the most important components may be lipids. A range in lipid content from less than 0.1 to 1.4 % has been reported in cereal starch (Buléon *et al.*, 1998; Abdel-Aal *et al.*, 2002), but most commonly the content ranges from 0.5 to 1 % (Hoover and Vasanthan, 1994; Sahlström *et al.*, 1998; Vasanthan and Bhatty, 1996; Buléon *et al.*, 1998; Andersson *et al.*, 1999). Quantitatively, lipids are thus the most important non-starch component in the starch granule. The lipids usually consist of free fatty acids and phospholipids (mainly lysophospholipids in cereals) that are associated with amylose, and palmitic and linoleic acids have been identified as the most common fatty acids found in the starch granule (Baldwin *et al.*, 1997). A significant portion of these lipids is found on the surface of the starch granule (Baldwin *et al.*, 1997). Lipid:starch complexes may influence digestion by reducing contact between enzyme and substrate. In addition, the amount of lipid:starch complexes are negatively associated with extent of swelling, probably due to increasing hydrophobity (Vasanthan and Bhatty, 1996). This may further impair digestibility both directly since water is necessary for enzymatic degradation and indirectly due to a lower extent of gelatinisation during processing of feed. It has been shown that complexes formed between fatty acids and amylose can reduce enzymic digestion of amylose (Crowe *et al.*, 2000).

Starch granules usually contain 0.3 % or less protein (Abdel-Aal *et al.*, 2002; Cornell *et al.*, 1994; Vasanthan and Bhatty, 1996; Hoover and Vasanthan, 1994). The proportion of protein increases towards the surface of the starch granule. In wheat, the 15 kDa protein friabilin found on the surface of starch granules has been associated with the important quality of endosperm hardness, affecting milling and baking qualities (Baldwin, 2001). Friabilin is found to be abundant on soft wheat starch granules, scarce on hard wheat starch granules, and absent in durum wheat (Greenwell and Schofield, 1986). Friabilin possibly hinders the bonding between starch granules and matrix protein, giving rise to a softer endosperm that fractures more easily upon milling and which produces finer-textured flours of lower starch damage. Surface proteins may thus affect availability of starch through interactions with milling conditions. Brennan *et al.* (1996) observed a dense protein matrix layer surrounding starch granules in poor malting barleys, while in good malting barleys, this layer was less apparent. Although this may indicate that the nature of the protein matrix and

the interactions between the protein matrix and starch may affect starch digestibility, it is important to take into consideration that protein digestion usually precedes starch digestion in the digestive tract, and thus protein layers would be expected to be significantly degraded before starch digestion takes place.

Non-starch components in the starch granule such as fat and protein may thus impair digestion both directly by reducing contact between digestive enzymes and starch, and indirectly through a reduced swelling of the starch granule and through interactions with milling and gelatinisation properties during processing of feeds. Results from experiments with potato starch granules have indicated that the surface of the starch granule may function as a boundary membrane (Fisher *et al.*, 1997). If these findings have relevance also for other starch granules, they may indicate that surface components can create a surface membrane that acts as a physical barrier to digestion. Endosperm hardness in wheat and barley is likely to affect starch digestibility through varying particle size distribution of the meal after feed processing and through the varying degree of starch damage upon milling.

It is generally accepted that a high degree of crystallinity will reduce the rate of starch digestion (Björck *et al.*, 2000). Thus, starch digestion for many species is strongly affected by gelatinisation of starch (Holm *et al.*, 1988; Kishida *et al.*, 2001). Despite a limited extent of gelatinisation (Skoch *et al.*, 1981), an increase in digestibility of starch (Ankrah *et al.*, 1999) or dry matter (Skoch *et al.*, 1983) has been observed after steam pelleting. Extrusion processing of feed gives a much more complete gelatinisation (Skoch *et al.*, 1983) and thus increases starch (Holm and Björck, 1988) and dry matter (Skoch *et al.*, 1983) digestibility. In addition, some plants may contain α-amylase inhibitors that reduce starch digestibility in the small intestine (Puszai *et al.*, 1995), and heat treatment may alter the effectiveness of these inhibitors and thus increase starch digestibility.

CONCLUSION

A low starch digestibility when broilers are fed certain feed sources could be caused by a feed intake and passage rate that exceeds the digestive capacity of the birds. Specific properties of the starch granule and associated components could be causes for a reduced digestibility rate, but more research is needed in this exciting area.

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GASTROINTESTINAL MICROBIAL POPULATIONS: BENEFICIAL MANIPULATIONS IN POULTRY

-K.A. DAWSON

Summary

Concerns over the risks associated with the use of antimicrobials as growth promotants has limited their use in strategies for beneficially manipulating the microbial population in the digestive tracts of poultry. However, new strategies that use low concentrations of specific substrates or exogenous enzyme supplements are now being adopted as tools for beneficially manipulating the gastrointestinal microbiota. These new supplementation strategies promise to provide more precise control of the composition and activities of the microorganisms that inhabit the digestive tract, and can provide new tools for enhancing poultry production in the absence of antimicrobial growth promotants.

I. INTRODUCTION

The gastrointestinal tract of domestic birds is known to harbor an extensive and diverse microbial population that may have significant effects on growth and health. However, the nature of the interactions between the bird and the microbial populations are generally poorly understood. As a result, there is little scientific basis for beneficially manipulating the composition and activities of these microbial communities (Mead, 1997). Despite this, strategies for beneficially modifying the microbial population have been used for years in modern livestock production systems. Probably the best examples of these strategies is the use of subtherapeutic concentrations of active antimicrobial substances in feeds. Such practices have clearly been associated with improved animal health and performance. Until recently, these have been the mainstay of supplementation strategies in modern livestock production systems in many areas of the world.

In recent years, the concern over the risks associated with the development of antimicrobial resistant bacteria have begun to limit the indiscriminant use of antimicrobials in animal feeds. As a result, there is renewed interest in developing new strategies for beneficially manipulating the interactions between the microbes in the gastrointestinal tract and the host animal. This presentation will examine two feed supplementation strategies for beneficially altering the composition and activities in the gastrointestinal tract.

II. ROLE OF THE GASTROINTESTINAL MICROBIAL POPULATION

In order to develop strategies for manipulating the microbial population in the gastrointestinal tract, it is important to understand some of the major interactions between these microbes and the animal. It is clear that the microbial populations in the gastrointestinal tract play many key roles in the normal physiological development, nutrition, and immunological processes of poultry. In general, we can classify these into four major areas. First, these organisms play a functional role in digestive processes. While these activities are often limited in birds by the relatively short residence time of digesta in the tract, it is clear that microorganisms can contribute to digestive processes in the hindgut. The short chain fatty acids produced by these bacteria have been reported to account for as much 30% of the maintenance energy required by some birds (Gasway, 1976). As a result, the role of

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microbial population can provide protection against toxins and antinutritive factors in the diet. Such activities not only impact the digestive processes, but may also serve to influence the overall health of the birds. The microbial population is also involved in the normal development of the immune system and disease resistance. This can be due to its ability to competitively prevent the growth of pathogenic or toxigenic organisms in the gut. These types of competitive exclusion have been used in the past as part of strategies for improving gut health and are generally associated with the Nurmi principle (Nurmi and Rantala, 1973). Disease resistance can also be induced indirectly by influencing the cellular or humoral immune systems. Finally, the microorganisms and the metabolites they produce in the digestive tract are critical to normal gut development (Gaskin, 1997). This is the result of an extremely complex interaction that also contributes to the disease defense mechanisms. Of particular interest may be the short chain fatty acids, particularly butyrate, that are key factors in modulating cytokine-mediated responses and stimulating tissue differentiation in the intestinal tract.

III. STRATEGIES FOR MANIPULATING THE MICROBIAL POPULATION IN THE GASTROINTESTINAL TRACT

Over the years, a number of strategies have been used to alter microbial activities in the gastrointestinal tract. These are basically procedures that influence some basic physiological factors within the gut and often depend on selective inhibition of certain types of bacteria. The objectives of these strategies are to alter the composition and the activities of the microbial populations in away that will beneficially influence the basic host/microbe interactions. While there are many management and nutritional strategies for altering the microbial population in the gastrointestinal tract, the use of subtherapeutic antimicrobials has been the most successful method for enhancing animal performance. Despite the success of these types of strategies, many of the basic mechanisms involved in their beneficial manipulations have yet to be fully elucidated. It is generally believed that the selective antimicrobial activities of many of these compounds can account for at least some of their beneficial effects.

While the selective activities of antimicrobials may be important to their overall effects on animal performance, there is concern that the selection for resistant bacteria contributes to the development of an increasing pool of antimicobial-resistant bacteria. As a result, the effectiveness of many antimicrobials is becoming limited and our ability to use such drugs in therapeutic settings to combat disease will decline. These concerns have led to new regulations that limit the use of antimicrobials for promoting the efficiency of animal production. In the absence of antimicrobial growth promotants, alternative strategies for beneficially modifying the gastrointestinal microbial population are needed.

IV. APPLICATION OF SPECIFIC SUBSTRATES TO BENEFICIALLY MANIPULATE MICROBIAL POPULATIONS IN THE GASTROINTESTINAL TRACT OF POULTRY

Currently, there are a number of strategies that use various indigestible carbohydrates or carbhydrate complexes to beneficially alter the composition of the microbial populations in the gastrointestinal tract. The “prebiotic” concept was developed in the mid-1990s to describe the use of nondigestible food ingredients that beneficially modulate the activities of one or a limited number of bacteria in the gastrointestinal tract (Gibson and Roberfroid, 1995). These strategies are used to improve animal health and performance.

Probably the best studied prebiotics are the fructanoligosaccharides. These are nondigestible carbohydrates composed of short chains of fructose linked together by a beta 2-1 linkage and are attached to a glucose terminal unit. While these materials are not digested by mammalian enzyme systems, they can be digested by certain groups of bacteria in the intestinal tract and have been used at low concentrations to beneficially alter the microbial population in the intestinal tract by increasing the concentrations of bifidobacteria (Gibson *et al*., 1995).

There has also been considerable interest in using the carbohydrate complexes associated with the yeast cell wall as materials for modifying the gastrointestinal microbiota. The yeast cell wall is composed of a complex mixture of carbohydrates that contains mixtures of mannan, glucan, chitin, and proteins. While this complex has a number of unique characteristics that suggest it can influence microbial growth in the digestive tract, there has been particular interest in the mannan-based sugars and mannoproteins. Many enteric pathogens use type-1-fimbriae to attach to the intestinal lining. These fimbriae specifically recognize mannan-based sugar residues and often define the ability of the organism to initially colonize the intestinal tract. In a screening study looking at bacterial attachment mechanisms, 66% of the strains of *Escherichia coli* tested expressed mannose-specific fimbriae (Finucane *et al.,* 1999b). The percentages of *Salmonella typhimurium* and *Salmonella enteritidis* strains that attached to mannose receptors were 80% and 67%, respectively. Low concentrations of mannose-type sugars and yeast-derived mannoproteins in the diet appear to block bacterial attachment by adhering to specific proteins of the bacterial cell surface, thereby reducing colonization. The effects of low concentrations of dietary mannan oligosaccharide on the gastrointestinal microflora have been investigated in a series of broiler and turkey studies, and significant reductions in both salmonella and pathogenic *E. coli* have been reported (Spring *et al.,* 2000). The ability of mannan oligosaccharides complexes in yeast cell walls to decrease the prevalence and prevent colonization of salmonella in the gastrointestinal tract has been well documented (Spring *et al.*, 2000).

Mannan oligosaccharides complexes from yeast cell walls have also been shown to have indirect effects on bacterial populations and colonization of organisms that are associated with growth depression in domestic poultry. This group includes the known toxigenic bacterium, *Clostridium perfringens*. In a recent turkey trial, a reduction in the prevalence of *Cl. perfringens,* was reported in response to a dietary mannan preparation (Finucane *et al.,* 1999a). The turkeys fed a yeast derived preparation tended to have greater cecal concentrations of beneficial anaerobic bacteria (Table 1). This decrease in the prevalence of Clostridia is not easily explained, since these organisms are not known to express type-1-fimbriae and do not appear to be subject to the antiadherence therapies associated with some of the fimbriae-containing enteric bacteria. It is possible that changes in clostridial concentration are brought about through indirect effects exerted by mannan complexes on the gut flora. Changes in the growth of beneficial anaerobes could competitively inhibit the growth and activities of the clostridia. These types of changes in microbial populations are consistent with the reported effects observed with antimicrobial supplementation and could account for some of the growth-promoting effects associated with the use of specific mannan oligosaccharide and mannan complexes in field trials.

Table 1. Effects of a yeast cell wall mannan oligosaccharide preparation (Bio-Mos®) or a growth promoting antimicrobial (bacitracin methylene disalycyate) on the concentrations (log_{10} CFU/g) of specific bacterial groups in the large intestines of turkeys (adapted from Finucane *et al.*, 1999).

Bacterial group	Treatment		
	Control	Bio-Mos	BMD
Coliforms	4.59	4.67	3.93
Lactobacilli	7.59	8.38	7.94
Bifidobacteria	7.09[a]	7.10[a]	8.18[b]
Aerotolerant anaerobes	7.92[a]	8.61[b]	8.47[ab]
Clostridia	4.22[a]	2.98[b]	1.82[c]
Streptococci	7.56	7.52	7.45

[a,b,c]Values in the same rows with different superscripts differ (P<0.05).

Another potential role for yeast-derived mannoprotein-based carbohydrates has recently become apparent and relates to its ability to influence the development of coccidiosis in poultry. Several studies looking at both induced and naturally-occurring *Eimeria* infections have demonstrated less severe health problems, less severe lesions, and improved animal growth in birds receiving a low concentration of a mannoprotein-based preparation from yeast (Table 2). These observations are consistent with other studies that have demonstrated up to a 50% reduction in fecal oocyst excretion in broilers supplemented with the mannoprotein preparation after challenge with *Eimeria acervulina*. Again, the mechanisms that explain these types of responses are not yet defined, but undoubtedly are related to the ability of these materials to modulate the activities of the microbial populations in the digestive tract. It is important to note that these responses are not as dramatic as those seen with some with commonly used coccidiostats. However, these data suggest that strategies using these types of products may be useful in coccidiosis control programs.

Table 2. Effects of a yeast cell wall-based natural feed supplement on the body weight and cecal lesion scores of broilers challenged with *Eimeria tenella*, *Eimeria maxima*, or a natural challenge in contaminated floor pens.

Treatment	Challenge		
	Eimeria tenella	*Eimeria maxima*	Natural contamination
Body weight (g) at 4 wk			
Unchallenged control	606	606[b]	
Challenged control	578	445[c]	1028[b]
Cell wall preparation (1g/kg)	605	537[b,c]	1245[c]
Antimicrobial[a]	544	579[b]	1187[c]
Cecal lesion scores at 4 wk			
Unchallenged control	0.83[b]	0.83[b]	
Challenged control	3.17[c]	2.33[c]	
Cell wall preparation (1g/kg)	1.33[b]	1.17[b]	
Antimicrobial[a]	1.50[b]	0.83[b]	

[a]Coban (50g/ton) was used in the *Eimeria* challenge studies and Avitec (90g/ton) was used in the natural challenge studies.
[b,c]Means in the same column within a given parameter with different superscripts differ (P<0.05).

V. THE USE OF LOW LEVELS OF EXOGENOUS ENZYMES TO MANIPULATE GASTROINTESTINAL MICROBIAL POPULATIONS

Traditionally, exogenous enzymes have been used in animal feeds to enhance digestive processes and address antinutritional factors associated with grain diets. In poultry, enzyme supplementation has been used to improve performance by addressing specific nutritional limitations in diets. This is typically accomplished by enhancing digestion and increasing the bioavailability of specific nutrients found in both traditional and non-traditional feedstuffs. Enzymes have also been used to address the antinutritional activities of various pentosans and glucan in grains. These non-starch polysaccharides increase the viscosity of the feed material in the digestive tract and decrease the efficiency of digestion and nutrient adsorption. Strategic use of carbohydrase enzymes can prevent the viscosity problems and improve animal performance on high grain diets. These strategies allow for reformulation of diets to provide for more efficient production and at lower feed costs. By increasing bioavailability of specific nutrients, enzyme systems may also be used to decrease the concentrations of specific nutrients in animal wastes and address environmental issues related to animal production.

In the last couple of years, evidence has begun to accumulate suggesting that low levels of enzymes can be strategically used to control substrate concentration in the gastrointestinal tract and may be useful for manipulating the composition and activities of the gastrointestinal microbiota. Such applications have been very successful in ruminant diets and require a new look at enzyme application strategies.

Microbial physiologists have known for many years that the growth rate of specific microorganisms is influenced by the substrate concentrations in their environment (Russell and Baldwin, 1979). The growth rate of bacteria will increase towards a maximum level as the concentration of a limiting substrate increases (Figure 1). This characteristic is a key biological determinant that will define which types of microorganisms will predominate in a mixture of organisms competing for limited nutrient resources. At high concentrations of substrates, rapidly growing organisms like the lactic acid-producing streptococci and lactobacilli will have a competitive advantage, while slower growing organisms like butyrivibrio will have a limited ability to maintain themselves in this competitive environment (Figure 1A). A different situation occurs at low substrate concentrations like those often found in the gastrointestinal tract. In this situation, the slower growing strains that have a high affinity for a limiting substrate at low concentrations will now have a competitive advantage and will be better able to compete for a limiting nutrient (Figure 1B). These theoretical illustrations suggest that one way to manipulate the composition of the microbial populations in the digestive tract is to control the concentrations of limiting substrate and to selectively enhance the growth of some of the more beneficial bacteria that tend to slow maximum growth rates. Preliminary studies have indicated that low concentrations of exogenous enzymes can be used to control carbohydrate concentrations in the digestive tract and may provide a tool for manipulating the microbial population. This has a tremendous implication, since similar tools for controlling microbial activities in the digestive tract have not been available in the past.

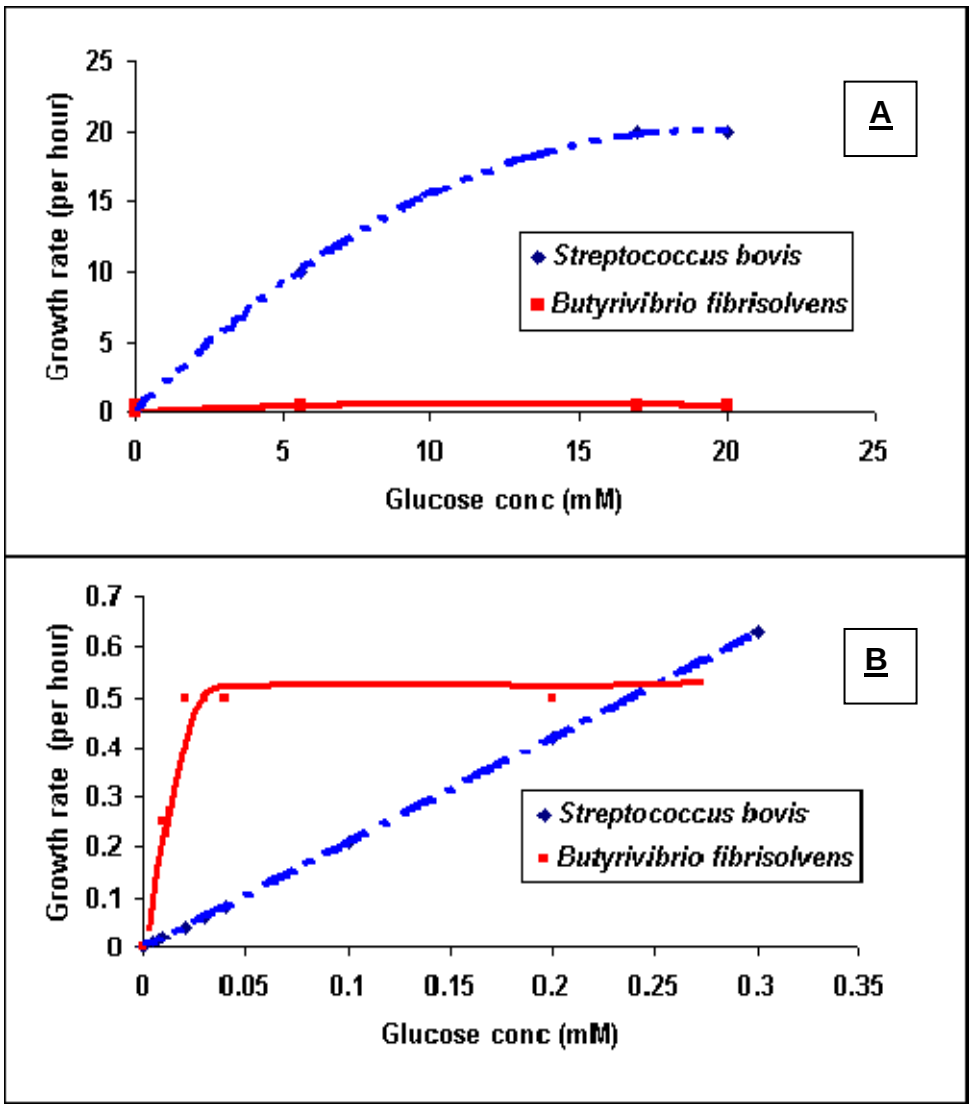

Figure 1. Effects of high and low substrate concentrations on growth rate of two representative gastrointestinal bacteria.

The ability of low concentrations of enzymes to selectively stimulate the growth of some representative gastrointestinal bacteria has been demonstrated in batch culture studies (Table 3). Representative strains of butyrivibrio, selenomanads and megasphaera were all stimulated by the addition of low fungal amylase concentrations (60 units/L) while the representative strain of the streptococci group was not. This suggests that the growth of some strains of bacteria can be enhanced with the use of low concentrations of fungal amylase while other strains are not stimulated. This could have considerable influence on the competition between microbes in the gastrointestinal tract and may provide a tool that is as powerful as antibiotics in altering the composition of the microbial populations.

Table 3. Effects of low concentrations (0.06 units/mL) of the growth rates of representative strains of gastrointestinal bacteria grown in batch culture.

Strain of bacteria	Growth rate , (h^{-1}) (Generation time (h))	
	Without enzyme	With enzyme
Butyrivibrio fibrisolvens strain D1	0.208 (4.81)	0.334 (2.99)
Butyrivibrio fibrisolvens strain 49	0.343 (2.92)	0.409 (2.45)
Selenomonas ruminantium strain GA192	0.347 (2.89)	0.729 (1.37)
Megasphaera elsdenii strain T81	0.150 (6.64)	0.324 (3.09)
Streptococcus bovis strain S1	0.988 (1.01)	0.957 (1.05)

Other studies have established that applications of low levels of enzymes can result in changes in representative gastrointestinal fermentations (Table 4). In these studies, a reduction in the relative amount of propionate produced by the mixed population of ruminal bacteria was accompanied by an increase in butyrate production when an exogenous enzyme preparation was added to the substrate. This is consistent with the selective activities suggested in the batch culture studies and is supported by increased productivity in both beef and dairy cattle. Enhanced butyrate production in the hind gut of poultry not only changes the relative amounts of energy available through microbial fermentation, but may also have a significant impact on the development of the wall of the gastrointestinal tract, villus growth and overall absorption mechanisms (Mosenthin, 1999).

Table 4. Manipulation of fermentation in a mixed population of ruminal bacteria in a continuous culture using low concentrations (60 units/L) of alpha-amylase to control glucose release.

Parameter	Treatment	
	Control (no enzyme)	Enzyme
Total short chain fatty acid (mM)	145.8 (10.5)[a]	143.3 (13.8)
Molar proportion (moles/100moles)		
Acetate	53.7 (0.8)	53.1 (0.4)
Propionate	23.6 (0.2)[b]	22.5 (0.3)[c]
Butyrate	16.9 (0.9)[b]	18.4 (0.5)[c]

[a]Values in parenthesis are standard deviations (n=4).
[b,c]Means within a row with different superscripts differ ($P<0.01$).

VI. CONCLUSION

Innovative uses of low concentrations of unique substrates and exogenous enzymes will provide some new tools for beneficially altering the microbial populations in the gastrointestinal tract. These new tools are different from any that were available in the past, but can be used in exciting new strategies for improving productivity and health in poultry production systems.

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MAXIMISING PRODUCTIVITY IN BROILER BREEDERS

P.M. HOCKING

Summary

Genetic selection for high growth rates results in an increase in multiple ovulations and disrupted egg formation. Feed restriction during rearing and laying controls multiple ovulation that leads to a substantial increase in the production of hatching eggs and a decrease in mortality. Experiments have shown that feed restriction must be tightly controlled before and after puberty to obtain the maximum production of hatching eggs. The key to managing fertility is the control of male body weight, but males must be allowed to gain adequate weight from 30 to 60 weeks of age. Low protein diets for males are not essential for maximising fertility in naturally mated flocks. Methods to alleviate hunger by manipulating growth curves and ration composition have not been successful to date. Genetic selection against multiple ovulation may lead to the development of broiler breeders that require less severe feed restriction than conventional lines.

I. INTRODUCTION

Broiler breeder females and males are floor reared, mate naturally and have ready access to nest boxes for laying. However, maximum feed intake is controlled for optimum health and productivity and concerns have been expressed that the degree of feed restriction is sufficiently severe to represent a welfare problem. Research at the Roslin Institute has investigated the relative roles of age, body weight and feed intake on aspects of productivity and welfare and the main research results will be outlined in this paper. Only limited reference will be made to feed quality as this has been considered elsewhere (Fisher, 1998).

II. MAXIMISING PRODUCTIVITY IN FEMALE BROILER BREEDERS

Selection for greater body weight gains in broilers has been associated with an increase in the number of ova (yolks) that are ovulated by adult females on any one day (Hocking and Robertson, 2000). The consequences of multiple ovulation are disrupted egg shell formation leading to soft shelled, misshapen and double-yolked eggs, and the loss of some ovulations into the body cavity where they are absorbed. The net result is that the production of hatching eggs is poor (Tables 1 and 2). It requires 6 to 7 days for a yellow follicle to mature, and an ovary of a table-egg laying bird at peak rate of lay contains 6 or 7 yellow follicles (Table 1).

Table 1. Numbers of yellow follicles at first egg and egg production from 24 to 27 weeks of age (from Hocking *et al.*, 1987).

Group	Body weight	Yellow follicles	Egg production, N shells/hd	
	kg	N	Sound	Defective
Layer	1.5	6.3	0.94	0.03
Restricted broiler	3.0	6.3	0.77	0.04
Ad lib. broiler	5.5	12.6	0.40	0.25

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Feed restriction during rearing reduces the ovulation rate of broiler breeders to little more than one yolk per day and egg numbers and shell quality are improved (Hocking *et al.*, 1987). The positive effects of controlling body weight during rearing on egg production are maintained by feed restriction during adult life (Hocking *et al.*, 2002a). The positive effects of feed restriction on productivity in modern broiler breeders are considerable (Table 2) and mortality is substantially decreased: mortality in birds fed *ad libitum* can reach 70% or more at the end of the breeding period (Katanbaf *et al.*, 1987).

Table 2. Productivity and mortality to 60 weeks of age in feed restricted and *ad libitum* fed broiler breeders (from Hocking *et al.*, 2002a).

Trait	Restricted	*Ad libitum*
Body weight (kg)	3.7	5.3
Mortality (%)	4	46
Eggs (N/hd)	157	44
Hatching eggs (N/hd)	140	35
Egg weight (g)	65	65
Fertility (%)	86	87
Hatchability (%)	86	43

The relationships between body weight, feed intake and follicle numbers were studied in a series of experiments. Initially we demonstrated that feed restriction decreased the number of yellow follicles only when it had occurred after 14 weeks of age (Hocking *et al.*, 1989). (It is interesting to note that this was also the age at which the ovaries of birds fed *ad libitum* began to show follicular activity.) The numbers of yellow follicles at the onset of lay were subsequently shown to be directly proportional to body weight and were not affected by the degree or age of restriction between 14 weeks and photostimulation (Hocking, 1993a). In other words, the numbers of follicles were directly proportional to body weight at photostimulation regardless of how the birds achieved that body weight. In a third experiment, the roles of body weight and feed allocation post-photostimulation were evaluated (Hocking, 1996). Broiler breeders from three rearing schedules (*ad libitum*, 0.7 and 0.4 of the body weight of birds fed *ad libitum*) were each given the allocation of feed corresponding to the consumption of the three groups at 18 weeks of age in a 3 x 3 experiment. The important results are presented in Table 3.

Table 3. Effects of body weight at photostimulation and feed allocation after photostimulation on yellow follicle numbers (percentage of multiples) in birds fed *ad libitum* (AL), 0.7 or 0.4 (commercial restriction) of *ad libitum* body weight (from Hocking, 1996).

Rearing	Feeding after photostimulation (g/d)		
	AL	140	115
AL	13.5 (87)	11.3 (68)	12.7 (78)
0.7	12.5 (73)	9.2 (53)	8.2 (49)
0.4	10.5 (61)	7.6 (34)	6.9 (19)

Feed restriction after photostimulation had little effect on ovarian function in birds fed *ad libitum* during rearing and the birds had at least 2 hierarchies of yellow follicles of similar size. Restricted birds fed *ad libitum* after photostimulation had a large increase in the numbers

of yellow follicles (1.7 to 2.0 hierarchies) compared with birds fed 145 g/d or 115 g/d (1.2 to 1.6 hierarchies). Clearly, while body weight had a major effect on the numbers of follicles, feed intake could modify the number, particularly if the birds were fed *ad libitum*. In a subsidiary experiment, conventionally restricted female broiler breeders were fed a limited quantity of feed or *ad libitum* after laying an egg, or they were restricted for 3 weeks and then fed *ad libitum*. Ovarian function was assessed in all three treatments 6 weeks after the onset of lay and the treatment means are presented in Table 4. *Ad libitum* feeding in restricted birds immediately after the onset of lay resulted in an increase in the number of follicles and the proportion developing as multiples to an extent that was comparable to the response before the onset of lay (Table 4).

Table 4. Body weight, number of yellow follicles and the proportion present as multiples in restricted broiler breeder females fed 125 g/day (R/R) after photostimulation or transferred to *ad libitum* feeding immediately (AL/AL) or 3 weeks after photostimulation (R/AL). The birds were examined 6 weeks after laying their first egg (from Hocking, 1996).

Trait	Treatment		
	R/R	R/AL	AL/AL
Body weight (kg)	3.1	3.7	4.0
Yellow follicle number	5.7	8.6	9.6
Multiples (%)	2	31	46

In an unpublished experiment we confirmed that the number of yellow follicles at the onset of lay was linearly related to the degree of feed restriction throughout rearing. Furthermore, the minimum body weight that permitted sexual maturity was associated with the optimum number of follicles and birds did not achieve sexual maturity if feed intake did not allow them to reach this body weight. The proportional reduction of body weight in restricted compared with *ad libitum* fed broiler breeders at the onset of lay was about 0.3 in male and female "control" lines maintained without selection for 20 years compared with 0.4 in the genetically selected commercial birds. It was concluded that the effective degree of restriction would increase, management would become more difficult, and welfare would decline with time as broiler breeders became still larger. Opportunities for improving the future welfare of broiler breeders will be considered further at the end of this paper.

Summarising, feed restriction during rearing controls multiple ovulation and enhances hatching egg production. The degree of feed restriction has to be sufficient to control body weight to about 0.4 of *ad libitum* during rearing and is only effective from 14 weeks of age. The number of yellow follicles is directly proportional to body weight but can be modified by increased feed allocation after photostimulation. In practical terms, achieving the proper body weight at photostimulation and careful feed allocation thereafter is crucial for the effective control of ovarian function and to maximise the production of hatching eggs. The results also emphasise the importance of uniform body weights (i.e. a low coefficient of variation): high body weight will lead to an increase in multiple ovulations and low body weight will result in a rate of ovulation that is less than optimum. Large and overweight females also have depressed fertility compared with lighter, leaner birds (Hocking *et al.*, 2002a).

III. MANAGING BROILER BREEDER MALES

Separate sex feeding for broiler breeders was introduced in the late 1980s and management of the male then became a real possibility. Results from experiments at Roslin with caged males and those housed on the floor with a group of females were not consistent (Hocking and Bernard, 1997b; Hocking and Bernard, 1997a), suggesting that research findings from caged birds may not be applicable to large commercial flocks. The major conclusions from our research with naturally mated males will be summarised and results from caged males will be mentioned only briefly.

Effective management of the male is all about controlling body weight through the allocation of food. When a flock ages fertility declines rapidly as male body weight moves further from the optimum (Figure 1).

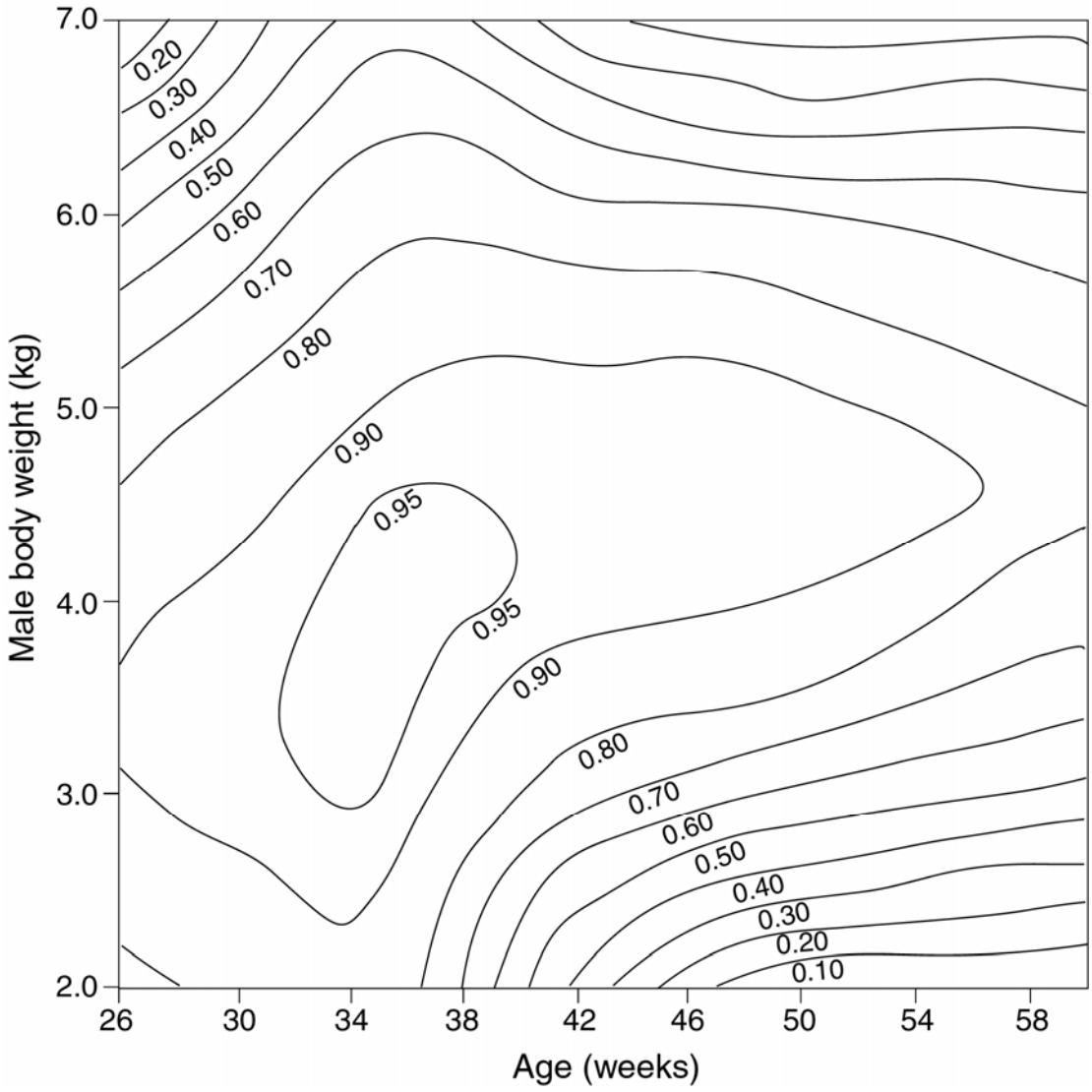

Figure 1. Fertility of naturally mated broiler breeder males at different ages and body weight (from Hocking, 1990).

Specifically, males over 5 kg body weight have poor fertility: in one experiment, 9 out of 48 males at 65 weeks of age were over 5 kg body weight and all had fertility less than 80%. Poor fertility in overweight males is related to the inability to copulate satisfactorily, and may be associated with excessive breast muscle development (Hocking and Bernard, 1997b).

Conversely, males with low body weight (less that 3.5 kg) may not be able to compete with larger males and have small testes that might further reduce fertility (Hocking, 1990; Hocking and Bernard, 1997b; Hocking and Bernard, 2000). Research suggests that males should be fed a gradually increasing amount of feed to gain at least 1.5 kg from 30 to 60 weeks for ease of management and optimum fertility (Hocking, 1990; Hocking and Bernard, 1997b; Hocking and Bernard, 2000). Clearly, uniformity of male body weight is just as critical as it is in females.

There is clear evidence in caged broiler breeders that high concentrations of dietary crude protein are associated with poor semen production (Wilson *et al.*, 1987a; Wilson *et al.*, 1987b; Hocking, 1989; Hocking and Bernard, 1997a). Experimental data from broiler breeder males fed a low protein diet and mated naturally showed no benefit in fertility compared with those fed a conventional female ration (Hocking, 1990; Hocking and Bernard, 1997b). In practice, feeding different diets is not simple and there is no strong evidence that a low protein male diet will enhance fertility in commercial flocks.

Male sexual activity is high at the start of the breeding period but does not result in greater fertility (Attia *et al.*, 1993; Hocking and Bernard, 2000). Fertility in young females was similar when they were mated to both contemporary and mature males and there were no detectable differences when old females were mated to old or mature males, although hatchability was depressed (Table 5). The results suggest that a significant decline in physiological fertility with age does not occur and confirm the importance of body weight control. However, measures of sperm transfer in commercial flocks with large numbers of males show an increasing proportion of eggs with no sperm and some with very high numbers suggesting that differential mating occurs (Wishart and Staines, 1995). Observations of sexual behaviour and female feathering are consistent with the view that some birds are over-mated and others do not mate at all. Methods of maximising female-male interaction should be investigated as a means of further improving broiler breeder flock fertility (see, for example, Jones *et al.*, 2001).

Table 5. Fertility and hatchability of male and female broiler breeders at different ages: 7-29 (young), 35-37 (mature) and 55-57 (old). From Hocking and Bernard (2000).

Male age	Female age	Fertility (%)	Hatchability (%)
Young	Young	95.9	90.7*
	Mature	96.0	93.0
Mature	Young	97.2	91.0*
	Mature	96.0	92.5
SEM		1.21	0.76
Mature	Mature	95.9	92.8
	Old	98.0	89.9*
Old	Mature	97.2	92.0
	Old	97.3	90.3*
SEM		0.62	1.10

* Significantly different ($P<0.05$) from mature females.

IV. WELFARE OF BROILER BREEDERS

The degree of hunger in feed restricted compared with *ad libitum* fed birds has been determined by regression methods (Hocking, 1993b) and in an operant conditioning

experiment (Savory *et al.*, 1993b). Both methods assume a linear relationship between hunger (i.e. the subjective psychological feeling of stress) and the measured variable. These results may be misleading and the degree of restriction should not be equated with the degree of hunger experienced by feed restricted birds. In general, comparisons of the degree of restriction may describe the programme of feeding but are probably of little value in assessing the welfare of restricted broiler breeders.

Virtually all the early work on the welfare of restricted broiler breeders has been conducted at the Roslin Institute by Hocking, Savory and co-workers (Maxwell *et al.*, 1990; Maxwell *et al.*, 1992; Savory *et al.*, 1992; Hocking, 1993b; Savory *et al.*, 1993a; Savory and Maros, 1993; Savory *et al.*, 1993b; Hocking *et al.*, 1996) and their results are summarised in Table 6. In general, feed restriction was associated with a change in behaviour (greater foraging, drinking and stereotypic behaviours, and less time resting), and an increase in the heterophil-lymphocyte ratio and the proportion of basophilic cells (recognised indexes of physiological stress). In most studies, an increase in plasma corticosterone concentrations was reported. There is little evidence that feed restriction compromises fundamental bodily functions, as indicated by several enzyme systems, or that immune function is adversely affected (Katanbaf *et al.*, 1989; O'Sullivan *et al.*, 1991; Hocking *et al.*, 1996).

Table 6. Changes in welfare criteria of broiler breeders fed *ad libitum* or restricted during rearing (+ = better; - = worse).

Criterion	Restricted	*Ad libitum*
Production	+	-
Health	+	-
Behaviour: activity	+	-
Behaviour: hunger	-	+
Fear	+	-
Corticosterone	-	+
Haematology	-	+
Organ function	+	-
Immunology	+	-

There is no single criterion of animal welfare: taken together, the data on welfare indexes, productivity, health and mortality suggest a curvilinear relationship between welfare and body weight (Figure 2). Mortality is high and productivity is low at the extremes, indicating poor welfare. Physiological indexes of welfare are little affected at intermediate levels (perhaps to 60% of *ad libitum* body weight) and increase steeply thereafter in larger or smaller birds (Hocking *et al.*, 1996).

There are two current methods that might be used to decrease the severity of feed restriction. These are dietary manipulation and genetic selection to decrease the potential for multiple ovulation. Manipulating dietary components by using indigestible material to induce satiety resulted in lower welfare, although excessive oral behaviour was abolished, and there was no apparent improvement in the motivation to feed (Savory *et al.*, 1996; Savory and Lariviere, 2000). Manipulating growth curves and feeding low protein rations were also ineffective in improving welfare and led to poor productivity (Hocking *et al.*, 2001; Hocking *et al.*, 2002a; Hocking *et al.*, 2002b). Furthermore, specific appetite suppressants were ineffective or had undesirable consequences (Hocking and Bernard, 1993; Savory *et al.*, 1996).

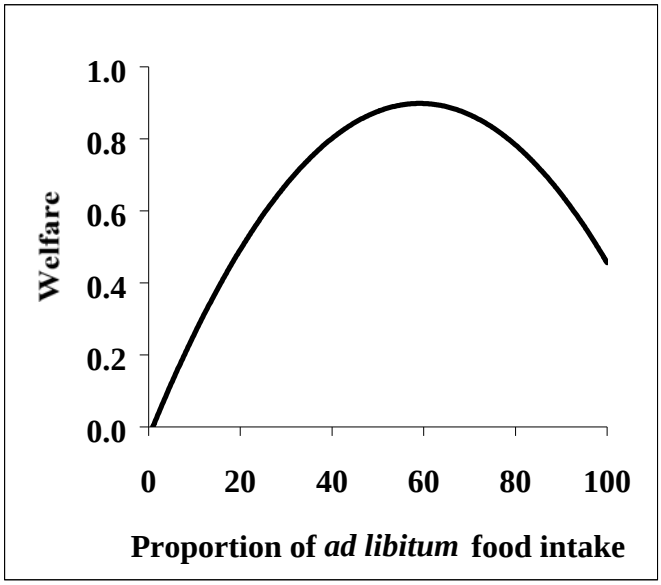

Figure 2. A model of welfare in feed restricted broiler breeders. The scale of welfare is arbitrary and represents a subjective integration of changes in physiology, immune function, behaviour, health, mortality and productivity in response to the degree of feed restriction based on the responses reported by Hocking *et al*. (1996 and 2002a).

We have recently examined the effects of increasing the fibre content of conventional rations for restricted broiler breeders and the results suggest that there may be some scope for decreasing the apparent hunger of these birds. However, the most promising long-term strategy is genetic selection to decrease the prevalence of multiple ovulation. If broiler breeders could be selected to ovulate a single ovum in each daily cycle, a more generous feed restriction programme that optimised the welfare of the birds could be adopted. The use of genetic markers for single ovulation is most promising in this respect and is a potential solution that we are pursuing at Roslin.

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BREEDING FOR DISEASE RESISTANCE: GENERAL PRINCIPLES AND LATEST RESULTS

F.W. NICHOLAS[1]

Summary

Genetic variation for resistance to disease is ubiquitous. Hence selection for resistance is possible, and there are many good examples of this in poultry. The genomics revolution has ushered in a golden age of genomic exploration that is identifying DNA markers linked to genes for resistance, and is beginning to identify the actual genes themselves. These discoveries will lead increasingly to indirect selection for resistance, via DNA markers, and ultimately to hitherto-unimagined non-genetic means of control of pathogenic disease. It is quite likely that selection for resistance will increasingly involve the maintenance of genetic diversity rather than simply increasing the frequency of genes conferring resistance.

I. INTRODUCTION

At the most recent World Congress on Genetics Applied to Livestock Production, held in France in August 2002, there was a section devoted to disease resistance, with a total of 46 papers, comprising 2 invited reviews (Bishop *et al*., 2002; Lantier *et al*., 2002) and 44 contributed papers, 5 of which concerned poultry. The reviews and the contributed papers provide an excellent snapshot of the current state of knowledge of genetic aspects of resistance to disease. This set of papers is complemented by the set of reviews in Axford *et al*. (2000), which covers all aspects of breeding for disease resistance. Recent reviews concerned specifically with poultry include those by Bumstead (1998a,b), Bacon *et al*. (2000, 2001), Kaufman (2000) and Burt (2002). The information summarised in the following paragraphs draws heavily on these reviews, and also mentions relevant results published since these reviews were written.

II. GENERAL PRINCIPLES

(a) Genetic variation in disease resistance is ubiquitous

The main review at the World Congress, by Bishop *et al.* (2002), started by confirming what has been acknowledged for many years, namely that wherever one looks, one is almost certain to find genetic differences in disease resistance among animals. In the case of chickens, Bishop *et al*. (2002) provided the following list of chicken diseases for which there is good evidence of genetic variation in resistance: Marek's disease, infectious laryngitracheitis, avian leucosis, infectious bursal disease, avian infectious bronchitis, rous sarcoma, Newcastle disease, pullorum, fowl typhoid, salmonellosis, coccidiosis, ascaris. This is an impressive list. In some cases, the genetic variation is between populations (breeds, strains); in other cases, it exists within populations. In either case, as noted by Bishop *et al*. (2002), the take-home message is that there is potential for selecting for resistance to all these diseases. And the same conclusion most likely applies to any other disease, as well.

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(b) Selecting for resistance to disease

One of the major challenges involved in conventional breeding for disease resistance is that deliberate and concerted selection for disease resistance requires deliberate and concerted exposure of animals to the disease-causing pathogen – a practice that presents many practical and ethical challenges. If only there were indirect or preferably direct markers for the genes contributing to genetic variation in resistance! It is interesting to note that in one situation where there are such markers, a national breeding program for resistance has been instigated – the National Scrapie Plan for Britain commenced in January 2002, based on molecular genotyping at three loci within the prion-protein gene (Arnold *et al*., 2002). Similar schemes are underway in France and the Netherlands.

When considering selection as a means of exploiting genetic variation in disease resistance, it is important to realise that the implications of selection can be wider than just its effect on the population undergoing selection. Much progress has been made in understanding these broader issues by the Edinburgh group of genetic epidemiologists, whose work is summarised by Bishop *et al*. (2002). Among other things, their broader approach embraces what they term genetic management strategies in controlling disease. One important aspect of this broader approach involves the recognition of pathways of infection.

(c) Pathways of infection

The bigger picture of infectious disease can involve various pathways of infection in relation to a host population and a reservoir of infection: there are pathways from reservoir to host, from host to host, and from host to reservoir. Not every pathogenic disease involves all pathways, but it is very useful to think in these terms when contemplating the overall impact of genetic management of disease. If, for example, selection in the host population can reduce not only infection within the host population, but also the flow of infection to the reservoir (as, for example, with selection for resistance to internal parasites whose life cycle involves some time outside the host), then the reservoir will become a less important source of infection; and selection for resistance can have an impact even on populations exposed to the same reservoir that have not been selected. In other words, these pathways help us to understand the epidemiological consequences of selection for resistance.

(d) Pathogen evolution

Common sense tells us that the more successful is selection for resistance in increasing the level of resistance in a population, the greater will be the natural selection imposed on the pathogen to evolve in such a way as to overcome the resistance. Bishop *et al*. (2002) argued that the greater the number of genes and mechanisms involved in resistance, the less likely is the pathogen to evolve to overcome the resistance. Another relevant argument has been put by John Gibson (ILRI, personal communication): resistance that has evolved naturally during the course of the evolution of a breed is likely to present greater challenges to a pathogen than resistance that has been created by artificial selection within a population during a relatively few generations. This latter argument raises the issue of introgression of resistance genes from naturally-resistant breeds into commercial populations.

Another issue is the extent to which genetic variation in itself is a buffer against effective evolution of pathogens. In one of the contributed papers at the World Congress, the Edinburgh group presented the results of a simulation study investigating the contribution of genetic diversity to the spread of infectious disease (Springbett *et al*., 2002). Their conclusions – that the lower the level of genetic diversity in the host population, the greater

the chance of extreme outcomes - provide a warning against aiming simply for selection for homozygosity at all loci affecting resistance.

(e) Candidate genes for resistance to disease

What genes could possibly be contributing to the ubiquitous genetic variation in resistance? Since resistance is commonly the result of innate immunity (the non-specific first line of defence) and/or acquired immunity (pathogen-specific responses involving antibodies and/or T cells, mediated via peptides (histoglobulins) encoded by the major histocompatibility complex, MHC), any gene involved in any type of immunity is an obvious candidate as a contributor to genetic variation in resistance. The list is long, including genes for antibodies, cytokines and histoglobulins. In addition, there are also genes for peptides that have nothing to do with the immune system, such as genes for receptors for pathogens.

From the 1930s onwards, researchers observed substantial variation between inbred lines of mice in susceptibility to salmonellosis and viral infections. Over the next few decades, segregation analyses applied to F_2 populations from crossing resistant and susceptible lines produced evidence of major genes for resistance, and ultimately led to the identification of six genes: *Ity*, *Bcg* and *Lsh*, for susceptibility to *Salmonella typhimurium*, *Mycobacterium bovis* and *Leishmania donovani*, respectively; *Lps* for response to the lipopolysaccharide component of bacterial membranes, *Xid* involved in regulation of antibody synthesis, and *Mx* for susceptibility to several viruses. In the 1990s, by which time powerful molecular tools were available, it was shown that the first three of these genes are actually a single coding sequence renamed *Nramp1* (natural resistance-associated macrophage protein 1), the importance of which was brought home by the clear-cut susceptibility of knockout mice (Vidal *et al*., 1995).

Once this and the other resistance genes had been cloned in mice, it was a trivial task to clone the homologous genes from other species. Later in this review, we shall see where this work has led in chickens.

(f) The golden age of genomic exploration

The discovery of the structure of DNA heralded in a new age of biological discovery. Fifty years on, we are now privileged to be reaping the enormous benefits of that discovery. Wave after wave of ever-more-powerful research tools has opened more and more black boxes, none more so than the black box of quantitative genetics. It was exciting enough to be able to use these tools to identify the coding sequences behind identifiable single genes (qualitative genetic variation), but now it is equally possible (albeit more challenging!) to identify the coding sequences that contribute to quantitative genetic variation. Previously known as polygenes, these coding sequences are now called quantitative trait loci (QTL).

The strategy of so-called QTL mapping is straightforward: take two populations of contrasting performance for quantitative traits of interest (e.g. disease resistance); cross them to produce F_2 and/or backcross populations; measure the performance of these populations for the traits of interest; genotype those same individuals for a set of DNA markers that cover all regions of all chromosomes (a so-called genome scan); and then conduct a joint analysis of the performance and marker data. The results of such an analysis indicate the regions of chromosomes containing QTL that contribute to the genetic differences that exist between the parental populations.

By measuring and genotyping generations derived from intercrossing the F_2s and backcrosses (thereby allowing for crossing over to reduce the size of the parental chromosomal segments) and/or judicious analysis of associations between markers and

performance at the population level (linkage-disequilibrium mapping), it is possible to map a QTL to a level of precision that is sufficient to enable the linked marker(s) to be used in marker-assisted selection (MAS) for the trait, and to narrow it down to one or a small number of clones in a DNA library, which can then be searched for coding sequences. At the same time, the enormous power of comparative mapping, which has revealed the surprising extent to which segments of chromosomes have been conserved among vertebrates (e.g. Burt *et al*., 1999), can be put to good use, especially now that the entire genomic DNA sequence of humans and mice is available (http://www3.ncbi.nlm.nih.gov/): by examining the coding sequences present in the regions of the human and/or mouse genome that are homologous to the QTL in the species of interest, it is possible to draw up a list of so-called positional candidate genes. An example of this approach in chickens is presented in section III.

III. RESULTS IN CHICKENS

(a) Selection for resistance

The potential for selection within populations is reinforced by the observation that the heritability for resistance (measured in a variety of ways) is relatively high. Gavora's (1990) summary of heritability estimates from field populations is still a useful guide. Consistent with these estimates, Cole and Hutt at Cornell developed resistant (C and K) and susceptible (S) strains by selection. Cole also used family selection to develop N (resistant) and P (susceptible) lines. Importantly, it appears that breeding for disease resistance is now a common feature of practical breeding programs in the commercial industry (McKay, 1998).

(b) Experimental selection with inbreeding

In addition to commercial breeding, there has been enormous effort devoted over many decades to experimental selection/inbreeding for resistance and susceptibility. Bacon *et al*. (2001) review of the lines created at the USDA's Avian Disease and Oncology Laboratory (ADOL) provides excellent examples of this type of research. Commencing in 1939, and overseen by such pioneers as Nelson Waters, Howard Stone and Lyman Crittenden, the ADOL work resulted in 15 highly inbred lines for resistance or susceptibility to viral-induced tumours, now classified as Marek's Disease (MD; induced by a DNA alpha-herpesvirus, MDV); lymphoid leucosis (LL; caused by an avian leucosis virus, ALV); myeloid leucosis (ML; also induced by an ALV); and reticuloendotheliosis (RE; also induced by yet another ALV).

Three of the 15 ADOL lines are still maintained: Line 6 (resistant to MD and LL), Line 7 (susceptible to MD), and Line 15 (susceptible to LL and MD). All three are highly inbred (in effect, 100% homozygous). Just as with the mice described in section IIe, these three lines have been used for the creation of many F_2s and backcrosses that have provided evidence of five systems of major genes influencing resistance to tumours:

1) *TV*S* (tumor-virus susceptibility). These genes encode receptors for ALV. As expected, presence of the receptor confers susceptibility, while absence creates resistance.
2) ALVE are endogenous genomic proviral (DNA) versions of the ALV RNA genome. Complete ALVE express the three genes of ALV (*env*, *gag* and *pol*). Incomplete ALVE express only *env* alone or *env* and *gag*. Some express nothing, i.e., are completely inactive. Interestingly, those that express *env* confer resistance to otherwise susceptible birds, because the *env* peptide attaches itself to the ALV receptor, preventing it from attachment by actual ALV.

3) MHC (see section IIId)
4) *TH1*, *LY4*, and *BU1;* non-MHC lymphocyte alloantigen loci, which were identified following reciprocal immunization between inbred lines with the same MHC haplotypes. The gene names reflect cross-immunisation of thymocytes, lymphocytes and bursal cells, respectively.
5) Immunoglobulin genes

(c) Congenic lines

To study further the genetic basis of the difference between the inbred lines, congenic lines have been created, in which one or more chromosomal segments from one line exist in a "background" of the genome of another line, e.g. lines 100 and 7.6 were created by repeated backcrossing between a resistant and a susceptible line, with the resistant line as recurrent parent, while selecting for susceptibility every generation. An extension of this strategy involves the creation of recombinant congenic lines, by full-sib inbreeding for ten generations from an F_2 or backcross generation, which results in a set of inbred lines each of which is homozygous for a different set of segments of chromosomes from the two parent lines. A set of recombinant congenic lines has also been created at University of California, Davis (UCD). The utility of both types of congenic lines is now being exploited by measuring them for the widest possible range of disease phenotypes, and then performing a genome scan with DNA markers. Results are awaited with great interest.

Congenic lines have also been established for a range of MHC haplotypes at ADOL and by Abplanalp and colleagues at UCD. These lines have proven to be very useful in characterizing the effect of the MHC on resistance.

(d) The Major Histocompatibility Complex (MHC)

A few years after the discovery of the chicken MHC came the first report of an association between the class I products (called histoglobulins) of this complex of loci and susceptibility/resistance to MD (Hansen *et al*., 1967). An enormous effort has been expended on MHC research in the last 40 years across a wide range of vertebrate species, because of its central role in recognition of pathogens – an essential first step in mounting an effective immune response. In humans and mice (the two most-studied species), the strong associations between particular histoglobulins and susceptibility/resistance to infectious disease that everyone hoped for, have not materialised. Instead, the major MHC associations in humans are with autoimmune diseases. In contrast, and in isolation from all other species studied, histoglobulins at the chicken MHC have been repeatedly shown to be strongly associated with susceptibility/resistance to pathogens such as MDV and Rous-sarcoma virus (RSV). In fact, these associations are by far the strongest and most convincing of any MHC-disease associations in any species. How can this be?

As reviewed by Kaufman (2000), Kaufman and colleagues argue that this is largely because the chicken MHC is far smaller (44 kb) and simpler than the mammalian MHC (4000 kb). The chicken MHC has only one class I locus that is expressed to any degree, which means that at most there are only two different class I histoglobulins available for recognition of pathogens and their subsequent presentation to the immune system. In contrast, the mammalian MHC has three expressed class I loci, each of which is highly polymorphic. For small pathogens in particular, such as RSV which produces only one relevant peptide, it is, therefore, far more likely that pathogens will be unrecognized by chicken histoglobulins than by their mammalian equivalents. But MDV is a large virus, producing around 80 different peptides, and yet shows the strongest of all MHC associations. Surely at least some

of its peptides would be recognised by one of the chicken class I histoglobulins? One interesting observation by Kaufman and colleagues is that unlike in mammals, the total quantity of class I histoglobulins expressed on the cell surface varies substantially among histoglobulins. Mysteriously, the most resistant histoglobulin, B21, has the lowest level of expression, while the most susceptible histoglobulin, B19, has the highest. Another point raised by Kaufman and colleagues is that the small size of the chicken MHC virtually precludes recombination within it, which means that the there is no opportunity for other MHC genes, such as the TAP genes whose products are responsible for transporting foreign peptide fragments to the cell surface, to evolve independently from the histoglobulin genes. This could seriously limit the number of options for the host to mount an effective immune response.

As if to emphasise the point made by McKay (1998) that commercial breeders should think twice before heading off in the direction of homozygosity for the most favourable alleles, Macklin *et al*. (2002) recently confirmed earlier reports that the allele conferring maximum resistance to MDV, namely B21, confers maximum susceptibility to cellulitis induced by *Escherichia coli*; and one of the alleles most susceptible to MDV, namely B13, confers maximum resistance to cellulitis. We are left to conclude that the MHC retains some intriguing mysteries.

(e) Candidate genes other than the MHC

As anticipated in section IIe, it was a straightforward task to clone the chicken *Nramp1* gene (Hu *et al*., 1996). There was great excitement when only one year later, Hu *et al*. (1997) showed that chicken *Nramp1* is closely linked to resistance to salmonellosis. Recently, Girard-Santosuosso *et al*. (2002) confirmed this result in an association study. In a similar vein, the chicken equivalent of *Lps* (now known as *TolR4*, for toll receptor 4) has been shown to influence resistance to salmonellosis in chickens (Hu *et al*., 1997). The chicken homologue of *Mx* has also been cloned, and a polymorphism involving an amino-acid substitution at position 631 has been shown to influence antiviral activity (Ko *et al*., 2002). It is too early to determine whether any of these results will have commercial significance. But they definitely indicate the value of studies with candidate genes.

(f) QTL mapping

In chickens, there have been two genome scans for resistance to Marek's disease, by the ADOL group (Vallejo *et al*., 1998; Yonash *et al*., 1999) and by the Compton group (Bumstead, 1998a). Both scans used segregating generations from a cross between ADOL lines 6 and 7. These lines were chosen because they differ markedly in MD resistance and yet are homozygous for the same MHC haplotype. This latter point meant that the genome scan would be searching for non-MHC genes affecting resistance. The ADOL study involved 272 F_2 birds genotyped for 135 DNA markers (microsatellites), and identified 14 possible QTL. The Compton study used a backcross to line 7, and identified several QTL, the most important of which, called *MDV1*, is located midway along chromosome 1. A comparative mapping analysis showed that the human and mouse chromosomal regions corresponding to *MDV1* contain a set of genes called the NK complex, that encodes receptors on natural killer (NK) cells, which are a vital component of the immune system; and, in mice, a gene for resistance to cytomegalovirus (*Cmv1*). Obviously, these are exciting results.

Because genome scans are very costly and time-consuming if all animals are genotyped for all markers, strategies have been developed by which only the animals in the tails of the distribution of the segregating generation are genotyped for all markers (called

selective genotyping). All animals are subsequently genotyped for markers that appear to be linked to genes affecting the trait(s) of interest. An extension of this strategy is to pool DNA from animals in the tail of the distribution, thereby requiring the genotyping of only two samples (high and low tails) rather than each animal in each tail. Technical problems associated with detecting differences in microsatellite frequency from densitometrical analysis of gels of pooled DNA have been overcome by Lipkin *et al*. (2002), who showed how standard half-sib families can be used for very cost-effective QTL mapping with pooled DNA. These workers demonstrated the effectiveness of this strategy by confirming two QTL for resistance to MDV.

Using selective genotyping for 124 microsatellites in a backcross between lines resistant and susceptible to salmonellosis, Mariani *et al*. (2001) identified a major QTL on chromosome 5, which they named *SAL1*. Mapping of further markers in that region narrowed the QTL down to 2 cM. In this case, comparative mapping revealed no obvious positional candidate genes. This multinational research group are now well advanced in creating a set of recombinant congenic lines that will be homozygous for different regions of *SAL1*, which will allow them to refine further the map position. In a recent report the likes of which we will see many times in the future, Wigley *et al*. (2002) investigated the possible mechanism of *SAL1* by studying details of immune response in the parental lines, and concluded that resistance is due to enhanced killing by macrophages. By excluding certain branches of immunity, this type of discovery substantially narrows the field of candidate genes.

Another example of a genome scan was described by Yonash *et al*. (2001), who created an F_2 and a backcross from high and low lines resulting from five generations of selection for antibody response to *E. coli* vaccination. Yunis *et al*. (2002) created a new F_2 and backcross after 11 generations of selection in the same parental lines, and genotyped these populations for 263 microsatellites. Using DNA pooling and selective genotyping, they have identified a QTL on chromosome 2.

Finally, in the World Congress, Siwek *et al*. (2002) reported a Dutch genome scan in an F_2 from high and low lines resulting from 17 generations of selection for antibody response to sheep red blood cells (SRBC). There have been several such selection experiments conducted over the years, in an attempt to investigate the extent to which generalized immunity confers immunity to specific pathogens. Wanting to maximize the effectiveness of their genome scan, these workers have measured a range of generalized immunity traits, namely primary and secondary response to SRBC, primary response to *Escherichia coli*, keyhole limpet haemocyanin-dinitrophenyl, and *Mycobacterium butyricum*; and cellular response to concanavalin A. Sadly, but indicative of a global trend towards protecting intellectual property, their paper contains no results from the QTL analysis.

Despite this sobering note, it is evident that much progress has been made in the search for genes underlying quantitative genetic variation in disease resistance. In some cases, there are already markers that could be used in selection programs. More importantly, there are many avenues for determining the actual mechanisms of inherited resistance right down to the molecular level. The commercial benefits that will emerge from this work are likely to be (a) not yet thought of, and (b) substantial.

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INNATE IMMUNITY: RECOGNITION AND RESPONSE

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Summary

Innate immunity is the front line in protection against pathogens, it is therefore crucial that this response rapidly detects and defines the appropriate protective immune response to a wide variety of pathogens. Innate cells use a series of Toll-like receptors (TLR) to identify pathogens and the engagement of these receptors by microbial products leads to the induction of particular cytokines vital to the protective response. As TLR are crucial to the nature of the immune response the identification and functional analysis of TLR in the chicken may provide a means to manipulate immune responses and augment protection. Furthermore, information about chicken TLR may be used to develop adjuvants to enhance existing vaccines or increase the efficacy of newly developed subunit vaccines. Similarly, approaches using cytokines directed at enhancing or exploiting features of the innate response to infections may provide alternative strategies for disease control. The availability of innate cytokines, such as ChIL-6 and ChIL-18, as pure, biologically active preparations will facilitate further exploration into their role as adjuvants and enhancers of protective immunity. It is, therefore, vital to understand the nature of this innate response and the associated cytokines to use these to enhance immune responses and improve the protective capacity of vaccines.

I. INTRODUCTION

Antibiotics have been very successful in the control of infectious diseases in food production animals; nevertheless, there is a growing perception by consumers of the need for the use of fewer chemicals, including antibiotics, in this process (Swartz, 2002). A possible outcome of a reduction in the use of antibiotics for the poultry industry then is increased incidence of bacterial infection and decreased productivity. With this in mind, there is increased pressure on the development of alternative strategies to manage infections in chickens. Moreover, there is greater emphasis on vaccine use and on the enhancement of existing vaccines to provide better long-term protection, particularly against emerging hyper-virulent strains. Adjuvants act to increase the immune response to the antigen they are administered with, however, many existing adjuvants can have deleterious effects (Vogel, 1995), such as local inflammation, which may result in the downgrading of meat quality and thus, lower profits (Lowenthal *et al.,* 2000). Therefore, if alternate adjuvants can be developed, the use of vaccination as an alternative to antibiotics would presumably be enhanced. The development of new strategies towards immunoenhancement of vaccines must be directed to understand and utilize the capacity of the immune response to differentially deal with the various pathogens it encounters. By analysing the nature of the immune response during infection we can determine the key factors associated with the initiation of disease and an ensuing protective response. These factors can then be exploited as potential adjuvants or therapeutics (McCluskie & Weeratna, 2001).

The immune response to pathogens incorporates two systems of recognition. The first line of defence is innate immunity and this is followed, if required, by the ensuing adaptive response (Aderem & Ulevitch, 2000). As the innate response is the first line of defence it is vital to understand its role in the induction and development of protective immune responses. The innate immune response is chiefly carried out by cells such as phagocytes and heterophils, and is

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a fundamental defensive weapon against pathogens as the primary point of contact (Qureshi, *et al*., 2000).

In the chicken, phagocytes such as monocytes and macrophages, are vital in the host defence against pathogens. The response of activated macrophages, which includes, migration and chemotaxis, phagocytosis and the production of reactive nitrogen and oxygen intermediates, is critical to inflammatory reactions and the containment of pathogens (Qureshi, *et al*., 2000). Furthermore, as an adjunct to their effector cell role, macrophages amplify this initial response by utilizing their role as antigen presenter cells. As an antigen-presenting cell, these cells activate T cells through co-stimulatory molecules and stimulatory cytokines (Rescigno, 2002). In response to this, the mutual interaction between the innate and adaptive immune response leads to the macrophage being further activated by the T cell predominantly by the actions of cytokines such as interferon-γ (IFN-γ) (Feng *et al*., 1999) (Figure 1). As the frontier of immune defence, these innate cells therefore represent an important stage in the detection and interaction with pathogens.

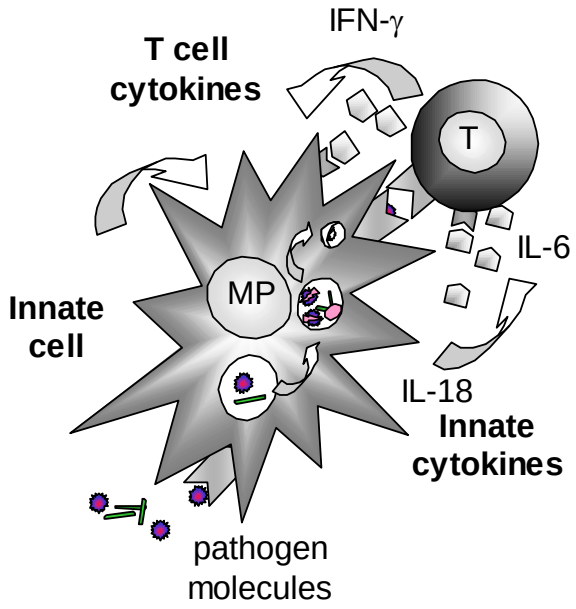

Figure 1: Interactions between innate cells and the adaptive response. Antigen presenting cells, such as macrophages (MP), interact with molecules from pathogens through their TLR. These molecules can be captured and processed for presentation to T cells. The interaction of the pathogen molecules with the TLR can activate the MP to produce nitrogen (NO) and oxygen (ROI) radicals as well as induce the expression of cytokines, such as IL-6 and IL-18 which subsequently influence the activation of T cells. Upon activation the T cell may produce cytokines to amply the activity of the MP.

II. INNATE-CELL PATHOGEN RECOGNITION

The immune response to pathogens, particularly acute infections, requires an immediate reaction to control growth and spread of infection. Fundamental to this rapid response is the recognition of the pathogen and the initiation of an appropriate protective response (Gordon, 2002). The initiation of an innate response entails the recognition of common components of pathogens not normally found in the host. In mammals much of the recent emphasis on studies of the innate immune system have centered on the mechanisms by which innate cells recognise invading pathogens (Gordon, 2002). This process of pathogen recognition involves germ-line-encoded pattern recognition receptors, particularly the toll-like receptors (TLR) (Medzhitov & Janeway, 2000). It has recently been shown that in the chicken the innate response involves a system of initial recognition of pathogens through pathogen pattern recognition receptors (Fukui, *et al.*, 2001). These TLR are homologues of Toll, a receptor that functions to establish dorso-ventral polarity in drosophila embryonic development. As in other animals, Toll in drosophila is also a component of the signaling pathway mediating the anti-fungal host defence (Imler & Hoffmann, 2002). Bacterial lipopolysaccharide provokes a vigorous activation of the innate immune response. The engagement of TLR with components of pathogens, such as

lipopolysaccharide from bacteria, induces the production of pro-inflammatory cytokines and reactive intermediates (Dil & Qureshi, 2002;Werling & Jungi, 2003). This capacity to potently activate innate and inflammatory responses means that LPS functions as an important molecule that alerts the host to potential bacterial infection. Recent studies have identified much of the LPS recognition and signalling receptors in mammalian innate cells. Initial recognition of LPS is by the mutual interaction between the LPS-binding protein, CD14 and the TLR4-MD-2 complex (Beutler *et al.*, 2001.; Guha & Mackman, 2001 ~~Shimazu et al., 1999~~). Recognition of LPS leads to the transcription of nuclear factors and the eventual activation of the inflammatory cascade (Takeuchi & Akira, 2001). Using antibody staining we have identified the expression of TLR4 on chicken peripheral blood leukocytes (PBL). Multiparameter labelling has identified these as MHC class II expressing cells, presumably monocytes and macrophages (Figure 2). As the TLR system of recognition activates the biocidal activity of innate cells it is, therefore, vital to understand the nature of the cytokines and other immune molecules produced to use these to enhance cell mediated and antibody responses to infection (Takeda & Akira. 2001). Approaches using cytokines directed at enhancing the role of the innate immune response or exploiting features of the innate response to infections may provide adjuvants to enhance vaccination or therapeutic to deal with this disease.

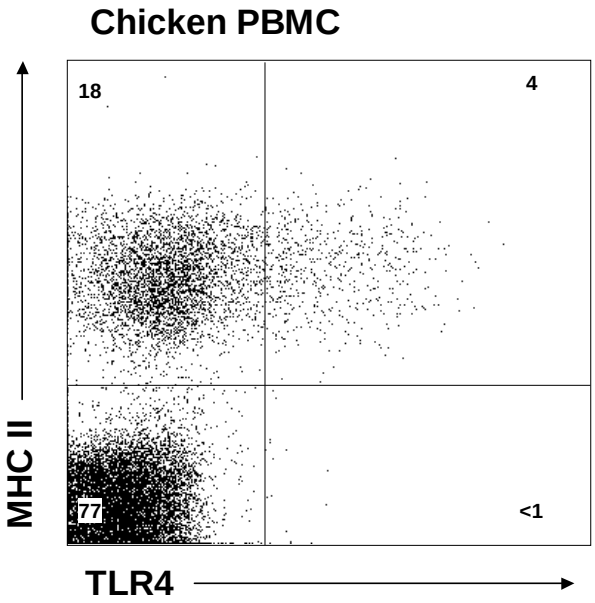

Figure 2: anti-TLR4 antibody stains a subpopulation of chicken peripheral blood mononuclear cells (PBMC). The FACS profile shows anti-TLR4 labelling against major histocompatability class II antigen staining on chicken PBMC. The percentages of positive cells are indicated within each quadrant.

III. INNATE-CELL CYTOKINE RESPONSE

Cytokines are regulatory proteins secreted by a variety of cell types that play a crucial role in controlling the immune system. Cytokines are classified in to several families such as chemokines, growth factors, neurotrophic factors, interleukins and interferons, each having specific action on specific cells. Cytokines are produced in response to infection and provide signals which help to direct the immune response towards either an antibody mediated response, often generalized as a T helper response type two (Th2) or cell mediated response, similarly referred to as T helper response type 1 (Th1) (Mosmann and Sad, 1996). A large number of mammalian cytokines have been described in detail but until recently less was known about avian cytokines. Recently, the cDNA sequences of several chicken cytokines have been identified (Staeheli *et al.*, 2001; Hilton *et al*., 2002). The increased identification, expression and characterisation of these genes provides the possibility to study the effectiveness of cytokine therapy to control disease in poultry. As cytokines are the major regulators of the immune response, they provide a natural, alternative strategy for enhancing the immune response to

infection or during vaccination. Cytokines such as interleukin-6 and -18 (IL-6 and IL-18) are made by innate cells early in the response to infection. It has been shown in mammalian models that these innate cytokines are critical to the early detection of pathogens and the direction of protective immune responses. This results in the enhancement of mucosal responses, cell mediated immunity and the maintenance of long-term protective immunity (Stevceva, *et al*.. 2000). Similar studies have not, as yet, been carried out in the chicken. With the recent discovery of the chicken homologues of these molecules there is now the potential to similarly exploit the immune enhancing potential of these cytokines.

(a) Chicken interleukin-18

Many cytokines have pleotropic activity and can act to protect the host against pathogens. Furthermore, these cytokines have vital a role in regulating the immune response but the recently described IL-18 plays a critical role in enhancing IFN-γ production. Studies on IFN-γ production in *Propionibacterium acnes*-infected mice led to the discovery of mouse IL-18 and its subsequent cloning and protein expression (Okamura *et al.*, 1995). Following the cloning of mouse IL-18 the human (Ushio *et al.*, 1996) and porcine (Muneta *et al.*, 2000) homologues have been cloned. Recently, chicken IL-18 (ChIL-18) cDNA was also cloned (Schneider *et al.*, 2000) and the protein was expressed and assessed for biological activity. Recombinant ChIL-18 induced the proliferation of cultured chicken splenocytes, and furthermore, also stimulated the production of ChIFN-γ by these cells. The scope of ChIL-18 activity suggests that this cytokine plays a significant regulatory role in cell-mediated immunity against foreign pathogens in chickens. Furthermore, LPS exposure stimulates an inflammatory response , therefore, LPS activation of cultured chicken PBL leads to the induction of ChIL-18 (Figure 3A). It is vital to study these effects in chickens in order to gain a better understanding of the role of IL-18 and its potential. Current research in our laboratory is directed toward providing evidence for this.

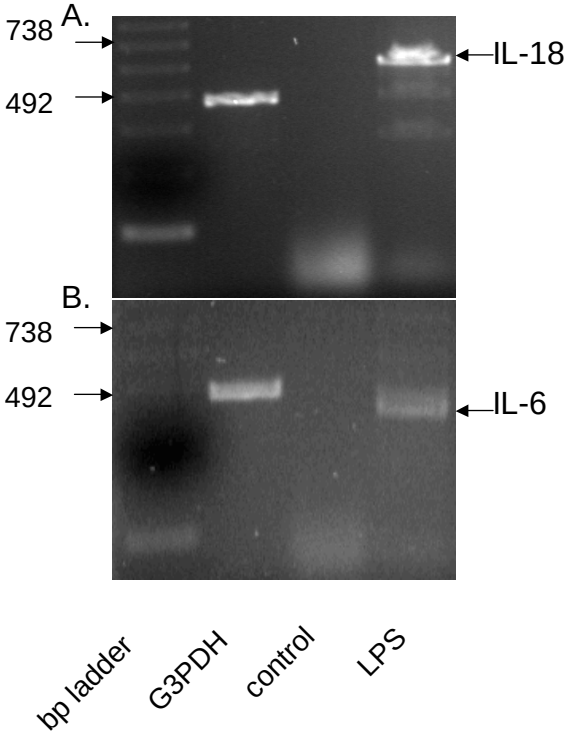

Figure 3: PCR shows IL-18 and IL-6 mRNA expression in LPS stimulated chicken peripheral blood mononuclear cells (PBMC).
Agarose gel showing the PCR products from primers designed to amplify chicken IL-18 (A) and chicken IL-6 (B). The left lane shows the 100 base pair (bp) ladder and bp sizes whilst the next lane shows a PCR control. The 3rd and 4th lanes show the PCR products for either IL-18 (A) or IL-6 (B) on control unstimulated cells or cells that have been cultured with LPS.

(b) Chicken IL-6

The functions of the various interleukins are diverse as they act to protect host cells against pathogens. The role of these interleukins in protection at the initial sites of pathogen contact, such as mucosal surfaces, is vital to protection against infection. The significance of this is exemplified by the fact that in chickens the majority pathogens invade via mucosal surfaces. Therefore, a specific interleukin which stimulates mucosal immunity could possibly be used to enhance mucosal vaccinations or as a therapeutic to protect against infections at mucosal surfaces. One such interleukin may be IL-6. IL-6 is a pleiotropic cytokine with a relative molecular weight of 21-27 kDa (Alderson *et al.*, 1989; Schneider *et al.*, 2001) and is produced in response to bacterial infection, particularly when innate cells respond to bacterial LPS. This LPS activates neutrophils, monocytes and macrophages to produce IL-6 (Figure 3B) (Svanborg *et al.*, 1999). Furthermore, bacterial infection also results in the production of other cytokines that influence IL-6 levels; for example IL-1, which further increases IL-6 levels (Strober *et al.*, 1988; Scholz, 1996). *In vitro* studies have found mucosal epithelial cell lines secrete IL-6 following contact with bacteria (Svanborg *et al.*, 1999). Moreover, *in vivo* studies support this finding as IL-6 concentrations in the gut have been found to increase following mucosal infections (Svanborg *et al.*, 1999). As a consequence, the IL-6 produced in response to bacterial infections is important in mucosal immune responses, due to its ability to enhance the terminal maturation of mucosal B-lymphocytes into immunoglobulin A (IgA) producing plasma cells (Bromander *et al.*, 1996; Boyaka *et al.*, 1999a). This is significant as IgA antibodies neutralise pathogens at mucosal surfaces, reducing infections (Ryan *et al.*, 2001). Therefore, the administration of IL-6, via gene or protein, may boost IgA levels, enhancing mucosal immunity (Husband *et al.*, 1996b). IL-6 has also been found to induce protective immune responses by directing proliferation and differentiation of T cells (Aarden *et al.*, 1992). There are conflicting views regarding the exact influence IL-6 has on T cells and whether it acts alone or in synergy with other cytokines. IL-6 has been shown to induce cytotoxic T cells to differentiate (Tosato *et al.*, 1988), although some studies have shown this is reliant on the synergistic action of IL-2 (Bass *et al.*, 1993). Moreover, in human T cell proliferation, IL-6 has been shown to act synergistically with IL-1, another proinflammatory cytokine (Holsti *et al.*, 1989; Grimble, 1998; Xie *et al.*, 2001).

The efficacy of vaccine adjuvants, particularly with regard to mucosal immunity, may be determined by their ability to immunoenhance the immune response in gut-associated lymphoid tissue. This activity is characterised by the expression of various costimulatory molecules and inflammatory cytokines. Thus, elucidation of the patterns of inflammatory cytokine expression and features of APC activation will help to facilitate the rational development of more efficacious vaccines.

IV. CONCLUSIONS

In the face of restrictions on the use of antibiotics, a greater emphasis has been placed on the development of new strategies to deal with pathogens. With this in mind, our ever-increasing comprehension of the innate immune response may provide a foundation for the treatment of a number of important poultry pathogens.

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MAREK'S DISEASE, CHICKEN INFECTIOUS ANEMIA, AND IMMUNOSUPPRESSION: A NASTY COMBINATION

K.A. SCHAT

Summary

Marek's disease (MD) herpesvirus (MDV) and chicken infectious anemia virus (CAV) are two important pathogens affecting the poultry industry. The former causes lymphomas, while the latter induces anemia and immunosuppression. Innate immune responses to MD include cytokine activation and nitric oxide (NO) production, but the latter is not necessarily beneficial for the host depending on the MDV strain. MDV-specific cytotoxic T cells (CTL) responses to a large number of MDV proteins have been described. However, concomitant infection with CAV impairs the CTL responses to MDV and REV. The pathogenesis of CAV is complex. For example, seroconversion in SPF flocks occurs during or after the onset of sexual maturity and viral DNA can be transferred to embryos. It is hypothesized that CAV can remain latent in embryonal tissues and gonadal tissues.

I. INTRODUCTION

Marek's disease (MD), caused by MD herpesvirus (MDV), has been characterized as a lymphomatous disease affecting chickens. The lymphomas consist mostly of $CD4^{+}CD8^{-}$ T lymphocytes expressing T cell receptor (TCR)αβ and major histocompatibility complex (MHC) class II antigens, although other phenotypes of T cells can also be transformed (Schat *et al*., 1991). Vaccination at embryo day (ED)18 or at one day of age with monovalent or polyvalent vaccines has provided an excellent protection against challenge. However, MD remains an economically important disease in chickens even in the face of these successful vaccination programs. The reasons for the continued economical importance are a combination of relatively high vaccination costs, a continued evolution of the MD virus (MDV) strains to ever more virulent strains (Witter, 2001a; Witter, 2001b), early MDV challenge prior to vaccine-induced protection, and the economical realities of broiler production. Condemnation rates for MD ("skin leukosis") of 1% are already considered "vaccine breaks" causing financial problems for the broiler industry. This is mostly caused by prolonged processing time resulting in increased labor costs. That most of the broiler flocks experience far less than 1% MD condemnations is actually a remarkable tribute to the efficacy of current vaccine strategies (Schat, in press).

The causes of the vaccine breaks are often difficult to identify. First of all, there is a lack of knowledge on the different aspects of vaccine-induced immunity and more importantly on the integration of innate and acquired vaccine-induced responses (Schat, 2001; Schat and Markowski-Grimsrud, 2001). Secondly, the apparently never-ending evolution towards more virulent strains of MDV is certainly a continuing concern for the industry. Not only are these strains able to cause vaccine breaks in chickens that are properly vaccinated with CVI988 or polyvalent combinations containing CVI988, but the pathology associated with these strains is also changing (Gimeno *et al*., 2001; Witter *et al*., 1999). Finally, immunosuppressive viruses, and especially chicken infectious anemia virus (CAV), are probably far more important than was previously believed. The pathogenesis of CAV is far more complex than previously suggested complicating eradication efforts in specific-pathogen-free flocks. In this review, current research in my laboratory on the pathogenesis of CAV, the current understanding of the MDV immunity,

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and the impact of CAV on MDV immunity will be briefly discussed. More detailed information can be found in several reviews recently published by our group addressing MDV immune responses (Schat and Markowski-Grimsrud, 2001; Schat and Xing, 2000) and CAV (Schat, 2003).

II. MAREK'S DISEASE IMMUNITY

To discuss MDV immunity, it will be important to briefly describe the pathogenesis of MD based on the "Cornell Model" as originally proposed by Calnek (1986) and Schat (1987) and updated by Schat and Xing (2000) and Schat (in press). Following this model, cell-free MDV infects the spleen within 36 hours probably transported by macrophages. The initial virus replication occurs in B lymphocytes resulting in the production of viral proteins including viral (v)IL-8 and cell death, but cell-free virus is not produced. Thus, in order to infect new B lymphocytes, there needs to be an intimate contact between the infected and the uninfected cells. The production of viral proteins will cause the activation of T lymphocytes, which become susceptible to infection with MDV. We have proposed that vIL-8 is a key factor in attracting B cells and activated T cells, especially because interferon (IFN)-γ is upregulated early after MDV replication starts. Activated T cells may undergo a lytic infection or become latently infected, which normally occurs around 5 to 7 days post infection. The actual onset of latency depends on a number of factors such as virulence of the MDV strain, age of infection, and genetic resistance. A secondary lytic infection has been reported in genetically susceptible chickens after the development of latency and it has been suggested that infected cells become transformed during or after the secondary cytolytic infection. However, latency may not really occur in chickens infected with vv+ strains independently of genetic resistance (Jarosinski *et al*., 2002). These birds may die as a consequence of lytic infection (early mortality syndrome) or the recently described neural syndromes.

Infection of MDV or vaccination against MDV activates several cytokines within 3 to 4 days post infection, especially IFN-γ is upregulated. The importance of other cytokines during the primary lytic phase of the pathogenesis has not been studied in detail and conflicting results have been reported [see Schat (in press) for details]. Inducible nitric oxide synthase (iNOS) is upregulated early after infection leading to the production of nitric oxide (NO) in the blood plasma. Xing and Schat (Xing and Schat, 2000) and (Djeraba *et al*., 2000) reported that the production of NO inhibits MDV replication suggesting that increased production of NO may be beneficial. It was also reported that genetically resistant strains produce more NO than susceptible strains. Jarosinski *et al*. (2002) found, however, that this may be the case when virulent strains (e.g., JM-16) as challenge virus are used, but that challenge with vv+ strains may actually lead to pathological levels of NO especially in the resistant strains. We have recently challenged offspring from a broiler line with RB-1B to examine the influence of NO on resistance. Offspring were selected from sire families based on the NO production of macrophages harvested from 20-day-old embryos and divided in low, intermediate, and high NO producers. Interestingly, chickens in the high NO producing group had a higher incidence of early mortality than the low and intermediate group (K.A. Schat, I. Pevzner, P.H. O'Connell, and C. Buscaglia, unpublished data). In addition to cytokine activation, NK cells are also activated early after infection. The true importance of this observation remains unclear, because NK cell activity is measured *in vitro* against an avian leukosis virus-transformed cell line (RP9). It will be essential to determine if these NK cells are able to lyse MD (glyco) protein expressing target cells.

Cytotoxic T cells (CTL) are the main component of the acquired immunity to MDV infected cells. In a series of experiments we have examined the CTL responses against a number of proteins (Schat and Markowski-Grimsrud, 2001; Markowski-Grimsrud and Schat, 2002).

Epitopes derived from several proteins are recognized, several of which had previously been associated with protective immunity (Table 1) (Schat, in press).

Table 1. MDV proteins recognized by cytotoxic spleen cells obtained from resistant N2a (MHC: $B^{21}B^{21}$) and susceptible P2a ($B^{19}B^{19}$) chickens 7 days post inoculation with serotype 1 or 2 MDV strains. Proteins associated with protective immunity are printed in bold (from: Schat, in press).

Marek's disease viral protein					
IE genes		Early genes		Glycoproteins	
N2a	P2a	N2a	P2a	N2a	P2a
ICP27	ICP27	**pp38**	**Pp38**	**gB**	**gB**
ICP4		Meq	Meq	**gI**	**gI**
				gC	gE
				gK	
				gH*	
				gL*	
				gM*	

* CTL were not demonstrated in all assays.

Antigen-specific CTL can be demonstrated at approximately 7 days post infection, at which time a primary infection is expected to be in latency and CTL are expected to be important in preventing reactivation from latency. However, if the CTL are the consequence of vaccination either at ED18 or at one day of age, these CTL are certainly expected to be important in curtailing subsequent replication of challenge virus once chickens are placed in the field. The impact of CAV infection on antigen-specific CTL will be discussed in section III.B.

III. CHICKEN INFECTIOUS ANEMIA VIRUS

CAV is a small DNA virus of the family Circoviridae that has recently been classified as the sole member of the genus Gyrovirus (Pringle, 1999). Its genome consists of negative sense, single stranded DNA encoding three viral proteins designated viral proteins 1 – 3 (VP1-3) (Noteborn *et al*., 1991). VP1 is the only protein found in the capsid. VP2 has protein phosphatase activity (Peters *et al*., 2001) and may also be a chaperone for protein folding of VP1 (Noteborn *et al*., 1998). Both VP1 and VP2 proteins are required to induce protective immunity (Koch *et al*., 1995). VP3 or apoptin causes cell death by apoptosis (Noteborn *et al*., 1994). CAV is highly resistant to different disinfecting methods, which contributes to the ease of infecting susceptible chickens. Infection of susceptible chickens, i.e., chicks younger than 2 to 3 weeks of age and lacking maternal antibodies, can result in anemia, immunosuppression, secondary bacterial infection, and mortality. Vaccination of breeders prior to sexual maturity has been used to induce uniform levels of maternal antibodies and protect their offspring to clinical disease. Research in my laboratory has led to new insights in the importance of this virus and has challenged conventional wisdom concerning the transmission and importance of this virus as an immunosuppressive agent. These aspects will be discussed in more detail in the next sections.

a) Transmission of CAV

Transmission of CAV occurs both by horizontal and vertical routes (reviewed by Schat, 2003 and references therein). The latter is especially important when adult birds become infected. In that case, virions can be transferred through the embryo during the viremic period

prior to the development of virus-neutralizing antibodies. Chicks hatching from these eggs develop anemia, immunosuppression, and secondary bacterial infections. These chicks will also be a source of CAV for horizontal transmission probably through the feces until neutralizing antibodies develop. It is almost a dogma that CAV spreads easily among chickens in a contaminated environment, especially because the extreme resistance of CAV to clean-up procedures would prevent effective disinfection of poultry facilities. These dogmas have been challenged recently by observations from the field and experimental data from our group. For example, 70% of grandparent flocks that were imported into Sweden and kept in quarantine remained seronegative until 16 weeks of age (Engström, 1999). Horizontal transmission may be also far less effective than previously suggested when chickens are housed in cages. Cardona and Schat (unpublished data) placed a rooster that was shedding CAV through the semen in a cage adjacent to cages containing antibody-negative hens. These birds did not seroconvert during an experimental two-month period. In addition, when specific-pathogen-free (SPF) birds of three genetic lines, maintained at Cornell University in the filtered-air, positive-pressure (FAPP) house, became accidentally infected with CAV these flocks did not seroconvert completely over a 60- to 80-week period. Interestingly, seroconversion started in most instances after onset of sexual maturity even while chicks were hatched and maintained in colony cages in a CAV-contaminated environment (Cardona *et al.*, 2000a; Miller *et al.*, 2001). We have continued to monitor the SPF flocks for seroconversion patterns without cleaning the FAPP house between generations. Interestingly, and unexplainable at the current time, flocks do sometimes show a very low pattern of seroconversion and marked differences between male and female birds (Figure 1A and B, respectively) (Miller *et al.*, 2001 and M.M. Miller, personal communication). Similar seroconversion patterns have been reported for commercial and noncommercial SPF flocks causing serious problems because the flocks are no longer considered CAV-free, thus reducing the economical value of these flocks (Schat, 2003). Interestingly, CIAV DNA could be detected in seronegative and seropositive birds by nested PCR assays of gonadal tissues and spleens, even in chickens that had been antibody positive for more than 40 wk (Cardona *et al.*, 2000b; Miller *et al.*, submitted). These birds can transfer CAV either DNA or virions to embryos, where it can be detected in the blastoderm and at 20-days of embryonation mostly in the gonads, lymphoid organs, and egg shell membranes. Other organs, eg., livers, can also be positive but with a significantly lower frequency in positive embryos than the gonads or lymphoid tissues (Miller *et al.*, submitted). Similar results have been obtained for vaccinated and non-vaccinated broiler breeder flocks (Brentano *et al.*, manuscript in preparation).

These results raise important questions on the mechanisms involved in maintaining viral DNA in gonadal tissues and embryonal tissues. McNulty (1991) speculated already that CAV could establish a latent infection and the current data seem to support this hypothesis. We propose the hypothesis that CAV is able to maintain its genomic DNA as latent, episomal double-stranded DNA in the gonads and that sexual maturity leads to activation of viral transcription leading to virus replication and subsequent seroconversion. We further hypothesize that viral DNA can be transferred to the embryo where it may undergo a limited replication at some time during embryonal development without inducing tolerance. Current research in our laboratory is directed toward providing evidence for these hypotheses.

b) CAV-induced immunosuppression

CAV infection of susceptible one-day-old chicks has long been associated with immunosuppression. Adair *et al.* (1991) reported that infection of one-day-old chickens resulted in decreased mitogen responsiveness of spleen cells and production of T cell growth factors

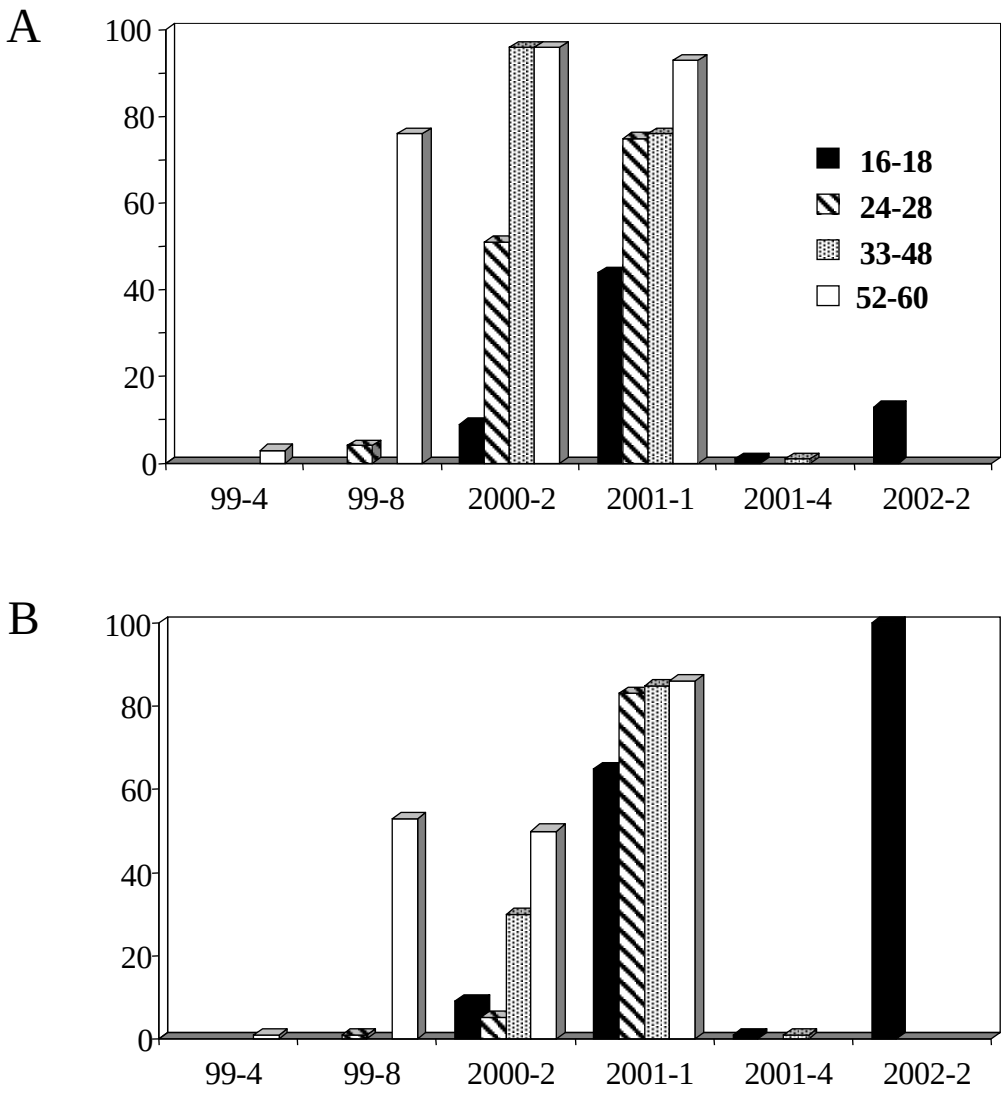

Figure 1. Antibody development to CAV in successive flocks of P2a chickens maintained in the FAPP house at Cornell University. Panel A: female chickens, panel B female chickens (adapted from Miller et al, 2001, and updated for 2001 and 2002 flocks, Miller personal communication). The legend indicates the age in weeks when sera samples were obtained.

[TCGF, presumably IL-2] at 8 and 15 days post infection (dpi). IFN production was increased at 8 dpi, but decreased between 15 and 29 dpi. Interestingly, infection in 3-week-old chickens also caused immunosuppression but this occurred in the absence of clinical disease. A decrease in IL-1, IL-2, and IFN-γ between 14 and 28 dpi was detected using bioassays. Suppression of macrophage functions was also reported when 3-week-old chickens were naturally exposed to CAV (McConnell *et al*., 1993a; McConnell *et al*., 1993b). We recently reported that CAV also affects the development or activity of MDV- and reticuloendotheliosis virus (REV)-specific CTL (Markowski-Grimsrud and Schat, submitted). REV-specific CTL were present in spleens 7 days post-infection of 9- to 30-day-old chickens that were positive for maternal antibodies to CAV at 9-17 days of age. Replication of CAV could not be demonstrated in these chickens using quantitative real-time PCR and RT-PCR assays. In contrast, REV-specific CTL failed to develop when chickens that were negative for CAV maternal antibodies at 9-17 days of age

were infected. Infection with CAV at 45 days of age also caused a decreased REV-specific CTL response. In these chickens increased levels of CAV DNA of up to 10^7 copy numbers per μg DNA and increased relative transcript levels of CAV by up to a factor of 10^6 were detected by quantitative real-time PCR and RT-PCR. IL-1β and IL-2 mRNA levels were not significantly affected by CAV infection at 7 or 14 dpi. Similar assays for IFN-γ transcripts demonstrated a ten-fold increase in IFN-γ mRNA levels at 7 dpi following REV or REV+CAV infection, while CAV alone caused a 2- to 4-fold increase.

The impact of CAV infection on the generation of antigen-specific CTL is a likely explanation of the impact of CAV on many diseases such as MDV, REV, and other viral diseases including infectious bronchitis (IB) as suggested by Dr. F Hoerr (personal communication). The latter would occur if mutations in the S1 gene causes suboptimal vaccine-induce protection through antibodies and IB virus-specific CTL become important (Collison *et al*., 2000).

IV. CONCLUSIONS

The knowledge of MDV immunity has progressed rapidly over the last few years especially in regard to the antigens that are recognized by CTL. The importance of NO for the protective immunity during the lytic infection is complex and it is likely that above a certain level NO may actually cause pathology. The pathogenesis of CAV is far more complex than previously reported. The possible transfer of latent CAV to offspring may complicate immune response studies especially if viral DNA would become activated during these studies. Latency of CAV in relation to sexual maturity raises important questions concerning the transmission of CAV. The impact of the immunosuppressive effects of subclinical infection of CAV has previously been underestimated.

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EFFECTS OF INCREASING LEVELS OF DIETARY "IDEAL PROTEIN" ON BROILER PERFORMANCE

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Summary

An experiment was conducted to investigate the effects of graded levels of dietary protein which was balanced according to the ideal protein (IP) concept on broiler performance. Ideal protein was increased up to 130% of current in full (CVB) recommendation and experimental diets were fed in the starter phase. In addition, effects of increased IP supply fed in both the starter and/or grower phase on performance were studied. Weight gain and feed conversion in the starter period and overall improved linearly with increased IP protein levels in the starter diet. Carcass quality also improved. Increased ideal protein levels in the grower phase resulted in additional improvements in performance.

I. INTRODUCTION

Previous experiments showed that increasing levels of ideal protein (IP) in grower and finisher feed substantially improved broiler performance and carcass quality (Wijtten *et al*., 2000). Moreover, Hoehler *et al*. (2002) reported, that the effects of feeding increased IP levels in consecutive phases effects on performance might be additive. Therefore, one objective of the present experiment was to evaluate the effects of increasing dietary IP levels up to 130 % of CVB recommendation (Schutte, 1996) during the starter phase on overall performance in male and female broilers. A second objective was to examine whether there is an interaction between dietary IP level (100%, 120% CVB) and growth phase (starter, grower) on overall performance. Thus two further treatments were included in order to achieve a 2x2 factorial design.

Table 1. Experimental design

Treatment*			Ideal protein-level (% of CVB standard)		
Objective I	Objective II	Sex	day 1 - 14	day 15 - 30	day 31 - 37
1	1	Male / Female	100	100	100
2		Male / Female	110	100	100
3	2	Male / Female	120	100	100
4		Male / Female	130	100	100
	3	Male / Female	100	120	100
	4	Male / Female	120	120	100

* each treatment comprised 6 replicates with male and 6 replicates with female broilers

II. METHODS

A total of 1440 male and female day old Ross 308 broilers were assigned to six dietary treatments (Table 1) to obtain a completely randomised block design. Each treatment

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comprised a total of twelve battery cages, six with 20 males and six with 20 female birds. The experimental starter, grower, and finisher diets (Table 2) were formulated according to the ideal protein concept suggested by Mack *et al*. (1999). Apparent digestible lysine levels of the 100% starter (10.5 g/kg), grower (10.2 g/kg), and finisher diets (9.9 g/kg) were based on the recommendation of CVB (Schutte, 1996). In order to obtain graded IP levels, the100% and 130% CVB starter diets fed from one to fourteen days of age (Table 2) were blended at different ratios. For the grower phase from 15 to 30 days of age , diets with 100% and 120% of CVB apparent digestible lysine recommendations were produced. Regarding the remaining nutrients and energy, diets were formulated to be adequate for each phase. The diets consisted mainly of corn, soybean meal, soy isolate, wheat, potato protein, fishmeal, and crystalline amino acids. Calculated nutrient contents were confirmed by analysis. Feed and water were available *ad libitum*.

Body weights and feed consumption were recorded for each phase. At 37 days of age the trial was terminated and five birds per pen were subjected to carcass evaluation. Data were analysed by ANOVA with subsequent comparison of means. For the latter $P<0.05$ was considered significant.

Table 2. Calculated AME (MJ/kg), crude protein (CP, g/kg) and apparent faecal digestible amino acid content (g/kg) of the experimental diets

	Starter		Grower		Finisher
Energy, CP and digestible amino acid content	100 % of CVB	130 % of CVB	100 % of CVB	120 % of CVB	100 % of CVB
AME_n	11.92	11.92	12.55	12.55	12.55
CP	205	266	199	239	197
Lys	10.5	13.7	10.2	12.2	9.9
Met	5.3	6.9	5.1	6.1	4.8
Met + Cys	7.9	10.2	7.7	9.2	7.4
Thr	6.6	8.6	6.4	7.7	6.3
Trp	2.2	2.9	2.2	2.6	2.2
Ile	8.0	10.4	7.7	9.2	7.6
Val	8.9	11.5	8.5	10.2	8.5
Arg	11.8	15.3	11.4	13.7	11.1

III. RESULTS AND DISCUSSION

As shown in Table 3 birds of both genders responded to increasing IP levels. Most of the responses followed a linear trend as illustrated for overall weight gain and feed conversion in Figure 1. In the starter period, weight gain and feed conversion improved by six to nine percent. A linear performance improvement due to increasing dietary IP levels has also been reported for grower and finisher broilers (Wijtten *et al*., 2000).

At day 37 male and female birds of the 130% CVB treatments were 58 g (2.4%) and 70 g (3.2%), respectively, heavier compared to those fed the 100% CVB diets which is in good agreement with the experiment of Hoehler *et al*. (2002). There, an increase in dietary IP from 100% to 120% CVB in the starter diets resulted in a 3.6 % higher final weight in male broilers. However, while there were hardly any performance differences between sexes at day 14, male birds performed clearly better regarding the overall results in the present experiment. Weight gain and FCR in males or females at 100% CVB was about 400g and 0.04 kg/kg, respectively, better compared to breeder's recommendations (Aviagen, 2000)

demonstrating the high potential of current broiler strains to respond to elevated ideal protein levels in feed.

Table 3. Effect of increasing levels of ideal protein in the starter phase on various performance criteria in male and female broilers 1 to 37 days of age

Starter IP-level, % of CVB	**100 %**	**110 %**	**120 %**	**130 %**
Male				
Gain, 1-14 days	460 [c]	466 [bc]	487 [ab]	496 [a]
Gain, 1-37 days	2443	2453	2501	2501
FCR, 1-14 days	1.248 [a]	1.217 [ab]	1.188 [b]	1.141 [c]
FCR, 1-37 days	1.570	1.564	1.562	1.550
Breast meat **	31.0	31.1	31.0	31.3
Abdom. fat **	2.12	2.11	1.95	1.98
Female				
Gain, 1-14 days	462 [c]	470 [bc]	491 [a]	487 [ab]
Gain, 1-37 days	2180 [b]	2212 [ab]	2215 [ab]	2250 [a]
FCR, 1-14 days	1.262 [a]	1.217 [b]	1.188 [c]	1.171 [c]
FCR, 1-37 days	1.647	1.641	1.652	1.632
Breast meat **	312 [b]	310 [b]	313 [ab]	320 [a]
Abdom. fat **	277	263	253	254

* Different superscripts (a,b,c) within row indicate significant differences ($P < 0.05$)
** expressed as proportion (g/kg) of carcass weight

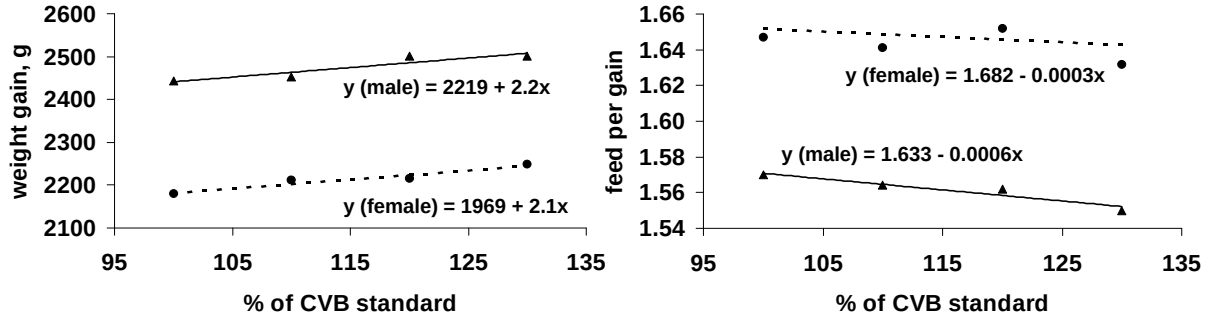

Figure 1: Effect of increasing ideal protein levels in the starter diets on weight gain and feed conversion in male (solid) and female (dotted) broilers 1 to 37 days of age

Fat pad proportion was higher in females than males but was linearly reduced by increasing IP levels in both sexes. In females, breast meat yield was improved in the 130% CVB group. In previous studies, breast meat yield was increased substantially when increased IP levels were fed in the grower-finisher phase (Wijtten *et al*., 2000). Conversely, in the experiment of Hoehler *et al*. (2002) an increase in IP from 100% to 120% of CVB recommendation in the starter diets did not affect breast meat yield whereas increased IP levels fed during the starter and grower phase increased breast meat yield. Data obtained in the present experiment also suggest that breast meat growth is especially influenced by amino acid supply during the grower phase (Table 4), although differences between 120%/100% and 120%/120% IP supply were not significant. In males abdominal fat content showed basically the opposite effect to breast meat while for females this effect was not as consistent. The results are in contrast to those of Kidd *et al*. (1998) who reported that dietary Lys levels above that considered adequate in grower-finisher diets were not able to completely compensate for inadequate Lys provision in the starter diet. However, in the present study

even the 100% CVB treatments did not represent an amino acid deficiency.

Similarly to breast meat yield, weight gain and feed conversion of male birds and feed conversion of female birds improved significantly when feeding 120% IP levels during the grower phase (Table 4). This is in line with findings of Hoehler *et al*. (2002).

Table 4. Effects of increasing levels of balanced protein in the starter and/or grower phase on various performance criteria in male and female broilers from 1 to 37 days of age

Starter IP-level	**100**	**100**	**120**	**120**
Grower IP-level	**100**	**120**	**100**	**120**
Male				
Gain, 1-14 days	460 ^{bc}	446 ^{c}	487 ^{ab}	495 ^{a}
Gain, 1.37 days	2443 ^{b}	2455 ^{ab}	2501 ^{ab}	2534 ^{a}
FCR, 1-14 days	1.248 ^{a}	1.252 ^{a}	1.188 ^{b}	1.173 ^{b}
FCR, 1-37 days	1.570 ^{a}	1.542 ^{b}	1.562 ^{a}	1.536 ^{b}
Breast meat **	310 ^{b}	318 ^{a}	310 ^{ab}	317 ^{ab}
Abdom. fat **	212 ^{a}	174 ^{b}	195 ^{a}	179 ^{b}
Female				
Gain, 1-14 days	462 ^{b}	462 ^{b}	491 ^{a}	479 ^{a}
Gain, 1.37 days	2180	2224	2215	2211
FCR, 1-14 days	1.262 ^{a}	1.245 ^{a}	1.188 ^{b}	1.182 ^{b}
FCR, 1-37 days	1.647 ^{a}	1.623 ^{b}	1.652 ^{a}	1.624 ^{b}
Breast meat **	312 ^{b}	321 ^{a}	313 ^{ab}	316 ^{ab}
Abdom. fat **	277	265	253	261

* Different superscripts (a,b,c) within row indicate significant differences ($P < 0.05$)
** expressed as a proportion (g/kg) of carcass weight

In conclusion, the experiment showed that increasing dietary IP levels in the starter diet improved performance of male and female broilers not only during the starter phase but also for the overall grow-out period. Increased dietary ideal protein given during both the starter and grower phases may result in further improvements in performance. Breast meat development appeared to be more influenced by amino acid supply during the grower than during the starter phase.

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INTERACTIONS OF DIETARY PROTEIN AND SUPPLEMENTS OF METHIONINE PLUS CYSTEINE ON BROILER PRODUCTION

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Summary

An experiment with two strains of male broilers (14-35 days of age) was conducted to investigate the effects of four levels of digestible methionine plus cysteine (Met+Cys) on various performance criteria at two dietary protein levels (20.5 and 26.0 g/kg crude protein (CP) containing 11.2 and 14.6 g/kg digestible lysine (Lys) respectively). Except for Met+Cys, the ratios between essential amino acids (EAA) and non-EAA were kept equal in all diets and were consistent with the ideal protein concept. Increasing the balanced protein level resulted in clear improvements in gain, feed conversion, breast meat yield, and abdominal fat content. The effects of increasing Met+Cys level (50, 62, 69, 77% Met+Cys : Lys ratio) followed non-linear or linear trends at both protein levels and for both strains. The results suggest an optimum Met+Cys : Lys ratio higher than 0.7777 for feed conversion and breast meat yield.

I. INTRODUCTION

The ideal protein (IP) concept is a tool enabling quick and easy adjustments to changing production conditions. Thus, knowledge only of optimum dietary lysine levels for certain conditions are needed while the ratios between all essential amino acids (EAA) and lysine are maintained the same. However, in an ideal protein all EAA are equally limiting and hence the reduction of one of the EAA must inevitably lead to impaired performance. In addition, it has recently been reported that broiler performance is substantially improved with increasing IP levels (Wijtten *et al*., 2000). The aim of the present study was to examine the effects of graded Met+Cys levels up to a Met+Cys : Lys ratio of 0.77 on broiler performance at both adequate and high IP levels.

II. METHODS

Day-old male Ross 308 and Cobb 500 chickens were raised until thirteen days of age by feeding a commercial starter diet. At day fourteen, birds were individually weighed and randomly distributed to 80 floor pens (40 pens/strain; 36 birds/pen). Birds of each strain were assigned to eight dietary treatments according to body weight to achieve a completely randomised block design. Dietary treatments comprised four levels of Met+Cys at two protein levels (adequate, high) (Table 1). Corresponding Met+Cys contents of both protein levels were expressed as Met+Cys : Lys ratio based on the IP concept of Mack *et al.* (1999).
All experimental diets were based on corn, corn starch, soybean meal, and poultry by-product meal. A high protein diet was formulated to contain 260 g/kg protein and 14.6 g/kg true fecal digestible Lys (Table 2). All remaining EAA were adjusted according to the IP concept of Mack *et al.* (1999) except for Met+Cys. Digestible Met+Cys content of the basal high protein diet was 7.3 g/kg which corresponded to a Met+Cys to Lys ratio of 0.50. The

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ratio between Met and Cys was 0.58 : 0.42. All other nutrients and energy met or exceeded requirements according to NRC (1994). The adequate protein diet (200 g/kg IP, 11.2 g/kg dig. Lys) was obtained by diluting the high protein diet with a non-protein containing mixture. This was mainly based on corn starch, soybean oil, and minerals. Increasing Met+Cys levels were achieved by supplementation with increasing levels of a premix containing Met+Cys in a ratio of 0.58 : 0.42 at the expense of an inert filler. Calculated amino acid contents were confirmed by analysis.

Table 1. Experimental design

Treatment	Protein (g/kg)	Met+Cys (g/kg)	Met+Cys to Lys ratio
1	205	5.6	0.50
2	205	6.9	0.62
3	205	7.8	0.69
4	205	8.6	0.77
5	260	7.3	0.50
6	260	9.0	0.62
7	260	10.1	0.69
8	260	11.2	0.77

Table 2. Measured protein and amino acid levels (g/kg) and calculated values of Metabolisable Energy (MJ/kg) and True Faecal Digestible Amino Acid content (g/kg) of the adequate and high protein diets

Measured protein and amino acids	High protein diet	Adequate protein diet	Calculated ME and digestible amino acids	High protein diet	Adequate protein diet
Crude protein	257	204	ME	12.8	12.8
Met	4.4	3.5	tr. dig. Met	4.2	3.2
Met + Cys	7.9	6.3	tr. dig. Met + Cys	7.3	5.6
Lys	16.0	12.6	tr. dig. Lys	14.6	11.2
Thr	10.9	8.5	tr. dig. Thr	9.8	7.6
Arg	17.5	13.7	tr. dig. Arg	15.7	12.1

In addition to recording body weights and feed consumption of all birds at weekly intervals, six birds per pen (30 birds/treatment) were randomly selected and slaughtered for carcass evaluation at day 35 of the experiment.

Data were analysed by ANOVA procedure and, where possible, by exponential regression analysis (PROC NLIN, SAS 8.01). For comparison of means, $P<0.05$ was considered significant.

III. RESULTS and DISCUSSION

As shown in Table 3 and Figures 1 and 2, all treatments had significantly influenced broiler performance. However, differences between strains must be interpreted carefully because initial weight at day 14 differed significantly ($P < 0.05$) between strains (Cobb 500: 434 g; Ross 308: 465 g). Hence, observed effects cannot be completely attributed to strain differences.

Increasing the IP level significantly improved weight gain, feed conversion, breast meat yield, and fat accretion. These observations confirm recent findings demonstrating substantially improved broiler performance at increased IP levels up to 27 % (Wijtten *et al*., 2000, Hoehler *et al*., 2002). In the present study, the increased IP level improved weight gain, feed conversion and breast meat yield and reduced abdominal fat by 13%, 8%, 5%, and 22%, respectively. These effects may indicate that current nutritional recommendations are not sufficient for realising the full genetic potential of current broiler strains.

Table 3. Effect of strain, ideal protein level and Met+Cys level on weight gain, feed conversion, breast meat yield, and fat pad percentage in broilers 14 to 35 days of age

	Weight gain	Feed per gain	Breast meat	Abdominal fat
	g		% of carcass	% of carcass
Cobb 500	1288	1.702	20.60	1.27
Ross 308	1346	1.710	20.72	1.45
260 g/kg Protein	1402	1.633	21.19	1.19
205 g/kg Protein	1233	1.779	20.13	1.53
Met+Cys/Lys: 50**	1180 ^{B}	1.845 ^{A}	19.47 ^{C}	1.69 ^{A}
Met+Cys/Lys: 62	1336 ^{A}	1.696 ^{B}	20.59 ^{B}	1.33 ^{B}
Met+Cys/Lys: 69	1381 ^{A}	1.649 ^{BC}	21.20 ^{A}	1.24 ^{B}
Met+Cys/Lys: 77	1372 ^{A}	1.634 ^{C}	21.38 ^{A}	1.18 ^{B}
Strain*	0.004	0.506	0.394	0.002
Protein	< 0.001	< 0.001	< 0.001	< 0.001
Met+Cys : Lys	< 0.001	< 0.001	< 0.001	< 0.001
C.V., %	6.49	3.17	2.98	17.08

* Significance level of main effects in ANOVA

** Means within a column with different superscripts are significantly (P < 0.05) different

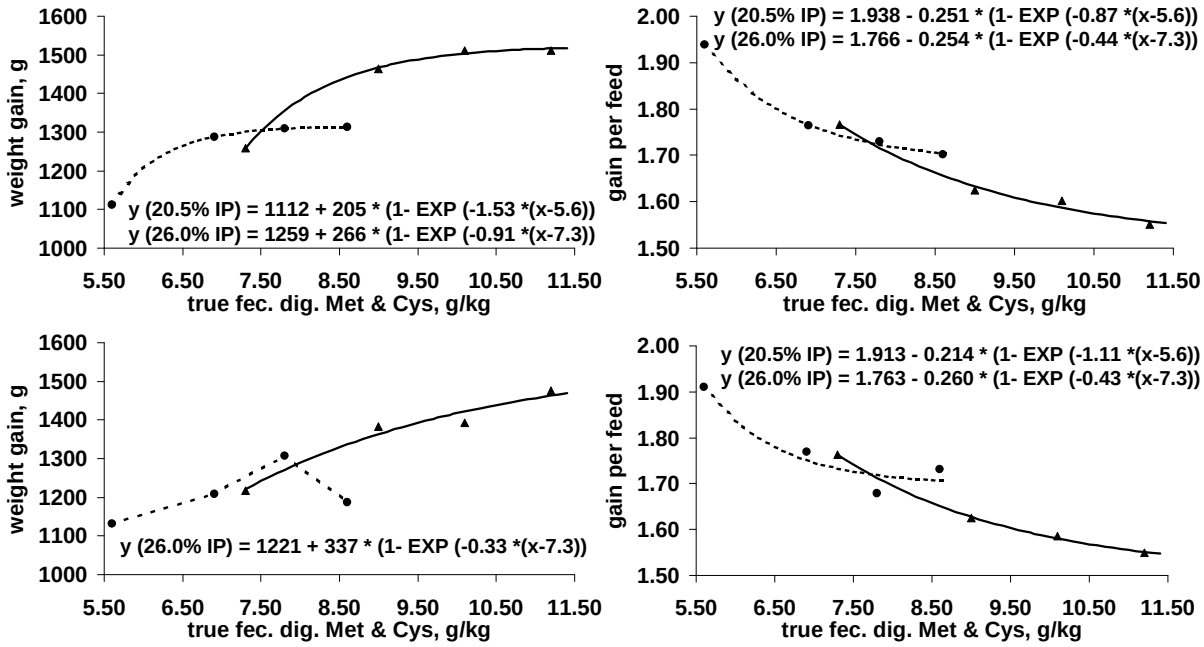

Figure 1: Effects of graded levels of Met+Cys on weight gain and feed conversion in male Ross 308 (top) and Cobb 500 (bottom) at adequate (dotted) and high (continuous) ideal protein levels.

Birds of both strains and protein levels responded markedly to increasing dietary Met+Cys. There is no explanation for the decrease in weight gain of the Cobb 500 birds at adequate IP level and highest Met+Cys supplementation. However, the majority of the dose-responses followed a non-linear or linear trend suggesting that reducing Met+Cys in the diet and thus lowering the Met+Cys : Lys ratio from 77% is immediately reflected by impaired performance.

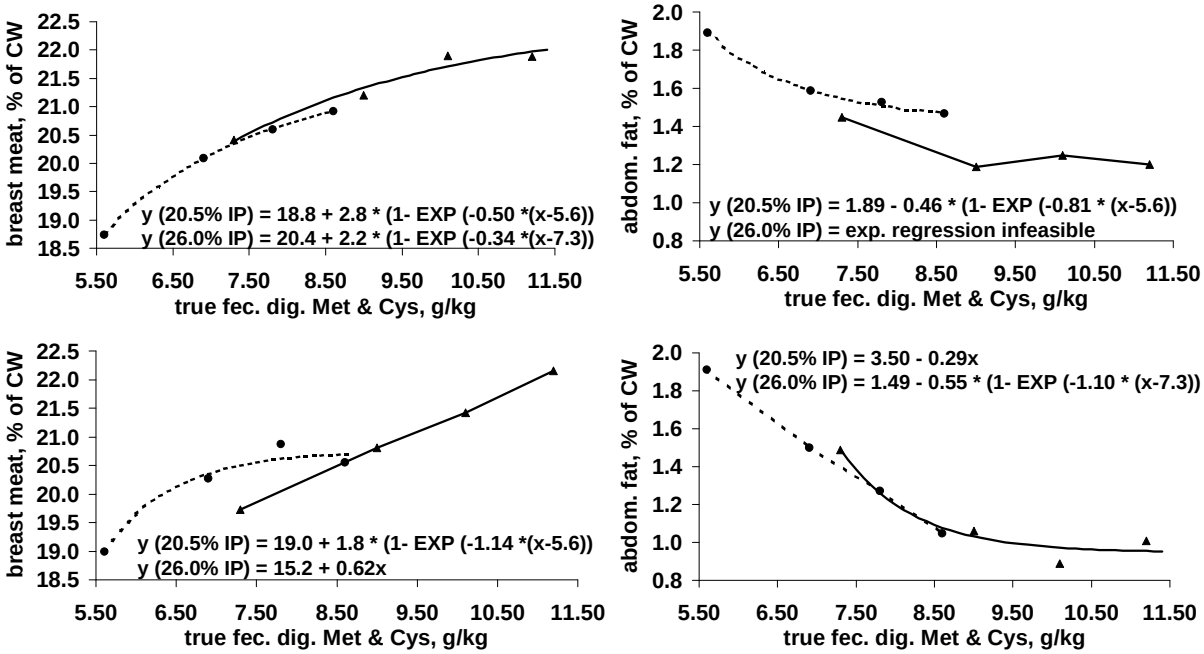

Figure 2: Effects of graded levels of Met+Cys on breast meat yield and abdominal fat content in male Ross 308 (top) and Cobb 500 (bottom) broilers at adequate (dotted) and high (continuos) ideal protein levels.

Moreover, taking 95% of the asymptotic response as the optimum performance level, the corresponding digestible Met+Cys level for most of the curves would be higher than maximum tested levels. Only weight gain data confirmed a sufficient Met+Cys supply at a Met+Cys : Lys ratio of 0.77, except for Cobb 500 at high IP supply. Thus, the findings indicate feed conversion, breast meat yield and fat pat percentage to be more sensitive to a (relative) Met+Cys deficiency than weight gain. This is in line with the findings of Schutte and Pack (1995), where weight gain was optimised at lower dietary Met+Cys levels than were required to optimise feed conversion or breast meat yield. The present data suggest a Met+Cys: Lys ratio higher than 0.77 to optimise feed conversion and breast meat yield.

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HYDROLYSIS AND ABSORPTION OF HMB OLIGOMERS ARE COMPLETE IN THE CHICKEN INTESTINE

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Methionine is the first limiting amino acid in poultry nutrition. A synthetic supplement is usually supplied either as DL-methionine (DLM) or as DL-HMB (hydroxy analogue, 2-hydroxy-(4-methylthio) butanoic acid). In both cases, D-methionine and DL-HMB have to be converted into L-methionine to be functional in chickens. Moreover, in solution, HMB forms oligomers that have to be hydrolyzed prior to conversion. The first study investigated both the capacity of the intestine to hydrolyze HMB oligomers as well as the absorption of HMB along the chicken small intestine. The second study determined the capacity of the chicken intestine to convert HMB to methionine and to use it as a substrate for taurine and cysteine synthesis.

The capacity of the intestine to hydrolyse HMB oligomers and the regional profile of monomer uptake along the chicken small intestine were compared with an HMB-product containing only monomer. *In vivo* HMB perfusion of the jejunum shows that the intestine hydrolyses the oligomers efficiently, and there were no significant differences between the two hydroxy analogue sources in monomer disappearance from the intestinal lumen or in plasma concentration. The results obtained in everted sacs showed that there were no statistical differences between the two substrates tested in monomer serosal appearance or tissue accumulation, and that a higher uptake for the jejunum and ileum than for the duodenum, but no significant regional differences in oligomer hydrolysis. Due to the high hydrolytic activity detected in the small intestine, it can be concluded that HMB oligomer hydrolysis is not limiting in the supply of methionine from this source.

In order to evaluate the efficacy of the conversion of HMB into methionine, the synthesis of taurine and cysteine was also evaluated. Conversion of HMB into L-methionine starts by a two step-mediated process which begins in the intestine for D-HMB. The concentration of these sulphur-containing amino acids was quantified in the serosal compartment of everted sacs from the chicken small intestine (D, duodenum, J, jejunum and I, ileum) incubated in the presence of 7 mM HMB or L-methionine on the mucosal side at two pH conditions (5.5 and 7.4). The results showed that, as previously described, HMB transport capacity is higher at pH 5.5 than at pH 7.4 (pH 5.5 *vs* 7.4: D, $P \geq 0.05$ and, J and I, $P < 0.05$ 1.6- and 1.3-fold, respectively). Methionine and taurine appearing in the serosal compartment showed a similar pH profile to HMB transport, whereas no pH effect was detected for cysteine (methionine pH 5.5 *vs* 7.4: D and I, $P < 0.05$ 1.7- and 1.6-fold and J, $P \geq 0.05$ and, taurine pH 5.5 *vs* 7.4: D, $P \geq 0.05$ and, J and I, $P < 0.05$ 1.8- and 1.7-fold). Regional comparisons showed that the jejunum and ileum had a higher HMB transport capacity than the duodenum ($P < 0.05$), but this behaviour is not reflected in the analysis of sulphur-containing amino acids. Taurine reached similar levels ($P \geq 0.05$) in HMB and L-methionine incubated sacs and cysteine had significantly higher values at pH 5.5, in HMB than in L-Met sacs (D, J and I, $P < 0.05$ 2-, 2.1- and 2.5-fold, respectively). We concluded that taurine and cysteine production are similar from both sources of methionine. Moreover, the ability of the chicken intestine to convert HMB to sulphur-containing amino acids is related to its capacity to transport this substrate.

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PREDICTION OF AMINO ACID DIGESTIBILITY OF COMPLETE BROILER DIETS

W.L. BRYDEN and X. LI

With the advantages of formulating diets using amino acid digestibility values (Ravindran and Bryden, 1999) there is a need to know the extent to which ileal digestibility values determined with individual feed ingredients or taken from published values predict amino acid digestibility of complete diets. In this study finisher diets comprising sorghum, wheat, canola meal, cottonseed meal, meat and bone meal and soyabean meal were formulated using either the RIRDC Booklet (Ravindran *et al*., 1998) values for digestible amino acids (Diet 1) or ingredients of known amino acid digestibility (Diet 2). Both diets were mixed using the same batch of ingredients for which digestibility values had been determined. The diets were each fed to 6 pens of 6 male broilers (Cobb) from days 28 to 42. On day 42, ileal samples were collected and amino acid digestibility determined. The predicted and determined apparent ileal digestibility coefficients for selected amino acids are shown in the Table.

Amino acid	Diet 1		Diet 2	
	Predicted	Determined	Predicted	Determined
Threonine	0.725	0.711	0.741	0.714
Alanine	0.798	0.770	0.781	0.785
Valine	0.752	0.715	0.747	0.731
Isoleucine	0.757	0.718	0.755	0.734
Leucine	0.792	0.773	0.796	0.790
Phenylalanine	0.789	0.775	0.795	0.788
Lysine	0.786	0.773	0.783	0.762

Although there were differences in apparent digestibility among amino acids, the results indicate that values are additive and that amino acid supply in a complete diet can be predicted from amino acid digestibilities of individual ingredients. The lower determined values may reflect greater endogenous amino acid secretion when complete diets are fed compared to the feeding of semi-purified diets in bioassays for determining digestibility of individual feed ingredients.

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COMPARISON OF ILEAL ENDOGENOUS AMINO ACID FLOWS IN BROILERS, ROOSTERS AND LAYERS

V. RAVINDRAN, W.H. HENDRIKS, D.V. THOMAS and B.J. CAMDEN

Published data comparing endogenous amino acid (EAA) losses in different classes of chickens is lacking. In the present study, the ileal EAA losses in broilers, roosters and layers were determined using the enzymatically hydrolysed casein (EHC) method. A test diet, based on dextrose and EHC to give a protein level of 180 g/kg and containing 3 g chromic oxide/kg as a digesta marker, was offered *ad libitum* to four pens (4 birds/pen) of male broilers (Ross; 39day old), 16 cages of individually-housed layers (Hy-line; 70-weeks old) or 16 cages of individually-housed roosters (Hy-line; 70-weeks old). For layers and roosters, four birds in adjacent cages were considered a replicate. Thus, there were four replicates per class of chicken. After four days on the diet, digesta contents from the lower half of the ileum were collected and pooled within a replicate. The ileal endogenous flow (related to the ingestion of 1 kg of dry matter; the units are mg/kg dry matter intake) was calculated according to the procedures of Moughan *et al*. (1992). The endogenous flows of nitrogen, selected amino acids and total amino acids are presented.

Parameter	Broilers	Roosters	Layers	Pooled SEM
Nitrogen	2477	2291	2285	114.4
Aspartic acid	1114	1136	1118	44.7
Threonine	968	996	1133	46.0
Serine	926[b]	1195[a]	962[b]	81.3
Glutamic acid	2098[a]	2164[a]	1271[b]	159.9
Proline	1203[a]	942[b]	839[b]	68.2
Glycine	584	519	624	35.0
Valine	714	783	742	30.8
Isoleucine	521[ab]	678[a]	468[b]	41.1
Leucine	729	675	679	50.0
Lysine	462	527	454	67.2
Cystine	310	307	351	27.5
Methionine	198[a]	160[b]	185[ab]	9.1
Total amino acids	12394	12180	11607	512.7

[a,b] Means in a row without a common superscript are significantly different (P<0.05).

The endogenous output of nitrogen and total amino acids in broilers, roosters and layers were similar (P>0.05). Endogenous flows of individual amino acids were also similar (P>0.05), except for glutamic acid, proline, isoleucine and methionine. The flow of glutamic acid in layers was lower (P<0.05) than in the other two bird classes. The flow of proline in broilers was higher (P<0.05) than with the other two bird classes and the flow of isoleucine in roosters washigher (P<0.05) than in layers. Methionine flow in roosters was lower (P<0.05) than that in broilers. It is known that individual sources of endogenous protein have different amino acid compositions and this may partly explain the observed differences between different classes of chickens.

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PRACTICAL APPLICATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY TO PREDICT AMINO ACIDS IN FEED INGREDIENTS

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Accurate knowledge of the amino acid content in feed ingredients is essential to produce precise and cost-effective feeds. Classical amino acid analysis requires oxidation and hydrolysis of the protein followed by ion-exchange chromatography. These time-consuming and expensive techniques are unsuitable for routine control of incoming raw materials in feed mills. For these reasons, table values or average values of amino acids in raw materials are commonly used in practical feed formulations.

Near-Infrared Reflectance Spectroscopy (NIRS), used for over 30 years to determine proximate analysis, can also be used to predict the amino acid content of feed ingredients (Fontaine *et al.* 2001, 2002). The advantages of NIRS are speed, easy and safe handling, simultaneous measurement of different nutrients and low costs. As high throughput is the major advantage of NIRS, more samples can be analysed. Analysing a large number of samples of a single raw material will help feed manufacturers to screen incoming feed ingredients on an on-going basis. For example, raw material quality can be compared between suppliers or by regions in which it was produced.

The practical advantage of using NIRS is illustrated below using meat and bone meal (MBM) as an example. A compound feed manufacturer sampled deliveries from two MBM suppliers over a 4-week period. A total of 45 samples were taken, 24 samples from supplier A and 21 from supplier B, and the contents of crude protein (CP), lysine (Lys) and total sulphur amino acids (TSAA; methionine + cystine) were determined using established NIRS calibrations. Table 1 shows the mean values and the coefficient of variation for CP, Lys and TSAA. for the total population and the two suppliers. .

	Total number n = 45	Supplier A N = 24	Supplier B n = 21
Crude protein,%	52.4	49.9	55.4
CV	*7.1*	*6.3*	*2.8*
Lysine,%	2.97	2.80	3.17
CV	*11.1*	*11.9*	*5.7*
TSAA,%	1.18	1.13	1.24
CV	*8.7*	*8.8*	*5.5*

It is clear that differences exist in the mean CP, Lys and TSAA contents of the two MBM suppliers. Even more important than reliable mean values is information on the variation of a raw material. If we consider only the coefficients of variation of supplier A and supplier B, it is readily apparent that splitting the population into two sub-populations has the effect of reducing the variation of MBM samples from supplier B by almost 50%, irrespective of the parameter observed. Reducing the raw material variation allows the nutritionist to reduce safety margins in ration formulation.

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[2]Degussa AG, Feed Additives Division, P.O. Box 1345, D-63403 Hanau, Germany.

INFLUENCE OF AGE ON ILEAL LYSINE DIGESTIBILITY OF FEEDSTUFFS IN BROILER CHICKENS

K. HUANG[1,2,4], X. LI[1,2], V. RAVINDRAN[1,3], L.I. HEW[1] and W.L.BRYDEN[1,2]

Lysine is normally the first amino acid to be considered in ~~th~~amino acid profiles in commercial broiler diets. Although there are reports of effect of broiler age on lysine digestibility in complete diets (Batal and Parsons, 2002), the influence of age on lysine digestibility of individual feedstuffs has not been established. In the present study, the apparent ileal digestibility (AID) of lysine in eight feed ingredients for broilers was determined at three ages (14, 28 and 42 days of post-hatching). The ingredients were assayed using the procedures described previously (Ravindran *et al*., 1998). Assay diets contained the test ingredient as the only source of protein. Celite was included in all diets as a digesta marker. Following overnight fasting, each assay diet was fed *ad libitum* to five pens (12 birds/pen at 14 days, 8 birds/pen at 28 days and 6 birds/pen at 42 days) of male broilers (Cobb) for three days. Chickens were killed for collection of digesta from the terminal ileum. Lysine digestibility values for the three age groups are shown below.

Ingredient	14 days	28 days	42 days	Pooled SEM	P value	LSD $_{0.05}$
Maize	0.69$^{b\ 1}$	0.77^{a}	0.80^{a}	0.018	0.002	0.054
Sorghum	0.69^{b}	0.68^{b}	0.77^{a}	0.011	0.000	0.035
Wheat	0.68^{a}	0.58^{b}	0.64^{ab}	0.019	0.006	0.058
Millmix	0.62^{b}	0.61^{b}	0.79^{a}	0.006	0.000	0.017
Soyabean meal	0.87^{b}	0.89^{b}	0.91^{a}	0.005	0.001	0.016
Canola meal	0.79	0.79	0.80	0.005	0.515	0.017
Cottonseed meal	0.53^{b}	0.51^{b}	0.60^{a}	0.010	0.000	0.030
Meat and bone meal	0.79^{b}	0.83^{a}	0.81^{ab}	0.009	0.026	0.028

[1] Means in a row bearing different superscripts are significantly different ($P < 0.05$).

The influence of age on ileal lysine digestibility varied with ingredients. Lysine AID increased with age ($P < 0.05$) in maize, sorghum, millmix, soyabean meal, cotton meal, and meat and bone meal. Broiler age had no effect ($P > 0.05$) on AID of lysine in canola meal. The digestibility value for wheat determined at 14-days was higher ($P < 0.05$) than that determined at 28-days, but similar ($P > 0.05$) to 42-days. The reason for this unexpected observation is unclear. It was previously shown (Angkanaporn *et al*., 1996) that amino acid digestibility in complete diets can be predicted from amino acid digestibility in individual feed ingredients. The values that were determined in this study could be used to predict lysine digestibility of broiler diets formulated from a range of feed ingredients for the different broiler age groups.

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BROILER PERFORMANCE AND METHIONINE AND LYSINE CONCENTRATIONS IN DIETS FORMULATED USING DIGESTIBLE AMINO ACID VALUES

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Results of a previous study demonstrated that formulating diets based on digestible amino acid values gave superior broiler performance when compared to birds fed diets based on total amino acid values (Li *et al.*, 2002). It was observed in this study that birds receiving higher dietary lysine and methionine levels had higher breast meat yield and lower abdominal fat content. The objective of this study was to determine the optimum dietary levels of digestible lysine and methionine for producing maximum breast muscle yield and minimal abdominal fat content when fed throughout the broiler growing cycle.

Diets consisting of sorghum, wheat, canola meal, cottonseed meal, meat and bone meal, soybean meal, minerals and vitamins were formulated to contain 230, 210 and 200g crude protein per kg of diet for starter, grower and finisher phases, respectively. The apparent metabolisable energy level of the finisher diets was approximately 13 MJ/kg diet. There were 12 experimental diets consisting of four levels of lysine and three levels of methionine. During the finisher phase the supplementary dietary levels of lysine and methionine were 11.5, 12.0, 12.5 and 13.0 g/kg and 3.8, 4.5 and 5.5 g/kg, respectively. Each diet was fed to 6 pens of 6 male broilers (Cobb); starter from days 1 to 14, grower from days 14 to 28 and finisher from days 28 to 40. Feed consumption and body weights were recorded throughout the study. On day 40 body measurements were made on 12 birds from each diet following a lethal injection of sodium pentobarbitone.

The results of the study show that methionine significantly increased liveweight gain and breast muscle yield, decreased abdominal fat content ($P<0.05$) but had no effect on feed intake. Feed conversion was significantly improved as methionine level in the diets increased ($P<0.05$). A similar positive response in performance was not observed with increasing levels of dietary lysine. There were no interactions between dietary lysine and methionine levels. It would appear from these results that a greater economic return can be expected from higher dietary sulphur amino acid levels when improvements in the most critical response parameters, FCR and breast meat yield, are taken into account. Moreover, the results of this and the previous study, suggest that amino acid requirements for broilers need to be reassessed for diets formulated using digestible amino acid values.

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OPTIMISING INFECTIOUS BRONCHITIS VACCINATION FOR LAYING HENS: EFFECT OF REGULAR REVACCINATION AND MOULT

A. SULAIMAN, J.R. ROBERTS and W. BALL

Summary

Different vaccination protocols with two vaccine strains (VicS and A3) for infectious bronchitis (IB) virus were administered to Isa Brown laying hens during rearing and half the birds were revaccinated regularly during lay. At 57 wks of age, half of the birds were placed into an induced moult for a period of 5 weeks (moulted prior to revaccination), all birds were then revaccinated for IB and the other half of the birds moulted (moulted following revaccination). Production was lower in the birds that were revaccinated regularly during lay and the control (no vaccination until 14 weeks) and VicS eyedrop groups. Egg shell quality was better in the birds that were revaccinated prior to moult. Excreta moisture following revaccination was higher in the birds that had been revaccinated regularly during lay and in birds that were moulted after revaccination.

I. INTRODUCTION

Infectious Bronchitis (IB) is an extremely contagious viral disease that affects the respiratory system, oviduct, and kidneys of chickens. The disease has the potential for serious economic impacts on layers where it may cause a reduction in the quantity and quality of egg production (Jordan, 1996). It is approximately 40 years since nephropathogenic strains of infectious bronchitis virus (IBV) were isolated in Australia by Cumming in 1963. Subsequently, several Australian researchers have focused on the study of the diverse factors involved in the pathogenesis of Australian IB (see review by Cumming and Chubb, 1988). Despite this knowledge of IB, there are several aspects that require further investigation in order to understand the level of perturbation and the mechanisms of adaptation evoked by IBV, taking into account that extrinsic influences such as temperature, nutrition, water quality and water management and intrinsic influences such as breed, age and IB strains play an important role in the pathogenesis of IB (Afanador and Roberts, 1994).

Vaccination programs will remain the cornerstone of the strategy for IB control (Lister, 2001). However, research that elucidates the pattern of the infectious bronchitis disease in poultry and investigates the effectiveness of current vaccines and vaccination methods against development of this disease need to be carried out.

Vaccination at one day old with Vic S-strain IBV provided a limited degree of protection against a heterologous challenge with T-strain IBV at 15 days of age in broilers (Afanador and Roberts, 1994). A study using VicS IB (Fort Dodge) vaccine strain with ISA brown cockerels found that vaccination at either day-old or two weeks of age, by eyedrop, coarse spray or water vaccination, protected birds against the effects of exposure to T strain IBV (Sulaiman *et al.*, 2001).

The current experiment investigated the effect of strain of vaccine, route of vaccine administration, regular revaccination for IB, and timing of moult in relation to revaccination late in lay, on production performance in laying hens.

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II. MATERIALS and METHODS

Day-old ISA Brown hens (625) were purchased from the Winton Hatchery near Tamworth, NSW and transferred to isolation pens at the University of New England, Armidale, NSW. The birds were reared according to standard commercial practice. There were seven experimental groups, each of 89 birds: Control (No vaccination), VicS eye (VicS vaccine by eye drop at day old), VicS spray (VicS by coarse spray at day old), VicS water (VicS in water at day old), A3 eye (A3 vaccine strain by eye drop at day old), A3 spray (A3 by coarse spray at day old), A3 water (A3 in water at day old). Blood samples were taken from ten birds from each group at four weeks of age and birds were then revaccinated with the opposite strain of vaccine to that used at day-old, via the same routes as day old. The Control Group remained unvaccinated. Blood samples were taken from ten birds per group at six weeks of age. At 14 weeks of age, all birds (including the Control birds) were revaccinated with VicS vaccine strain by eye drop. At 15 weeks of age, all birds were transferred to two poultry isolation sheds equipped with three-bird commercial-style cages. One-half of the birds from each treatment group were allocated to each shed, two birds per cage. The birds in one shed were revaccinated every eight weeks with VicS vaccine strain by coarse spray, whereas the birds in the other shed were not revaccinated beyond 14 weeks of age.

At 57 weeks of age, birds were moved to individual cages for revaccination either before or after an induced moult. Half of the birds were moulted at 57 weeks by removal of artificial light and feeding whole grain barley and shell grit for a period of 5 weeks. At 62 weeks of age, all birds were revaccinated by coarse spray with VicS IBV. Birds that had been moulted were then placed on a normal commercial layer diet and the other half of the birds were placed on whole grain barley and shell grit for a period of five weeks (until 67 weeks).

There were now 28 groups in total: seven initial vaccination treatments, birds revaccinated regularly during lay and those which were not, as well as late revaccination of all birds either before or after an induced moult. For each group, egg production, egg weight and the external appearance of the eggs were recorded daily. Faecal moisture was measured one and two weeks post revaccination. Every four weeks, 21 eggs of each group from each shed were collected for egg and egg shell quality measurements (egg weight, shell reflectivity, shell breaking strength, deformation, shell weight, shell thickness, percentage shell, albumen height, Haugh Units, yolk colour score). Blood samples were taken, from five birds from each group, three weeks after revaccination for determination of antibody titres.

Analysis of Variance was used to test the effect of vaccination treatment, regular revaccination during production and the timing of moult on each measured parameter. Fisher's protected LSD was utilized to separate means when significant effects were observed. Statements of statistical significance were based on $P<0.05$.

III. RESULTS

Hen-day Production

Overall, there were significant main effects on hen-day production of initial vaccination treatment and regular revaccination from 57 to 73 weeks of age. The birds that had been revaccinated regularly for IBV during lay had slightly lower production at 57-73 weeks (57.9 eggs/hen/100 days) than the birds that had not been revaccinated (59.2 eggs/hen/100 days). Control (no vaccination until 14 weeks) and the VicS eye group had lower production than the other groups (Table 1). There was a significant interaction between initial vaccination treatment and the timing of moult with production being more

variable for the birds that had not been revaccinated regularly. There was also a significant interaction between whether or not birds had been revaccinated regularly during lay and the timing of the moult. For birds that had been revaccinated regularly during lay, production was higher for those moulted after revaccination at 62 weeks, whereas for birds not revaccinated regularly, production was higher when moult preceded revaccination. However, there was no significant main effect of timing of moult on overall hen-day production.

Table 1, Effect of initial vaccination treatment on hen-day production (eggs/hen/100 days) before, during and after an induced moult at 57 weeks.

Control	VicS eye	VicS spray	VicS water	A3 eye	A3 spray	A3 water
^{d}55.8	^{d}55.8	^{ab}60.3	^{c}58.2	^{ab}60.3	^{bc}58.6	^{a}60.6
± 1.85	± 1.79	± 1.83	± 1.77	± 1.89	± 1.91	± 1.91

Egg and Egg Shell Quality

Only one of the moult treatment groups was sampled for the egg collections at 62 weeks (birds moulted after revaccination), 64 and 68 weeks (birds moulted prior to revaccination). At these times, there were very few statistically significant effects on egg and egg shell quality. However, at 72 and 78 weeks of age, eggs were collected from all birds. A general finding was that egg and egg shell quality were better in the birds that were moulted after revaccination, than in birds that were moulted prior to revaccination (Table 2). The improved breaking strength and Haugh Units in birds moulted after revaccination were seen also at 72 weeks of age. If the two groups were compared at 10 weeks following moult (72 weeks of age for the birds moulted prior to revaccination, 78 weeks of age in the birds moulted following revaccination), the latter group had significantly better shell breaking strength, shell weight, percentage shell and shell thickness. There were also some effects at 72 and 78 weeks of initial vaccination treatment with breaking strength being generally higher in the control birds and those vaccinated initially with A3 strain.

Table 2. Effect of timing of moult on egg and egg shell quality at 78 weeks (Mean±SE)

	Moult Before Revaccination	Moult After Revaccination
Shell Reflectivity %	^{a}35.6 ± 0.6	^{b}31.9 ± 0.4
Shell Weight g	^{b}6.18 ± 0.05	^{a}6.33 ± 0.05
Percentage Shell %	^{b}9.46 ± 0.08	^{a}9.82 ± 0.07
Shell Thickness	^{b}427.8 ± 2.8	^{a}440.4 ± 2.8
Albumen Height	^{b}7.39 ± 0.14	^{a}7.80 ± 0.13
Haugh Units	^{b}82.9 ± 1.0	^{a}86.1 ± 0.8
Yolk Colour	^{b}10.02 ± 0.08	^{a}10.25 ± 0.09

Faecal Moisture, IB Titre Levels and Blood Electrolytes

Faecal moisture was measured in samples collected over a 24 hour period, one and two weeks following revaccination. Excreta moisture was significantly higher for the birds that had previously been revaccinated regularly (78.3%) than for the birds which were not revaccinated (74.5%). Excreta moisture was significantly higher in the birds that were moulted after revaccination (83.8%) than those moulted before revaccination (69.8%). There was also a significant effect of initial vaccination treatment with excreta moisture being highest in the control and VicS spray groups.

There was no significant effect of initial vaccination treatment, regular revaccination or timing of moult on titre levels. However, titres were significantly higher for all treatment

groups at 65 weeks of age (558) than at 58 weeks (379), with 77 weeks being intermediate (473).

Haematocrit and the plasma concentrations of sodium, potassium and calcium were significantly affected by the age of the birds, during the moult experiment. Haematocrit decreased from 58 to 77 weeks of age, at the same time as the plasma concentrations of sodium, potassium and ionized calcium increased.

IV. DISCUSSION and CONCLUSIONS

In the present study, up until 56 weeks of age, it was found that vaccination treatment, including regular revaccination for IB, affected egg production and regular revaccination had some deleterious effects on egg shell quality (Sulaiman *et al*., 2002). For older birds, revaccination following an induced moult resulted in significantly better egg shell quality than revaccination prior to an induced moult. This finding was consistent when birds were compared at the same ages or at the same duration following moult. Shell breaking strength was better late in lay for birds that had been vaccinated initially with the A3 strain.

Regular revaccination during lay resulted in higher excreta moisture following revaccination late in lay. However, the higher excreta moisture found in the birds which were moulted following revaccination would be due mainly to the fact that those birds were consuming whole grain barley.

The antibody titres were relatively low for all groups and were affected only by the revaccination at 62 weeks which resulted in slightly elevated titres in all treatment groups.

Results suggest that there is little advantage in regularly revaccinating laying hens for IB virus, provided that they have received appropriate vaccination during the rearing phase. IB vaccination in moulted birds is best given after the moult. However, more information is required about the correlation between blood IB titre levels and protection against intercurrent IB infection before recommendations can be made to the Australian industry.

V. ACKNOWLEDGEMENTS

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AN EPIDEMIOLOGICAL STUDY OF THE ASCITES SYNDROME IN BROILER CHICKENS IN A NORTHERN REGION OF INDIA

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A world-wide ascites survey has shown that ascites has become a major economic problem in modern broiler operations globally (Maxwell and Robertson, 1997). A study was conducted on 100 broiler farms (51 Ascites Syndrome affected, 49 unaffected) in 46 villages of two districts of a northern state of India. Information on Ascites Syndrome (AS) in broiler chickens and the epidemiological factors existing at the farms were collected using a questionnaire devised for this purpose. Additionally, one sample each of feed, for the estimation of sodium chloride and aflatoxin, and water, for determination of sodium chloride, carbonates and bicarbonates, were collected from each of the 100 poultry farms.

AS was found prevalent during November to March with morbidity and mortality rates of 8.26% and 4.86% respectively and the case fatality rate was found to be 9.61 per cent.The economic loss in the form of medication cost and mortality was estimated as Rs. 788305/- . Various epidemiological factors found to be significantly associated ($P<0.01$) in the causation of AS included production of smoke inside the shed, use of air-tight plastic curtains around sheds and on vehicles during transportation, source of feed from local manufacturers, pelleted feed, high salt, chloride and bicarbonate content of underground water (> 500mg/litre) and salt content of feed (> 0.5%). Other factors found to be associated significantly ($P<0.05$) with AS were: history of ascites at the same farm, source of drinking water (underground water), stocking rate, furazolidone supplementation in feed, aflatoxin (AFB1) in feed (> 30 ppb), age of birds(11-40 days), concurrent respiratory problem, number of chicks (> 75 per box during transportation) and distance of transportation (> 50 km). Factors not found to be significantly associated with causation of AS were: source of birds, type of floor of poultry shed and level of carbonates in water. The results of this study should help poultry farmers reduce their losses from Ascites Syndrome.

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STUDIES ON ULTRASTRUCTURAL PATHOLOGY AND PATHOGENESIS OF HYDROPERICARDIUM SYNDROME IN EXPERIMENTALLY INFECTED CHICKENS

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Hydropericardium syndrome (HPS) is an important disease affecting the chicken population particularly on the Indian subcontinent. The present investigation was conducted with a view to study the pathology and pathogenesis of HPS in experimentally infected chickens. The disease was reproduced experimentally in broiler chicks by inoculation of ID_{50} (0.087 ml of 20 per cent HPS infected liver suspension) subcutaneously at 21 days of age. The clinical signs observed were dullness, depression, reluctance to move, ruffled feathers and chest resting posture with closed eyes. The most conspicuous gross lesion was hydropericardium noticed as early as 36 hours post inoculation (h PI). The liver was markedly enlarged congested/pale with petechiae. Lungs exhibited varying degree of congestion and oedema. Microscopical lesions in liver were characterized by cloudy swelling, hydropic changes, fatty changes, necrosis and hepatitis. The basophilic intranuclear inclusion bodies were conspicuous in hepatocytes. The predominant changes in the heart included separation and atrophy of cardiac muscle fibres and interstitial oedema with congestion, haemorrhage, mild myocarditis. In the lungs there was interlobular oedema with infiltration of mononuclear cells and congested blood vessels. The spleen exhibited depletion of lymphocytes in white pulp and reticuloendothelial cell hyperplasia. The bursa of Fabricius showed depletion of lymphocytes in medulla and increased interfollicular connective tissue. Similar lesions (gross and microscopic) have been described by other workers in HPS infected chicks (Asrani *et al*., 1997).

Electron microscopic studies of the liver revealed degenerative lesions in hepatocytes as swelling of mitochondria with loss of cristae and viral particles were seen as electron dense spherical granules in the nucleus. In the heart, electron semilucent lipid bodies, autophagic vacuoles, swollen mitochondria with loss of cristae and disintegration of Z-lines were seen in cardiomyofibril cells. The chromatin material was marginated in the nucleus of pericardial mesothelial cells.

Ingue *et al.* (1977) have observed that elevation of serum creatine phosphokinase (CPK) levels is indicative of myocardial damage in human beings. Biochemical assays revealed a significant increase in the serum levels of lactate dehydrogenase and CPK indicating degenerative changes in tissues, particularly the liver and heart. Further evidence of myocardial damage was demonstrated in heart slices of infected birds stained with triphenyl-tetrazolium chloride (Jolly *et al*., 1984). Serum antibodies to HPS were detected by an agar gel precipitation test as early as 120 h PI in infected chicks and turkey poults.

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EFFECT OF CYCLOPHOSPHAMIDE ON PATHOLOGY OF HYDROPERICARDIUM SYNDROME IN EXPERIMENTALLY INFECTED CHICKENS

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Cyclophosphamide is a known immunosuppressive agent affecting primarily the humoral immune response (Glick, 1971). The present investigation was conducted to study the pathology of hydropericardium syndrome (HPS) in cyclophosphamide-immuno-suppressed chickens. Twenty, day-old broiler chicks were divided into two groups A and B. Group A birds were given cyclophosphamide at 2mg per chick (0.1 mL of a 2% solution) intramuscularly daily for four days. Group B was given 0.1 mL normal saline per chick intramuscularly for same duration. At 21 days of age the chicks in both the groups were inoculated with ID_{50} (0.5 ml, 10^{-3} dilution of 20% HPS infected liver suspension).

No clinical signs except sudden mortality were observed in the first few cases. However, in other cases the bird became debilitated, dull and depressed, with ruffled feathers followed by resting the head on the chest with eyes closed. Mortality was observed as early as 36 hours post infection (h PI) in group A (total 90%) while in group B mortality (total 40%) was observed only after 48 h PI.

The most prominent gross lesions included hydropericardium and marked enlargement of the liver along with petechaeal haemorrhages on the liver surface. Kidneys were pale and swollen. The spleen and bursa of Fabricius were highly atrophied in group A whereas only mild changes were seen in group B. Histopathological studies revealed cellular degenerative changes in hepatocytes along with necrosis and hepatitis with mononuclear cell infiltration. Basophilic intranuclear inclusion bodies were observed in hepatocytes. Cardiac muscle fibres revealed congestion and haemorrhage along with lymphocytic and heterophilic infiltration. The spleen and bursa of Fabricius showed severe depletion of lymphocytes in the treated group and mild to moderate depletion was observed in the untreated group. There was increased mortality, more pronounced clinical signs and more gross and microscopic lesions in the cyclophosphamide-treated group.

A role for immunosuppression in precipitation and aggravation of HPS under field conditions has been suggested by several workers (Cowen *et al*., 1996; Toro *et al*., 2000), but sparse experimental information is available in the literature on the effect of immunosuppressive agents on the pathology of HPS. The present study clearly demonstrates that treatment with cyclophosphamide increases the severity of HPS in chickens.

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GENETIC STUDIES OF IMMUNOCOMPETENCE AND ECONOMIC TRAITS IN A SYNTHETIC DAM LINE OF BROILER CHICKENS

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Summary

Least squares means of 6 wk body weight and in vivo response to sheep red blood cells (HA titre) and PHA-P (CMI), serum Lysozyme (LLG) and IgG levels estimated on 225 broiler chickens from a synthetic dam line were 1282±20 g, 6.01±0.25, 0.42±0.012 mm, 1.86±0.075 µg/ml and 6.47±0.27 mg/ml, respectively. The analysis of variance revealed a significant ($P<0.05$) effect of sire on 6 wk body weight and HA titre. Hatch had no significant effect on any of the traits studied. Males had significantly ($P<0.01$) higher 6 wk body weight than females. The h^2 of 6 wk body weight and all the immunological traits except CMI were low to medium. With the exception of the r_p between HA titre and LLG, the r_p between all traits were very low. The r_g of 6 wk body weight with immunological traits showed no specific trends. The r_g of HA titre with CMI, LLG and IgG were low to medium. The r_g of IgG with CMI and LLG were medium to high but had large standard errors. The results suggest that the immunocompetence status of the broiler line might be improved by selective breeding.

I. INTRODUCTION

India ranks 18th in the world in broiler production. Several broiler lines/ strains having genetic potential for rapid and higher growth have been developed. A synthetic broiler dam line (SDL) has been developed at this Institute, which has undergone several generations of selection for broiler traits. In order to economically economise broiler production by reducing the cost of inputs such as vaccination and medicines and in consideration of consumer awareness about drug residues in the poultry meat, there is a need to develop specialized lines/ strains which have high production potential and genetically improved immunocompetence. Knowledge of genetic and phenotypic parameters of the immunocompetence traits and their associations with broiler economic traits is a prerequisite for the formulation of a suitable breeding plan to achieve the above goal. Hence, the present study was carried out to evaluate the immunocompetence traits in SDL birds, mode of inheritance of such traits and their associations with broiler economic traits (Siegel and Gross, 1980 and Dunnington *et al*., 1996).

II. MATERIALS AND METHODS

Two hundred and twenty five chicks from two hatches aged from 5-6 weeks, the progeny of 19 sires from the SDL broiler line, maintained in the experimental broiler farm of this Institute were assayed for four important immunological traits. Body weight at 6 weeks of age (BW6) was also recorded on these birds. The chicks were fed with a starter diet (11.7 MJ ME and 220 g CP/kg DM) until 6 weeks, with a grower diet (10.9 MJ ME and 150 g CP/kg DM) up to 16 weeks and then on breeder ration (10.9 MJ ME and 150 g CP/kg DM).

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(a) Response to Sheep RBC

The in vivo response to Sheep RBCs was determined by Haemoagglutuination (HA) test on 5 dpi (days post immunization) after injecting (i/v) each bird with 1 ml of 1% Sheep RBCs (Siegel and Gross, 1980). The titre was expressed in $\log_2 n$, where n is the titre in HA test.

(b) Response to PHA-P

The Cell mediated immune response was assessed by estimating the response to mitogen (Phytohaemagglutinin-P) using 'Foot web index' method (Corrier and Deloach, 1990). It was measured as the difference in the thickness of foot web (between 3rd and 4th digit) at 0 hour (prior to PHA-P injection) and 24 hours after injecting the PHA-P @ 100 µg/ bird.

(c) Serum lysozyme level

The serum Lysozyme level was estimated by the 'Lysoplate method (Lie *et al*., 1986). The serum Lysozyme concentration (LLG) was expressed as the $\log_2$.

(d) Serum IgG level

The serum immunoglobulin G (IgG) was estimated by single radial immunodiffusion (SRID) method (Manicini *et al*., 1965) as modified by Fahey and McKlevey (1965).

(e) Statistical analysis

The data collected on above immunological traits and six weeks body weight was subjected to least squares analysis of variance (Harvey, 1975) employing paternal half-sib correlation method incorporating sire as random and hatch and sex as fixed effects in the mixed model.

III. RESULTS

Least squares analysis of variance (Table1) revealed significant effects of sire ($P<0.05$) on body weight and HA titre and sex ($P<0.01$) on BW6 only. Hatch had non-significant effect on all the traits studied. Males were significantly heavier than females. Males also had higher values for all immunological traits than females.

Table 1. Least squares analysis of variance for body weight and immunological traits in broiler chickens

Sources of Variation	d.f.	Mean sum of squares of				
		BW6	Ha titre	CMI	LLG	IgG
Sire	18	79486.596*	12.033*	0.0273	1.0995	14.271
Hatch	1	1275.648	0.0946	0.0093	1.8584	0.5380
Sex	1	1552981.511**	11.537	0.0033	0.0271	13.0944
Error	195	40920.891	6.437	0.0254	0.6919	9.6244

* Significant at $P<0.05$, ** Significant at $P<0.01$

Least squares means of BW6, HA titre, CMI, serum lysozyme levels of LLG and IgG are given in Table 2. The only significant difference between the sexes was for BW6.

Table 2. Least squares means (± SEM) of body weight and immunological traits in the broiler chicken line

Classes	BW6 (g)	HA Titre	CMI (mm)	LLG (µg/ml)	IgG (mg/ml)
Overall (µ)	1282	6.01	0.419	1.86	6.47
	±20.4	±0.251	±0.012	±0.075	±0.272
Hatch					
First	1279	5.99	0.425	1.95	6.42
	±24.5	±0.303	±0.016	±0.094	±0.341
Second	1284	6.036	0.412	1.76	6.52
	±25.2	±0.312	±0.016	±0.097	±0.353
Sex					
Male	1373[a]	6.26	0.423	1.87	6.74
	±27.0	±0.335	±0.018	±0.105	±0.383
Female	1190[b]	5.76	0.415	1.85	6.21
	±23.3	±0.288	±0.015	±0.089	±0.321

Means with different superscripts in a column within a subclass differed significantly ($P<0.05$)

The genetic and phenotypic parameters are shown in Table 3. The heritability estimates were medium for body weight and low to medium (0.01±0.13 to 0.29±0.20) for the four immunological traits. The phenotypic correlations (r_p) of BW6 with immunological traits were positive and very low in magnitude however, with lysozyme, it was low and negative. The genetic correlations (r_g) of body weight with HA titre and lysozyme were negative but positive and low with IgG. HA titre had positive and low to medium r_g with other immunological traits. The r_g of IgG with CMI and lysozyme were high. However, all estimates were associated with large standard errors.

Table 3. Heritability (diagonal), Phenotypic (below diagonal) and genetic (above diagonal) correlations among body weight and immunological traits in SDL of broiler chickens

Traits	BW6	HA Titre	CMI	LLG	IgG
BW6	0.31±0.21	-0.23±0.49	>1	-0.98±0.54	0.08±0.60
HA	0.06	0.29±0.20	0.26±1.48	0.02±0.58	0.40±0.61
CMI	0.08	-0.08	0.03±0.13	<1	0.34±1.79
LLG	-0.06	0.15**	-0.08	0.20±1.18	0.56±0.67
IgG	0.04	-0.01	-0.06	0.02	0.16±0.17

** Significant at $P<0.01$

IV. DISCUSSION

The present findings of least squares means of HA titre (5dpi) were comparable to those of Dunnington *et al*. (1989) and Nath (1999). The non-significant sire effect for CMI and IgG in the present study was in agreement with the report of Saxena (1993) and Nath (1999). Contrary to the present findings, Cheng and Lamont (1988) and Saxena *et al*. (1997)

reported significant sire differences on immunological traits, which may be due to the difference in the genetic structure of the populations and environmental conditions. The non-significant effect of sex on HA titre, CMI, LLG and IgG was also reported by Saxena (1993), Saxena *et al*. (1997) and Nath (1999). Pal (1992) also noticed non-significantly higher lysozyme activities in male than female guinea fowl.

The heritability estimate of SRBC response (0.29±0.20) was identical to the estimate (0.29) reported by Van der Zijjp (1983). It is evident that SRBC response is under the control of additive gene action. Hence, selection may be useful for improvement of humoral immunity in the SDL broilers. The low to medium h^2 estimates associated with high standard errors for CMI, LLG and IgG may be attributed to sampling variation due to small sample size. Saxena (1993) also found low h^2 estimate with high standard errors for CMI in guinea fowl.

The phenotypic correlations (r_p) between BW6 and the immunological traits were, with the exception of LLG, positive but were very low in magnitude. Siegel and Gross (1980) however reported a negative correlation between body weight and SRBC response in divergent lines. Similar to the r_p of SRBC with other immunological traits in the present study, Saxena (1993) also reported inconsistent r_p of SRBC response with CMI and IgG in guinea fowl.

The negative r_g of six weeks body weight with response to SRBC indicated that genetic selection for six weeks body weight might bring reduction in the latter. The medium r_g of SRBC response with other immunological traits except LLG suggested that selection for SRBC response might bring improvement in other traits. Given the large standard errors associated with the small sample size, further investigation using a much larger sample size should be carried out on these aspects before designing an appropriate breeding plan.

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INFLUENCE OF SELECTION FOR RESPONSE TO SHEEP RED BLOOD CELLS ON TURKEY POULT MORTALITY

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Summary

The reproductive traits viz., fertility and hatchability and mortality patterns were studied in lines of turkeys, derived from a local Black variety of turkey, selected for high response to sheep red blood cells for one generation (high SRBC line) or at random (control line). Percent fertility and percent hatchability (TES and FES) did not differ significantly between eggs produced by the parents of the high SRBC response (85.57, 58.33 and 68.29% respectively) and the Control (85.34, 58.24 and 67.95% respectively) lines. Similarly, percent poult mortality during 0-2, 2-4 and 0-4 weeks of age did not differ significantly between the High SRBC (10.71, 10.00 and 19.64, respectively) and Control (11.32, 10.64 and 20.72% respectively) lines. The present findings are indicative of improvement in the non specific resistance of turkey without any adverse effect on fertility, hatchability and mortality through genetic selection for high humoral immunity.

I. INTRODUCTION

Turkey farming is gaining attention in India as well, but yet to be exploited with proper scientific approaches. Besides development of turkeys for high production, improvement in non specific disease resistance of turkeys will facilitate economic production. Lines selected for high immune response to sheep RBCs have been reported to be immunologically more responsive to certain bacterial, viral and parasitic disease causing agents in domestic fowl (Siegel and Gross, 1980 and Van der Zijpp and Leenstra, 1980). However, reports on these aspects are scanty in turkeys. In order to design a genetic improvement program for non specific resistance to diseases in turkeys, knowledge of the influence of selection for high humoral immunity on fertility, hatchability of eggs and mortality of poults is a pre-requisite. Hence, the present study was initiated to select the birds of a Black variety of turkeys, maintained at this Institute, for high response to Sheep RBCs and to evaluate some reproductive traits and poult mortality patterns in selected and control lines.

II. MATERIAL AND METHODS

(a) Variety

A Black variety of turkey maintained at the Turkey Research Unit of this Institute was used in the present study. The birds had not been selected previously for any economic or immunological traits. The variety of birds was derived from the descendents of Broad Breasted Bronze turkeys imported from Europe and North America during the early years by the Christian missionaries. They are generally regarded as the local Black variety of Turkeys.

Molecular Genetics Laboratory, Central Avian Research Institute, Izatnagar-243 122, India.

(b) Screening of base population

One hundred and four adult turkeys (28 males and 76 females) were screened at about 10 months of age for immune response to SRBC during the first year of production using a slightly modified method of Siegel and Gross (1980). Each bird was injected (i/v) with 1 ml of 5 percent SRBC suspension. The antibody titre (n) in response to SRBC was determined at 5 dpi (post days immunization) by Haemagglutination test using 1% SRBC suspension and expressed as $\log_2 n$ values. The males and females were ranked (from high to low) on the basis of titre.

(c) Mating system

Four males and forty females at the high extreme of response to SRBC were selected as parents of the GI generation. The selection differential in the HA titre applied for development of the high SRBC line was 2.196 in males and 1.63 in females (Kumar, 2002). A contemporary random bred control population consisting of 2 males and 20 females was also maintained for comparative studies. Pen mating (1 male: 10 females) was done to produce the GI generation.

(d) Incubation and hatching

Fertile eggs were collected for ten consecutive days and were stored in a cool chamber until their setting in an incubator. Two hatches were obtained for each of the two lines. Total numbers of eggs set were 134 and 153, respectively in the two hatches of High SRBC line. The corresponding numbers in the control line were 48 and 43.

(e) Management of GI progeny

Day old poults were wing banded and reared in a brooder house. They were provided with a starter diet (10.9 MJ ME and 280 g CP/kg). Mortality was recorded up to 4 weeks of age. Standard and uniform management practices were followed.

(f) Statistical analysis

Data collected on fertility and hatchability in the two parental groups and and poult mortality in the two lines were subjected to Normal deviate test.

III. RESULTS

(a) Fertility and hatchability of eggs

The fertility and hatchability percent of eggs produced by the parent birds of the two matings are presented in table 1. Normal deviate test revealed that the high SRBC and random bred control groups did not differ significantly in fertility or hatchability.

Table 1. Fertility and hatchability percentages in eggs produced by the parents of the high SRBC and Control lines of turkey

Group	Fertility (%)	Hatchability (%)	
		Total eggs set (TES)	Fertile eggs set (FES)
High SRBCs	85.57	58.33	68.29
Control	85.34	58.24	67.95

(a) Poult mortality

The percent mortality of poults during different periods in both the lines are presented in table2. The mortality of poults during various periods in the high SRBC lines did not differ significantly (P>0.05) from those in control line.

Table 2. Percent mortality in high SRBC and control lines of turkeys over three intervals

Line	Mortality (%) during		
	0-2 weeks	2-4 weeks*	0-4 weeks
High SRBCs	10.71	10.00	19.64
Control	11.32	10.64	20.72

*Calculated on the basis of the total number of poults surviving after 2 weeks.

IV. DISCUSSION

The present findings showed non-significant differences in fertility and hatchability between eggs produced by the high SRBC and control line parent birds. This suggests that correlated response in the reproductive traits to selection on the basis of SRBC rersponse, will be minimal. This is supported by the results of Prusinowska *et al*. (2000) who observed non-significant differences in fertility and hatchability between the high and low lines of turkeys after three generations of selection based on serum lysozyme activity. However, in WLH chickens, Shivakumar *et al*. (2002) reported a significant decrease in fertility and hatchability (TES) in the high SRBC line compared to the low line after one generation of divergent selection.

The non-significant (P>0.05) difference between the high SRBC and control lines in percent poult mortality observed in the present study is likely associated with limited response to only one generation of selection. It is possible that mortality may be reduced after a few generations of selection for high SRBC response, but there are no studies in the literature to evaluate the likelihood of this. Prusinowska *et al*. (2000) reported that poult mortality in birds from a line selected for high lysozyme activity was lower than in their counterparts from the low lysozyme activity line after three generations.

The present findings suggest that selection for high humoral immunity are unlikely to have an adverse effect on fertility, hatchability and poult mortality. Further studies after more generations of selection for high SRBC response are suggested to provide more definitive information about the effect of such selection upon reproductive performance and poult mortality.

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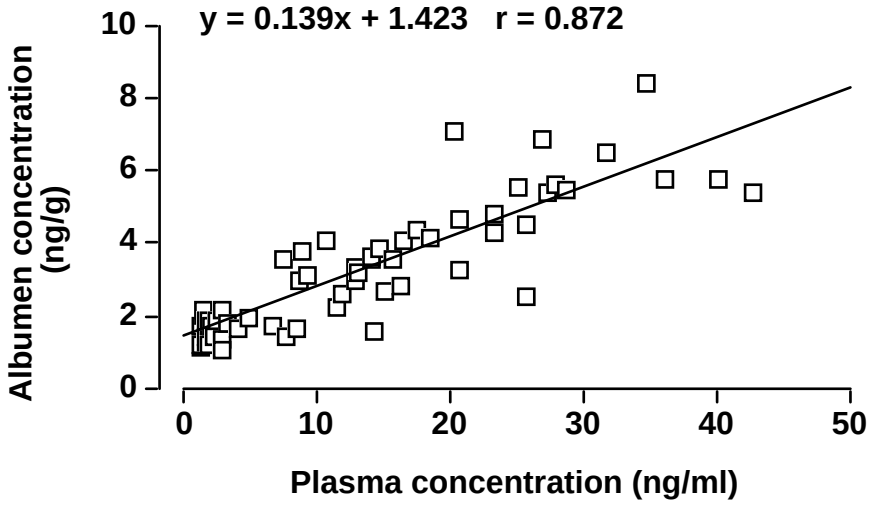
THE RELATIONSHIP BETWEEN PLASMA AND EGG ALBUMEN CORTICOSTERONE LEVELS

J.A. DOWNING [1] and W.L. BRYDEN [2]

Many stresses result in activation of the hypothalamic-adrenal axis with consequent changes in the plasma and tissue levels of glucocorticoids and catecholamines. Corticosterone is the main glucocorticoid secreted by the adrenal gland in hens. Measuring plasma corticosterone levels is difficult because sampling procedures are themselves stress inducing. A non-invasive means of measuring corticosterone would alleviate this problem (Downing *et al*., 2001). The gradual accumulation of albumen over 5-6 h during egg formation potentially provides an accurate reflection of circulating hormone levels over this time. The purpose of the study was to determine if there is a relationship between plasma and egg albumen corticosterone levels.

Isa Brown hens (63 weeks of age) were used in the study. At 0600 h, 20 hens for each treatment were given a subcutaneous injection of 5 or 10 mg of corticosterone suspended in 1 ml of peanut oil or 1 ml of peanut oil alone. Between 1600-1700 h, a 1 ml blood sample was taken from each hen and the plasma harvested and stored until assayed. All eggs laid on the day following the injection were collected, broken open and the albumen separated and stored until assayed. Corticosterone levels in plasma and egg albumen were determined by radioimmunoassay.



The injection of corticosterone resulted in a significant increase in plasma corticosterone levels. There was a significant positive relationship between the level of plasma corticosterone and the level in the egg albumen. The data suggest that in hens, corticosterone levels in egg albumen provide a non-invasive measure of plasma corticosterone levels.

Downing, J.A., Fraser, D.R. and Bryden, W.L. (2001). *Proc. Aust. Poult. Sci. Symp.* Ed D. Balnave. **13**: 232.

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INFLUENCE OF PULLET WEIGHT ON PRODUCTION AND CLOACAL HAEMORRHAGE IN LAYERS

M. NG, G.B. PARKINSON and P.H. CRANSBERG

Summary

Laboratory model studies undertaken in cages have indicated that oviduct haemorrhage and/or picking behaviours in commercial layers are not uniformly distributed across the life of the laying flock. These problems are accentuated at stages that correspond to periods of high metabolic pressure (peak production and peak egg mass). Comparative studies with individually housed birds indicate that approximately 50% of the cloacal haemorrhage can occur independently of picking behaviours. Furthermore, the incidence of cloacal haemorrhage appears to be correlated with low body weights in early lay and the production of disproportionately large eggs. Birds that experience cloacal haemorrhage in early lay can continue to manifest the problem, whilst some birds repair the damaged oviduct very rapidly. Superior management of the transition from the rearing to the laying phase and more attention to body weight management is likely to reduce the extent of cloacal haemorrhage.

I. INTRODUCTION

Anecdotal evidence from studies of commercial laying flocks suggests an important link between body weight, production, cloacal haemorrhage and mortality. High levels of blood stained shells, recorded in many under weight flocks, seem likely to reflect significant levels of cloacal haemorrhage/trauma and may be important in the health and mortality of a flock. Cloacal or oviduct haemorrhage could be linked to oviduct prolapse, salpingitis/egg peritonitis and cannibalism.

In most of the research to date the relationship between bodyweights, production, and mortality have been performed on flocks with bodyweights close to optimum. The main conclusions drawn from these studies relate to feed efficiency, egg size, and egg mass (Balnave, 1984; Harms *et al.*, 1982; Leeson and Summers, 1987). Very little research has examined the thresholds of body weight, below which mortality may be manifested. Furthermore, there is relatively little empirical knowledge about the causes of mortality in under-weight flocks or birds that have been clearly pushed below the genetically defined standards for body weight.

The experiment described in this paper attempts to model the farming of brown egg layer pullets that are substantially below the appropriate body weight standards. Eighteen- week-old birds from a commercial flock were selected based on body weight. The light (L) group contained birds weighing less than 1.27kg and the heavy (H) group consisted of birds weighing more than 1.50kg (breed standard is 1.33kg) The flocks were housed in single bird cages, in a temperature-controlled shed, under 16 hours of light. The pullets were fed *ad libitum* a commercial diet containing 170 g protein, 11.7 MJ metabolisable energy, and 37 g calcium/ kg. Body weight, egg weight, egg production, feed intake, and cloacal haemorrhage and blood stained eggs were monitored from 18 to 36 weeks of age. The experimental birds had no opportunity to undertake picking or cannibalism behaviours, so that oviduct haemorrhage could be studied independently of social behaviours.

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II. RESULTS AND DISCUSSION

Statistical analysis of the data revealed that average body weight at 36 weeks of age of L was significantly lighter than H ($P<0.01$). The bodyweight patterns of the two groups are shown in Figure 1. The light group had body weights 5-30% below accepted body weight standards at 18 weeks. At 36 weeks of age the majority of L birds were between 10 and 30% below breed standards, however, two of the birds exceeded the breed standard weight. There was a clear increase in predisposition to cloacal haemorrhage in the light group. Despite obvious damage to the oviduct, as indicated by the higher number of blood stained eggs, the incidence of cloacal haemorrhage in the L group was not associated with prolapse or mortality.

Regression analysis confirmed that body weight at 18 weeks of age was strongly correlated with body weight at 36 weeks of age ($r = 0.811$, $P< 0.001$). This finding indicates that 66% of the variation in bodyweight at 36 weeks of age is accounted for by initial weight at 18 weeks of age, and is similar to the findings of Balnave (1984). This study indicates that the relationship between weight at 18 weeks and weight at 36 weeks applies over a broad range of initial body weights, and in birds housed in single-bird cages without social competition. Again, this study supports the findings of Harms *et al.* (1982), Balnave (1984) and Leeson and Summers (1987) that body weight differentials are maintained throughout the laying period.

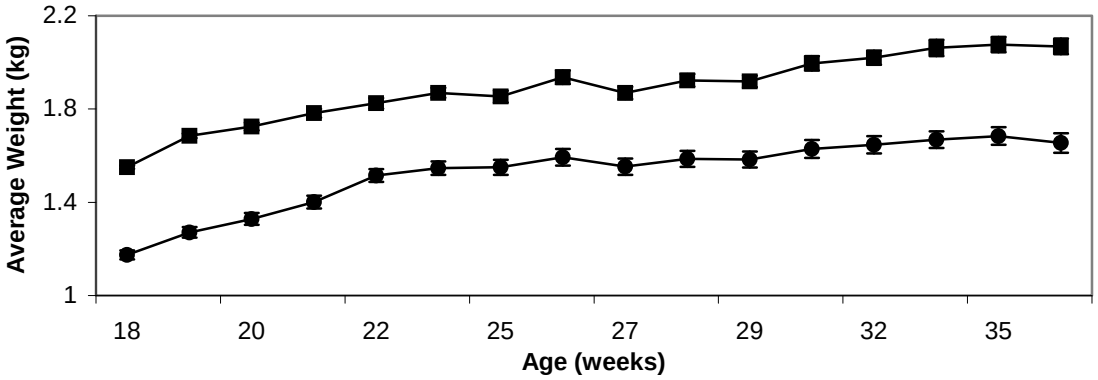

Figure 1. Body weight patterns of light (●) and heavy birds (■) from 18 to 36 weeks of age. Error bars are SEM's.

Comparison of total production between each of the body weight groups indicated that the H group produced significantly more eggs between 18 and 36 weeks of age compared with the L group (Figure 2). In contrast, Harms *et al.* (1982) and Balnave (1984) found that pullet bodyweight did not significantly influence production. This difference may be a reflection of the low body weight range (1.18 kg at 18 weeks of age) used in the current experiment. Leeson and Summers (1987) also found that low body weights in 18-week-old White Leghorns (1.1kg) also reduced egg production between 19-25 weeks by approximately 5%.

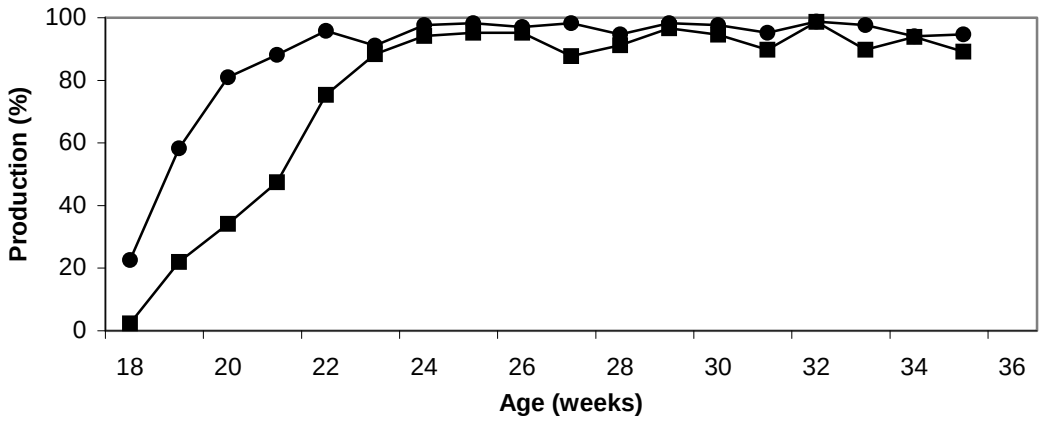

Figure 2. Comparison of egg production (% rate of lay) between the heavy (●) and light (■) groups to 35 weeks of age.

Egg size was not significantly different between the two groups (Table 1). In fact, egg weight was well below breed standards in both groups. This lower egg weight may have reduced the incidence and severity of cloacal haemorrhage in both groups.

Table 1. Production comparisons between the two bodyweight groups from 18 to 36 weeks of age.

Group	N	Egg size At 36 weeks of age (g)	Average daily food intake (g)	Number of birds laying blood-stained eggs	Proportion of blood-stained eggs (%)	Egg:body weight ratio at 36 weeks of age (g egg/kg body weight)
L	21	54.5(1.18)	95.06(2.06)[a]	9[a]	1.23	3.37(0.085)[a]
H	24	56.3(0.75)	113.00(2.43)[b]	3[b]	0.22	2.75(0.057)[b]

Means in the same column without a common superscript are significantly different, $P<0.05$.

The L group laid substantially more blood stained eggs than the H group. Approximately 40% of the L group produced at least one blood stained egg whereas only 10% of the H group produced a blood stained egg (Table 1). In the L group, 15% of birds produced multiple blood stained eggs, and 10% produced eggs with severe blood staining on the shell surface.

The egg:body weight ratio at 36 weeks of age was significantly larger ($P<0.05$) in the L group than in the H group. This finding suggests that low body weight increases egg:body weight ratio, despite a slight though non-significant decrease in egg size.

III. CONCLUSION

Overall, these findings suggest that extremely low body weights at 18 weeks of age are associated with a reduction in total production to 36 weeks, with a significant increase in the number of birds laying blood stained eggs, and with an increased proportion of eggs that are blood stained. The significance of cloacal haemorrhage to problems such as cannibalism and salpingitis remains to be determined, but it could be hypothesised that severe cloacal haemorrhage frequently observed in under-weight flocks or flocks with a large proportion of under-weight birds, could be a critical factor(s) in determining flock mortality. More work is

also needed on the tolerance of both under-weight and normal body weight flocks to changes in average egg weight induced by nutritional strategies.

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EGG AND EGG SHELL QUALITY GUIDELINES FOR THE AUSTRALIAN EGG INDUSTRY

J.R. ROBERTS and W. BALL

Summary

Studies were conducted to provide realistic commercial guidelines on egg internal quality and egg shell quality for the Australian Egg Industry. Eggs were obtained from commercial operations both from flocks that were followed throughout their laying life ("longitudinal studies") and also from flocks that were sampled at only one age of bird from a flock but across a range of ages of flocks ("cross-sectional studies"). Eggs were analysed for internal quality and egg shell quality as soon as possible after collection. The data collected indicate that there is considerable variation among flocks in egg and egg shell quality measurements, although there is little difference among the three main brown egg layer strains. However, consistent findings in relation to bird age were: an increase in egg weight and shell weight up to 45 and 40 weeks of age, respectively, after which these weights remained relatively constant, reduced shell colour, shell breaking strength, deformation, percentage shell, and reduced albumen height and Haugh Units.

I. INTRODUCTION

Problems with egg and egg shell quality continue to be of concern to the poultry industry in Australia and around the world. The egg shell quality seminar organised by the Egg Industry Research and Development Council (now the Egg Program of the Rural Industries Research and Development Corporation), in July 1988, concluded that approximately 10% of eggs are downgraded because of problems with shell quality alone. Noting that the gross value of production of the Australian egg industry was of the order of $337 million per annum in 1998-1999 (AEIA Statistics), this represents a loss well in excess of $10 million per annum to the egg industry. The exact losses are not easy to estimate because of difficulty in determining the net value of the industry. Information obtained recently from egg grading facilities indicates that 10% is still a valid working figure for the percentage of eggs lost to egg shell quality problems.

Additional losses result from egg and egg shell quality problems in breeder birds. In addition to shell quality problems, egg internal quality is increasingly important to the egg industry, with supermarkets setting minimum standards for albumen quality. It is critical to the Australian Egg Industry to have available guidelines on the levels of egg shell quality and egg internal quality that can be realistically achieved in the commercial setting. Such guidelines can then be used to negotiate quality guidelines with purchasers such as the major supermarket chains.

This study documents the relationship between laboratory measurements of egg and egg shell quality, and what happens in the commercial situation. Egg internal quality and egg shell quality are described for the three brown egg layer strains commonly in use in the Australian Egg Industry, in relation to bird age.

II. MATERIALS AND METHODS

Two different types of study were conducted. The first type was the longitudinal study where individual flocks were studied at different ages. The second type was the cross-

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sectional study where eggs from flocks of known backgrounds were sampled, mainly from commercial grading floors. In all, 36 flocks were sampled in the longitudinal studies (9 HyLine Brown, 12 HiSex and 14 Isa Brown) with a total of 185 sampling occasions (37 HyLine Brown, 57 HiSex and 91 Isa Brown). Eighty-six flocks were sampled for the cross-sectional studies (one sampling from each flock – 35 HyLine Brown, 25 HiSex, 17 Isa, 6 Lohmann, 1 HyLine W36, 1 HyLine Gray, 1 Ingham Tint). This resulted in a total of 271 samples from flocks, each of 90 eggs – a total of 24,390 eggs (16,650 from the longitudinal studies and 7740 from the cross-sectional studies). The flocks sampled were mainly from three strains of birds: Isa Brown, HyLine Brown and HiSex. However, limited data were collected from Lohmann Brown, HyLine W36 (white egg layers), HyLine Gray (tinted eggs), Ingham Tint (tinted eggs). Of the 271 samplings of eggs, 221 were from New South Wales, 37 from Queensland, 2 from Victoria and 11 from South Australia. Only data from the three commonly used brown egg layer strains (Isa Brown, HyLine Brown, HiSex Brown) are included here.

All eggs were analysed for egg internal quality and egg shell quality within 2 days of dispatch for eggs in the longitudinal study and an average of 4.4 days for the cross-sectional study. Eggs were candled and any defects or cracks noted. Eggs were then subjected to the following measurements for egg shell quality: egg weight; shell colour (measured by percentage reflectivity – the higher the reflectivity, the lighter the colour of the shell); egg shell breaking strength (by quasi-static compression). The eggs were then broken out for measurement of internal quality as albumen height (from which Haugh Units were calculated) and yolk colour score (Roche scale). All measurements were made using equipment from Technical Services and Supplies (TSS), U.K. Egg shells were then washed carefully and dried. Shell weight was measured and the ratio of shell weight to egg weight (percentage shell) was calculated. Three small pieces of shell were taken from around the equator of the egg shell and shell thickness (including shell membranes) measured using a Mitutoyo Dial Comparator gauge.

III. RESULTS

Egg weight increased up until about 50 weeks of age and then remained relatively constant (Figure 1) whereas shell weight increased to 40 weeks and then remained constant (Figure 2).

Figure 1: Egg weight vs hen age

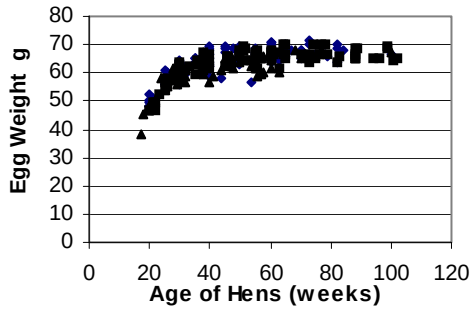
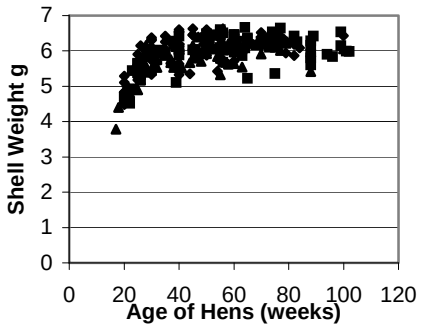


Figure 2: Shell weight vs hen age



Shell breaking strength declined with age (Figure 3) as did shell deformation (Figure 4). Shell reflectivity increased with hen age (Figure 5), indicating that shell colour became lighter. Although both egg weight and shell weight increased with age up to a point, the ratio of shell weight to egg weight, the percentage shell, decreased with age (Figure 6).

Figure 3: Shell breaking strength vs age

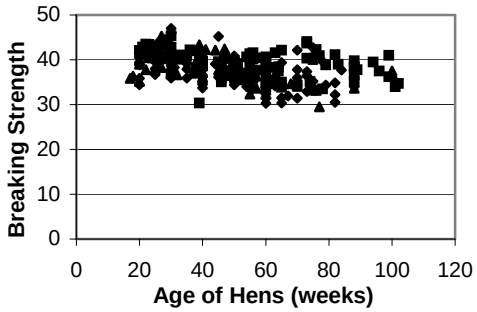


Figure 4: Shell deformation vs age

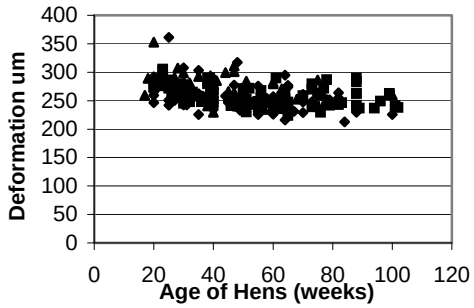


Figure 5: Shell reflectivity versus age

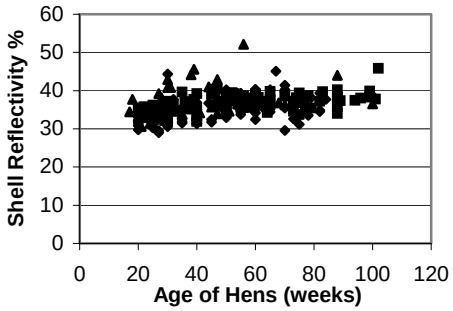
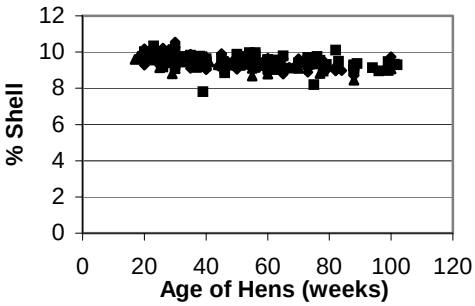


Figure 6: Percentage shell versus age



Albumen height and Haugh Units calculated from albumen height and egg weight decreased with age of hen (Figure 7). However, the age of the egg itself (Figure 8) and the storage conditions affect albumen height and Haugh Units. Therefore, Haugh Units will be influenced by hen age, age of egg and storage conditions.

Figure 7: Haugh Units versus hen age

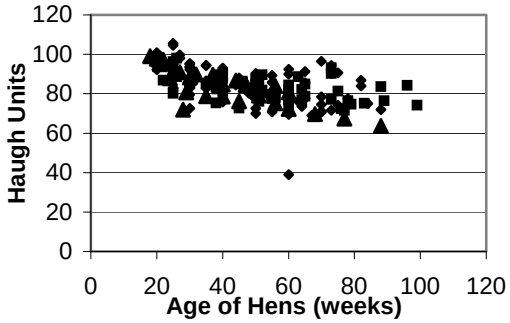
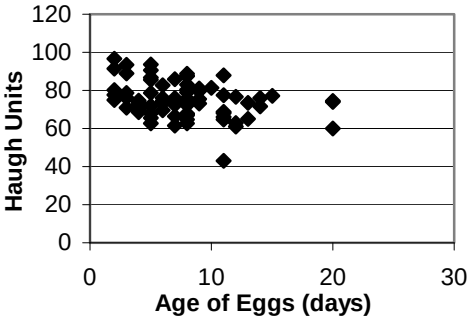


Figure 8: Haugh Units versus age of egg



The effect of storage temperature on Haugh Units is illustrated in Figure 9. Weight loss in eggs stored at the same temperatures and relative humidities is shown in Figure 10. While storage temperature is the main determinant of Haugh Units, weight loss from the egg is significantly influenced by relative humidity.

Figure 9: Haugh Units vs storage temperature

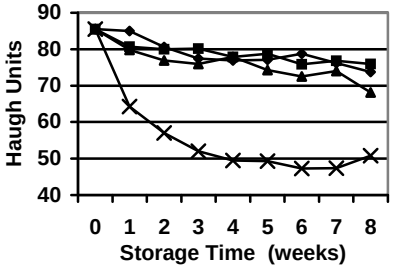
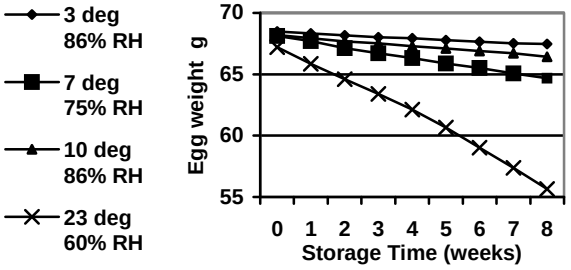


Figure 10: Egg weight vs temperature and relative humidity



IV. DISCUSSION AND CONCLUSIONS

Considerable variation in the indicators of egg internal quality and egg shell quality exists within commercial flocks in Australia. Despite this variation, it is clear that there is an increase in egg weight and a decrease in egg shell quality (egg shell breaking strength, deformation, percentage shell) and internal quality (Haugh Units) as hens get older. Previous studies (Roberts, 1998, 1999; Roberts and Ball, 1998) have described the changes in egg internal quality and egg shell quality that occur as hens age in small commercial-style research flocks. They have also distinguished between Australian-bred and imported strains of bird. However, a systemic collection of such data from commercial flocks has not been attempted recently in Australia.

Scientific studies have investigated many of the factors that affect egg shell quality (see Wells and Belyavin, 1987). Factors which contribute to the variation reported from this study include: age of bird, strain of bird, climate, season of year, diet, rearing conditions, type of cage and housing.

This study will result in the publication of a practical booklet which will provide graphs of egg internal quality and egg shell quality for different strains of birds at different ages, under different conditions.

V. ACKNOWLEDGEMENTS

This project is supported by the Egg Program of the Rural Industries Research and Development Corporation. The generous cooperation and assistance by members of the Australian Egg Industry is gratefully acknowledged. This study would not have been possible without them.

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XYLANASES WITH DIFFERENT SUBSTRATE AFFINITIES IN BROILER DIETS

M. CHOCT[1], A. KOCHER[1], D.L.E. WATERS[2], G. ROSS[3] and D. PETTERSSON[4]

Summary

Three xylanase products (Enzymes A, B and C) were examined for their effect on the apparent metabolisable energy (AME), growth rate and digesta characteristics of broilers fed two Australian wheats (Currawong and Harvey). Enzymes increased (P<0.001) weight gain regardless of wheat type. There was an overall increase in AME values of both wheats due to enzyme inclusion. The AME value of the Currawong wheat was not affected by the enzymes, whereas Enzymes A and C markedly (P<0.05) improved the AME value of the lower ME Harvey wheat. Enzyme A reduced (P<0.05) excreta moisture regardless of wheat type. A wheat type x enzyme interaction (P<0.001) on the viscosity of the jejunal and ileal digesta showed different responses of digesta viscosity depending on wheat type. Whilst both Enzymes A and B increased the soluble non-starch polysaccharides (NSP) levels in the small intestine, their effect on digesta viscosity was contrasting. Enzyme A decreased digesta viscosity (P<0.05), but Enzyme B elevated (P<0.05) it. These responses were most noticeable with the lower ME wheat. Enzyme C had no effect on the soluble NSP levels, but significantly (P<0.05) reduced viscosity in the small intestine.

I. INTRODUCTION

Glycanases, including enzymes such as xylanases, cellulases, ß-glucanases, amylases and pectinases, can be produced from a range of plants, animals and microorganisms. Although particular types of enzymes, like xylanases, share a common substrate, arabinoxylan, their substrate affinities and activities can differ widely (Biely *et al.*, 1997). Some xylanases can cleave the arabinose side chains, whereas others only act on the main xylan chain when there are certain numbers of unsubstituted xylose units on the xylan backbone. The practical implications of these characteristics of xylanases in poultry production are not understood, but there are two schools of thought on the significance of substrate affinity of feed glycanases. First, the effect of enzymes on the nutritive value of grains is related to their ability to partially depolymerise soluble NSP to reduce digesta viscosity (Burnett, 1966; Choct and Annison, 1992). The consequences of high digesta viscosity are: (a) a reduced mixing of digestive enzymes with their substrates, leading to impaired digestion; (b) an increased thickness of the mucosal layer of the intestine, leading to a decreased rate of absorption, and (c) a delayed emptying of digesta, leading to depleted oxygen level in the gut and proliferation of fermentative microflora in the small intestine (Johnson and Gee, 1988; Choct *et al.*, 1996). Second, the effect of enzymes on the nutritive value of grains is related to their ability to breakdown the cell walls (which mainly consist of insoluble NSP) to release encapsulated nutrients, exposing them to digestion by the endogenous enzymes (Pettersson and Åman, 1989).

The current study was conducted to examine the importance of reducing viscosity, breaking down cell walls or a combination of both on the AME value of wheat for broilers.

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II. MATERIALS AND METHODS

Male Cobb broiler chicks were raised on a commercial starter crumble to 24 days when the birds were transferred to metabolism cages in controlled temperature rooms for classical AME studies involving quantitative measurements of feed intake and excreta output. The two wheats were selected from 9 wheats tested in a preliminary study. Wheat 1 (Currawong) had a measured AME of 12.5 MJ/kg DM and Wheat 2 (Harvey) had a measured AME of 11.5 MJ/kg DM and on this basis could be classified as low-ME wheats. Wheat was included at 78.4% in a semipurified diet with casein as the main protein source. Three xylanase products were obtained from a commercial supplier (Novozymes, Australia) and were added to the diet at the recommended dosage rates (Enzyme A at 200mg/kg; Enzyme B at 250mg/kg; Enzyme C at 426mg/kg). The diets were fed for 7 days (chickens 24-31 days of age) with six replicates of six birds each. Birds were weighed in groups at the end of the 7-day period. Two birds per cage were killed to provide ileal digesta. Digesta were stored in ice until centrifuged at 12,000 *g* for 15 min and then frozen. Viscosity was determined using a Brookfield DVIII viscometer at 25^{o}C with a shear rate of 5-500 s-1. Dry matter (DM) contents of samples of pelleted and milled feeds were measured. Gross energy was determined using a DDS CP500 Automatic calorific processor (Digital Data Systems, Johannesburg, South Africa). Celite was added at 20g/kg as a source of acid insoluble ash, which was determined using the procedure of Choct and Annison (1992). The soluble NSP level was measured following the alditol acetate method of the AOAC (Method 994.13).

III. RESULTS

(a) Effects of enzymes on weight gain, AME and excreta moisture

Enzymes increased ($P<0.001$) weight gain regardless of wheat type. There was a numerical increase in the AME values of both wheats due to enzyme inclusion, but the effect on the AME value of Currawong wheat was not significant. On the other hand, Enzymes A and C improved ($P<0.05$) the AME value of the lower ME wheat (Harvey). Enzyme A reduced ($P<0.05$) excreta moisture content of birds regardless of wheat type (Table 1).

(b) Digesta viscosity and soluble NSP levels in the intestine

Wheat type as well as enzyme supplementation had marked ($P<0.01$) effects on digesta viscosity in all sections of the intestine. The significant ($P<0. 01$) wheat type x enzyme interaction on the viscosity of the jejunal and ileal digesta showed different enzyme responses on digesta viscosity depending on wheat type. Thus, similar responses to Enzymes A and C in viscosity of the jejunal and ileal digesta were obtained regardless of wheat type, whereas the response to Enzyme B was more pronounced for the Harvey wheat than the Currawong wheat. Enzyme supplementation had a significant effect on the relative amount of soluble NSP both in the jejunum and ileum, but wheat type had no effect. Enzyme A increased ($P<0.05$) the relative amount of soluble NSP in the jejunum, but not in the ileum, of birds fed Currawong wheat. It, however, significantly elevated ($P<0.05$) the soluble NSP levels in both the jejunum and ileum of birds fed the Harvey wheat. This is manifested by a significant enzyme x wheat interaction in the ileum. On the other hand, Enzyme B markedly increased ($P<0.01$) the soluble NSP levels in the jejunum of birds fed the Harvey wheat and in the ileum of birds fed both wheats. Enzyme C had no effect on the relative levels of soluble NSP regardless of the section of the gut or the wheat type (Table 1).

Table 1. Apparent metabolisable energy (AME) values of wheats, excreta moisture contents and weight gains of broiler chickens fed diets containing Currawong or Harvey wheats with or without enzyme supplementation. Digesta viscosity in the duodenum, jejunum and ileum as well as the relative amounts of soluble non-starch polysaccharides (NSP) in the jejunum and ileum are also shown.

Wheat	Diet	Weight gain (g/bird/week)	AME (MJ/kg DM)	Excreta Moisture (%)	Viscosity (mPa.s)			Soluble NSP (mg/g marker)	
					Duodenum	Jejunum	Ileum	Jejunum	Ileum
Currawong	Control	345±33^{c}	13.7±0.5^{ab}	77.1±1.6^{a}	3.3±0.5^{c}	8.0±1.2^{c}	23.3±4.0^{bc}	488±25^{c}	510±64^{cd}
	Enzyme A	392±20^{ab}	14.5±0.4^{a}	73.4±1.9^{b}	2.2±0.3^{d}	4.1±0.7^{d}	6.8±1.1^{c}	641±73^{ab}	547±43^{c}
	Enzyme B	395±29^{ab}	13.9±0.9^{ab}	76.1±3.6^{ab}	3.6±0.5b^{c}	12.3±2.4^{b}	39.7±6.7^{b}	617±271^{cb}	807±40^{a}
	Enzyme C	383±33^{ab}	14.2±0.7^{ab}	75.0±2.2^{ab}	2.3±0.6^{d}	3.3±0.8^{d}	7.7±4.2^{c}	428±217^{bc}	540±33^{c}
Harvey	Control	369b±24^{c}	12.7±0.6^{c}	77.4±3.1^{a}	4.0±0.8^{b}	9.4±0.8^{c}	28.3±5.8^{b}	492±127^{c}	509±23^{cd}
	Enzyme A	410±31^{a}	13.6±0.9^{ab}	73.0±2.4^{b}	2.4±0.4^{d}	5.2±1.8^{d}	8.3±2.7^{c}	705±59^{a}	610±76^{b}
	Enzyme B	385±12^{ab}	13.3±0.4^{bc}	75.1±1.5^{ab}	4.7±0.6^{a}	18.4±4.5^{a}	84.1±42.6^{a}	716±101^{a}	809±56^{a}
	Enzyme C	401±23^{ab}	13.9±0.8^{ab}	74.7±2.4^{ab}	2.2±0.6^{d}	3.4±0.6^{d}	7.2±2.0^{c}	472±83^{c}	465±66^{d}
Level of Significance									
Wheat		NS	***	NS	***	***	**	NS	NS
Enzyme		***	***	***	***	***	***	***	***
Wheat x Enzyme		NS	NS	NS	NS	***	***	NS	*

All data are shown as Means ± SD. Data were analysed using ANOVA and multiple comparisons were made using Duncan's Test.

[a-d] Values in a column without a common superscript differ significantly ($P<0.05$).
*** $P<0.001$; ** $P<0.01$; ** $P<0.05$.

IV. DISCUSSION

Xylanases differing in their substrate affinity can certainly have contrasting effects on digesta viscosity and their ability to release soluble NSP *in situ*. For example, Enzyme A, a xylanase with a strong affinity for both soluble and insoluble arabinoxylans, increased the soluble NSP levels in the jejunum of birds regardless of wheat type, and those in the ileum of birds fed the Harvey, the lower ME wheat. However, the increases in soluble NSP did not lead to elevated digesta viscosity, suggesting that whilst the enzyme attacked the insoluble cell walls of wheat to release soluble NSP into the digesta, it also effectively depolymerised the released NSP. On the other hand, Enzyme B, a glycanase capable of attacking insoluble NSP, increased the soluble NSP levels in both the jejunum and ileum regardless of wheat type, the increases in soluble NSP levels, whilst comparable to that in birds fed diets containing Enzyme A, were manifested in elevated digesta viscosity. This clearly suggests that Enzyme B had no effect on the soluble NSP. As expected, Enzyme C, a xylanase having affinity for only soluble NSP, effectively reduced digesta viscosity, presumably by cleaving the large molecules into smaller fragments. The current data confirm the conclusion that the ability of NSP to increase digesta viscosity depends on their solubility, molecular size and tertiary structures (Fincher and Stone, 1982), and quantitative measurements, such as the NSP level or digesta viscosity, should not be used as a sole indicator of the anti-nutritive activity of NSP in poultry diets. Indeed, all three enzymes improved the growth rate of the birds and increased the AME value of the lower ME Harvey wheat despite their different effects on gut viscosity. The only marked difference between the three enzymes on production parameters was a significant reduction in excreta moisture by Enzyme A.

A change in the physicochemical environment of the gut can drastically alter the gut microflora with contrasting outcomes (Bedford and Apajalahti, 2001). For example, use of viscosity-reducing xylanases led to a large reduction in fermentation in the small intestine in general (Choct *et al.*, 1996), and in the number of *C. perfringens* in particular (Sinlae and Choct, 2000). Modulation of the gut microflora of birds by *in situ* production of xylo-oligomers with xylanases is a clear possibility (Chesson and Stewart, 2001). Although the three xylanases used in this study differed widely in their effect on the physicochemical environment of the gut, due to the short-term nature of the experiment it is not possible to draw conclusions from the current study on the comparative advantages of the three xylanases with respect to their benefits throughout the production cycle of broiler chickens.

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EFFECTS OF EXOGENOUS XYLANASES ON GROWTH PERFORMANCE OF BROILERS OFFERED STEAM-PELLETED DIETS FROM 1-40 DAYS POST-HATCH

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Summary

Overall, adding xylanases to wheat-based diets enhanced weight gain and feed efficiency of broilers, although growth performance was highly satisfactory. The beneficial effects of the NSP enzymes were most pronounced during the grower phase (15-28 days post-hatch). Differences in the magnitude of growth performance responses between exogenous enzymes were recorded and it appeared that full inclusion rates of two xylanases were excessive in the finisher phase. Losses in the order of 3.5% from 'Splay Leg' were observed, which may have been exacerbated by rapid growth rates.

I. INTRODUCTION

The inclusion of non-starch polysaccharide (NSP) degrading feed enzymes in wheat-based broiler diets is routinely practiced and, as reviewed by Bedford and Schulze (1998), the addition of 'NSP enzymes' to poultry diets has been extensively investigated. Numerous feed enzymes with predominantly endoxylanase activity have been developed to counter the antinutritive properties of particularly soluble arabinoxylans, which increase gut viscosity and are associated with the low ME wheat phenomenon (Mollah *et al.,* 1983). The purpose of this study was to compare the effects of five exogenous xylanases on the performance of broilers in a practical context.

II. MATERIALS AND METHODS

On the basis of body weight, 1440 male Cobb chicks were allocated into 48 deep litter pens in an environmentally controlled facility and were offered *ad libitum*, starter, grower and finisher diets, from 1-14, 15-28 and 29-40 days post-hatch respectively. Body weights and feed intakes were determined at the conclusion of the three phases, mortalities were monitored daily and weights recorded to correct feed conversion ratio calculations. The basal diets (Table 1) had been steam-pelleted (~90°C) and contained anti-coccidial and anti-microbial feed additives. As the wheat was considered to be of good quality, rye offal was included (40 g/kg) in the diets to increase their NSP content because of its high soluble arabinoxylan component (Antoniou *et al.,* 1981).

Liquid NSP feed enzymes were diluted and immediately sprayed onto diets in a horizontal paddle mixer according to manufacturers' recommendations. The feed enzymes included Avizyme 1310, Biofeed Wheat, Natugrain Blend, Natugrain Wheat and Rovobio. Both Biofeed Wheat and Natugrain Wheat were added at 'half' (50%) and 'full' (100%) inclusion rates, which is consistent with their directions for use. Thus seven diets supplemented with NSP feed enzymes and a negative control comprised the eight dietary treatments (6 pens of 30 birds per treatment). Experimental data was subject to analyses of variance with general linear model procedures and linear regressions using 'SPSS 8.0 for Windows' software (SPSS Inc. Chicago, IL) Where the effects of treatment had a probability of less than 5%, least significant differences (LSD) were calculated to compare treatments.

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Table 1. Composition and nutritional specifications of experimental diets.

Item	Starter diet	Grower diet	Finisher diet
Ingredient (g/kg)			
Wheat	649.70	703.50	702.90
Rye offal	40.00	40.00	40.00
Meat & bone meal	81.00	65.00	60.00
Soyabean meal	87.50	89.00	112.50
Cottonseed meal	35.00	-	-
Canola meal	70.00	70.00	57.50
Tallow	20.00	12.50	12.50
Limestone	-	2.50	2.50
Salt	0.50	0.50	1.00
Sodium bicarbonate	3.50	3.50	2.50
Lysine monohydrochloride	4.60	4.70	3.15
Methionine	3.15	3.40	2.85
Threonine	-	0.15	-
Choline chloride	0.05	-	-
Potassium carbonate	1.00	1.25	-
Premix	4.00	4.00	2.00
D.O.T. 25% premix	-	-	0.60
Specification (%)			
Protein	21.425	19.803	20.11
Calcium	0.976	0.899	0.846
Total phosphorus	0.793	0.695	0.673
Available phosphorus	0.548	0.452	0.427
Metabolisable energy (MJ/kg)	12.13	12.13	12.18
Lysine	1.313	1.237	1.151
Available lysine	1.190	1.131	1.047
Methionine	0.568	0.570	0.530
Methionine + Cystine	0.921	0.905	0.868
Stenorol (g/kg)	0.50	0.50	-
Virginiamycin (g/kg)	0.02	0.02	0.0375
Zinc bacitracin (g/kg)	0.03	0.03	0.0375
3-Nitro (g/kg)	0.05	0.05	-
D.O.T. (g/kg)	-	-	0.150

III. RESULTS

Bird performance was highly satisfactory with an overall growth rate of 2,643 g/bird and feed efficiency of 1.795 from 1-40 days post-hatch (Table 2). The 3.96% mortality rate was not related to treatments ($P > 0.40$) and most of the losses were due to 'Splay Leg'. In the starter and finisher phases, NSP enzymes did not significantly alter growth performance. However, in the grower phase (15-28 days post-hatch), NSP enzymes significantly enhanced ($P < 0.001$) both weight gain and of feed efficiency. The most pronounced responses were observed with Enzyme F for gain (1,012 vs. 1,134 g/bird, 12.1%) and Enzyme I 100% for feed conversion (1.73 vs. 1.54, 11.0%). From 1-40 days post-hatch, NSP feed enzymes improved ($P < 0.01$) growth rates and feed efficiency, the most pronounced responses were 6.8% for growth rate (Enzyme F) and 5.4% for feed efficiency (Enzyme G 50%). In contrast, Enzyme J did not improve gain or

conversion and Enzyme H did not improve gain ($P > 0.05$) and there were significant differences between certain enzymes for growth and conversion responses.

DISCUSSION

Few comparative assessments of NSP feed enzymes have been published; however, Beasley *et al.,* (1996) compared Avizyme, Biofeed Plus and Rovobio but did not find any real differences in growth performance responses between the three feed enzymes. In contrast, in the present study, significant differences between exogenous enzymes were observed. However, as the soluble and total NSP in wheat are highly variable (Choct *et al.*, 1999) and the various NSP feed enzymes differ, the pattern of responses in a duplicated experiment may not be similar.

In the present study significant improvements in performance to NSP enzymes were confined to the grower phase, although younger birds are usually considered to be more responsive. Interestingly, Bedford (1997) drew a distinction between caged birds and birds on deep litter in considering age in relation to NSP feed enzyme responses. He argued that responses to xylanase are mainly driven by changes in gut microflora and the relatively greater microbial challenge to birds kept on deep litter leads to performance responses from NSP enzymes being observed in older birds. Increased bacterial fermentation in the small intestine, which is facilitated by higher gut viscosities, may contribute to the anti-nutritive properties of NSP in poultry (Choct *et al.,* 1996). Thus, in the present study, it is possible that NSP enzymes reduced microbial fermentation, via decreasing gut viscosity, leading to responses being observed during the grower phase following an accumulative microbial challenge from deep litter.

The addition of Enzymes G and I at 50% and 100% inclusion rates generated significant, linear responses in gain and conversion from 1-28 days post-hatch. However, in the finisher phase, numerically greater responses in gain and conversion were observed at the lower inclusion rate with both feed enzymes and consequently linear relationships were not evident. The inference is that full inclusion rates were excessive from 29-40 days post-hatch. This may have resulted from the complete hydrolysis of NSP to pentose sugars, which can negatively influence performance (Schutte, 1990; Schutte *et al.,* 1992), as opposed to partial polymerisation of NSP by xylanase. The incidence of 'Splay Leg' in the present study was in the order of 3.5% and it is understood that Cobb birds are genetically predisposed to this condition. 'Splay Leg' may be caused by partial slipping of the tendon at the hock leading to distortion of the tibiotarsus and is associated with poor bone mineralisation. The incidence is higher than usual (1.0% or less) and may have been exacerbated by the rapid growth of the trial birds.

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Table 2 Comparative effects of non-starch polysaccharide-degrading feed enzymes on growth performance of male chicks from 1-14 days, 1-28 days and 1-40 days post-hatch (6 replicates of 30 birds per treatment).

Treatments	1-14 days post-hatch			1-28 days post-hatch			1-40 days post-hatch		
	Gain (g/bird)	Feed intake (g/bird)	Feed:Gain (g/g)	Gain (g/bird)	Feed intake (g/bird)	Feed:Gain (g/g)	Gain (g/bird)	Feed intake (g/bird)	Feed:Gain (g/g)
Control	408	566	1.39	1420^{a}	2312	1.63^{a}	2528^{a}	4695	1.86^{a}
Enzyme F	432	564	1.31	1566^{d}	2340	1.50^{cd}	2699^{d}	4771	1.77^{c}
Enzyme G 100%	422	561	1.33	1522^{bcd}	2284	1.50^{bcd}	2646^{bcd}	4689	1.77^{c}
Enzyme G 50%	411	550	1.34	1507^{bcd}	2273	1.51^{bcd}	2677^{cd}	4725	1.76^{c}
Enzyme H	391	547	1.41	1475^{ab}	2266	1.54^{bc}	2603^{abc}	4644	1.79^{bc}
Enzyme I 100%	421	553	1.32	1554^{cd}	2299	1.48^{d}	2687^{d}	4826	1.80^{bc}
Enzyme I 50%	396	541	1.37	1491^{abc}	2307	1.55^{bc}	2633^{bcd}	4717	1.79^{bc}
Enzyme J	406	557	1.38	1473^{ab}	2289	1.56^{b}	2597^{ab}	4741	1.83^{ab}
SEM	11.827	9.405	0.0292	24.846	28.017	0.0208	27.867	51.252	0.0154
Significance (P =)	0.242	0.543	0.130	0.004	0.660	0.000	0.001	0.346	0.001
LSD (P < 0.05)	-	-	-	71.02	-	0.0595	79.65	-	0.0440

^{abcde} Within columns means without common superscripts are significantly different (P < 0.05).

Linear effects of Enzyme G. 1-28 days post-hatch: gain r = 0.651 (P = 0.003), feed:gain r = –0.780 (P = 0.000).
29-40 days post-hatch: gain r = 0.139 (P = 0.583), feed;gain r = -0.088 (P = 0.728).

Linear effects of Enzyme I. 1-28 days post-hatch: gain r = 0.670 (P = 0.002), feed:gain r = –0.730 (P = 0.001).
29-40 days post-hatch: gain r = 0.203 (P = 0.418), feed;gain r =-0.387 (P = 0.113).

INFLUENCE OF XYLANASE SUPPLEMENTATION AND WHOLE WHEAT INCLUSION ON THE PERFORMANCE AND GIZZARD WEIGHTS IN BROILERS

Y.B. WU, W.H. HENDRIKS and V. RAVINDRAN

Whole grain feeding for broilers has received attention in recent years due to the associated economic benefits. The objective of the present study was to examine the influence of an exogenous xylanase (Allzyme® PT; Alltech Inc., Nicholasville, Kentucky, USA) and whole wheat inclusion on the performance and gut characteristics of broiler chickens fed wheat-soy diets. The experiment was conducted as a 3 x 2 factorial arrangement of treatments, involving three wheat forms and two enzymes doses (0 or 1000 xylanase units /kg diet). The control diet (GW) contained 648 g/kg ground wheat and was cold-pelleted. In the experimental diets, whole wheat replaced ground wheat at 200 g/kg and was included either prior to (WW1) or after (WW2) cold-pelleting. Each of the six dietary treatments was fed to six pens of eight male broilers from days 1 to 21 post-hatching. Pen body weight and feed intake were recorded at weekly intervals and two birds per pen were euthanased on day 21 for digestive tract measurements. The results are summarised below.

Treatment		Weight gain, g/bird[1,3]	Feed intake, g/bird[2]	Feed/ gain, g/g[2,3]	Gizzard, g/kg body weight[2,3,4]
Wheat form	Enzyme				
GW	-	785	1252	1.595	10.0
	+	816	1282	1.584	10.1
WW1	-	821	1259	1.536	10.4
	+	841	1270	1.513	9.8
WW2	-	810	1225	1.513	18.8
	+	825	1209	1.479	16.2
Pooled SEM		13.2	18.8	0.012	0.55

[1]Wheat effect, P<0.10; [2]wheat effect, P<0.05; [3]enzyme effect, P<0.05; [4]interaction, P<0.10.

Weight gains were improved (P<0.05) and feed/gain was lowered (P<0.05) by xylanase supplementation, irrespective of the wheat form used. On average, xylanase addition improved weight gains by 2.6% and lowered feed/gain by 1.5%. Inclusion of whole wheat tended (P<0.10) to improve weight gains and lowered (P<0.05) feed/gain. Feed/gain of birds fed diets with whole wheat (WW1 and WW2) was lowered by 4.1 to 5.8%, respectively, compared to those birds fed the GW diet. The relative gizzard weights in birds fed WW2 diet were higher (P<0.05) than those in birds fed GW and WW1 diets. Interestingly, pre-pelleting inclusion of whole wheat in the ration (WW1) had no effect on relative gizzard weight. Improvements observed in the feed efficiency and the relative gizzard weights of birds given the whole wheat are in general agreement with previous reports (Preston *et al.*, 2000). The exact mechanism of the beneficial effect of feeding whole wheat is unclear, but may be related to a more extensive grinding of food within the gizzard and the resultant improvements in nutrient digestion. The results suggest that substituting whole wheat for ground wheat in broiler rations is economically advantageous and that this benefit can be further enhanced with xylanase supplementation.

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POTENTIAL OF ENZYMES IN LAYER DIETS

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Summary

Benefits of using Non Starch Polysaccharide (NSP)-enzyme supplementation in layer diets are highlighted based on results of trials involving metabolisable energy, laying performance and egg quality measurements. The addition of a NSP-enzyme produced by *Penicillium funiculosum* (Rovabio™ Excel) to wheat- or barley-based diets improved metabolisable energy by 0.13 – 0.70 MJ/kg, and improved feed efficiency by 2.55.6 %. Layers fed maize-based diets also benefitted from NSP-enzyme supplementation, with 2 to 2.5 % improvements in feed efficiency being observed. The results suggest that it is possible to reformulate a maize-soy diets allowing a 0.28 MJ/kg reduction in metabolisable energy.

I. INTRODUCTION

NSP-enzymes, such as xylanase and β-glucanase, have long been used in broiler diets based on wheat or barley to overcome the anti-nutritional effects of soluble NSP components in these cereals. It has often been assumed that adult birds are less sensitive to viscosity problems and thus to soluble NSP. The sticky excreta associated with soluble NSP, however, could lead to dirty eggs and economic losses in the layer industry. Research during the recent years have demonstrated some advantage on performance, particularly on feed efficiency, when layer diets are supplemented with NSP-enzymes. .

The present paper reviews results from a number of trials conducted to determine digestibility measurements, laying performance and egg quality parameters in layer diets based on wheat, barley or maize when supplemented with Rovabio™ Excel, an enzyme product produced by *Penicillium funiculosum* containing xylanase, β-glucanase and cellulase (Geraert *et al*., 2003).

II. INFLUENCE ON NUTRIENT DIGESTIBILITY

The improvement of energy utilisation with this NSP-enzyme has been clearly demonstrated, using the European reference method for Apparent Metabolisable Energy (AME) evaluation adapted to laying hens (Lessire *et al*., 1995). The method is based on *ad libitum* feeding and total excreta collection for seven days. Hens were fed diets containing varying levels of wheat (160-670 g/kg) or barley (400-460 g/kg). The results showed that the improvements in AME ranged from 0.19- 0.49 MJ/kg and 0.37-0.70 MJ/kg in wheat and barley diets, respectively (Table 1).

Interestingly, no linear relationship was noted between the dietary inclusion level of wheat and increases in AME or nutrient digestibility. The enhancement of nutrient digestibility was determined rather by wheat characteristics and dietary sources of protein and lipid.

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Table 1. Influence of ROVABIO™ EXCEL on the AME and nutrient

digestibility of wheat and barley for laying hens

Inclusion level (g/kg)	AME (MJ/kg DM)		Increase in apparent digestibility over the control	
	Unsupplemented control	Increase over the control	Protein	Lipid
Wheat				
650	12.66	+ 0.49	+ 0.6	+ 0.5
500	12.75	+0.29	+ 0.6	+ 1.2
485	12.90	+0.33	+ 1.8	+ 1.6
300	12.51	+0.13		
160	12.87	+0.19	+ 0.9	+ 1.1
Barley				
460	12.73	+0.70		
400	12.71	+0.37		

Similar improvements in digestibility in layer diets with NSP-enzyme addition have been reported by Barrier-Guillot *et al*. (1995) and Francesch and Perez-Vendrell (1996).

III. INFLUENCE ON LAYER PERFORMANCE

The improved digestibility of energy, protein and lipids were reflected in enhanced layer performance, especially in FCR. FCR was improved by 2.7 % in birds fed wheat diets and by 4.2 % in those fed barley diets (Table 2). Research data also show that, with enzyme supplementation, it is possible to use up to 35 % rye or 45 % wheat bran without adverse effects on performance, with savings in feed cost.

To benefit from the use of enzymes, reformulation of diets is often recommended. In trials performed in Mexico, the energy value of a sorghum-soybean meal based diet was decreased by 0.10, 0.21, 0.31 MJ/kg . Results demonstrated that a 0.31 MJ/kg reduction of AME in the specification could be totally compensated by enzyme addition with improvements in feed conversion and egg mass. In a maize-soybean meal based diet, up to 0.28 MJ/kg in AME level was fully compensated by enzyme addition without affecting either feed conversion or egg mass output (Table 2).

Unlike what is normally observed in broilers, improvement in AME in layers is often not accompanied by a reduced feed intake but rather by an increased egg mass. Indeed, modern layers often exhibit limited eating capacities to achieve their genetic potential and a reduced feed consumption would impose a drastic imbalance on nutrient intakes. Moreover, the specific appetite for nutrients like calcium carbonate in relation with oviposition would limit the interest of decreasing feed intake.

Table 2. Effect of ROVABIO^{TM} EXCEL on the performance of layer hens fed maize-, barley-or wheat-based diets between 22 and 42 weeks of age (expressed as improvements over unsupplemented control)

Diet type	Rate of lay (%)	Egg weight (g)	FCR (g/g)	FCR[1] (%)
Barley	+ 3.2	+ 0.46	- 0.092	- 4.2
Wheat	+ 1.5	+ 0.37	- 0.058	- 2.7
Maize standard[2]	+ 0.2	+ 0.78	- 0.05	- 2.5
Maize reformulated[3]	+ 0.1	- 0.88	- 0.04	- 2.0

[1] Percentage improvement over unsupplemented control [2] Between 18 and 36 weeks : maize-soybean diet formulated using standard energy values. [3] Maize-soybean diet reformulated using an assigned value of 0.28 MJ/kg for the enzyme.

IV. ENZYME EFFECT MAY DEPEND ON FEED FORM

Laying efficiency may be improved as a result of better feed conversion ratio due to an increase in egg mass or a reduction of feed intake. It is important to understand the basis of improved laying efficiency with enzyme supplementation. ISA brown laying hens were fed a wheat-based diet for 12 weeks. The diet was presented either in crumbles or mash-form. Egg production, feed intake and egg weight were recorded at weekly intervals, and egg mass and feed conversion were calculated. Results are presented in Table 3.

Table 3. Effect of Rovabio^{TM} Excel on performance of laying hens fed mash or crumble wheat-based diets between 25 and 36 weeks of age

	Mash		Crumbles	
	Control	With enzyme	Control	With enzyme
Feed Intake (g/day)	108.0 ± 9.2^{a}	108.7 ± 10.6^{a}	121.0 ± 14.0^{c}	114.7 ± 12.1^{b}
Rate of lay (%)	94.5 ± 6.5	95.4 ± 4.3	94.0 ± 12.7	95.3 ± 4.8
Mean egg weight (g)	57.3 ± 3.7^{a}	58.2 ± 3.4^{ab}	60.5 ± 3.9^{c}	59.9 ± 3.1^{bc}
Egg mass (g/hen/day)	54.2 ± 5.5^{a}	55.6 ± 3.7^{ab}	56.6 ± 8.7^{ab}	57.1 ± 4.4^{b}
FCR (g/g)	2.00 ± 0.20^{a}	1.95 ± 0.14^{b}	2.13 ± 0.29^{c}	2.01 ± 0.14^{a}
Probabilities		Feed form[1]	Enzyme	Feed*Enz
Feed Intake		<0.001	0.098	0.035
Rate of lay		0.756	0.140	0.633
Mean egg weight		<0.001	0.601	0.091
Egg mass		<0.001	0.349	0.185
FCR		0.001	0.002	0.250

[1] Feed form:mash or crumbles.
[a,b,c] Mean values in a row with no common superscripts are significantly different ($p<0.05$).

Feed efficiency was improved by 2.5 and 5.6 % in birds fed mash and crumble feeds, respectively. In mash-fed hens the improvement was due to an increased in egg mass while in crumble-fed hens, the reduced feed intake is largely responsible for the improved efficiency. The response to NSP-enzymes therefore depends on feed form. Hens appeared able to adjust their feed intake with crumbles but not with mash diets. .

V. INFLUENCE ON EGG QUALITY

An improvement in yolk colour has been reported (Le Ny, 1996) due to improved lipid digestibility with enzyme supplementation. Such an improvement will save on carotenoid incorporation. However, no significant effect of enzyme supplementation was observed on egg characteristics (Table 4). Similar observations have been reported by Francesch *et al*. (1995) in layers fed a barley-sunflower meal based diet.

Table 4. Effect of ROVABIO^{TM} EXCEL on egg quality parameters of hens fed a wheat (600 g/kg)- based diet (mean of 60 eggs per treatment)

Parameter	Control	With enzyme	Significance
Mean egg weight (g)	62.6	63.11	NS
Yolk (%)	27.7	27.2	NS
Albumen (%)	60.8	61.3	NS
Shell weight (g DM)	5.9	6.1	NS
Shell index (g/100 cm^2)	8.1	8.2	NS
Shell thickness (mm)	0.34	0.35	NS

VI. CONCLUSIONS

The addition of NSP-enzyme to layer feeds containing wheat, barley or maize has the potential to improve the nutritive value s and laying efficiency. .

Modern layers often exhibit limited eating capacities to achieve their genetic potential for production efficiency. By improving nutrient availability, exogenous NSP-enzymes can compensate for this feed intake limitation.

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THE ADDITION OF FEED ENZYMES TO LAYER DIETS BASED ON WHEAT OR WHEAT PLUS RYE

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Summary

Four different commercial enzyme products were added to either a standard commercial wheat-based layer diet or the same diet with 20% of the wheat substituted with cereal rye. Diets were fed to Isa Brown laying hens from 50 to 65 weeks of age. Measurements of egg and egg shell quality, apparent metabolisable energy and excreta moisture were conducted at five-weekly intervals. Digesta viscosity was measured at the end of the trial. The AME of the diets was similar and relatively stable. Excreta moisture was not affected by the addition of feed enzymes. Egg and egg shell quality varied significantly as the birds grew older and was significantly better for the wheat+rye diet as compared with the wheat diet. There were few effects of enzymes on egg and egg shell quality except that Kemzyme resulted in lower albumen quality and lighter shell colour. Production was not affected by type of diet but the Roxazyme treatment had the highest production. Both type of diet and the addition of enzymes affected egg and egg shell quality.

I. INTRODUCTION

Enzymes are added to commercial layer diets to increase the digestibility of feed ingredients, reduce the incidence of wet droppings resulting from non-starch polysaccharides in the diets (Acamovic, 2001) and increase the availability of feed microingredients which influence egg shell quality (Hurwitz, 1987). A recent study showed that addition of commercial enzyme preparations improved egg shell quality in wheat- and barley-based layer diets but that there were some negative effects on shell colour and Haugh Units (Roberts and Choct, 1999; Roberts *et al*., 1999). In Australia, wheat is a common ingredient in layer diets. However, the quality and composition of Australian wheats are variable (Hughes and Choct, 1999). The present study was therefore conducted to investigate the effect of dietary enzymes and dietary non-starch polysaccharide levels on egg and egg shell quality in Isa Brown laying hens.

II. MATERIALS AND METHODS

Two basal diets were formulated to standard commercial specifications. The control diet contained 670 g/kg wheat whereas, in the experimental diet, 20% of the wheat was substituted with cereal rye. The other ingredients were identical in the two diets. The basal diets were each used to prepare five experimental diets by adding one of four commercial feed enzyme preparations according to the manufacturers' instructions; a control diet of each type (no enzyme added), Bio-Feed Wheat (175 g/tonne), Avizyme 1302 (265 g/tonne), Roxazyme G2 granular (100 g/tonne), or Kemzyme W dry (600 g/tonne). The diets were fed from 50 to 65 weeks of age to 760 Isa Brown laying hens which were maintained, 2-3 to a cage (26 replicates), in a commercial poultry house at the University of New England "Laureldale" Poultry Farm. The different treatment groups were randomised to avoid effects due to position in the poultry house. Egg and egg shell quality were assessed at 5 weekly intervals.

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At each age, 300 eggs were collected, 30 from each of the ten treatment groups. Egg and egg shell quality analyses were completed within 24 hours of the eggs being laid. Measurements taken to assess egg shell quality were egg weight, shell reflectivity (an indication of the colour of the egg shell), egg shell breaking strength (measured by quasi-static compression), deformation (the distance that the egg shell is depressed by the shell breaking strength machine before the shell cracks) and shell weight. The percentage shell was calculated as the ratio of shell weight to egg weight, expressed as a percentage. The internal quality of the eggs was assessed as albumen height and Haugh Units as well as yolk colour.

Apparent metabolisable energy (AME) and excreta moisture were measured every 5 weeks from 50 to 65 weeks of age. AME was determined by the conventional total collection procedure. Birds had received the experimental diets for at least 5 weeks prior to the AME assays which were conducted over 4 days. Feed intake was measured and all excreta collected daily. Feed samples were dried at 105ºC for 16 hours. Excreta were dried in a fan-forced oven at 80ºC for 36 h and excreta from each replicate were pooled over the collection period for the determination of gross energy (GE). AME of diets was calculated as:

$$\frac{\text{(g of feed eaten x GE of feed) – (g excreta voided x GE excreta)}}{\text{g feed eaten}}$$

At the end of the trial, digesta were collected from 5 birds from each diet, centrifuged and the viscosity of the supernatant determined using a Brookfield DVIII Model viscometer. Data were analysed by ANOVA with bird age, grain base and enzyme treatment as independent variables. Differences between means were assessed by Fisher's (Protected) Least Significance Difference test. Significance was assumed at $P<0.05$.

III. RESULTS

The extract viscosity was higher for the diet based on wheat+rye and this diet was higher in soluble, insoluble and total non-starch polysaccharides (Table 1). Digesta viscosity was significantly higher for the wheat+rye diet than for the wheat diet in both the jejunum (wheat 1.95 cP, wheat+rye 3.19 cP) and the ileum (wheat 3.57 cP, wheat+rye 8.88 cP) but was not affected by the addition of commercial enzyme preparations (Table 2).

Table 1. Extract viscosity of diets and grains

Sample	Insoluble NSP g/kg	Soluble NSP g/kg	Total NSP g/kg	Extract viscosity cP
Feed based on wheat	73.8	5.8	79.6	3.40
Wheat	82.6	6.8	89.4	5.38
Feed based on wheat + rye	82.9	7.8	90.7	10.60
Rye	77.7	24.9	102.6	347.47

Table 2. Jejunal and ileal viscosity of laying hens fed diets containing wheat and wheat plus rye, with or without enzyme supplementation (Means ± SE)

Enzyme treatment	Wheat diet			Wheat + Rye diet		
	n	Jejunum	Ileum	n	Jejunum	Ileum
Control	5	2.26 ± 0.22	5.50 ± 0.90	4	2.75 ± 0.71	8.08 ± 2.66
Bio-Feed Wheat	5	1.82 ± 0.23	2.19 ± 0.20	5	2.24 ± 0.16	5.98 ± 1.21
Avizyme	5	1.85 ± 0,12	3.39 ± 0.44	5	2.51 ± 0.34	15.02 ± 7.08
Roxazyme	5	1.99 ± 0.30	2.82 ± 0.40	5	5.32 ± 1.95	8.03 ± 3.89
Kemzyme	5	1.84 ± 0.17	3.65 ± 0.59	5	3.04 ± 0.66	7.27 ± 2.04

Table 3. Effect of hen age on AME (MJ/kg DM) (Means ± SE)

Age	50 weeks	55 weeks	60 weeks	65 weeks
AME	[a]13.52	[c]13.26	[ab]13.47	[bc]13.31
MJ/kg DM	±0.12	±0.08	±0.15	±0.12

Means with no common superscript differ significantly (P<0.05)

AME remained relatively constant during the trial and was not affected by diet type (Table 3). Feed intake and excreta moisture of birds on the two diets were also similar, as was production. The addition of commercial enzyme preparations had no significant effect on AME or excreta moisture. Production was higher than the control for Roxazyme (Table 4).

Table 4. Effect of enzyme treatment on production (eggs/100 hens/day) (Means ± SE)

Control	Bio-Feed Wheat	Avizyme	Roxazyme	Kemzyme
[bc]84.08	[ab]85.72	[c]83.29	[a]86.68	[bc]83.92
±0.98	±0.86	±0.97	±0.97	±0.90

Means with no common superscript differ significantly (P<0.05)

For egg and egg shell quality measurements, there were statistically significant main effects of age on most variables. As hens became older, egg weight, shell weight, shell thickness and yolk colour increased whereas shell colour, shell breaking strength, albumen height, Haugh Units, and percentage shell decreased. The wheat+rye diet resulted in better egg quality than wheat alone (Table 5). Enzyme type and/or inclusion had significant effects on shell colour and albumen quality (Table 6), with Kemzyme addition resulting in lighter shell colour and lower albumen quality.

Table 5. Effect of diet (wheat or wheat+rye) on egg and egg shell quality (Means ± SE)

Diet	Breaking strength N	Shell wt g	% Shell	Albumen Ht mm	Haugh units	Yolk colour
Wheat	[b]32.5±0.4	[b]6.23±0.03	[b]9.05±0.04	[b]7.98±0.07	[b]85.7±0.5	[a]11.79±0.3
Wheat+rye	[a]33.9±0.4	[a]6.35±0.03	[a]9.20±0.04	[a]8.36±0.06	[a]88.4±0.4	[b]11.65±0.4

Means within columns with no common superscript differ significantly (P<0.05)

Table 6. Effect of enzyme treatment on egg and egg shell quality (Means ± SE)

Measurement	Control	Bio-Feed Wheat	Avizyme	Roxazyme	Kemzyme
Reflectivity %	[b]34.1±0.4	[b]34.3±0.4	[b]34.6±0.4	[b]34.9±0.4	[a]36.1±0.4
Albumen Ht mm	[a]8.31±0.12	[a]8.35±0.09	[a]8.09±0.11	[a]8.36±0.10	[b]7.74±0.12
Haugh Units	[a]87.7±0.8	[a]88.4±0.6	[a]86.6±0.8	[a]88.3±0.6	[b]84.5±0.8

Means within rows with no common superscript differ significantly (P<0.05)

IV. DISCUSSION

Cereal rye was added to the diet of the experimental group to increase the non-starch polysaccharide levels of the diet. It was expected that this would increase the extract viscosity of the experimental diet, simulating the situation that has been observed previously for “new season” wheats (Hughes and Choct, 1999). The extract viscosity of the whole grain cereal rye was very high and difficult to measure accurately, having approximately 60 times

the extract viscosity of whole grain wheat. At the same time, the extract viscosity of the diet based on wheat+rye was 2-3 times that of the diet based on wheat alone. This discrepancy may be due in part to the presence of endogenous enzymes in other feed ingredients. Digesta viscosity was increased by the addition of rye but was not affected by enzyme addition. It is possible that enzyme inhibitors may be involved (Brufau *et al.*, 2002). The addition of commercial enzyme preparations had no effect on the AME value of the diets, nor was there any significant effect on excreta moisture levels. Production was affected only slightly with Roxazyme resulting in improved production.

Egg and egg shell quality, in general, deteriorated with the age of the hens. The age-related changes in egg and egg shell quality are similar to those reported previously (Roberts and Ball, 1998). The wheat diet containing 20% cereal rye resulted in better egg quality than diets containing only wheat despite the higher non-starch polysaccharide levels and extract viscosity of the wheat+rye diet. The type of grain on which diets are based appears to have direct effects on egg quality. It appears that factors other than the levels of crude protein and non-starch polysaccharides are involved but these factors have yet to be clearly identified.

The improved shell breaking strength observed in the previous study in eggs from birds given enzymes (Roberts and Choct, 1999; Roberts *et al.*, 1999) was not observed in the present study. This may be due to differences in feed ingredients used in the two experiments. However, the decrease in shell colour and reduced albumen in response to dietary enzyme inclusion, was consistent across the two studies. Kemzyme had negative effects on shell colour and albumen quality.

In conclusion, both type of diet and addition of commercial feed enzyme preparations affected egg and egg shell quality.

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EFFECTS OF PHYTASE ON APPARENT METABOLISABLE ENEGRY IN BROILER DIETS BASED ON WHEAT OR SORGHUM

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The benefits of phytase addition to poultry diets in improving P availability, growth performance, digestibility of crude protein and individual amino acids are well established and documented (Kies *et al*., 2001). In contrast, the effects of phytase addition on the metabolisable energy (ME) are less well understood. Ravindran *et al*. (1999) reported a positive effect of phytase addition on apparent ME in diets with wheat, whereas Biehl and Baker (1997) showed no effect of phytase addition on true ME_N. The present study reports on the effects of two commercially available phytase products on the apparent ME (AME) in wheat-based diets with or without xylanase and in sorghum-based diets without xylanase.

Nine experimental diets based on wheat/soybean meal sorghum/SBM were formulated to commercial standards with reduced Ca (0.84%) and available P (0.36%) levels. Two phytases were added at their recommended dosage as granulates prior to pelleting (P1 at 300 ppm and P2 at 100 ppm). All diets were cold-pelleted (60^OC). The xylanase product was sprayed onto the feed after pelleting. Feed intake and excreta output of 225 male and 180 female broiler chickens (5birds/cage and 9 reps/diet) were measured over a 4-day period. Gross energy of the excreta and feed was determined by bomb calorimetry and the AME of each diet was calculated.

Table AME (MJ/kg DM) of broiler chickens fed wheat and sorghum based diets with or without phytase (P) and xylanase (Xyl) (wheat based diets only).

Grain	Control	Xyl	P1	P2	Xyl+P1	Xyl+P2
Wheat	14.88^c	15.65^b	15.07^c	14.85^c	15.45^b	15.50^b
Sorghum	16.15^a	---	16.15^a	16.20^a	---	---

^{a,b,c} Means without a common superscript are significantly different ($P<0.05$). SEM = 0.070

The inclusion of phytase in either the wheat-based or sorghum-based basal diets had no effect on the AME of the diet. Wheat diets supplemented with xylanase had a significantly higher AME compare to the control diet or diets with phytase only. These results are in contrast to the findings of Ravindran *et al.* (1999) who showed an improvement of 6.3 and 4.5%, respectively, for AME value of wheat with phytase. The present results suggest that assignment of a fixed energy value to phytase in feed formulation is questionable since there exists some doubt on the energy response to added phytase in broiler diets.

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PHYTASE AND XYLANASE DOSE TITRATION AND BROILER PERFORMANCE ON WHEAT-BASED DIETS

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Arabinoxylan and phytate contents of wheat negatively influence nutrient utilisation and performance of broiler chicks. The combined inclusion of phytase and xylanase feed enzymes in broiler diets has been evaluated (Ravindran, *et al*., 1999), but optimum inclusion rates have not been defined. Therefore, the effects of a range of inclusion rates of Natuphos® phytase (5,000 FTU/g) and Natugrain® Wheat xylanase (28,000 EXU/g) in a phosphorus-deficient (0.25% non-phytate P) wheat-based broiler starter diet on performance, apparent metabolisable energy (AME) and nitrogen retention were investigated. Nine dietary treatments were offered *ad libitum* to six pens (6 birds/pen) of male chicks (Cobb) from 4 to 19 days post-hatch. Feed intake and body weight were recorded, and feed conversion rate (FCR) was calculated. All excreta were collected from 15 to18 days post-hatch to determine AME and N retention.

Treatment	Wt gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	AME (MJ/kg DM)	N retention (% DM)
Basal diet (B)	539.1[a1]	740.8[ab]	1.376	13.74[a]	70.96
B + 600 FTU/kg phytase	583.8[b]	795.6[c]	1.364	14.00[abc]	72.14
B + 5,600 EXU/kg xylanase	540.1[a]	718.6[a]	1.331	14.23[c]	72.54
B + 600 FTU + 5,600 EXU	565.3[ab]	765.1[bc]	1.355	14.14[c]	74.16
B + 450 FTU + 4,200 EXU	588.4[b]	778.8[c]	1.335	14.14[c]	72.17
B + 300 FTU + 2,800 EXU	587.3[b]	793.4[c]	1.352	14.24[c]	73.21
B + 150 FTU + 1,400 EXU	578.4[b]	777.6[c]	1.345	14.15[c]	73.70
B + 500 FTU + 2,800 EXU	585.9[b]	793.7[c]	1.355	14.06[bc]	72.20
B + 300 FTU + 4,650 EXU	569.1[b]	767.6[bc]	1.349	13.82[ab]	71.49
SEM	9.697	11.288	0.015	0.112	1.102
P value~~s~~	0.001	0.000	0.576	0.027	0.553
LSD (P =0.05)	27.62	32.15	0.043	0.319	3.140

[1]Means in a column bearing different superscripts are significantly different ($P < 0.05$).

Overall, the addition of phytase and xylanase, individually and in combinations, to low P diets significantly increased body weight gain ($P < 0.05$), feed intake ($P < 0.05$) and AME ($P < 0.05$) by up to 9.1%, 7.4%, and 0.50 MJ, respectively, but did not alter ($P > 0.05$) feed efficiency or N retention. Phytase alone significantly increased weight gain and feed intake, whereas xylanase alone increased AME and the combinations showed significant responses to all three parameters. It appears that when used in combination the inclusion rates of the enzymes may be reduced below the individual recommended levels without compromising responses in the assessed traits. However, responses to phytase plus xylanase supplementation of P deficient diet did not exceed those of phytase alone. It is possible that the inadequate non-phytate P of the basal diet limited growth performance responses to exogenous enzymes.

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COMPARISON OF *IN VITRO* NUTRIENT RELEASE BY THREE ENZYME PREPARATIONS IN WHEAT- AND MAIZE-BASED DIETS

Y.B. WU[1], J. PIERCE[2], W.H. HENDRIKS[1] and V. RAVINDRAN[1]

Summary

The release of phosphorus, reducing sugars and α-amino nitrogen by three phytase sources from wheat- and maize-based diets was compared using an *in vitro* model. A phytase produced by solid state fermentation, and containing side enzyme activities, released more ($P<0.05$) phytate-bound phosphorus (11.0% and 7.8% in wheat- and maize-based diets, respectively) and α-amino nitrogen (1.7% and 6.2% for wheat- and maize-based diets, respectively) than a source of pure phytase produced by submerged liquid fermentation. The phytase produced by solid state fermentation also released 2.9% more reducing sugars in the wheat-based diet. The superiority of this phytase product in releasing nutrients in both types of diets may be due to activities of other enzymes present, but these *in vitro* results need to be confirmed in *in vivo* studies.

I. INTRODUCTION

The usefulness of microbial phytase in releasing phytate-bound phosphorus and improving phosphorus availability in poultry and pig diets is now well documented. Several commercial microbial phytase products are currently available and two distinct fermentation technologies are used to produce these products - one involving submerged liquid fermentation and the other based on solid state fermentation. Because of the technology employed, the phytase produced by solid state fermentation also contains several side enzyme activities, including protease, amylase, cellulase, xylanase and β-glucanase. Studies have shown that phytase produced by solid state fermentation is effective in enhancing the utilisation of nutrients in a range of diet types for broiler chickens and these responses were, in part, attributed to side-enzyme activities present (Ravindran *et al*., 2001; Wu and Ravindran, 2002). The trial designs used in these studies, however, did not permit any conclusion on the benefits of the side activities.

In vitro simulation models have been recently developed and successfully used to predict the release of nutrients by exogenous enzymes for turkeys (Zyla *et al*., 1995) and broilers (Zyla *et al*., 1999a; 2000). The objective of the present study was to examine whether or not there are beneficial effects from the side activities present in a phytase produced by naturally selected *Aspergillus niger* in solid state fermentation by determining *in vitro* nutrient release in wheat- and maize-based diets. The influence of graded levels of this phytase product (phytase A; 0, 250, 500, 750 and 1000 phytase units (PU)/kg diet) was compared with two levels of a synthetic enzyme blend (phytase B, prepared by blending pure sources of phytase, fungal protease, fungal amylase, cellulase, xylanase and β-glucanase to match the enzyme activities in phytase A; 500 and 750 PU/kg diet) and one level of a commercial source of pure phytase produced by genetically modified *Aspergillus niger* in submerged liquid fermentation (Phytase C; 500 PU/kg diet).

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II. MATERIALS AND METHODS

Phytase A was analysed for the following enzyme activities: phytase, fungal protease, fungal amylase, cellulase, xylanase and Beta glucanase. The phytase activity exceeded product guarantees at 1216 PU/g. Phytase B was formulated, using pure sources of enzymes, to contain a similar enzyme profile to phytase A. Phytase C was determined to contain 3645 PU/g phytase activity and had no detectable side enzyme activities.

The *in vitro* digestion model described by Zyla *et al.* (1999a) was employed, except for modifications in the pH values used for incubation, to determine the release of phosphorus, reducing sugars, and α-amino nitrogen in wheat-soy and maize-soy diets. Based on a preliminary *in vivo* study with broilers fed the same wheat-soy and maize-soy diets, the pH values used to simulate digestion in crop, gizzard and small intestine were adjusted to 5.7-5.9, 2.7-2.9 and 5.9-6.1, respectively. The composition of the wheat-soy and maize-soy diets has been previously reported (Wu and Ravindran, 2002). The diets were formulated to meet or exceed recommended specifications for all nutrients, except phosphorus and calcium, for broiler starters. The non-phytate phosphorus and calcium levels in the diets were 2.5 and 6.9 g/kg, respectively. Six milliliters of the dialysate samples were taken after 2 and 4 hours of incubation and analysed for inorganic phosphate (Shieh *et al*., 1969), reducing sugars (Miller *et al*., 1960) and α-amino nitrogen (Moore and Stein, 1954). The experimental data were collected in three replicate samples.

The data were statistically analysed by the General Linear Model (GLM) procedure (SAS, 1990). Linear and quadratic effects on dialysable phosphorus, reducing sugars, and α-amino nitrogen were tested with five levels of phytase A using the contrast statement in the GLM. For the comparison of phytase sources, mean differences were separated by least significant difference. Significant differences were considered at $P<0.05$.

III. RESULTS AND DISCUSSION

Although the dialysable samples were taken at 2 and 4 hours of incubation, only the data at 4 hours are reported. The nutrient release data at 2 hours, in general, followed a similar trend to those at 4 hours.

a) *In vitro* dialysable phytate-bound phosphorus

The dialysable phosphorus levels increased quadraticaly with increasing levels of phytase A in both wheat- ($R^2 = 0.99$; $P<0.001$; Figure 1) and maize-based diets ($R^2 = 0.99$; $P<0.001$; Figure 2). These results are consistent with previous *in vitro* data (Zyla *et al*., 1999 a,b; 2000) reported for other commercial phytase products, including phytase C.

Compared to phytase C, phytase A at 500 PU/ kg diet released more ($P<0.05$) phytate-bound phosphorus (11.0 and 7.8% for wheat- and maize-based diets, respectively). Compared to phytase B, phytase A released more phosphorus ($P<0.05$) at 500 and 750 PU/kg diet in both types of diets (Table 3). The latter observation in difficult to explain since phytase B was blended to contain similar enzyme activities as phytase A.

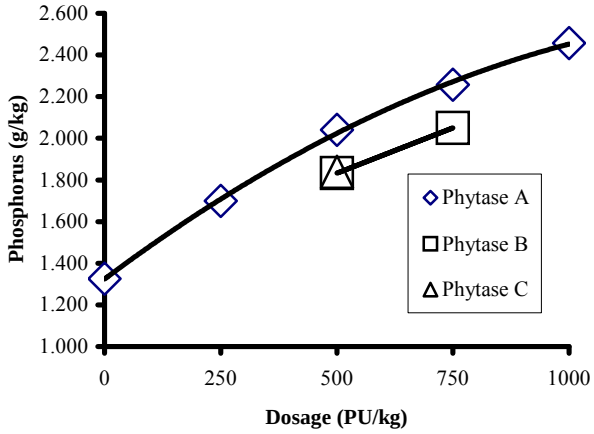

Figure 1. Effect of source and concentration of phytase on *in vitro* release of dialysable phosphorus in the wheat-soy diet.

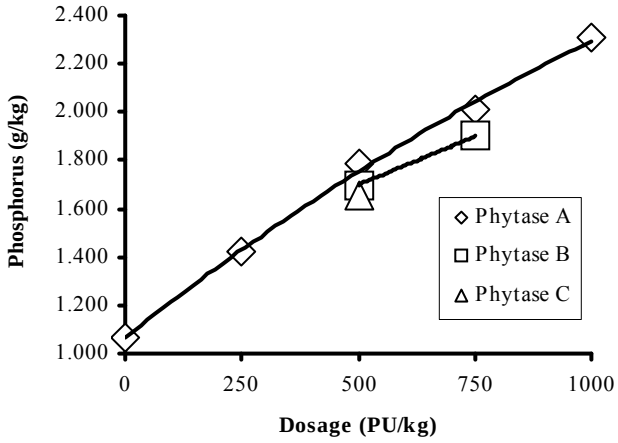

Figure 2. Effect of source and concentration of phytase on *in vitro* release of dialysable phosphorus in the maize-soy diet.

b) *In vitro* dialysable reducing sugars

Dialysable reducing sugar levels increased linearly (R^2 = 0.85; P<0.001) with increasing levels of phytase A in the wheat-based diet. Phytase A supplemented at 500 PU/ kg diet released 2.9% more (P<0.05) reducing sugars than phytase C supplemented at the same level of activity. No significant differences were observed between phytases A and B. In the maize-based diet, increasing levels of phytase A had no effect (P>0.05) on the reducing sugar levels and no differences (P>0.05) were observed between the three phytases at 500 PU/ kg diet. The responses to added phytases in the maize-soy diet are in general agreement with *in vivo* results (Wu and Ravindran, 2002) that wheat-based diets are more responsive to microbial phytase than maize-based diets.

c) *In vitro* dialysable α-amino nitrogen

Increasing levels of phytase A had no effect (P>0.05) on the α-amino nitrogen levels (expressed as glycine per kg diet) in the dialysate in the wheat-based diet (Figure 3), but quadratically increased the α-amino nitrogen levels in the maize-based diet (R^2 = 0.71; P<0.001; Figure 4). The lack of response in the wheat-based diet was unexpected and difficult to explain. Compared to phytase C, phytase A at 500 PU/kg diet released 1.7% more α-amino nitrogen in the wheat-based diet, but the differences were not significant (P>0.05). In the maize-based diet, Phytase A at 500 PU/kg diet released 6.2% more (P<0.05) α-amino nitrogen than phytase C. No significant differences were observed between phytases A and B in either wheat- or maize-based diets.

The results of this *in vitro* study showed that phytase A, a product with side enzyme activities, produced better response in terms of nutrient release than phytase C, a source of pure phytase. It should be, however, noted that these *in vitro* results may not be directly applicable to *in vivo* situations and therefore should be considered only as crude indicators of the relative efficacy of the enzymes evaluated.

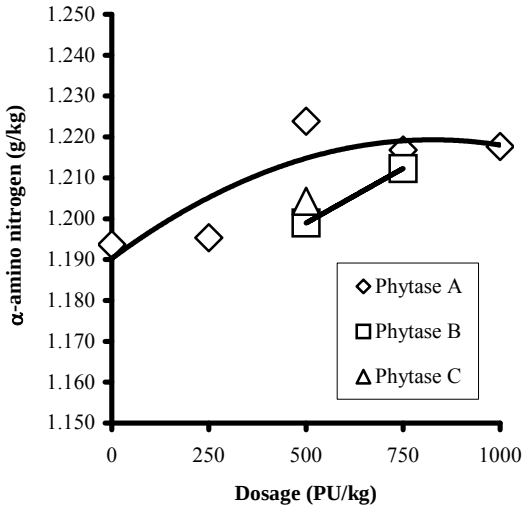

Figure 3. Effect of source and concentration of phytase on *in vitro* release of α-amino nitrogen in the wheat-based diet.

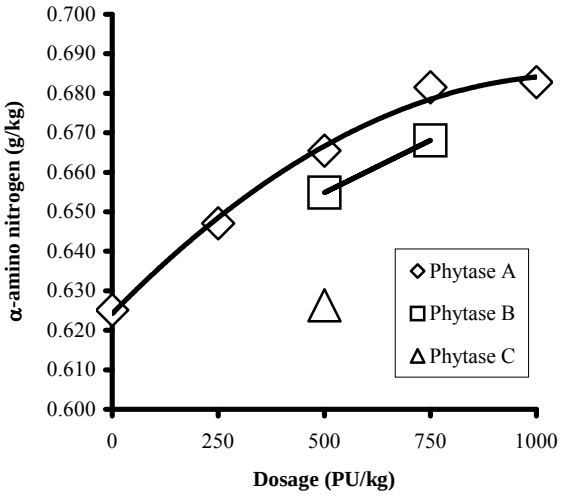

Figure 4. Effect of source and concentration of phytase on *in vitro* release of α-amino nitrogen in the maize-based diet.

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DRY POST-PELLET APPLICATION OF HEAT-LABILE PRODUCTS TO LIVESTOCK DIETS

J.L. PIERCE, C.A. MORAN and A.E. SEFTON

Summary

Post pelleting application of heat-labile enzymes and bacteria was evaluated in a commercial setting. A product containing phytase and *Lactobacillus plantarum* was applied to pellets after fat coating. The fines were separated from the whole pellets in order to determine the amount of product that adhered to the pellets. In this study, 98.6% of the enzyme activity remained adhered to the pellets while only 1.4% was associated with the fines. When the feed was separated into fines and pellets, there was no difference between *L. plantarum* counts in the complete feed and in the screened pellets.

I. INTRODUCTION

Many nutrients, enzymes, and microorganisms currently available are not stable through the conditioning and processing conditions associated with pelleting and extrusion of feeds for livestock and poultry (Chae and Han, 1998; Vanderval, 1979). Vitamins, such as folic acid and niacin, are the first to be destroyed as pelleting temperature reaches 60°C. Many enzymes tend to lose activity past 75°C. However, temperatures above 95°C are often applied to reduce bacterial load in the feed and make harder pellets. There are many factors to consider when evaluating whether an enzyme or microbe survives feed processing as described by Spring *et al*. (1996). The major variables include: temperature, moisture, time, pH, pressure, and feed composition. To overcome the loss of enzyme activity, post-pelleting (processing) application techniques have been used in recent years.

Engelen and van der Poel (1999) state that the only way to fix additives to feed is to spray them on as a liquid. They argue that a powder will not adhere to a feed and will result in separation of the additive into the fines. With those thoughts being shared by many, it is not surprising that the most successful application method to date is liquid spray. However, there are many disadvantages to liquid spray applications such as the effects of temperature on liquids, clogging of spray nozzles and calibration of minute amounts of liquid onto relatively large volumes of feed. The possibility of adding dry enzymes to feed post pelleting was explored by Edens *et al*. (2002). In their study, they used a simple device to apply enzyme to feed in either dry form or with the addition of an oil spray. Even with relatively high CV's for application, the bird performance in terms of feed conversion, Phosphorus (P) reduction in excreta, and growth rate were equal to that of a liquid-sprayed phytase when compared across experiments.

The objective of this study was to determine the post-pellet adhesion of a powdered phytase product and bacteria to pellets.

II. MATERIALS AND METHODS

The test material contained 1000 PU/g of phytase activity and 8.7 $\log_{10}$ cfu *Lactobacillus plantarum* /g. Product was applied immediately after fat coating into a screw conveyor at a rate of 250 g/min. The feed flow rate was approximately 15 tonne/h. A control blank sample was taken prior to treatment with the enzyme/bacterial preparation. Ten replicate samples were taken at each of three treatment locations:

Alltech Inc., 3031 Catnip Hill Pike, Nicholasville, Kentucky 40356, USA.

1. Complete feed (CF) as it entered a storage bin at the end of the screw conveyer;
2. Screened pellets from load out, and
3. Fines screened from load out.

The feed contained 1.3% fines. The feed was assayed for phytase activity under conditions of pH 5.5 and 37°C in the presence of phytic acid for 60 minutes. Phosphorus release was determined colorimetrically. One PU is the μmol of P released per minute under the conditions of the assay.

Lactobacillus plantarum (*L. plantarum*) counts were enumerated after appropriate dilutions, in peptone water, using the pour-plate technique in MRS agar and incubated aerobically for 3 days at 30ºC. The number of lactobacilli was expressed as the $\log_{10}$ per ml of feed sample. Bacterial counts were log transformed to fit a normal distribution prior to analysis by a univariate general linear model analysis of variance (GLM-ANOVA). Significant differences ($P<0.05$) between the feed samples were compared by Tukey's post-hoc test (Zar, 1999).

III. RESULTS AND DISCUSSION

The complete feed samples were slightly lower in phytase activity than the load out, though not significantly so (Table 1). A total of 98.7% of the enzymatic activity remained on the pellets (Table 2). The activity in the fines was not as high as has been reported with spray systems which have been shown to have fines with three times the amount of activity as the screened feed (Engelen and van der Poel, 1999). The coefficient of variation was highest in the complete feed most likely because this was nearest the application point. As the feed proceeded to load out and was further mixed, there was a reduction in the variability of enzyme activity. Traditionally there had been a rule-of-thumb 15% acceptable C.V. for enzyme application. This was proven to be an unreliable, arbitrary number in a study demonstrating that birds were still able to perform satisfactorily with an application C.V of 103% (Harter-Dennis, 2000).

Table 1. Phytase activity in complete feed and components (Means of 10 duplicate samples)

	Complete feed	Load out	Fines
PU/kg	1770[a]	1979[a]	2209[b]
CV., %	20.7	12.8	1.9

Means in row with different superscript differ ($P<.05$)

The feed evaluated in this study was of very high pellet quality as indicated by its high percentage of pellets (98.7%) (Table 2). The weighted phytase activity in the pellets and fines is calculated simply by the percent of each fraction multiplied by the activity in each and divided by 100. The liquid addition evaluated by Engelen and van der Poel (1999) showed that nearly 24% of the enzyme activity was in the fines. However, in the current study, only 1.4% of the total activity in the feed was "lost" in the fines (Table 2). It is clear that pellet quality has a major influence on distribution of the enzyme activities in the fines and the pellets, which probably explains the vast difference between the current results and that of Engelen and van der Poel (1999).

Table 2. Weighted phytase activity with feed separated into pellets and fines.

	Pellets	Fines	Feed
Feed Distribution, %	98.7	1.3	100.0
Activity, PU/kg	1953	29	1982
% of Phytase Activity	98.6	1.4	100.0

In their study, Engelen and van de Poel (1999) hypothesize that the high amount of fines shown in their study (8.9%) may have been due to the addition of water and thus resulting in higher water activity. This, in turn, resulted in a decrease in pellet hardness and a concomitant decrease in pellet durability. They concluded that based on the three-fold increase in enzyme activity in the fines compared with the pellets that enzyme absorption in the pellet is very small when applied as a liquid.

In addition to enzyme activity, the feed was also analyzed for bacterial adhesion to the pellets. The test material contained 8.7 $\log_{10}$ cfu *L. plantarum* /g (SE = 1.18) and therefore, theoretically the pellets should have received 5.7 $\log_{10}$ cfu *L. plantarum* /g. Counts of *L. plantarum* in the complete feed and load out did not differ significantly (SE = 0.09) (Figure 1). A higher recovery was identified in the fines (P = 0.01) due to the greater surface area available. The control feed, which did not receive the treatment, contained 3.8 $\log_{10}$ cfu lactic acid bacteria /g, which was significantly lower ($P < 0.05$) than treated feed.

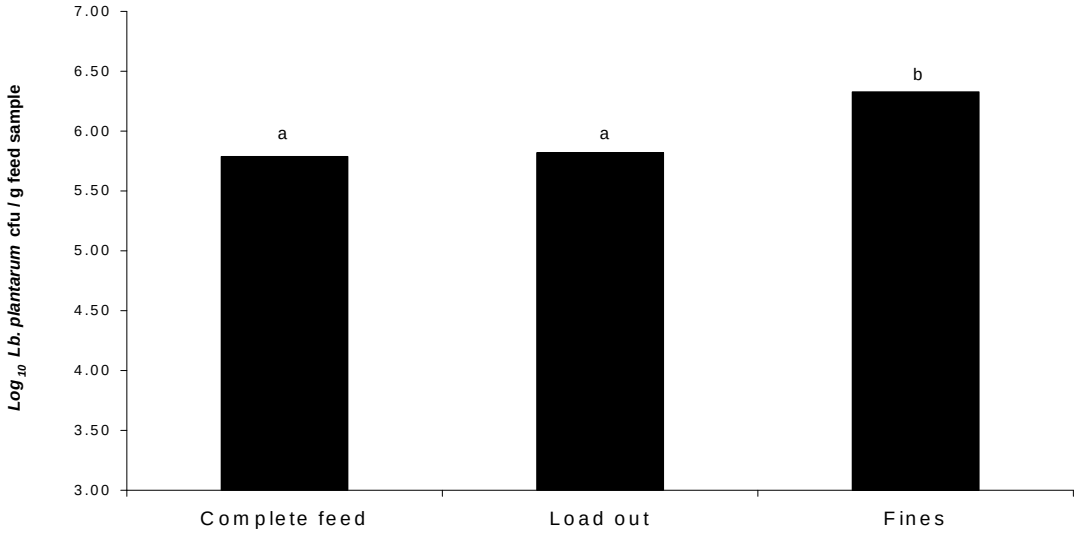

Figure 1. *L. plantarum* counts ($\log_{10}$ cfu g^{-1}) from feed samples (n = 10) taken at three locations during the feed pelleting process.

IV. IMPLICATIONS

Based on these findings, it is anticipated that a variety of other important, heat-labile, feed additives, such as vitamins, may be applied to pelleted or extruded feeds with this technology. The addition of dry enzymes and microorganisms to post-pelleted feeds opens opportunities for probiotics, competitive exclusion bacteria, and yeast cultures to be effectively applied to animal feed.

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ENZYMES AND NON-STARCH POLYSACCHARIDES : A BETTER MATCH IMPROVES EFFICACY

P.A. GERAERT, S. MAISONNIER, K. LIU and P. DALIBARD

Summary

This paper describes the structures of non-starch polysaccharides (NSP) in commonly-used feed ingredients and the adapted enzyme profile required for their hydrolysis. Under the term “xylanases”, a wide range of proteins with different substrate affinities is often quoted. Choosing the correct enzyme product can be a difficult process. Future developments of enzymes will require a better knowledge of the spectrum of enzyme secreted by fungi.

I. INTRODUCTION

Identification of NSP can be confusing. The terms pentosans, xylans and arabinoxylans cover different biochemical structures thus making it difficult for a non-specialist to understand. Indeed, arabinoxylans are polymeric structures of arabinose-furanosyl residues branched on a xylose-pyranosyl backbone. Pentosans include arabino-xylans but also arabino-galactans and hetero-xylans. Moreover, there is confusion between the general name of an enzyme and the likely mode of action of this enzyme. The endo-1,4 β-glucanase, also called cellulase and hence able to hydrolyse cellulose, is different from the endo-1,3(1,4)-β-glucanase which is only able to hydrolyse the complex barley β-glucans. This paper discusses the importance of matching the activities of the enzymes to the structure of NSP substrates for efficacy of depolymerisation and associated improvements in growth performance and feed efficiency of the flock.

II. NSP ARE COMPLEX STRUCTURES

The main NSP of raw materials commonly used in poultry feed are arabinoxylans, mixed-linked β glucans, cellulose and pectins (Table 1). ~~Th~~Arabinoxylans are constituted from a xylose backbone substituted by arabinose, either as mono- or di-substitution. There is a diverse range of NSP in different raw materials and within the same raw material. For example, arabinose to xylose ratios range from 0.48 to 0.72 according to the type of raw material (Izydorczyk and Biliaderis, 1995) and from 0.32 to 0.69 according to the wheat cultivar (Anderson *et al*., 1994).

III. DEGRADATION OF NSP-REQUIRES A WIDE RANGE OF ACTIVITIES

The complex structure of the NSP requires the action of various enzyme activities to break it down. The endo 1,4-β-xylanase cuts the endo-1,4-β linkages between the xylose units when there are no substitutions around these linkages. Therefore, on highly substituted arabinoxylans, the arabinofuranose must be released by the α-arabinofuranosidase in order to allow the action of the endo-1,4-β-xylanase. Some commercial products, for example Rovabio^{TM} Excel, contain a wide range of enzyme activities (Table 2).

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Table 1. Main NSPs of raw materials used in poultry feed

Nature	Structure		Required enzymes
	Main chain	Side chain	
Arabinoxylan	(β(1–>4) D Xyl*p*)$_n$	α(1–>3) L Ara*f*, α(1–>2) L Ara*f*	endo-1,4-β-xylanase, α-arabinofuranosidase, β-xylosidase, feruloyl est.
Mixed linked β-glucan	{(β(1–>4) D gluc*p*)$_{2-5}$ (β(1–>3) D gluc*p*)$_1$}$_n$	-	endo-1,3(4)-β-glucanase, cellobio-hydrolase, β-glucosidase.
Cellulose	(β(1–>4) D gluc*p*)$_n$	-	endo-1,4-β-glucanase, cellobio-hydrolase, β-glucosidase.
Pectins	((GalA-Rha)$_n$-GalA$_n$)	Variable	Rhamnogalacturonase, polygalacturonase, pectin esterase, pectin acetylesterase.

Table 2. Enzyme activities (in μmol/min/mg protein) in Rovabio Excel

Enzyme	Activity	Enzyme	Activity
Xylanases		Pectinases	
endo-1,4- β-xylanase	30,000	Pectinase	1,000
α-arabinofuranosidase	50	Polygalacturonase	1,000
β-xylosidase	100	Pectin Me-esterase	80
feruloyl esterase	<10	Proteases	
endo-1,5 α-arabinanase	150	Aspartic protease	15
β-glucanases		Metallo protease	15
endo-1,3(4)- β-glucanase	40,000	Others	
β-1,3-glucanase (laminarinase)	1,500	Endo-1,4 β-mannanase	200
endo-1,4- β-glucanase	20,000	β-mannosidase	20
cellobiohydrolase	200		
β-glucosidase	2,000		

Activity of NSP enzyme products is often more important on the unsubstituted xylan either water soluble or water insoluble such as on oat xylan (Table 3). However, commercial NSP enzyme products are also able to breakdown substituted xylan (arabinoxylan) and particularly water soluble arabinoxylan (Table 3). Their greater activity on water soluble than on water insoluble arabinoxylan is linked with their effect on reducing *in vivo* viscosity.

Table 3. Activity (nkat/ml) of different NSP enzyme products on xylan according to the rate of substitution and water solubility

Substrates	Product A	Product B	Product C
Xylan (oat spelt)			
Water insoluble	12000	11000	17000
Water soluble	11500	13000	17000
Wheat arabinoxylan			
Water insoluble	3500	1000	7800
Water soluble	7500	5500	8500

Mathlouthi *et al*. (2002) demonstrated the benefits of a multi-enzyme product on the reduction of the viscosity of wheat and maize (Figure 1). The viscosities of wheat and maize were reduced by the addition of the endo 1,4-β-xylanase by approximately 90 and 15%, respectively, but the viscosity was lower with the incorporation of additional enzyme activities (multi enzyme preparation). The debranched activities in the multi enzyme preparation allows breakdown of the highly substituted arabinoxylans and explains its greater efficacy.

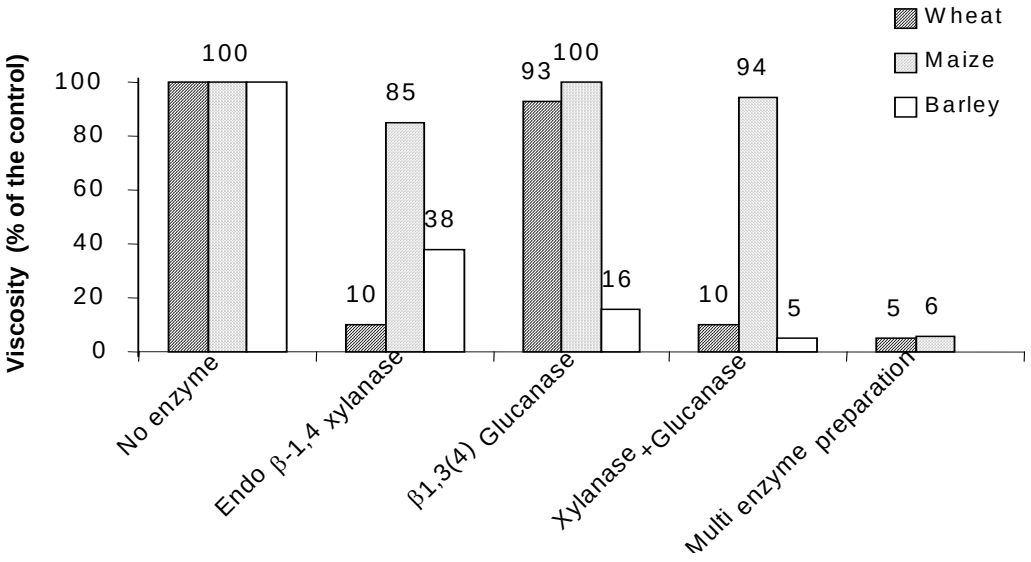

Figure 1. Effects of pure enzyme and multi-enzyme preparation on the viscosity value of cereals (Mathlouthi *et al*., 2002).

Even when considering endo-1,4-β-xylanase, this term can cover a range of proteins with different molecular size, and different affinities for substrates either soluble or insoluble. Indeed, several xylanase proteins have been identified in *Penicillium funiculosum* differing in their molecular size (15 to 45 kDa), their optimum pH and their affinity to insoluble or soluble substrates. To have an efficient action *in vivo*, a large spectrum is required to function from stomach (gizzard) to intestinal level.

Most of the commercial enzymes are produced by fungi from different species such as *Aspergillus niger*, *Trichoderma reesei*, and *Penicillium funiculosum*, which are known to have the ability to degrade plant cell walls. However, plants have also developed the ability to counteract the action of the fungal enzymes as a protective mechanism. Anti-enzyme factors recently identified include XIP-1 (Xylanase Inhibitor Protein factor 1), TAXI-I, TAXI-II (Triticum aestivum xylanase inhibitor I and II) (Rouau and Surget, 1998; Debyser *et al*., 1999). Inhibitors of β-glucanase and pectinase also exist. The proof of their role has been demonstrated *in vitro* and in the breadmaking process. Xylanases are added to assist the raising of dough. However, their action can be inhibited by these protein factors. The role of

enzyme inhibitors in animal feeding has not yet been evaluated but inhibition may explain the absence of effect on some wheat cultivars.

IV. HOW TO COMPARE PRODUCTS

When comparing the wide range of enzyme products available on the market, even when taking into account the recommended dose of incorporation, the differences remain large enough for feed formulators to make an informed choice. Indeed, when comparing commercial products on their efficacy to reduce feed conversion ratio in relation with their xylanase activity, Liang and Liu (1999) showed that there was no correlation between feed conversion and xylanase activity.

Measuring the activity with the same analytical procedure will not help, as there is no standard reference method available, because each fungus and thus each of its enzymes secreted have different pH and temperature optima (Sabatier and Fish, 1996). One possibility is to use a wide range of substrates to determine the possible range of enzyme activities. The absence of activity on a particular substrate may indicate that the product does not possess that particular enzyme activity, although it is possible that the activity cannot be expressed in the absence of enabling activities to expose the targeted site on the substrate. Finally, the best comparison of enzyme products remains the *in vivo* assay: let the animal be the judge!

V. CONCLUSION

NSP structures are complex and cannot be efficiently hydrolysed by just a few enzyme activities. Indeed, a wide range of enzymes, from debranching ones to enzymes involved in hydrolysis of oligomeric structures, is required to remove anti-nutritional factors and to improve the nutritive value of the grain. Even enzymes targeting insoluble components, which are not anti-nutritional but merely act as diluents, might be helpful, especially for so-called highly digestible corn or sorghum-soybean based diets.

It can be argued that the versatility of a multi-enzyme activity product stems from its ability to target wheat, barley, corn, sorghum, and soybean meal raw materials. In addition, its versatility is related to its ability to adapt to the different physiological conditions of the digestive tracts of poultry and pigs. Future developments of enzymes will require a better knowledge of the spectrum of enzyme secreted by fungi.

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THE EFFECT OF ENZYMES ON *CAMPYLOBACTER* AND *SALMONELLA* IN BROILERS

G. JIN[1] and M. HRUBY[2]

Summary

The action of exogenous enzymes resulted in a reduction in zoonotic bacteria, *Campylobacter jejuni* and *Salmonella enteritidis*, in the caeca of broilers fed either wheat- or corn-based diets. The feed ingredient type and the level of starting pathogenic challenge influenced~~a~~ the effect of enzymes on microbial population change. The reductions in *Campylobacter* and *Salmonella* observed in the reported studies indicated that exogenous enzyme supplementation, through its effect on intestinal environment, offers a useful addition to other management practices presently employed to improve food safety of poultry meat.

I. INTRODUCTION

Most countries with systems for reporting cases of foodborne diseases have documented significant increases over the past few decades in the incidence of diseases caused by micro-organisms in food including *Salmonella spp.*, *Campylobacter jejuni*, *Listeria monocytogenes* or *E. coli O157* among others (FOA/WHO, 2002).

Camplylobacter spp. induced enteritis continues to be a significant public health problem throughout the world. In the USA alone, it is estimated that more than 2.4 million cases have been occurring annually, of which 80% are considered to be foodborne (Stern, 2002). A study in New Zealand found that campylobacteriosis occurrence was strongly associated with consumption of chicken meat (Eberhart-Phillips *et al*., 1997). In the UK, poultry contamination levels with campylobacter currently average 50% (Lister, 2002).

Compared to the control of *Salmonella spp.*, there is limited information available on methods for decontamination of poultry carcasses or a use of other management practices during poultry growth and processing to reduce *Campylobacter* contamination. In feed, for example, Wagenaar and Jacob-Reitsma, (2002) and Mead (2002) reported that there is no existing competitive exclusion product affective against *Campylobacter*.

Bedford (2000) reported that exogenous enzymes might play a role in microbial population changes in broilers. Choct *et al.* (1999) reported that the anti-nutritive effect of soluble non-starch polysaccharides (NSP) present in wheat and barley is related to their ability to increase digesta viscosity along the gut of broilers, which in turn causes changes (types and levels) in gut microflora. Exogenous carbohydrases have been shown to reduce intestinal viscosity and improve nutrient digestibility in broilers fed different diet types. Against this background it seems that exogenous enzymes may indirectly influence the microbial activity, including zoonotic bacteria, in the birds gastrointestinal tract.

II. METHODS

Fifteen studies were conducted to evaluate the effect of exogenous enzyme supplementation either on *Campylobacter jejuni* or *Salmonella enteritidis* in wheat- and corn-based broiler diets. One-day-old broiler chicks (Ross-1) were randomly assigned to different treatments (wheat +/- enzyme or corn +/- enzyme) and kept in floor pens (12 to 36 broilers per treatment depending on a study). The chicks were challenged orally with variable suspension dilutions of *C. jejuni* or *S. enteritidis*. Diets were given *ad libitum* from day one.

Danisco Animal Nutrition, Singapore[1] and Marlborough, UK[2].

Exogenous enzymes (Avizyme® 1300 in wheat diets, Avizyme® 1500 in corn diets) were used at the standard recommended dose rates of the appropriate product and no antibiotics or coccidiostatics were used (Table 1).

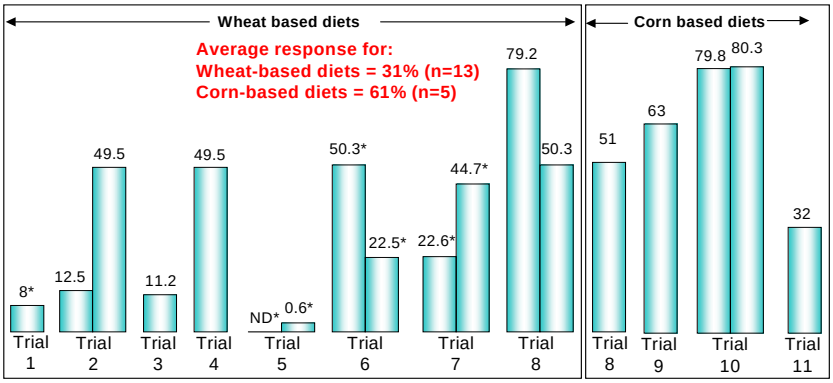
Table 1. Ingredient composition (g/kg) of experimental diets

Ingredient	Wheat diet	Corn diet
Wheat	546.3 - 547.3	-
Corn	-	541.5 - 542.5
Soybean meal 48%	348.9	376.7
Soy oil	42.6	17.5
Tallow	20.0	20.0
Salt	3.8	4.1
DL Methionine	1.7	1.5
Limestone	12.2	12.2
Dical Phosphate	13.5	15.5
Vit/Min premix	10.0	10.0
Avizyme 1300 (wheat) or 1500 (corn)	+/- 1	+/- 1

In the trials investigating *Campylobacter*, broiler chicks were challenged orally with the bacteria at four or five days of age, and population numbers were measured between 12 and 33 days of age. In the trials investigating *Salmonella* the broiler chicks were challenged orally at one day of age and measurements were recorded between 14 and 17 days of age. The contents of caeca were sampled aseptically and inoculated onto campylobacter- or salmonella-selective media. The trials were part of a joint research project between the Dep. of Clinical Vet. Science at the University of Bristol, UK and Danisco Animal Nutrition.

III. RESULTS

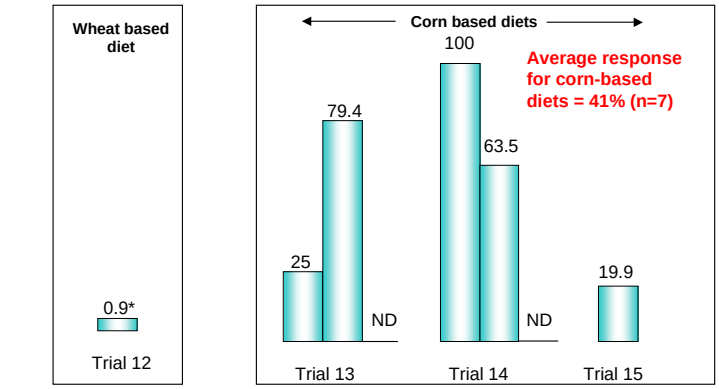
In the eight wheat-based trials, there was, on average, a two thirds reduction in the number of *Campylobacter* found in birds fed the enzyme supplemented diet, and in the four corn-based trials there was a reduction of over a third in birds fed the enzyme treated diet, compared with the control (Figure 1).



*P<0.05; ND = not detected
Data for birds inoculated with ≤ 10^6 colony forming units (cfus)
More than one column for a specific trial means that variable strengths of inoculation were evaluated

Figure 1. Proportion (%) of *Campylobacter jejuni* numbers in the caecum of broilers given diets containing enzymes compared to those given control diets without enzymes

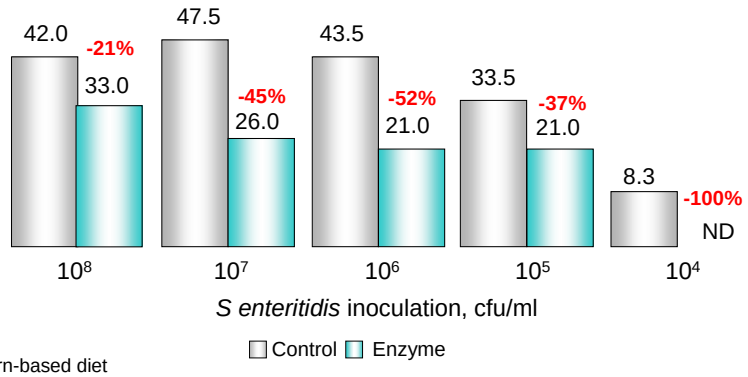
In the three corn-based trials, there was, on average, a reduction of almost 60% in the number of *Salmonella* found in birds fed the enzyme treated corn-based diet (Figure 2).



*P<0.05; ND = not detected
Data for birds inoculated with ≤ 10^6 colony forming units (cfus)
More than one column for a specific trial means variable strengths of inoculation were evaluated

Figure 2. Proportion (%) of *Salmonella enteritidis* numbers in the caecum of broilers given diets containing enzymes compared to those given control diets without enzymes

Additionally, it was found that significantly fewer birds fed the enzyme treated corn-based diets tested positive to *Salmonella*, when compared with the control (Figure 3).



Corn-based diet
Birds inoculated with 10^4 - 10^8 *S. enteritidis* at 1 day of age
*Defined as a bird with >10^5 cfu/g ND = not detected
Reference: Trials 13 & 14

Figure 3. Proportion (%) of *Salmonella enteritidis*-positive birds given diets containing enzymes compared to those given control diets without enzymes

There was also a tendency for birds inoculated with less *Salmonella* to show higher responses to the enzyme addition.

IV. DISCUSSION

A dietary enzyme supplementation initiates fermentation changes in the gastrointestinal tract of broilers (Choct *et al.*, 1999). These effects are likely related to a reduce undigested substrate reaching terminal ileum and lower gastrointestinal tract due to a viscosity reduction and/or improved nutrient digestibility (Annison and Choct, 1991, Zannella *et al.*, 1999 Burrows *et al.*, 2002) and production of short chain sugars (from fibre degradation) (Apajalahti and Bedford, 1999).

As a result of such improvements in diet digestibility, there is a significant change in the substrate quality and quantity available to the intestinal microflora in both the upper and

lower gut. Sohail *et al.* (in press) reported that a multienzyme mixture (protease, amylase, xylanase) addition into a corn/soy-based layer diet changed the microbial profile in the caeca by measuring the percentage of guanine and cytosine of a total microbial DNA profile in digesta. Cowieson *et al.* (2000) found that the abundance of bacteria with a GC% (guanine and cytosine %) between 20 and 40 was decreased while the abundance of bacteria with a GC% between 40 and 60 was increased in birds fed the control diet supplemented with enzymes.

More specifically, Francis *et al.* (1999) reported a significant reduction in colony forming units of *salmonella* and *clostridia* species measured in caeca with dietary supplementation of the multienzyme mixture product. They also reported an increase in acetic acid concentration in birds fed the enzyme supplemented diets supporting the effect of exogenous enzymes on volatile fatty acid production potentially through the exogenous enzyme effect on production of short chain sugars preferred by some beneficial microflora.

The current results suggested that exogenous enzymes may promote an environment in the intestine that is unfavourable for zoonotic microflora such as *Campylobacter* and *Salmonella.* It is possible that a number of different modes of action were responsible for the results observed. Reduced viscosity, improved nutrient digestibility, increased digesta passage rate and increased production of short chain sugars were likely the most important factors contributing to the overall microflora reduction/changes.

The reductions in *Campylobacter* and *Salmonella* observed in the reported studies indicated that exogenous enzyme supplementation offers a useful addition to other management practices presently employed to improve food safety of poultry meat.

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A CULTURE-INDEPENDENT APPROACH TO THE ANALYSIS OF THE GUT MICROFLORA OF BROILERS

W. McBURNEY[1], G.W. TANNOCK[1] and V.RAVINDRAN[2]

Summary

Denaturing gradient gel electrophoresis of DNA fragments obtained by polymerase chain reaction amplification was used to define the microflora profile in the ileal contents of healthy broilers. The birds were fed maize-soy diets without or with an in-feed antibiotic (zinc bacitracin; 100 mg/kg diet) in a 6-week trial. Contents from the terminal ileum were obtained on Days 1 and 2, and then at weekly intervals. The microflora profile progressed from a simple collection of bacterial species containing enterococci and *Escherichia coli* at day 1, to a profile in which lactobacilli were predominant from week 3. Zinc bacitracin supplementation had no effect on the composition of the ileal microflora, except at day 2 when *Clostridium perfringens* was detected in the untreated birds but not in the treated birds.

I. INTRODUCTION

In common with the gut microflora of other animal species, a large proportion of the bacteria that reside in the distal gut of broilers have not yet been cultivated in the laboratory (O'Sullivan, 1999; Zhu *et al*., 2002). This has necessitated the application of nucleic acid-based, culture-independent methods of analysis of the gut microflora. The gut microflora is recognised as an important factor in animal husbandry because of its impact on the nutrition of the host animal. Antibiotics are commonly included in broiler diets to suppress *Clostridium perfringens* which causes necrotic enteritis outbreaks in flocks and adversely affects the performance of the birds (Elwinger *et al*., 1998). It is predicted that, due to consumer pressure, the use of antibiotics in poultry feeds may be banned in the future. Therefore new approaches to prevent necrotic enteritis and optimise broiler production will be required.

The aim of the present study was to define the gut microflora of healthy broilers raised under New Zealand farming conditions. With this information as a benchmark, the composition of the gut microflora in birds that have been fed different diets or have been raised under varying management conditions will be compared. The variations in the gut microflora would probably be entirely predictable if their causes were better understood. By comparing the changes in gut microflora profiles to the performance and general health of the broilers, it should be possible to define an optimal gut microflora that would provide maximum protection from pathogenic organisms and support efficient broiler growth. In this paper, initial screening of the ileal microflora of broilers with respect to age and in-feed antibiotic (zinc bacitracin; Albac^{TM}; 100 mg/kg diet) administration is described.

II. MATERIALS AND METHODS

Two hundred and forty day-old male broiler chicks (Ross) were obtained and divided into two groups. Each group was placed in separate, but identical, environmentally-controlled rooms (120 chicks per room) with 24-hour fluorescent lighting. The rooms were fumigated and sanitised prior to the introduction of chicks. The birds were raised on floor at a stocking density of 20 birds/m^2 and managed according to normal commercial practices. Two diets

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based on maize and soyabean meal, without or with an in-feed antibiotic (zinc bacitracin; Albac^{TM}; 100 ppm) were formulated and cold-pelleted (65 – 70 ^{0}C). The two diets were fed to bird groups in separate rooms to avoid any cross contamination. Ten birds from each treatment group were killed by cervical dislocation on Days 1 and 2, and then at weekly intervals during the 6-week trial, and digesta samples were obtained from the terminal ileum. Samples were collected in sterile tubes, immediately frozen and stored at –20 ^{0}C until analysed for microflora profile.

Bacterial DNA was extracted from each ileal sample and the V2-V3 regions of the 16S ribosomal RNA gene were amplified by Polymerase Chain Reaction (PCR) using bacterial primers HDA1-GC and HDA2 (Tannock *et al*., 2000; Walter *et al*., 2000). The 16S rDNA fragments in the PCR products were separated by denaturing gradient gel electrophoresis (DGGE) to generate a profile of the bacterial community in each ileal sample (Tannock *et al*., 2000). DNA fragments of interest were cut from the DGGE gel and sequenced to permit bacterial identification (Requena *et al*., 2002).

III. RESULTS AND DISCUSSION

The impact of age and bacitracin administration on the composition of the ileal microflora is shown in Figure 1 and Table 1. The microflora profile progressed from a simple collection of bacterial species containing enterococci and *Escherichia coli* at day 1, to a profile in which lactobacilli were predominant from week 3. Particularly noticeable in birds aged one week of age were DNA fragments representing gram-positive cocci (enterococci/streptococci), the appearance of *Lactobacillus aviarius* in the ileum from week four, and *Lactobacillus salivarius* from week 3. The segmented filamentous ileal organism that attaches to the ileal mucosa was particularly apparent in birds aged one week. The impact of zinc bacitracin on the composition of the ileal microflora was negligible except at day 2 when *C. perfringens* was detected in the untreated birds but not in the treated birds.

The results demonstrate that PCR/DGGE is a useful analytical procedure that enables a large number of gut samples to be investigated in a comparative manner. All of the members of the microflora are included in the analysis because it is nucleic acid-based and does not require the culture of the bacteria. The analytical laboratory does not have to be in the same locality as the poultry research facility because fresh samples are not required. The samples can simply be frozen soon after collection and dispatched, still frozen, to the laboratory. This means that national and international collaborative investigations can be carried out.

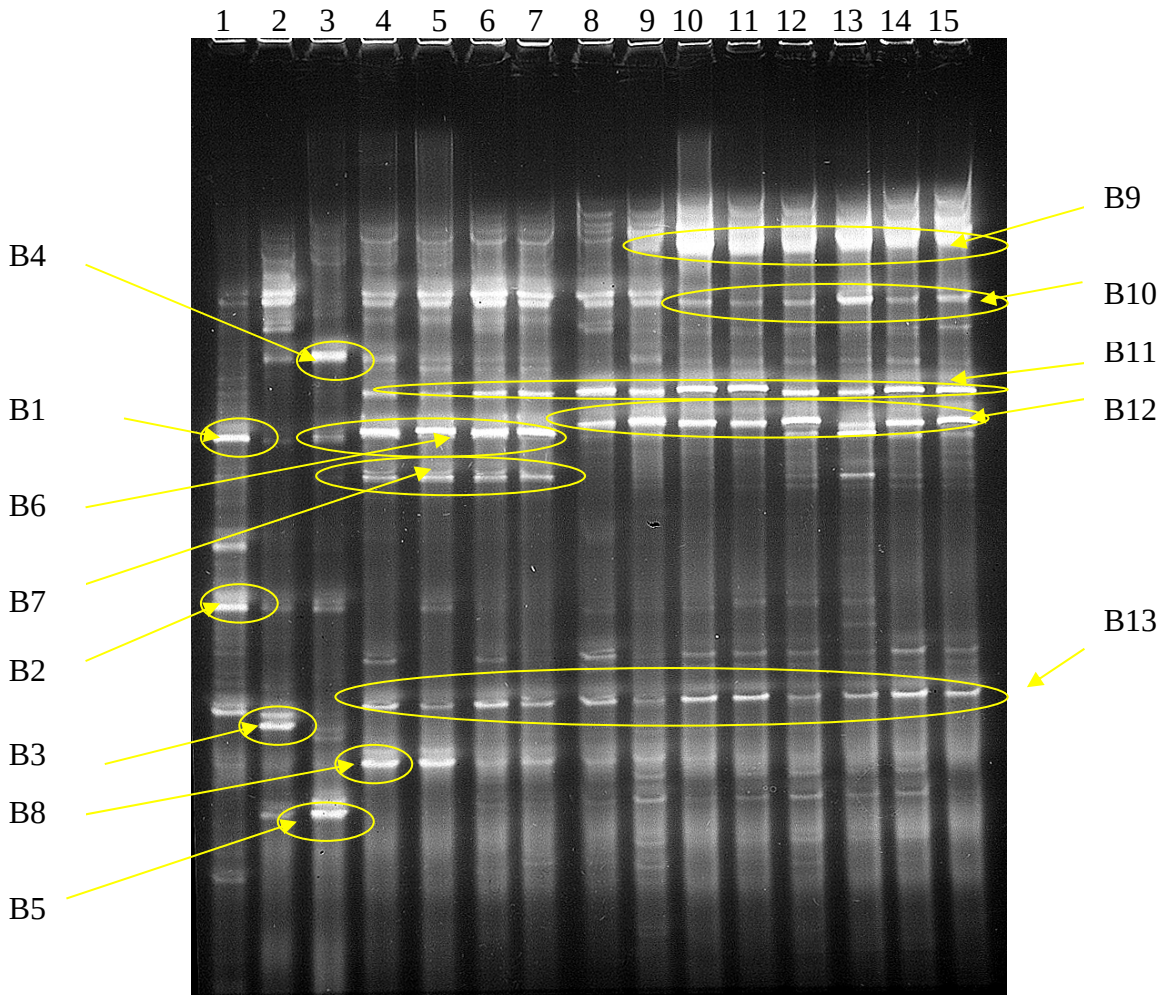

Figure 1. DGGE profiles obtained from pooled DNA samples from ileal contents of broiler chickens. Lane 1, Day 1; lane 2, Day 2 (no antibiotic treatment); lane 3, Day 2 (antibiotic treatment); lane 4, Week 1 (no antibiotic treatment); lane 5, Week 1 (antibiotic treatment); lane 6, Week 2 (no antibiotic treatment); lane 7, Week 2 (antibiotic treatment); lane 8, Week 3 (no antibiotic treatment); lane 9, Week 3 (antibiotic treatment); lane 10, Week 4 (no antibiotic treatment); lane 11, Week 4 (antibiotic treatment); lane 12, Week 5 (no antibiotic treatment); lane 13, Week 5 (antibiotic treatment); lane 14, Week 6 (no antibiotic treatment); lane 15, Week 6 (antibiotic treatment). See Table 1 for identification of B1 – B13.

Table 1. Identification of DNA fragments excised from DGGE profile of ileal samples of broiler chickens.

Bacterial species (primary identification)	Bacterial species (alternate identification)	Code (see Figure 1)
Enterococcus faecalis		B1
Escherichia coli		B2
Clostridium perfringens	Uncultured bacterium	B3
Enterooccus species	Uncultured bacterium	B4
Eubacterium moniliforme	*Clostridium* species	B5
Enterococcus species	Uncultured bacterium	B6
Streptococcus species		B7
Segmented filamentous organism		B8
Lactobacillus aviarius		B9
Lactobacillus species		B10
Lactobacillus species		B11
Lactobacillus salivarius		B12
Lactobacillus species		B13

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THE RELATIONSHIP BETWEEN PHYSICO-CHEMICAL PROPERTIES OF FIBRE AND THEIR EFFECTS ON THE GUT WEIGHT OF CHICKENS

S. HARTINI, M. CHOCT, G. HINCH and J.V. NOLAN

Summary

Two experiments were conducted to observe the effects of different types of soluble and insoluble non-starch polysaccharides (NSP) on digesta viscosity and gastrointestinal weight of laying hens. In experiment 1, four diets were offered, viz, wheat-based (wheat), millrun-based (millrun), barley-based (barley), and barley diet with added enzyme (barley + enzyme). Gut viscosity was high ($P<0.01$) on birds fed wheat and barley diets. Birds fed the barley diet had a higher ($P<0.05$) caecal weight. Addition of enzyme to the barley diet reduced ($P<0.01$) jejunal viscosity and caecal weight. There was no significant effect of diet on feed intake (FI) and egg production. In experiment 2, six different diets were used. The first four diets were: wheat-based (wheat); oat-based (oats), millrun-based (millrun), and rice hull-based (ricehull). Diets 5 and 6 were the wheat diet plus 0.2% manno-oligosaccharides (MOS) and 0.2% fructo-oligosaccharides (FOS), respectively. There was no effect of diet on gut viscosity and egg production. The oat diet had a higher ($P<0.05$) FI and gizzard weight. Soluble NSP and physical structure of oats seemed to be responsible for the increase in FI and gizzard weight.

I. INTRODUCTION

Dietary fibre consists of up to 90% NSP that can be either soluble or insoluble, plus lignin (Englyst and Hudson, 1987). The negative effects of a high level of dietary NSP for monogastrics often increase the viscosity of intestinal digesta, which could stimulate the proliferation of microorganisms (Choct *et al*., 1996). A high microbial count in the small intestine could not only disturb the ecology of the gut (Eggum *et al*., 1982) but also change its morphology (Viveros *et al*., 1994), and impair nutrient absorption. An enlargement of digestive organs and the pancreas was observed when birds ingested viscous polysaccharides (Ikegami *et al*., 1990). These suggestions are supported by the finding that the *in situ* degradation of ß-glucans, the main types of NSP in barley, reduced the gastrointestinal weight in broiler chickens (Brenes *et al*., 1993). The present experiments were conducted to investigate the effect of NSP in different cereal grain products on intestinal viscosity and gastrointestinal weight.

II. METHODS

Experiments were conducted in a conventional layer shed at the Laureldale Research Station, University of New England, NSW, Australia. Individual battery cages (25cm x 50cm) equipped with a feed trough placed outside the cage and an automatic drinker located at the top back of cage were used in these experiments. Birds were held under a natural light regime supplemented by artificial light (>80 lux) to a total of 16h/day.

Experiment 1.

ISA Brown birds (n=32) at 42 weeks of age were housed in single battery cages and allocated at random to four diets: wheat-based (wheat), millrun-based (millrun), barley-based

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(barley), and the barley diet plus enzyme (barley + enzyme). A commercial β-glucanase (Novozymes Pty Ltd, Australia) was added (300g/t). There were 8 replicates per diet. All diets were formulated to be isonitrogenous and approximately isoenergetic according to commercial specifications and were produced at a commercial mill (Ridley AgriProducts, Tamworth, NSW). Feed and water were given *ad libitum* for 28 days. Feed intake and egg production were recorded weekly. At the end of 4 weeks, all birds were weighed and then killed by cervical dislocation. The digestive tract, including gizzard, was removed immediately and then cut into segments: gizzard, crop, jejunum, ileum, and caecum. The gut sections were weighed before and after removal of digesta. The empty gut weights were expressed as percents of final body weight (%BW). The digesta from the jejunum and ileum were individually stored for later estimation of viscosity. The contents of the soluble and insoluble NSP were analysed for the diets according to the uppsala method by Theander and Westerlund (1993).

Experiment 2.

ISA Brown laying hens (n=24, 20 weeks of age) were housed in individual cages and randomly allocated to six dietary treatments with four replicates per diet. The first four diets were: wheat-based (wheat); oat-based (oats), millrun-based, and rice hull-based (Ricehull). Diets 5 and 6 were the wheat diet plus 0.2% manno-oligosaccharides (MOS) and 0.2% fructo-oligosaccharides (FOS), respectively. The experiment lasted 56 days. The birds had free access to feed and water. Feed intake and egg production were recorded weekly. At the end of the experiment, birds were weighed and then killed by cervical dislocation. Gut weight and digesta viscosity were determined as described for Experiment 1. Variables measured include digesta viscosity, feed intake (FI), egg production, and gut weight (gizzard, duodenum, jejunum, ileum and caeca) per 100g body weight (%BW). The levels of soluble and insoluble NSP were measured for the diets.

Data from Experiment 1 and 2 were analysed using ANOVA.

III. RESULTS

Experiment 1.

The barley diet had the highest soluble NSP content ($P<0.01$) whereas its insoluble NSP content was not different to that of the millrun diet but both diets were higher than the wheat diet ($P<0.01$). Addition of the enzyme to the barley diet greatly reduced ($P<0.01$) its soluble and insoluble NSP contents. Birds fed the wheat diet had higher digesta viscosity ($P<0.01$) both in the jejunum and ileum and those fed the barley diet had markedly higher digesta viscosity in the jejunum ($P<0.01$) than those on the other diets. Only the caecal weight relative to body weight was affected by diet, with birds given the barley diet having the heaviest caeca ($P<0.05$). Enzyme addition to the barley diet reduced ($P<0.01$) caecal weight and jejunal digesta viscosity. FI and egg production did not differ ($P>0.05$) between diets.

Experiment 2.

The results are summarised in Table 2. The soluble NSP content was higher ($P<0.01$) in the oat diet than in the other diets which did not differ significantly ($P>0.05$). The insoluble NSP content was highest in the millrun and rice hull diets, and lowest in the FOS diet ($P<0.01$). Diet did not affect viscosity and egg production but did affect FI, with birds fed the oat and rice hull diets having higher intakes than those on other diets ($P<0.05$). In addition, the relative gizzard weight ($P<0.01$) was higher in birds on the oat diet and jejunal weight also tended ($P<0.08$) to be higher on the oat diet.

Table 1. Soluble and insoluble NSP contents of diets, jejunal and ileal viscosity, and relative caecal weight (%BW) of laying hens fed different dietary fibres (Experiment1)

Experiment Diet	Soluble NSP (g/kg)	Insoluble NSP (g/kg)	Jejunal viscosity (mPa.s)	Ileal viscosity (mPa.s)	Caecal weight (%BW)
Wheat	4.95[a]	67.22 [a]	3.2 [b]	9.5 [b]	0.53 [a]
Millrun	6.47 [b]	115.63 [c]	2.3 [a]	5.6 [a]	0.50 [a]
Barley	20.26 [d]	112.52 [c]	3.0 [b]	4.8 [a]	0.61 [b]
Barley+enzyme	8.59 [c]	82.41 [b]	2.1 [a]	3.5 [a]	0.52 [a]
P-value	<0.01	<0.01	<0.01	<0.01	<0.05

[a-d]Mean values within a column with no common superscripts differ significantly (P<0.05)

Table 2. Soluble and insoluble NSP contents of the diets, intestinal digesta viscosity, feed intake (FI), relative gizzard and jejunum weights (%BW) of laying hens fed different dietary fibres (Experiment 2)

Experiment Diet	Soluble NSP (g/kg)	Insoluble NSP (g/kg)	Viscosity (mPa.s)	FI (g/bird/day)	Gizzard weight (%BW)	Jejunal weight (%BW)
Wheat	6.04[a]	68.94[bc]	3.0	100[a]	1.40[a]	1.11
Oats	10.44[b]	67.54[ab]	2.5	109[c]	2.38[b]	1.26
Millrun	5.88[a]	93.38[d]	2.9	101[ab]	1.48[a]	1.21
Rice Hull	5.06[a]	92.53[d]	2.9	108[bc]	1.39[a]	1.16
MOS	5.24[a]	71.47[c]	2.7	103[abc]	1.15[a]	0.94
FOS	5.62[a]	64.85[a]	2.3	99[a]	1.32[a]	0.96
P-value	<0.01	<0.01	NS	<0.05	<0.01	<0.08

[a-d]Mean values within a column with no common superscripts differ significantly (P<0.05), NS not significant

IV. DISCUSSION

The anti-nutritive activities of cereal NSP in chickens are well documented. The β-glucan, the main soluble NSP in barley, and pentosan, the main soluble NSP in wheat, are believed to act in a similar manner, through increased intestinal viscosity. This has been shown for partially purified NSP (Ikegami *et al*., 1990) as well as for compounds derived from normal feed ingredients (Annison and Choct, 1991). In general, the data support previous work with barley and wheat where birds fed these grains had elevated digesta viscosity. An exception was that a higher soluble NSP content in the millrun diet did not increase digesta viscosity. The solubility of NSP depends on their chemical structure and association with the rest of the cell wall components (Choct, 1997). In addition to having a higher soluble NSP, the millrun diet also had a high insoluble NSP content. The bulking properties of insoluble NSP would increase the rate of feed passage in the gastrointestinal tract of birds (Roberfroid, 1993). Thus, it was likely that the markedly different generic effects of soluble and insoluble NSP in the gut are further modified by their interaction with other cell wall components. Choct (1997) suggested that perhaps at an appropriate ratio between soluble and insoluble fractions, the anti-nutritive effect of soluble NSP may be

minimised. That the higher soluble NSP content in the oat diet did not increase intestinal digesta viscosity, again, indicated the possibility of an interaction between soluble NSP and other cell wall components.

Caecal weight was highest in birds fed the barley diet. Microorganisms tend to be present in highest numbers in the caeca and large intestine (Tasaki and Kibe, 1959). The soluble NSP are easily fermented by microorganisms providing energy for microorganism proliferation (Choct *et al*., 1996). This may result in changes to gut morphology (Viveros *et al*., 1994) and eventually increases in gut weight. A larger gizzard in birds fed the oat diet probably related to the physical structure of oats. Feed intake was higher on the oat diet. Hetland and Svihus (2001) proposed that physical structure of oat hulls initiated the increase of gut capacity and rate of feed passage consequently allowing increased feed consumption.

Addition of β-glucanase in the barley diet reduced its soluble and insoluble NSP content presumably by a partial depolymerisation of the β-glucans, and this resulted in decreased digesta viscosity and lower caecal weight of the birds. This was probably due to a reduction in fermentative substrates, and a reduction in microbial influence on the digestive tract of the birds (Choct *et al*., 1996; Brenes *et al*., 1993).

It can be concluded that soluble NSP had a more profound effect on the caecal weight of birds than insoluble NSP, but the actions of both soluble and insoluble NSP effects may be modified by their interaction with other cell wall components.

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EFFECTS OF DIETARY CEREAL CHANGE ON HINDGUT pH, ORGANIC ACIDS AND A "COLITIS-LIKE" RESPONSE IN LAYERS AT PEAK PRODUCTION

R.D. TAYLOR

Summary

Layer strain birds were given layer diets based on either wheat, sorghum, barley or rice as the single cereal, for 48 h following continuous feeding on commercial diets. Fresh excreta pH was measured at 12 h intervals to monitor changes after substitution of the commercial feed with the test diets. pH decreased over 48 h irrespective of cereal type.

Caecal digesta volatile fatty acid (VFA) concentrations did not differ with the type of cereal. The time of digesta collection may affect total VFA and lactate concentrations measured in layers due to the diurnal variation in feed intake. The rice-based diet produced higher ileal lactic acid concentrations than the wheat, sorghum or barley-based diets. Excreta changes were scored prior to and during the test period. Dietary change caused diarrhoea and production of mucus and fresh blood. The presence of faecal occult blood was confirmed by a diagnostic test. The results suggest a reduction in digesta pH and changes in organic acid production in the hindgut of layers may precipitate symptoms associated with gut inflammation.

I. INTRODUCTION

The variation in pH throughout the digestive tract of poultry was reviewed by (Hill, 1971). Jayne-Williams and Fuller (1971) outlined the effects of pH on gut function and micro-organism activity. These reviews suggest a broad range in pH in any gut section, but that this is relatively fixed in the individual bird. The almost continuous eating and production of digesta in broiler chickens may generate a narrow range of pH in any organ or section of the gut. The generally longer dark periods and interruptions to feed consumption (e.g. oviposition) in laying hens may lead to digesta that is moving through the gut in varying amounts throughout the day. Similarly, total daily feed intake may alter rapidly in laying birds at physiologically important points such as point-of-lay and peak egg/egg mass production. In turn, this may be exacerbated in broiler-breeders on heavily restricted feeding programs, especially as feed allowances increase dramatically between pre-lay to peak egg production.

In ruminant and monogastric animals, pH can be altered markedly following changes in the composition of the diet (Clayton, 2000). Typically this involves a decrease in pH. This has been associated with immediate, negative effects on animal production and health (Clayton, 2000). A decrease in pH by as little as 0.5 units over a few days has also been associated with a severe inflammatory response in the hindgut tissue of mice (Clayton and Buffinton, 2000). The role of pH reduction in the normal gut function of layer type birds, through alterations to the activity of α-amylase, irrespective of source of the enzyme, was highlighted by Taylor and Jones (2000). A sudden change in dietary cereal, with attendant alterations in carbohydrate constituents, could effect a short-term reduction in digesta pH in the hindgut. The effects of such dietary change is relevant to the poultry industry, particularly given recent commercial developments of pH stable enzymes for use in poultry

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diets and restrictions of available cereal types and/or gradings resulting from adverse weather.

The caeca are recognised as the major site of digesta fermentation in the chicken (Jayne-Williams and Fuller, 1971). Much work has focussed on fermentation in the broiler chicken (Corrier *et al*., 1990; Williams *et al*., 1997). The patterns of feed intake in broilers and layers differ markedly. Although commercial diets may be based on blends of different cereals, rapid substitution of cereal types can occur, usually driven by financial considerations. Such changes in feed type may affect fermentation patterns as different carbohydrate constituents alter enzyme, micro-organism and physical conditions in the gut.

Some of the major fermentation metabolites, VFA and lactate, can accumulate in considerable concentrations in the lower gut after dietary change. The role of fibre in gut health has been long investigated and conflicting evidence has been presented in relation to effects of changing fibre types or proportions in the diet. Changes to patterns of fermentation of dietary carbohydrate may have putative benefits such as increased butyrate production (Cummings, 1983) or negative effects (Jacobs and Lupton, 1984). One particular concern is the increased production of acetate with fibre fermentation (Cummings, 1983). Mild solutions of acetate have been used to study inflammatory response in the lower gut of rats (Empey *et al*., 1993).

This paper describes the responses of layer birds given a range of single-cereal based diets, in hindgut function as measured through changes in digesta and excreta pH and organic acid concentrations and symptoms associated with gut inflammation.

II. METHODS

Single-cereal, wheat, rice, sorghum or barley- based diets were formulated (Table 1) to meet the breeders' specifications for commercial layers (AZTEC 007/101). Prior to the trials, the birds were grown on commercial starter, grower and layer crumbles (Weston Animal Nutrition), with all management as per commercial recommendations.

Table 1. Ingredient and nutrient composition (g/kg) of commercial and single-cereal experimental layer diets (g/kg).

Raw	Layer	Wheat	Rice	Sorghum	Barley
Rice (80g/kg CP)			600.0		
Wheat (120 g/kg CP)		673.3			
Sorghum (90g/kg CP)	577.4			600.0	
Barley (100g/kg CP)	50.0				600.0
Calculated specifications					
DM	878.0	898.3	888.2	888.8	902.6
Protein	160.0	182.7	180.3	180.4	179.9
AME chick MJ/kg	11.30	11.50	11.52	11.53	11.52

During laying, birds were caged individually and at peak production (24 weeks) were fed for 48 h on the test diets which were single-cereal diets cold-pelleted from hammer-milled grain. At 12 h intervals, fresh excreta were collected for immediate pH measurement until slaughter at 48 h when digesta was removed from the hindgut sections and the pH measured. Digesta pH data were analysed using the GLM procedure, with repeated measures of excreta pH analysed by the MIXED Model procedure, of SAS (SAS Institute, 1996). After 48 h on the diets, the birds were euthanased and digesta collected to measure VFA and

lactate levels. VFA's were measured by capillary gas chromatography. The concentrations of lactic acid were measured (r-Biopharm GmbH) and data were analysed using the GLM procedure of SAS (SAS Institute, 1996). Prior to and during the experimental period, at 12 h intervals, excreta were scored for the presence or absence of blood. To confirm the presence of blood, fresh excreta were analysed using the Hemo FEC® test (Boehringer Mannheim GmbH). The blood scores were tested by analysis of deviance using the GLM (family="binomial") model of R (Ihaka and Gentleman, 1996).

III. RESULTS

At 24 weeks, mean excreta pH (i.e. irrespective of the cereal) decreased ($P<0.05$) from 12 to 24 h then again from 36 to 48 h (Table 2). Mean excreta pH was higher ($P<0.05$) on the wheat than rice and barley diets, which were similar ($P>0.05$); pH on the sorghum diet was intermediate to that on the wheat and barley diets, but higher ($P<0.05$) than on the rice diet (6.94 a, 6.56 c, 6.86 ab and 6.67 bc, for wheat, rice, sorghum and barley, respectively). Ileal digesta pH (Table 2) was lower ($P<0.05$) on the rice diet than on the other three diets which were similar; caecal pH was unaltered ($P>0.05$) by grain type but colon pH was higher ($P<0.05$) on the wheat than rice and sorghum diets, with an intermediate pH on the barley diet.

Table 2. Excreta and digesta pH (LS Mean ± SE) of birds fed ground, cold-pelleted, wheat, rice, sorghum or barley-based grower diets for 48 h at 24 weeks of age.

Feed	Excreta pH over time (h)					Gut section digesta pH		
	0	12	24	36	48	Ileum	Caeca	Colon
Mean pH	7.29	7.49 a	6.87 b	6.75 b	5.93 c			
SE			0.092					
Wheat	7.28	7.35	7.26	6.90	6.26	7.99 a	6.21	7.60 a
Rice	7.34	7.40	6.50	6.54	5.79	7.03 b	6.60	6.62 b
Sorghum	7.12	7.85	6.85	6.93	5.79	7.67 a	6.38	6.97 b
Barley	7.44	7.34	6.86	6.62	5.87	7.85 a	6.52	7.05 ab
SE			0.187			0.225	0.141	0.226

Diet change at 24 weeks of age did not alter ($P>0.05$) VFA concentrations (Table 3) across the four cereals. Lactic acid concentrations in the plasma were not influenced ($P>0.05$) by grain type, however the D-lactate concentration increased substantially to 48 h and the time x grain type interaction approached significance ($P=0.082$). Digesta lactate concentrations (Table 3) were quite substantial throughout the gut and the rice diet produced greater ($P<0.05$) concentrations of both isomers in the ileum. Caecal lactate was not influenced ($P>0.05$) by grain.

Table 3. Caecal digesta VFA and ileal and caecal lactic acid concentrations (mMol) of layer birds fed ground, cold-pelleted, wheat, rice, sorghum or barley-based layer diets for 48 h at 24 weeks of age.

Feed	Caecal VFA (mMol)				Lactic acid (mMol)			
	C2	C3	C4:0	Tot C2-C7	Ileal L-	Ileal D-	Caec L-	Caec D-
Wheat	21.7	1.7	3.3	27.2	9.0 b	6.5 b	2.1	2.0
Rice	23.8	3.0	3.6	31.0	17.8 a	18.6 a	2.5	2.4
Sorghum	32.6	3.2	5.8	42.3	9.6 b	4.7 b	0.9	0.9
Barley	24.5	2.2	5.7	33.1	7.5 b	3.6 b	1.6	1.6
SE	3.81	0.45	1.18	5.00	2.57	3.39	0.90	0.88

The single-cereal diets, fed at peak production, produced a mean increase ($P<0.05$) in blood in excreta (Table 4) over 48 h, but with no differences ($P>0.05$) across the four cereals.

Table 4. Probability estimates (± SE) for the presence of blood in excreta of layers at 24 weeks of age offered wheat, rice, sorghum or barley-based diets for 48 h.

Feed	- 12 h	12 h	24 h	36 h	48 h
Wheat	0.09 ± 0.139	0.14 ± 0.101	0.20 ± 0.078	0.29 ± 0.084	0.39 ± 0.113
Rice	0.12 ± 0.127	0.23 ± 0.089	0.40 ± 0.070	0.60 ± 0.080	0.77 ± 0.106
Sorghum	0.07 ± 0.158	0.11 ± 0.115	0.17 ± 0.088	0.25 ± 0.092	0.35 ± 0.123
Barley	0.08 ± 0.153	0.11 ± 0.112	0.16 ± 0.089	0.22 ± 0.097	0.29 ± 0.129

IV. DISCUSSION

An abrupt change to the cereal base of the diet to layers resulted in a decline in excreta pH within 24 h. Comparisons of four different cereals resulted in similar pH reductions over the 48 h of exposure to the test diets. In other words, a main effect of cereal, irrespective of type, was observed. The decrease in pH was in the order of 1.0 unit. This degree of pH reduction has been associated with short-term negative effects on the health of both ruminant and monogastric animals (Clayton, 2000) and inflammation of the hindgut of mice (Clayton and Buffinton, 2000).

Digesta pH was found to vary inconsistently across the cereals fed to the birds in the layer phase. A significant decrease in the pH of ileal and colonic digesta was found with the rice diet compared with the other cereals but all the pH values reported were in the normal range (Hill, 1971; Jayne-Williams and Fuller, 1971). Change in fresh excreta pH may provide a useful tool for simple, rapid and non-destructive monitoring of complex changes occurring in the hindgut of the bird associated with changes in digesta flow and fermentation in response to sudden dietary changes.

Consideration of relative feed intake, intake patterns and subsequent digesta flow in the different bird types and with age should be made with regard to VFA concentrations. The current data were from digesta of birds killed approximately 60 min after lights-on and subsequent feeding. The lactate results provided some insight into how sudden alteration of the diet constituents may influence fermentation. Starch and other carbohydrate fractions vary in volume, type and form and are altered by different feed processing methods. Adaptation of the gut may take a considerable time as suggested by Jones and Taylor (2001). Lactate accumulation has negative effects on gut health (Clayton, 2000) and some evidence of short-term lactate accumulation was generated over the course of this experiment.

A sudden change in the diet provided to these layers produced symptoms consistent with gastro-intestinal inflammation resulting in blood loss. The blood loss was almost always associated with increased secretion of mucus and diarrhoea. The results are similar to the changes noted when colitis was induced in the hindgut of rats (Jacobs, 1986). Clayton and Buffinton (2000) induced colitis in mice and found both diarrhoea and rectal bleeding which they attributed to a lowering of faecal pH.

The short-term responses found in this study may be of little concern as the birds in these trials did not display short-term reductions in feed intake, egg production or in egg shell quality. Nevertheless, long–term damage to the gut tissue has been shown in rats after short-term, induced colitis (Sharon and Stenson, 1985). Therefore the possibility of such damage should not be ignored in layers. The differences in grain components across the four cereals were not measured in detail, so the results cannot be attributed to any particular fraction. However, the response of the gut to the rice-based diet was notable. Although of little immediate importance to poultry production, rice has, in recent times, been used in considerable quantities in some areas. Rice, with little structural carbohydrate content, may allow for rapid alteration to gut fermentation patterns, through changes to the site(s) of starch metabolism. In turn, this may lead to excessive fermentation, resulting in a significant reduction in pH (Cummings *et al.*, 1987). Clayton and Buffinton (2000) concluded that a transient mild decrease in hindgut pH in mice could induce tissue damage as evidenced by colitis. The present evidence of a colitis-like response to dietary change in layers argues for further investigation.

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COMPARISON OF WEIGHT LOSS OF BROILERS TRANSPORTED ON CONVENTIONAL OR CONTROLLED ENVIRONMENT TRAILERS

T. M. BYWATER[1, 2], J. G. DINGLE[1] and S. McGOLDRICK[2]

Summary

The loss of liveweight during transportation of meat chickens from the farm to the factory was compared using two different trailer designs (conventional (open sided) versus tunnel ventilated (close sided)) and over six transportation distances (ranging from 20km to 109km). The loss of liveweight of the birds was greater in the conventional trailer than in the tunnel ventilated trailer. The birds from the more distant farms had a greater liveweight loss than those from the closer farms. The prediction of liveweight loss of birds transported on the conventional trailer was Weight loss (g) = 5.22*distance (km) – 5.23* travel time (min) + 96.42. The linear relationship between weight loss and vehicle speed on the conventional trailer was Weight loss (g)= 3.36*speed (km/hour) – 95.87. Weight loss in the conventional trailer at 48km/h was equal to the weight loss in the tunnel ventilated trailer. It is recommended that the tunnel ventilated trailer be used where the average speed of the trailer would be greater than 48km/h.

I. INTRODUCTION

Once broiler chickens have attained the desired mean weight and spread of weights, they are caught, placed into crates and transported by truck from the growing site to the processing plant. The type of trailer used to transport the birds from the farm to the processing plant differs depending on the individual poultry organisation. Depending on the trailer design, the birds may be exposed to extreme ambient environmental conditions including high and low temperatures and high wind speeds during transportation from the farm to the factory (Kettlewell et al., 1993). The distance of the journey from the farm to the processing plant can range from 20km to 230km and the duration can range from 20 minutes to 4 hours (Freeman, 1984).

The handling and transportation of meat chickens from the point of collection on farm to the point of receival at the processing plant results in the loss of bird liveweight (Verkaamp, 1986). Verkaamp (1986) estimated broiler weight loss at a rate of 0.2-0.5% of bodyweight per hour for broilers in a shed without feed and water.

The loss of liveweight of broilers from the point of collection on farm to receival at the processing plant may be influenced by a number of factors. Three of these factors include the design of the trailer, the distance that the birds are transported from the farm to the factory and the duration of the journey. It is in the interest of the grower and the meat chicken company that the volume of liveweight loss under Australian environmental conditions is quantified and the development of improved management procedures be implemented to minimise liveweight loss. Minimising the loss of liveweight will improve bird wellbeing, avoid negative public perception, improve some environmental aspects of the chicken meat industry and have a financial benefit.

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II. METHODS

The individual liveweights of 480 Ross broiler birds were recorded using a Weltech digital scale at the point of collection on farm and, following transportation, on receival at the processing plant. Six Bartter Enterprises Pty. Ltd. broiler contract farms in SE Queensland were selected at random. Eighty birds from one shed on each broiler farm were selected at random for the measurements. The distance of each farm in relation to the Bartter Enterprises Pty. Ltd. processing plant were: farm 1 '109km', farm 2 '105km', farm 3 '90km', farm 4 '45 km', farm 5 '35 km' and farm 6 '20 km'. The average journey times from each farm over these distances were: farm 1 '108 min', farm 2 '97 min', farm 3 '84 min', farm 4 '40 min', farm 5 '39 min' and farm 6 '30 min'. A Tamdev mechanical harvestor was used to collect and crate the birds within the sheds. Timing of the collection of birds was dependent on the specific weights and the spread of weights within each shed; however, all birds used in this trial were approximately the same age (49 ± 1 days). The birds were collected on farm at 23:00 and the average ambient environmental temperature was 16°C. Feed was withdrawn from the birds three hours prior to pick-up and water was withdrawn immediately prior to pick-up. Once the birds were collected and measurements conducted on farm, the birds were placed into four modules positioned at mid height along the length of each of the two trailers, a conventional trailer and a tunnel ventilated trailer. The conventional trailer was an open sided trailer and the tunnel ventilated trailer was a closed trailer, each holding 32 modules. The modules were held in place by steel bars that were pulled down over the modules. The tunnel ventilated trailer was covered by curtains held in place by buckles. To maintain ventilation for the birds, there were four tunnel ventilation fans located at the rear of the trailer that extracted air from the trailer. The air entered the trailer through an inlet area located at the front of the trailer. Each trailer was loaded simultaneously at the farm, left the farm at the same time and both trailers followed the same route to the processing plant. There were two trailers (a conventional and a tunnel ventilated) used at each farm; six farms or distances and four replicate modules of birds on each trailer. Ten birds were measured in each module, giving 40 measurements for each of the 12 treatments.

III. RESULTS

The mean loss in liveweight of the birds from the point of collection on farm to the point of receival at the processing plant was 83.2 g per bird equating to a mean liveweight loss of 3.1% of the birds' initial mean liveweight. The loss of liveweight of the birds from the point of collection on farm to the point of receival at the processing plant following transportation was significantly different ($P<0.05$) for: (1) the trailer type, (2) the farm, and (3) the interaction between the trailer type and the farms.

(1). The birds transported on the conventional trailer lost a mean liveweight of 101g per bird or 3.8% of the birds' initial mean liveweight. This was significantly ($P<0.05$) more than the mean loss of 66 g per bird or 2.5% of the initial mean liveweight for the birds transported on the tunnel ventilated trailer.

(2). The birds from some of the more distant farms had significantly ($P<0.05$) greater liveweight loss during transportation than the birds from some of the farms closer to the processing plant (Table 1).

(3). There was a significant ($P<0.05$) interaction between farm of origin and the type of transportation vehicle used, in relation to the loss of liveweight of the birds from the point

Table 1. The combined effect of journey duration (min), distance (km) and speed of transport (km/h) of chickens on mean liveweight loss from the point of collection on farm to the point of receival at the processing plant

Farm	Journey Duration	Distance	Speed	Mean weight loss from collection to receival at plant (g/bird)	Mean weight loss from collection to receival at plant (%)
1	108	109	60.5	75.0 (b)	2.5 (d)
2	97	105	64.9	108.0 (a)	3.9 (ab)
3	84	90	64.3	85.0 (b)	3.3 (bc)
4	40	45	67.5	108.0 (a)	4.3 (a)
5	39	35	53.8	54.0 (c)	2.1 (d)
6	30	20	40.0	69.0 (bc)	2.6 (cd)
SEM				6.1	0.24

a, b, c, d : means within a column with a different postscript are significantly different ($P<0.05$). SEM = standard error of mean

of collection on farm to the receival at the processing plant. In five out of the six farms, there was significantly ($P<0.05$) less liveweight loss in the tunnel ventilated trailer. The situation was reversed for the birds transported from the closest farm (see Figure 1).

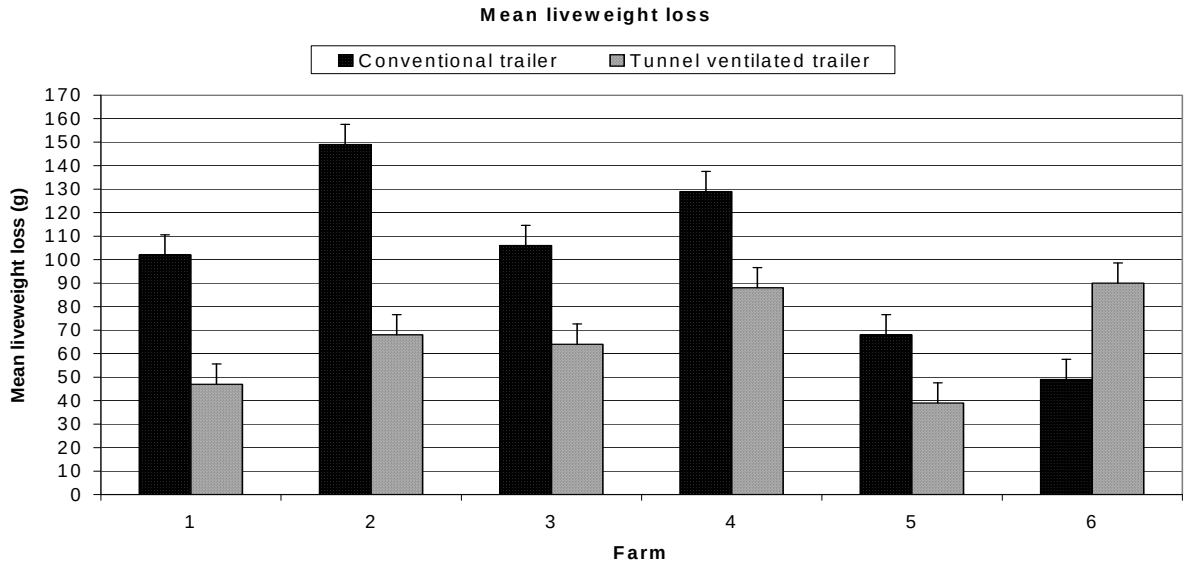

Figure 1 The loss in liveweight of birds transported on conventional and tunnel ventilated trailers from six farms in SE Queensland (Mean±SEM)

There were significant correlations and regressions of liveweight loss in relation to transportation distance and time. The regression of liveweight loss of birds on transport distances using the conventional trailer was Weight loss (g) = 5.22*distance (km) – 5.23*travel time (min) + 96.42. As speed is a function of distance and time, a second regression analysis was conducted and the linear relationship between weight loss and average speed of the conventional trailer was Weight loss (g) = 3.36*speed (km/hour) – 95.87. Weight loss in the conventional trailer at 48 km/h was equal to the weight loss in the tunnel ventilated trailer.

IV. DISCUSSION

The effect of trailer type on mean loss of liveweight of meat chickens during transportation from the farm to the factory thus appears to be influenced by transport distance. Although the results suggested there was greater weight loss for the birds transported on the tunnel ventilated than for those on the conventional trailer from farms located closer (approximately 20km) to the processing plant, the reverse was the case for the longer transport distances. The average distance that the company transports birds is approximately 45 km and at that distance the weight loss in the conventional trailer was 33% greater.

The use of the tunnel ventilated trailer would improve bird wellbeing, avoid negative public perception, improve some environmental aspects of the chicken meat industry and have a financial benefit. The financial saving that would be gained by the company using the tunnel ventilated trailer is considered substantial. Transporting meat chickens on a tunnel ventilated trailer would minimise bird exposure to the environment. However it has been suggested that closed ventilated trailers may restrict ventilation too much and excessive heat and moisture levels may build up around the birds (Kettlewell et al., 1993). Further research into the interaction between season and environmental conditions within the tunnel ventilated trailer is required. A thorough review of the prevention of ventilation failure would be necessary before replacement of conventional trailers with tunnel ventilated trailers. Transporting meat chickens in a closed ventilated trailer removes the birds from public view. This would allow meat chickens to be transported during the day minimising negative public perception and would enable processing on arrival at the factory which would further decrease weight loss. There is also a potential environmental benefit of transporting meat chickens in a closed ventilated system because faecal matter and feathers can be retained within the trailer. This material can then be disposed of at an appropriate disposal facility.

The recommendation of introducing tunnel ventilated trailers may be incorporated into the management procedure in a number of ways. These methods may include: (1) total use of the tunnel ventilated trailer to transport the birds from the farm to the processing plant; the benefits would outweigh the negatives for this company because there are only three farms in SEQ closer than 40km to the processing plant; (2) using the tunnel ventilated trailer to transport birds from the farms further from the processing plant and maintaining some conventional trailers to transport birds from the farms closest to the processing plant. Should the conventional trailer continue to be used, the trailer should be driven at a speed as close as possible to 48km/h to minimise liveweight loss but still maintain adequate airflow.

V. ACKNOWLEDGEMENTS

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EARLY NUTRITION FOR BROILERS - A TWO EDGED SWORD?

Z. AO and M. CHOCT

Summary

The effects of holding time after hatch and carbohydrate supplementation were investigated by offering four diets, one control and three test diets supplemented with glucose, manno-oligosaccharides (MOS) and fructo-oligosaccharides (FOS). Three feeding regimens included (a) immediate access to both feed and water; (b) immediate access to water but access to feed 36 h later, and (c) access to both feed and water 36 h post-hatch. Birds that had access to feed and water immediately after hatch were heavier ($P<0.05$) at 14 days of age, but this effect became less apparent as the birds got older. Birds given glucose, FOS or MOS tended to be heavier, and more efficient ($P<0.05$) in feed conversion at day 35 regardless of holding time. There was no effect of diet or holding time on bursa weight at 7 days of age. Bursa weight was heavier ($P<0.05$) for birds that had immediate access to water but 36 h delayed access to feed at 35 days of age. MOS supplementation significantly ($P<0.01$) increased spleen weight at 7 days of age, but this effect did not persist as the birds got older. Bird with immediate access to water but 36 h delayed access to feed post hatch drank more water ($P<0.01$) throughout their life. The same was true for birds given MOS. Also, birds that did not have access to both feed and water for 36 h post-hatch or those given MOS in their drinking water had a numerically lower mortality rate.

I. INTRODUCTION

Early access to feed and water post-hatch has a major impact on immediate and long-term development of chicks (Uni, 1998). However, in many cases chicks are held for 24-48 h before placement, without access to feed and water (Noy and Sklan, 1999). Klasing (1998) suggests that first week post-hatch is a critical period and the nutritional deficiencies may impact on the development of immune system. Delayed access to water and feed post-hatch dehydrates the chicks (Thaxton and Parkhurst, 1976), resulting in depressed immune response (Casteel *et al*., 1994), increased early mortality and reduced overall performance (Fanguy *et al*., 1980). Sklan *et al.* (2000) reported that immediate access to nutrients by chicks upon hatch improved breast muscle yield and the uniformity of chicks. Two types of oligosaccharides, fructo-oligosaccharides and manno-oligosaccharides are widely used in the feed industry as prebiotics, but most of the work with these carbohydrates was done following inclusion in diets rather than administering them in drinking water to newly hatched chick. In this study, two oligosaccharides, Raftilose 95 (a commercial FOS product, Orafti, Belgium) and Bio-MOS (a commercial MOS product, Alltech, USA), were given in the drinking water, to determine the effects of early treatment with oligosaccharides and time of administration on the development of the immune system and the long-term growth and health performance.

II. MATERIALS AND METHODS

A 4x3 factorial design was used in this trial. Four dietary treatments consisted of a control, glucose, manno-oligosaccharides (MOS) and fructo-oligosaccharides (FOS), respectively. The birds were fed under three feeding regimens, immediate access to both feed

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and water after hatch (0, 0), immediate access to water but 36 h delayed access to feed post-hatch (0, 36), and 36 h delayed access to both feed and water post-hatch (36, 36). MOS and FOS were given throughout the first 4 weeks at a concentration of 0.05% in drinking water. Glucose supplemented birds received a one-off treatment of 2% glucose in drinking water with the first offer of water. The birds were fed commercial starter crumbles during the first 4 weeks and then changed to a sorghum-based diet. All the diets were free of antibiotics.

Three hundred and twelve (312) day-old Cobb male broiler chicks were allocated to 2 replicates of 13 birds per treatment. At the end of the second week, all the birds were transferred into AME cages. The birds from one brooder cage were randomly allocated into 4 AME cages to make 3 birds per cage. All the birds were fed *ad libitum* throughout the experiment. Body weight, feed intake and water consumption by cage were recorded weekly. Body weight gain, feed conversion ratio and mortality rate were determined weekly.

III. RESULTS

The supplementation of glucose, MOS and FOS all significantly improved feed efficiency at 35 days of age ($P<0.05$) (Table 1). Birds with immediate access to both water and feed had significantly improved body weight at 14 days of age, compared to that of the other two feeding regimens ($P<0.001$). Birds with 36 h delayed access to both feed and water had lower FCR at 14 days of age, compared to the other two feeding regimens ($P<0.001$). However, the effects on body weight and FCR at 35 days of age became less apparent.

Table 1. Effects of glucose, MOS and FOS on body weight, feed conversion ratio of birds on day 14 and day 35[1].

Treatment	14-d		35-d	
	Body Weight (g)	FCR	Body Weight (g)	FCR
Diet				
Control	344.8	1.347^{a}	1720.5	1.711^{a}
Glucose	353.9	1.305^{b}	1754.2	1.660^{b}
MOS	350.9	1.310^{b}	1806.4	1.669^{b}
FOS	353.5	1.342^{a}	1811.8	1.655^{b}
Pooled SEM	2.9	0.012	31.5	0.014
Holding Time				
0, 0	367.3^{a}	1.357^{a}	1815.6	1.668
0, 36	344.6^{b}	1.342^{a}	1752.3	1.674
36, 36	340.4^{b}	1.279^{b}	1751.8	1.680
Pooled SEM	2.6	0.010	27.3	0.012
F test and level of significance[2]				
Diet type	2.04	3.77*	1.92	3.26*
Holding time	32.14***	18.97***	1.81	0.21
Diet x Holding time	1.91	2.29*	1.24	2.03

[1]Values are means of 24 replicates for dietary treatments and 32 replicates for holding time treatments.

[2]Levels of significance: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

^{a,b}Means within columns with different superscripts are significantly different ($P<0.05$).

Birds given MOS had numerically lower mortality (1.28%), than the control group (3.85%) throughout the experiment. FOS supplementation did not affect mortality rate (3.85%) whereas glucose supplementation increased mortality rate (7.69%) numerically.

Birds with immediate access to both water and feed had numerically higher mortality rate (5.77%), except those fed diets with MOS, while the birds with 36 h delayed access to both feed and water had numerically lower mortality rate (1.92%).

MOS supplementation increased the spleen weight as a percentage of body weigh at 7 days of age ($P<0.01$) (Table 2). The birds with immediate access to water but 36 h delayed access to feed had increased bursa weight as percentage of body weight at day 35 ($P<0.01$).

Table 2. Effects of glucose, MOS and FOS on bursa and spleen weight as a percentage of body weight of birds on day 7 and day 35[1].

Treatment	7-d		35-d	
	Bursa/BW (%)	Spleen/BW (%)	Bursa/BW (%)	Spleen/BW (%)
Diet				
Control	0.1334	0.0911[b]	0.2191	0.1294
Glucose	0.1402	0.0924[b]	0.2221	0.1210
MOS	0.1443	0.1093[a]	0.2374	0.1119
FOS	0.1534	0.0917[b]	0.2008	0.1264
Pooled SEM	0.0065	0.0041	0.0106	0.0071
Holding Time				
0, 0	0.1441	0.0977	0.2099[b]	0.1254
0, 36	0.1385	0.0947	0.2452[a]	0.1198
36, 36	0.1458	0.0959	0.2044[b]	0.1214
Pooled SEM	0.0057	0.0036	0.0092	0.0062
F test and level of significance[2]				
Diet type	1.63	4.53[**]	2.00	1.16
Holding time	0.45	0.18	5.78[**]	0.21
Diet x Holding time	1.41	8.37[***]	2.68[*]	0.92

[1]Values are means of 12 replicates for dietary treatments and 16 replicates for holding time treatments.
[2]Levels of significance: [*]$P<0.05$; [**]$P<0.01$; [***]$P<0.001$.
[a, b]Means within columns with different superscripts are significantly different ($P<0.05$).

The MOS supplemented birds drank more water compared with other birds, especially during the first two weeks ($P<0.001$) (Data not shown). The birds with immediate access to water but 36 h delayed access to feed had the highest water intake/feed intake ratio throughout the entire experimental period, over the other two feeding regimens ($P<0.01$).

IV. DISCUSSION

Data indicate that early nutrition is responsible for immune development of young chicks (Dibner, 1998). The bursa of Fabricius, a primary immune organ, plays a major role in creating antibody diversity (Uni, 1998), and inhibition of bursal development was found to result in failure of normal spleen development, the secondary immune organ (Glick, 1967). Dibner *et al.* (1998) found that chicks that had early access to feed and water had a heavier

bursa as a percentage of body weight, and better disease resistance. This is also supported by Wyatt *et al*. (1986) who concluded that early stress to young broiler chicks alters the immune competency and growth rate later in life. In this study, birds with immediate access to feed and water did not show a heavier bursa and spleen as a percentage of body weight, which is contrary to previous studies. However, MOS supplemented birds had a significantly heavier spleen at 7 days of age and bursa at 35 days of age, as a percentage of body weight, with a concomitant lower mortality rate. These results suggest that MOS supplementation of broiler diets may improve immune competency. Also, birds from the same hatch but fed diets containing antibiotics had a lower bursa and spleen weight as a percentage of body weight (data not shown), which suggest that carbohydrates and antibiotics influence the immune system via different mechanisms. There was significant diet x holding time interaction on 7 days spleen weight and 35 days bursa weight, which was complex and the reason is unknown.

The quality and quantity of feed, water intake, and overall nutrient digestibility influence the early growth and long-term performance of broiler chickens. In this study, holding time and MOS supplementation altered the water drinking pattern of birds throughout the entire experiment. Information on these areas of research is limited, especially the relationship between water consumption and immune competence. The importance of water intake/feed intake ratio for growth performance and feed efficiency is not understood. Birds with access to water and feed 36 h post hatch were more efficient in feed conversion at 14 days of age. This effect disappeared as the birds got older and the reason is not clear.

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N-3 AND N-6 FATTY ACIDS: DIFFERENT DIETARY RATIOS AFFECT ABDOMINAL FAT PAD MASS IN BROILER CHICKENS

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Previous studies have shown that feeding fish oil (n-3 fatty acids) and sunflower oil (n-6 fatty acids) reduces deposition of adipose tissue in broiler chickens compared to feeding saturated fatty acids (tallow) (Newman *et al*., 2002). Little is known about the effects of feeding different ratios of n-3 and n-6 fatty acids (FA's). This study investigated different dietary ratios of n-3 and n-6 FA's on abdominal fat pad mass (FP) and breast muscle mass (BM).

Day-old male broiler chickens (Cobb) were randomly divided into groups and allocated to one of 6 experimental diets (6 birds/pen and 7 replicates/treatment). The chickens were reared in brooders, fed starter diets for 18 days, then transferred to grower cages and fed grower diets for a further 24 days. The birds were fed *ad libitum* isonitrogenous and isoenergetic diets based on wheat and sorghum. These diets differed in the type and proportion of fat and were prepared by adding varying ratios of fish oil (FO) and sunflower oil (SO) as shown in the table. A control diet was also prepared by adding 60 g/kg of tallow. At day 43, 12 birds/treatment were selected for similar body weight (BW), slaughtered and BM and FP recorded. Fatty acid profiles of the diets were analysed by gas chromatography. Statistical examination of treatment effects was determined by ANOVA and Tukey-Kramer multi comparison test.

Treatment (g/kg)	Σ n-3 (g/kg)	Σ n-6 (g/kg)	BW (g)	FP (g)	BM (g)
FO/SO 60/0	1.77	1.75	1961 ± 93.6	20.8 ± 2.4^{a}	294 ± 16.6
FO/SO 40/20	1.21	2.91	1969 ± 57.6	24.7 ± 1.5^{ab}	292 ± 13.3
FO/SO 30/30	0.92	3.58	2010 ± 103.1	27.7 ± 2.2^{ab}	281 ± 14.0
FO/SO 20/40	0.72	3.89	1980 ± 82.4	32.1 ± 3.2^{b}	282 ± 19.1
FO/SO 0/60	0.28	4.73	2059 ± 103.9	29.8 ± 3.4^{ab}	313 ± 16.2
Tallow 60	0.27	1.58	1963 ± 70.8	29.4 ± 2.3^{ab}	298 ± 14.8

Means (± SEM) within a column without a common superscript differ significantly ($P<0.05$).

Breast muscle mass was not affected by dietary treatment. However, the data shows that increasing the proportion of dietary n-3 to n-6 FA's results in a linear decrease in FP ($R^2=0.76$; $P<0.06$). This decrease may be caused by suppressed expression of endogenous fatty acid synthase, the activity of which is modulated by dietary n-3 FA's through transcription factors that regulate lipid synthesis (Moon *et al*., 2002). Further research is required to elucidate this mechanism in broiler chickens.

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THE EFFECT OF MATERNAL DIETARY OMEGA (ω)-3 FATTY ACIDS ON HATCHABILITY AND GROWTH OF BROILER CHICKENS

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Summary

The effects of different maternal dietary ω-3 fatty acids on hatchability and post-hatch development of the broiler chickens were investigated. Incubated eggs were Low (Lω3), Medium (Mω3) or High (Hω3) in ω-3 fatty acids. The eggs were produced by feeding broiler breeder hens with a wheat-soybean meal diet containing (g/kg) 50 g of sunflower oil (Lω3); 25 g sunflower oil + 25 g fish oil (Mω3); or 50 g fish oil (Hω3). Hens fed the Mω3 diet had highest ($P<0.05$) fertility and hatchability values 78.26 and 65.35% compared to 69.01 and 47.18% for the Hω3 fed group. However, hens fed the Lω3 diets produced eggs that were significantly ($P<0.01$) larger than eggs from the Mω3 and Hω3 fed group. Consequently, the day-old chick live-weight were significantly different ($P<0.01$) with chicks hatched from the Lω3 > Mω3 > Hω3 fed groups. Best performance was obtained from the Mω3 fed group, which might indicate an optimum level of ω-3 for hatchability and growth.

I. INTRODUCTION

The physiological, biological and neurological importance of ω-3 fatty acids in human and livestock nutrition is well documented in the literature (Sim, pers. comm.). Consequently poultry products are enriched with nutritionally desirable ω-3 fatty acids by the manipulation of dietary fatty acids composition. In recent years the "hen-egg-embryo-chick" avian model has been used extensively to study nutrient accretion, in-particular ω-3 fatty acids metabolism during embryogenesis (Cherian *et al.,* 1997) and post hatch fatty acid composition of heart, brain and spleen (Ajuyah *et al.,* 2002).

Operators of commercial hatcheries are seeking to improve fertility, hatchability and reduce embryonic mortality while broiler producers expect minimal culls, improved feed conversion and weight gain of broiler chickens. Some studies have shown that the alteration of yolk fatty acid composition can have undesirable effects on fertility, hatchability embryonic survival and post hatch growth (Donaldson and Fites, 1970; Aydin *et al.,* 2001). Recently Halle (1999), reported that when broiler breeder hens were fed diets containing high levels of oleic acid (C18:1) or linoleic acid (C18:2), fertility was significantly affected, however embryonic mortality, hatchability and weight of the day old chicks were not significantly affected by the fatty acid composition.

This study was designed to examine the effects of different maternal dietary ω-3 fatty acids on egg weight, fertility, hatchability, early and late embryonic mortality, and post hatch traits such as live weight, weight gain, feed intake and feed conversion ratio of broiler chickens from hatch to slaughter. These traits are of economic importance to the Canadian broiler industry.

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II. MATERIALS AND METHODS

Eggs with low (Lω3), medium (Mω3) and high (Hω3) ω3 fatty acids were produced by feeding broiler breeder hens with the following diets: - a wheat-soybean meal based diet containing (g/kg) 50 g of sunflower oil (Lω3); 25 g sunflower oil + 25 g fish oil (Mω3); or 50 g fish oil (Hω3). The diets were calculated to contain the same amounts of protein (CP, 160 g/kg) and energy (11.8 ME MJ /kg). Vitamin E (27-mg/kg diet) was added to the Mω3 and Hω3 diets to protect the highly unsaturated fish oil fatty acids from undergoing auto-oxidation.

The breeder hens were 26 weeks old at the start of the experiment and each treatment had 40 females in individual cages with free access to feed and water. To facilitate the collection of fertile Lω3, Mω3 and Hω3 eggs, the hens were artificially inseminated weekly, two weeks after the introduction of the experimental diets. Eggs were collected and stored in a cold room (13-15 ^{o}C) for 6 weeks prior to incubation in a forced-draft 5000 egg capacity incubator, with automatic hourly turning (model PT-100, serial number H-A0019) and temperature set at 37.5^{o}C (dry bulb) and 29.4^{o}C (wet bulb). At 18 d of incubation the eggs were transferred to a 5000 egg unit hatcher (model PT-100, serial number H-A0014) with temperature set at 37.2^{o}C (dry bulb) and 29.4^{o}C (wet bulb). At the end of incubation (21 d) all the un-hatched eggs were cracked open to determine the number of clear eggs and the incidence of early and late embryonic mortality.

Post-hatch chicks were raised on deep-litter floor (pen size = 6.89 m^2) at a stocking density of 0.07 m^2 per bird. All the birds were fed the same commercial broiler starter (1-3 weeks) and finisher (3-6 weeks) diets, and water was provided *ad-libitum.* The design of the growth trial was completely randomized and the lighting regime employed throughout the experimental period was a light:dark (L:D) cycle of 23L:1D. Subsequently chicks hatched from Lω3, Mω3 and Hω3 diets were re-designated as groups 1, 2 and 3 respectively (n=100 birds per replicate and 5 replicates per group). The following data was collected: - number of total eggs incubated, post incubation clear eggs, early dead in shell (EDIS) and late dead in shell (LDIS) embryos, for determining percent fertility, hatchability and early and late embryonic mortality. In addition post-hatch traits such as live weight, weight gain, feed intake and feed conversion ratio of the broiler chickens from hatch to slaughter were also determined.

The chemical analysis of the fatty acid composition of the diet and eggs were determined using the method of Wang *et al.* (2000), and all the data were then subjected to analysis of variance (Genstat, 1997). The differences between means were determined by Least Significance Difference (LSD).

III. RESULTS

Table 1 shows the selected fatty acid composition of maternal diets and corresponding egg yolk (mg/g). Fertility was expressed as percent of total eggs less total clear eggs (F), while hatchability values were expressed for both total eggs (HTE) and fertile eggs (HFE). Hens fed the Mω3 diet had highest F, HTE and HFE (Figure 1). The early (EDIS) and late (LDIS) embryonic mortality were expressed as a percentage of total fertile eggs. EDIS was highest (26.3%) in the Hω3 group which, however, had the lowest LDIS (0.41%, data not shown). Percent culls for all groups were less than 5% ranging from 4.9% in the Hω3 group 3.6% in the Mω3 group (data not shown).

Hens fed the Lω3 diets (table 2) produced larger ($P<0.01$) eggs than Mω3 and Hω3 – fed hens, which were not significantly different. Consequently the day-old chick live-weight

for the Lω3, Mω3 and Hω3 groups were significantly different (P<0.01). The effect of maternal diet on live-weight and weight gain persisted for 3 weeks. However at 6 weeks of age there were no significant differences between all the groups for live weight, weight gain and feed conversion ratio. The correlation coefficient between egg weight and day-old chick weight was 0.987, and between day-old chick weight and 3 and 6 weeks live-weight were 0.955 and 0.928 respectively. The correlation coefficient between 3 and 6 week live weight was 0.776.

Table 1. Selected fatty composition of maternal diets and corresponding egg yolk (mg/g)

	Lω3	*Mω3*	*Hω3*
Diet			
C18:0	2.17	1.64	1.40
C18:1	10.03	7.82	7.01
SAFA	8.80	11.57	15.04
MUFA	10.31	10.48	12.47
ω-3	1.04	5.00	9.43
Egg-yolk			
C18:0	28.14	25.23	25.03
C18:1	103.78	112.00	114.41
SAFA	111.51	110.17	118.29
MUFA	113.50	125.46	133.23
ω-3	2.59	17.56	25.68

Lω3 = Low, Mω3 = Medium; Hω3 = High;
SAFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids.

Table 2. Effect of different maternal diets on egg weight, post hatch live weights and feed conversion efficiency of broiler chickens fed similar diets from hatch to slaughter

	Lω3	*Mω3*	*Hω3*	*P*
Egg wt. (g)	59.7[a]	54.5[b]	52.5[b]	**
Live weight (g)				
0 day	45.60[a]	43.60[b]	42.01[c]	**
3 weeks	677.4[a]	662.8[a]	631.2[b]	*
6 weeks	2258	2192	2188	NS
FCR				
3 weeks	1.63	1.50	1.52	NS
6 weeks	1.95	1.95	1.94	NS
Weight gain (g)				
3 weeks	631.8[a]	619.2[ab]	589.2[b]	*
6 weeks	1581.0	1529.0	1556.5	NS

* = P< 0.05; ** = P < 0.01; NS = not significant
[a-c] Means within rows with same superscript are not significantly different.
FCR = Feed conversion ratio.

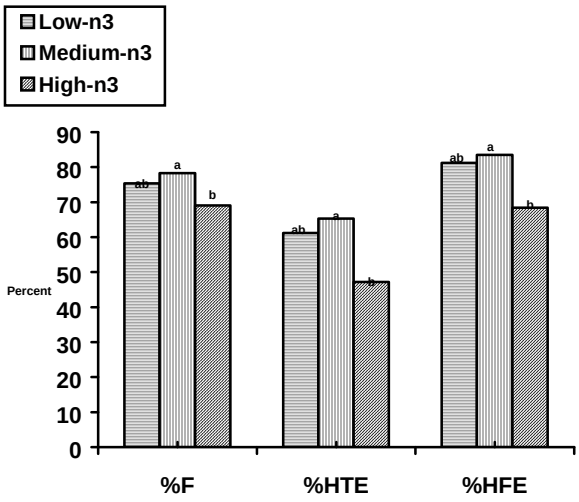

Figure 1. The effect of maternal diet on percent Fertility (F), Hatchability of Total Eggs (HTE) and Hatchability of Fertile Eggs (HFE).

IV. DISCUSSION

Studies have shown that alteration of yolk fatty acid composition can affect fertility, hatchability embryonic survival and post hatch growth of chicks. Aydin *et al.* (2001) attributed high embryonic chick mortality to decreases in yolk C18:1 and total monounsaturated fatty acids with associated increases in saturated fatty acids in the egg yolk. Donaldson and Fites (1970) observed that high levels of C18:0 and low levels of C18:1 induced embryonic mortality in quail. The current investigation suggests that high concentrations of maternal dietary ω-3 fatty acids may reduce egg size, fertility, hatchability, and early chick growth. Recently Halle (1999), reported that when broiler breeder hens were fed diets containing high levels of oleic acid (C18:1) or linoleic acid (C18:2), fertility was significantly affected, however embryonic mortality, hatchability and weight of the day old chicks were not significantly affected by fatty acid composition. Yolk fat as a source of energy and essential nutrients has been shown to play a crucial role in the avian embryonic development (Noble and Cocchi, 1990).

The maternal effects and high correlation between egg size, day-old chick and 3 week live-weight agree with the findings of numerous studies (e.g. Burke *et al*., 1997) which show a reduced influence of egg weight on body weight as growth proceeds. Peebles *et al.* (2002) reported that age, maternal dietary energy levels and fat types influenced chick live weight to 43 days of age.

V. CONCLUSION

Our studies suggest that changes in maternal dietary ω-3 fatty acids had an impact on pre and post hatch development of the broiler chickens, indices that are of economic importance to the commercial broiler industry. We agree with the observation of Hill (1993), who concluded that egg composition can have important consequences on chick survival by influencing body size at hatch and suggested that the practice of using egg size alone as a

measure of egg "quality" needs to be broadened to also consider internal composition. The specific influences of egg nutrients, in-particular fatty acids, on pre and post embryonic growth and development require further study. In the current study, the causes of low fertility, poor hatchability and high embryonic mortality in the Hω3 group is unknown. However, the relatively high fertility and hatchability of the Mω3 group indicates an optimum dietary fatty acid balance for reproductive performance.

IV. ACKNOWLEDGMENTS

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STORAGE OF GREEN OSTRICH SKIN

C.A. LUNAM[1], K.A. WEIR[1], J. RUIZ[1] and J.L. PLUMMER[2]

Green ostrich skins may be stored for four weeks prior to tanning. Degradation of the skins during storage would decrease skin quality. Tanned ostrich skin is comprised of the grain and corium layers. To assess the effects of different storage conditions on green ostrich skins, the thickness of these layers was compared between pre (at slaughter) and post storage. Ostriches were electrically stunned and killed by bleeding from the carotid artery. The skin was dry-plucked before removal from the carcass. Twelve skins were cut in half along the midline and each skin-half allocated to a different storage group so each skin-half served as control for the other half. Four whole skins were allocated to one of the four storage groups, with two samples taken from each skin. Prior to storage each skin or skin-half was liberally coated with hide salt and each group of skins stacked on separate palettes. All skin samples were taken from the upper belly region and the thickness of the combined grain and corium layers measured in histological sections using a calibrated graticule. Skin thickness data were analysed by random effects regression.

Group 1: Immersed in cooled hide salt solution (25 g/L) for 30 mins, stored at 22°C
Group 2: As Group 1 stored at 4°C
Group 3: Immersed in cooled hide salt solution (25 g/L) and Busan 85® (1.5g/L) bactericide solution for 30 mins, stored at 22°C
Group 4: As Group 3 stored at 4°C

Skins treated with bactericide prior to storage at 22°C for four weeks were significantly thinner compared to those without bactericide treatment (P=0.027). Bactericide had no effect on skins stored at 4°C (P=0.073). Prior to storage there were no differences in skin thickness between the groups (P=0.38): Pre-storage means ± SEM (μm) were: Group 1, 1715 ± 117; Group 2, 1798 ± 165; Group 3, 2017 ± 190 and Group 4, 1920 ± 111.

Skin (grain + corium) thickness (μm). Post – pre storage

Temperature	Bactericide	
	no bactericide	bactericide
4°C	-367 ± 152	-11 ± 149
22°C	497 ± 209	54 ± 201

Mean change ± SEM change

The data indicate that bactericide treatment is essential to prevent the alteration in the thickness of the ostrich skins stored for 4 weeks at room temperature. Denaturation or unraveling of the collagen fibres would likely result in a thicker connective tissue layer comprised of a decreased density of collagen bundles. As this change is prevented with bactericide treatment we suggest that denaturation of the collagen is likely to be caused by bacterial infection rather than lysosomal autolysis. The effect of increased thickness of the dense connective tissue layers on the physical properties of the skin is currently being investigated.

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OPTIMISING STORAGE CONDITIONS OF EMU SKIN

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The aim of this work was to determine the optimal storage conditions for emu skin prior to transport to the tannery. This work forms part of a series of experiments focused on improving the quality of Australian emu skins. Skins from 14 emus of known sex and age were obtained from a commercial abattoir in Keith, SA. The birds were euthanased by electrical stunning and bleeding from the carotid artery and dry-plucked before skin removal. All skins were cut in half and samples were taken from the rump region for microscopic analysis. Each skin half was then placed into one of four treatment groups.

Group 1: Rinsed in a cool solution of hide salt (25 g/L) for 30 minutes and stored at room temperature (25 °C) (6 half-skins).

Group 2: Rinsed in a cool solution of hide salt (25 g/L) for 30 minutes and stored at 4°C (7 half-skins).

Group 3: Rinsed in a cool solution of bactericide (Busan 1021, 1.5 g/L) and hide salt (25 g/L) for 30 minutes and stored at room temperature (25 °C) (6 half skins).

Group 4: Rinsed in a cool solution of bactericide (Busan 1021, 1.5 g/L) and hide salt (25 g/L) for 30 minutes and stored at 4°C (7 half-skins).

To test the effect of freezing the remaining two half-skins were stored at –17.5 °C. For storage the skins were stacked on palettes with hide salt between each layer (groups kept separate). Samples were taken from the rump region for light microscopy after storage for 4 weeks. The thickness of the grain layer, corium and dense connective tissue portion of the dermis were measured for each sample using a calibrated graticule under the light microscope.

		Without bactericide		With bactericide	
Thickness (µm)	Temp.	0 weeks	4 weeks	0 weeks	4 weeks
Grain	4 °C	43 ± 3	45 ± 2	46 ± 3	51 ± 6
	25 °C	43 ± 2	31 ± 4	49 ± 6	36 ± 2
Corium	4 °C	393 ± 35	453 ± 37	373 ± 27	405 ± 33
	25 °C	451 ± 30	212 ± 24	366 ± 19	336 ± 58
Dense connective	4 °C	436 ± 36	499 ± 37	419 ± 29	456 ± 37
tissue (DCT)	25 °C	494 ± 30	243 ± 27	415 ± 23	372 ± 59

Means ± SE, n ≥ 36 measurements, pooled from 6 half skins. Temp × time interaction ($P<0.01$) for Grain; Temp × time × bactericide interaction ($P<0.05$) for Corium and DCT.

Skin thickness data were analyzed by random effects regression. Both time ($P<0.01$) and temperature ($P<0.01$) have a significant effect on grain thickness. Grain thickness was reduced over time in skins stored at room temperature but not in skins stored at 4 °C. The presence of bactericide in the rinse solution had no effect on the thickness of the grain layer. The thickness of both the corium and dense connective tissue portion of the dermis was reduced over the storage period when skins were stored at room temperature but not at 4 °C. Addition of bactericide to the rinsing solution prior to skin storage reduced this thinning. Preliminary evidence suggests storage at –17.5 °C results in a reduction in thickness of all layers measured. The effect of the variation in thickness of these layers on the quality of the tanned product is currently being examined.

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USE OF ERGOT AFFECTED SORGHUM IN LAYER DIETS: QUALITY ASSURANCE OF EGGS

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Summary

Dihydroergosine (DHES) is the principal alkaloid produced by sorghum ergot (*Claviceps africana*). DHES in animal feed can cause significant production losses in cattle and pigs, but chickens are more tolerant. Hens were fed diets containing up to 19 mg DHES/kg for six weeks and eggs were collected daily. Eighty eggs from ergot-fed birds and 80 from control birds were assayed by an ELISA that is specific for DHES. The ELISA had a detection limit of 0.005 mg DHES/kg, but DHES was not detected in any egg. Forty eggs from the ergot-fed birds were also assayed by HPLC with fluorescence detection, also with negative results (<0.02 mg DHES/kg). Only 45% of ingested DHES was recovered in the excreta, suggesting that DHES was rapidly degraded in the stomach or intestine. The regulatory limit for ergot in feed for laying hens might be raised from 0.3% to 1% (about 1 mg DHES/kg to about 5 mg DHES/kg) without significantly increased risk of residues in eggs.

I. INTRODUCTION

Sorghum ergot (*Claviceps africana*) is a fungus that invades sorghum flowers preventing the development of the grain while developing a fungal body (the sclerotia, or ergot) that is of similar size to a sorghum grain. The fungus has recently become widespread in sorghum growing areas of Australia but its incidence varies depending on environmental conditions during flowering.

Livestock consuming ergot-contaminated sorghum develop various conditions caused by the alkaloids produced by the fungus. These alkaloids are dihydroergosine (DHES), which usually constitutes over 80% of total alkaloids, festuclavine and dihydroelymoclavine. The two minor alkaloids lack the peptide side chain of DHES. In turn, DHES differs from the main rye ergot (*C. purpurea*) alkaloid, ergotamine, in that the 9,10 position in the ergosine nucleus is saturated in DHES (hence dihydro-), and in a small difference in their peptide side chains. Ergotamine has been known for centuries to cause vasoconstriction in humans (St Anthony's fire) and its less toxic 9,10 dihydro-derivative (dihydroergotamine) is used pharmacologically as a treatment for migraine. Although sorghum ergot was previously considered non-toxic due to the dihydro-nature of its alkaloids, recent studies have shown that the sorghum ergot alkaloids have at least some actions in common with rye ergot alkaloids (Blaney *et al*., 2000).

As with rye ergot alkaloids, chickens are more tolerant than other livestock species to sorghum ergot alkaloids (Blaney *et al*., 1998). Effective use of ergot-infected sorghum might be achieved by feeding it to chickens or laying hens. However, if layers secreted any alkaloid in the eggs, this avenue could not be used. This trial tested whether laying hens fed ergot-infected sorghum would secrete ergot alkaloids into their eggs.

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II. METHODS

Four basal diets were formulated to contain sorghum as the sole grain with projected ergot alkaloid contents of 24, 12, 6 and 0 mg DHES/kg diet. Diet 1 was assayed and found to contain 19 mg DHES/kg. The nutrient content of the diets were 195 g crude protein, 12 ±0.6 MJ metabolizable energy, 3.7 g calcium, 3.8 g available phosphorus, 7.9 g available lysine, and 6.4 g methionine per kg of diet. Half of each of the basal diets was mixed with a binding agent, Mycosorb®, (Alltech Biotechnology P/L, Victoria) which had been found to decrease the effects of sorghum ergot alkaloids when fed to young chickens (Deo, 2000).

The eight treatment diets were each fed to 12 ISA Brown hens 60 weeks old, housed 2 per cage in 6 randomly allocated cages in a semi-controlled environment room for a period of 6 weeks. Average room temperature was 20 °C and light dark period was set at 16:8 hours. Eggs were collected daily for six weeks and stored in a refrigerator at 4 °C. At the end of the six weeks, dry matter digestibility was calculated by measuring dry matter consumption and dry matter excretion over 24 hours.

The presence of ergot alkaloids in whole egg contents was tested using ELISA and HPLC techniques. The competitive ELISA was based on DHES–specific mouse monoclonal antibody (Molloy *et al.*, 2003). Eighty eggs from hens fed 19 mg DHES/kg and 80 eggs from hens fed the control diet were analysed by ELISA. Forty eggs from hens fed 19 mg DHES/kg and 10 from control hens were analysed for ergot alkaloids by reversed phase HPLC with fluorescence detection (Blaney *et al*., 2003).

III. RESULTS

None of the eggs contained any trace of alkaloid that exceeded the background non-specific interference level of 0.005mg DHES/kg in the ELISA, nor exceeded the detection limit of 0.02mg DHES/kg in the HPLC analysis.

The excreta of birds fed the high ergot alkaloid diet contained 38mg DHES/kg DM. The diet contained 21mg DHES/kg on a DM basis and the DM digestibility was measured as 76%, indicating that only 45% of the ingested DHES was present in the excreta.

IV. DISCUSSION

None of the ingested ergot alkaloid was found in eggs despite 55% of the ingested ergot alkaloid in the high ergot diet disappearing from the gut. This is similar to the result found with pigs (Whittemore *et al*., 1976).

The greater resistance of monogastrics, especially poultry, to the effects of DHES compared with ruminants might be related in part to the low pH and high pepsin activity of the monogastric stomach, which perhaps is able to attack the cyclopeptide part of the molecule leading to de-toxification prior to absorption, whereas it might be absorbed undissociated from the rumen. However, more investigations would be required to test this and other hypotheses. Another question that should be addressed is whether residues can be detected in any of the tissues of hens fed ergot.

There was a small but significant decline in egg production in the birds fed the highest alkaloid concentration. Birds fed the lower alkaloid concentrations did not produce differently from the controls. The highest concentration of DHES used in this trial would rarely be found in bulk grain in practice, so it appears that there is no justification for concern about egg residues from laying hens fed moderate levels of ergot affected sorghum. The results suggest that the present regulatory limit for sorghum ergot for laying hens of 0.3% of

the diet could be raised to at least 1% (i.e. from about 1 mg DHES/kg to about 5 mg DHES/kg) without significantly increasing the risk of producing eggs containing ergot alkaloid residues.

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PERFORMANCE EVALUATION OF BROILER CONTRACT GROWERS IN FIJI

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Summary

An evaluation of 34 broiler contract farms was conducted in Fiji Islands using the Performance Indicator Factor (PIF) to determine variation in production performance. Results showed an overall range in PIF values from 79-210. The major causes of variability in PIF on contract farms were location, number of drinkers and feeders and water source. Collectively, these factors significantly ($P<0.05$) influenced final market weight of birds. Using an Australian PIF value as a standard, the best contract farms in Fiji need to improve by 16% to match the Australian performance while poorer performing farms in Fiji need to improve by 69%. This survey suggests that PIF measurements are appropriate to evaluate performance of contract broiler growers in Fiji and will lead to a uniform regional standard of performance evaluation for broiler contract farmers.

I. INTRODUCTION

The broiler industry in Fiji is one of the most developed in the South Pacific region and comprises the island countries of Samoa, Cook Island, Tonga, Solomon Islands, Vanuatu, Tuvalu, Niue, Kiribati, Tokelau, Marshall Islands and Nauru (Ajuyah and Umar, 2001). The total number of commercial broiler chickens in Fiji is approximately 6.2 million with an estimated value of $35.3 million Fijian dollars and per capita consumption of 12.9 kg broiler meat (MAFF, 2000).

In Fiji contract broiler farms are supplied all the inputs of production by commercial companies. These companies are in most cases vertically integrated and own the feed mill, parent breeder farms, hatchery, slaughter and processing plant, sales and marketing divisions. The average contract farmer manages 3,500-4,500 broiler chickens. Farmers are paid on the basis of a pre-determined remuneration package. Recent increases in the number of contract farms has resulted in considerable variability in broiler performance particularly final live weight and dressed carcass weight.

Performance Indicator Factor (PIF) is defined as growth rate/FCR x Live% x 100, where growth rate=weight(kg)/age(days), FCR=feed conversion ratio (kg feed/kg liveweight) and Live%=number of birds alive at the end of the grow out/number of birds housed x 100). PIF values were used by Barnett *et al.* (2002) to rank the performance of broiler producers (near Melbourne, Australia) involved in an evaluation of a broiler welfare audit. Because of the significant disparity in income and variation in productivity observed among contract broiler producers in Fiji, this survey was conducted to evaluate and rank contract broiler growers and also to develop an objective standard for performance evaluation.

II. METHODS

A total of 34 contract broiler farms were randomly chosen for the survey from approximately 80 farms in10 locations in the three agricultural zones of the Central and Eastern Division of Viti Levu, the biggest of the Fiji islands. The questionnaire was pre-

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tested on 3 farms prior to adoption and application to the rest of the farms. The questionnaire encompassed the following parameters; (a) farmer (age, gender, education and ethnicity); (b) stock (number, market weight and feed intake); (c) management (labor, brooding, drinkers, feeders, water source, manure and dead carcass disposal) and (d) land (type, zone and location). Data on feed efficiency, growth performance and mortality rates were obtained from company records. The duration of this study was for one batch of 4000 birds. Stocking density (0.081m^2/bird) and shed sizes (11 x 30 m) were the same on all farms. Also, feeders and drinkers were of the same size and capacity.

The PIF values were calculated for each farm and producers were ranked according to the following four PIF classes; PIF 1 (180-210); PIF 2 (139-179); PIF 3 (98-138) and PIF 4 (< 97). Analysis of variance (Genstat, 1997) was used to analyze the effect of farmer, stock, management and land parameters on PIF. Simple correlation coefficients (*r*) among productivity parameters were obtained using the Minitab Statistical Package (Minitab Inc., 1996).

III. RESULTS

The 34 broiler contract farms were from 10 locations within the three agricultural zones and 85% of these farms were located on leased land, 3% on freehold land and 12% on a combination of leased land and freehold land tenure systems. Values for growth rate, feed efficiency, flock size, mortality rates and PIF values are presented in Table 1.

Table 1. Performance of broilers on contract grower farms in the Fiji Islands.

Parameter	Zone A	Zone B	Zone C
Growth rate, kg/day			
Mean	0.027	0.031	0.036
SD	0.008	0.002	0.003
CV%	30	6	8.3
FCR			
Mean	2.05	2.10	1.94
SD	0.28	NA	0.16
CV%	11	NA	8
Live, %			
Mean	93.4	94.7	93.9
SD	2.05	NA	1.46
CV%	2	NA	1
Mortality, %			
Mean	5.58	5.30	5.4
SD	0.25	NA	0.86
CV %	4	NA	16
PIF			
Mean	128.7	142.05	175.8
SD	48.3	9.55	24.7
CV %	37	7	14.0

NA=not available

Table 2. Correlation coefficient (r) among selected productivity parameters.

	Growth rate	FCR	Live%	Mortality%
FCR	-0.503			
Live%	-0.031	0.196		
Mortality%	0.008	-0.364	-0.258	
PIF	0.964	-0.689	-0.025	0.136

Table 3. Significance of selected parameters for PIF on broiler contract grower farms in the Fiji Islands.

Performance parameter	*Significance*
Age	NS
Location	*
Education	NS
Zone	**
Flock size	NS
Brooder heat	NS
Drinkers	NS
Feeders	**
Water source	*
Market weight	**
Manure disposal	NS
Carcass disposal	NS

NS=Not significant; * = P<0.05; ** = P<0.001

PIF was significantly (P<0.05) affected by the location, zone and source of farm water supply (Table 1). Farmers who obtained their water supply from a stream had better PIF values compared to farmers using water supplied by the Public Works Department. For farms, the desirable numbers of drinkers and feeders for optimum productivity appeared to be 50 and 35 respectively. PIF values were highly correlated with growth rate and feed efficiency (1/FCR) but showed low to negative correlations with other productivity variables (Table 2).

Age, gender, education, ethnicity, number of staff, land type, stock number, source of heat during brooding, manure management and methods of carcass disposal had no significant (P>0.05) effects on PIF values (Table 3). The final market weight of the broiler chickens was related to overall PIF values as contract growers in the highest PIF class (180-210) produced the heaviest birds (>1.8 kg).

IV. DISCUSSION

Variation in performance and PIF values observed on Fiji contract broiler grower farms could be ascribed to a combination of management and environmental factors. Farms located in the highlands (Muaniweni and Lomaivuna) of Viti Levu had higher PIF values compared to those in the lowlands (Verata and Naselai). In the highlands, broiler houses are generally cooler and less humid than in the lowland areas where farmers typically resort to the use of fans to reduce problems related to heat and humidity. Also, plastic flaps are used to

prevent rainwater from entering the sheds and causing disease problems associated with wet litter. Only 6 parameters (zones, location, market weight, drinkers, feeders and water source) significantly affected the PIF values. Using the Australian PIF value (Barnett *et al.,* (2002) as a standard, it is evident that the best contract farmer in Fiji needs to improve PIF values by 16% to match the Australian performance while poorer performing farms in Fiji need to improve PIF values by 69%.

The lack of a significant ($P>0.05$) effect of the educational level of farmers on PIF values is indicative of the effectiveness of screening, training and monitoring programs offered by the parent companies. The higher PIF observed in farms with less than 3 staff indicates that higher levels of staffing is counter productive in view of the small sizes of the contract farms (3,500-4,500 birds). In addition, too many feeders and drinkers led to overcrowding which in turn lowered PIF values.

A major innovation is the use of kerosene as an alternative source of energy for brooding. Compared to electricity and natural gas, no differences in brooding performance were observed between these heat sources. Furthermore, kerosene is cheap, dependable and readily available in most remote areas of Fiji. It was observed that some farmers did not adhere strictly to company guidelines regarding flock management. The cumulative effects of differences in management and environment resulted in only 41% of farms producing birds heavier than the standard pick up live weight of 1.8kg.

All thirty-four contract farms had the same inputs and access to management and production support from the parent company. However, significant variability in performance was observed among farms as indicated by the wide range of PIF values (66-210). It is important to note that most of the farms surveyed have only been in operation for approximately two years. A follow-up study is recommended to determine if improvements are being made on contract broiler farms.

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IDENTIFYING QUANTITATIVE TRAIT LOCI FOR GROWTH, MUSCLING AND FATNESS TRAITS IN A BROILER X LAYER CROSS

P.M. HOCKING and D.W. BURT

Summary

Quantitative trait loci (QTL) for body weight, weight gain, muscling and fatness at 2 kg live weight were detected in the F_2 progeny of a broiler x layer cross. Genetic effects were largely additive for growth and muscling but significant dominance effects were detected for fat traits. Effects accounting for more than 4% of the phenotypic variation were identified on 6 chromosomes and 1 to 6 QTL affected specific traits. The largest single QTL was for body weight at 2 kg accounting for 249g or 12.5% of the mean of the F_2. The largest effect for abdominal fatness accounted for 10 g fat or 0.7 phenotypic standard deviations. Analysis of weight gains from 0 to 3, 3 to 6 and 6 to 9 weeks suggested that different QTL affected growth at different ages. Relatively few QTL account for a substantial proportion of the differences in body weight and fatness between broilers and layers.

1. INTRODUCTION

The location of Quantitative Trait Loci (QTL) through the use of genetic markers is the first step towards identifying the genes for economic traits and the study of the genetic architecture underlying quantitative traits. DNA markers for QTL can also be used to improve the productivity, health or welfare of commercial flocks by marker assisted selection or the introgression of desirable alleles from one population to another. The objectives of this study were to identify QTL for growth, muscling and fatness traits in a broiler x layer cross as a demonstration of the technology and as a prelude to basic understanding of the genetic changes underlying the massive increase in growth rates in modern broilers compared with the original breeds.

II. MATERIALS AND METHODS

Two males and 2 females from each of a White Leghorn egg layer and a broiler male line were crossed. F_1 progeny were reared and mated in a balanced fashion. Over 500 F_2 progeny were bred in 5 hatches and housed at random in 20 floor pens. The birds were fed *ad libitum* on a high energy, high protein ration based on conventional ingredients. A brooder lamp provided local heat throughout the experiment and the ambient temperature was 16^{o}C. The birds were weighed at 3, 6 and 9 weeks of age. At 9 weeks they were slaughtered, plucked and eviscerated. The carcass and abdominal fat were weighed. The carcasses were stored at –20^{o}C and subsequently dissected. The weights of breast meat, leg, thigh and wings were recorded. The breast, legs and thighs were dissected into skin, muscle and bone and the parts were weighed. Genotyping was conducted with microsatellite markers that covered 70-80% of the genome. After edits there were data from 466 F_2 birds that were genotyped for 102 markers in 27 linkage groups. Marker-QTL associations were analysed by the method of Haley *et al*, (1994) using the QTL Express software (Seaton *et al*., 2001). The model included additive and dominance genetic effects and terms for pen, family, sex, and a covariate (carcass weight or breast muscle weight) where appropriate.

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Table 1. Additive and dominance effects and their standard errors (SE), additive and dominance effects as multiples of the residual standard deviation (SdE) and

the proportion of the total phenotypic variance contributed by the additive and dominance effects of statistically significant QTL for body weight and carcass traits at 9 weeks of age.

Chromosome	Additive effect (g)			Dominance effect (g)			Proportion of phenotypic variance
	Mean	SE	SdE[1]	Mean	SE	SdE[1]	%
Live weight							
1	76	2	0.3	87	35	0.4	3.9
2	79	19	0.3	-57	29	-0.2	4.1
4	249	4	1.0	42	138	0.2	7.6
8	186	48	0.8	73	184	0.3	3.4
13	106	26	0.4	12	53	0.1	3.6
27	86	20	0.4	-11	27	-0.1	4.0
Abdominal fat, g[2]							
1	-3.1	0.9	-0.2	2.6	1.4	0.2	3.00
5	4.4	1.4	0.3	3.7	1.6	0.3	4.25
7	10.1	2.2	0.7	-4.1	6.7	-0.3	4.51
28	-4.3	1.2	-0.3	5.4	2.2	0.4	3.84
Breast muscle, g[2]							
8	13.5	3.1	0.5	-9.3	7.3	-0.4	4.0
Drum and thigh muscle, g[2]							
1	7.2	2.7	0.4	-23.4	7.2	-1.2	3.6
6	-5.3	1.4	-0.3	3.4	2.1	0.2	3.3
13	2.0	2.0	0.1	-15.6	3.7	-0.8	4.1

[1] Standardized effect (the mean additive effect divided by the residual standard deviation).
[2] Weight adjusted by covariance analysis to mean carcass weight.

III. RESULTS

QTL effects for weight and carcass quality at 2 kg live weight are presented in Table 1. Genetic effects for body weight and muscling traits were generally additive and positive (the broiler alleles increased weight and muscling). The effects of these QTL accounted for 0.2 to 1.0 phenotypic standard deviations. There were no family x QTL interactions, suggesting that QTL were not segregating in the parental lines.

An examination of the confidence intervals for body weight at different ages suggested that there were a least three QTL on chromosome 1 (Sewalem *et al.*, 2002), a conclusion that was consistent with multi-trait analyses (X. Yu, unpublished). We therefore analysed weight gains from 0 to 3, 3 to 6 and 6 to 9 weeks of age to discount the cumulative effects of previous growth on apparent genetic effects at a particular age. The results are presented in Figure 1 and show clearly that there were different QTL on the same chromosome that affected weight gain at different ages.

Significant QTL for abdominal fatness were both positive and negative and important dominance effects were also observed (Table 1). QTL for skin and subcutaneous fatness (skin fat weight adjusted for carcass weight) were found on chromosomes 3 and 28 and for fat distribution (abdominal fat weight adjusted for skin fat weight) on chromosomes 5, 7 and 15. The magnitudes of the QTL effects were similar to the body weight QTL and represented 0.2

to 0.7 phenotypic standard deviations or 3-5% of the residual phenotypic variation (Ikeobi *et al.*, 2002).

IV. DISCUSSION

Different QTL affected early and later growth and we speculate that genes affecting early growth control the development of essential organs and the skeleton whereas later growth is dominated by QTL that influence muscle deposition.

In contrast to the QTL for body weight, where only positive alleles were found in the broiler lines, both positive and negative alleles for fatness were identified in both lines. Interactions with family or sex were not significant. The results suggest that QTL for fatness were not segregating in the parental lines and that both positive and negative QTL were fixed in the two lines. The lack of any sex interaction with QTL for fat traits was surprising given that abdominal fatness, corrected for carcass weight, was greater in females than in males (0.042 vs. 0.034, se. 0.001). The differences in fatness were not large in this experiment and may have been too small to detect a sex interaction.

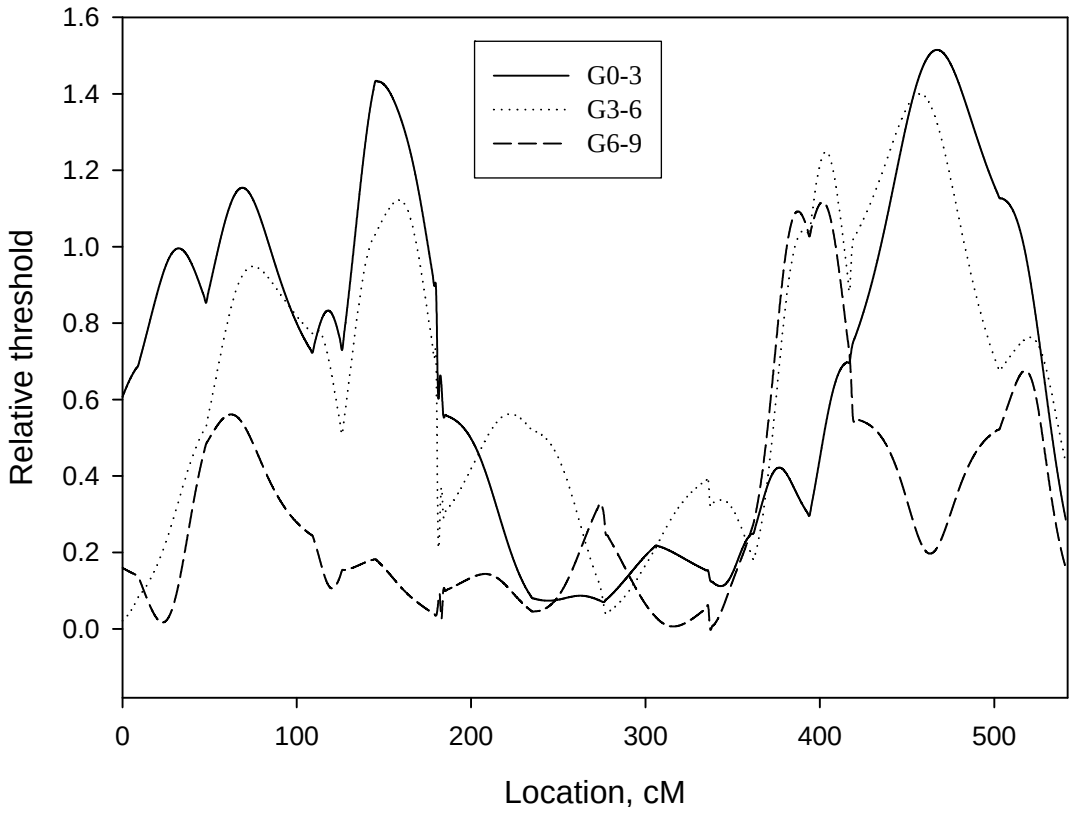

Figure 1. Relative significance thresholds for weight gain from 0 to 3 (G0-3), 3 to 6 (G3-6) and 6 to 9 (G6-9) weeks of age for QTL at different locations along chromosome 1 in a broiler layer cross (X. Yu, unpublished results). Threshold values greater that 1.0 are significant ($P<0.05$).

The techniques developed in this research are being applied to commercial populations to determine the importance of the QTL identified from this wide cross to those segregating within populations. The confidence intervals for the QTL in this study are relatively large and further refinement will be necessary to provide the close linkage

necessary for marker assisted selection and is an essential step towards cloning the gene(s) underlying the observed effects. We will approach this in two ways: firstly, by developing more markers in the region of the QTL and secondly by mapping the traits in an advanced intercross population (AIL). The development of markers will be based on single nucleotide polymorphisms (SNP's), which occur at a high frequency of 1-2/100 bp, about 10-fold higher than in human populations. The isolation of SNP's will be facilitated by the complete genome sequence of the chicken that is due for completion in the autumn of 2003.

In summary, QTL mapping of diverse crosses has proved that QTL of moderate to large effects for traits of economic significance can be detected. Multiple QTL for the same trait can be found on a single chromosome and QTL can have pleiotropic effects. The sum of the additive QTL effects at 6 weeks of age accounted for up to 75% of the line difference in body weight and we conclude that a few QTL account for much of the additive genetic variance between broilers and layers. Finally, it is clear that the distribution of QTL effects in a population is not that of a large number of QTL of small effect.

ACKNOWLEDGEMENTS

Our research was funded by BBSRC, EU and DEFRA and we are grateful for the many contributions of colleagues.

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SEX AND THE SINGLE CHICKEN

R.J. HUGHES

Summary

This paper discusses results from some recent studies that point to the existence of fundamental differences between males and females in metabolism of energy. It is apparent that gender can influence the digestive capacity of chickens through endogenous energy losses, gut structure and function, and metabolic activity of gut microflora. This raises the question "Is there sexual dimorphism in other physiological and biochemical systems also?" There are important scientific and commercial implications should such differences exist. Firstly, future research should include an examination of any gender-related influences. Secondly, the commercial implications are that males and females may have different nutrient requirements, and may respond differently to feed additives such as prebiotics, probiotics and feed enzymes. Hence, single-sex feeding and management programs may be desirable for optimisation of growth, carcass yield and carcass composition within each sex.

I. INTRODUCTION

The effect of the gender of the individual chicken on its functional capacity to digest and absorb nutrients has received little attention by researchers until recently. Hughes (2001) noted that much of our current knowledge of nutrient utilisation and nutrient requirements of broiler chickens was gained by study of males only. Experiments designed around chickens of the same sex may have some advantages, however, it is possible that only half of the true story will be revealed, or less, if underlying interactions involving sex go undetected.

This paper examines some recently published results which indicate that gender can influence the digestive capacity of chickens in several different ways involving endogenous energy losses, gut structure and function, and metabolic activity of gut microflora.

II. SEX INFLUENCES ENERGY METABOLISM

Hughes *et al*. (2000) observed that apparent metabolisable energy (AME) of a wheat-based diet was significantly affected ($P<0.05$) by an interaction between cleanliness of the rearing environment and sex of chickens. Males had lower AME than females (15.15 vs 15.32 MJ/kg DM) when reared in a dirty environment but there was no difference between males and females (mean value 15.29 MJ/kg DM) reared in a clean environment.

Hughes *et al*. (2001) reported that chickens of two different breeds showed variable responses in energy metabolism when given a diet containing a high concentration of soluble non-starch polysaccharide (NSP), with males more affected than females. The breed effect (14.4 vs 14.2 MJ/kg dry matter) was not significant, whereas females were superior to males (14.6 vs 14.0 MJ/kg dry matter). The plot of individual data points shown in Figure 1 points to a higher degree of variability in males than in females, irrespective of breed, with a relatively large proportion of males showing a poor capacity for uptake of energy.

Wu *et al*. (2002) noted that AME values in males tended to decrease when dietary P was increased, whereas no effect was observed in females. They also reported that apparent ileal

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nitrogen digestibility increased in males given a P deficient diet but decreased in females.

III. SEX AND ENDOGENOUS ENERGY LOSSES

Johnson (1987) and King (1998) pointed out that endogenous energy loss (EEL) could be a large source of error in measurements of AME and TME in assays involving the allocation of fixed amounts of test diet. The size of the EEL error relative to AME becomes minor in fully-fed birds.

An estimate of EEL can be obtained from the value α in the linear relationship:-

$$EE = \alpha + \beta \times GEI$$

where α = energy voided at fasting, and β = rate of increase in energy excreted as gross energy intake increases (King, 1998). The data of Hughes *et al*. (2001) shown in (Figure 1) were plotted in this manner (Figure 2).

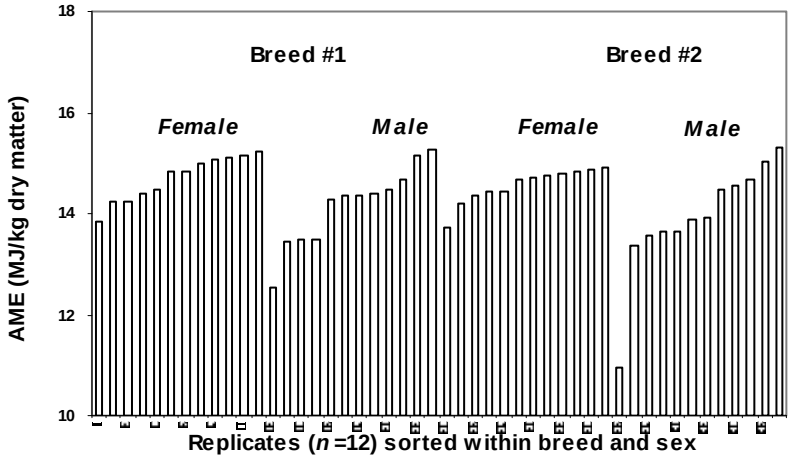

Figure 1. Variability in apparent metabolisable energy (AME) of a wheat diet given to male and female chickens of two commercial breeds. Each bar in the figure represents the result for a single chicken.

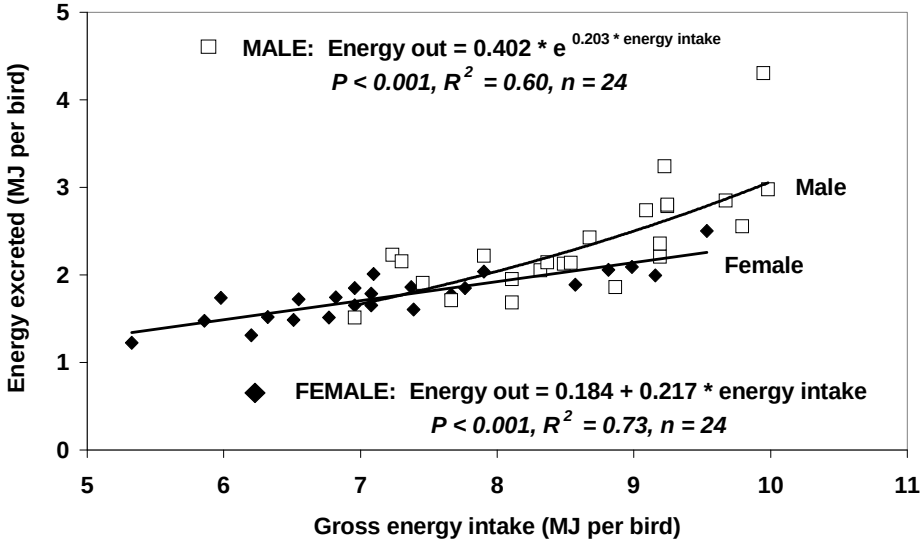

Figure 2. Relationship between energy excreted and gross energy intake for male and female

Analysis of covariance was used to determine whether the linear coefficients of regression for EE on GEI differed between the two breeds and between males and females. Breed was unimportant ($P>0.05$) but there was a significant difference ($P<0.05$) due to sex. It was also evident from observation of the plot of data points for males (Figure 2) that the

relationship was not linear. Various curvilinear functions were fitted to the data from male chickens. The best fit (as determined by R^2 value) indicated an exponential increase in excreted energy with increase in gross energy intake. Put simply, this points to fundamental differences between males and females in their digestive physiology. This conclusion is supported by recent data published by Yaghobfar (2001) who demonstrated differences in EEL according to sex and type of chicken. The estimates for energy voided at fasting were 46 KJ/bird/day for females, and 101 KJ/bird/day for males (Figure 1). These estimates should be verified by further testing at lower levels of energy intake to reduce errors associated with extrapolation.

IV. SEX INFLUENCES GUT STRUCTURE AND FUNCTION

Hughes (2001) estimated that up to one third (33%) of the variation in AME shown in Figure 1 was associated with physical features of the small intestinal mucosa. Ileal crypt depth was the single most important feature of the small intestinal mucosa associated with variation in AME. The breed and sex of chicken significantly affected villus heights of the mucosa in the jejunum and ileum, respectively. Re-modelling of the villus/crypt axis, presumably in response to dietary NSP in the wheat, differed in male chickens depending on breed, but there were no differences observed in female chickens.

Iji *et al*. (2001) observed a greater *in situ* expression of α-glucosidase in jejunal mucosa in female chickens compared with males, irrespective of whether the diet contained a commercial enzyme product with xylanase, glucanase and pectinase activities.

V. SEX AND METABOLIC ACTIVITY OF GUT MICROFLORA

AME and ileal digestible energy (DE) values for a selection of samples of barley, oats, sorghum, triticale and wheat were reported by Hughes *et al*. (2001). AME values for barley and oats exceeded ileal DE by about 0.4 MJ/kg, whereas for sorghum samples, ileal DE was approximately 0.3 MJ/kg higher than AME. Furthermore, the responses differed between males and females. They concluded that microbial fermentation of undigested carbohydrate influenced these results. Hughes *et al*. (2001) reasoned that if microbial overgrowth of viscous digesta in the small intestine can be avoided by use of feed enzymes, then therapeutic use of antibiotics in the feed should have a similar effect by eliminating gut bacteria.

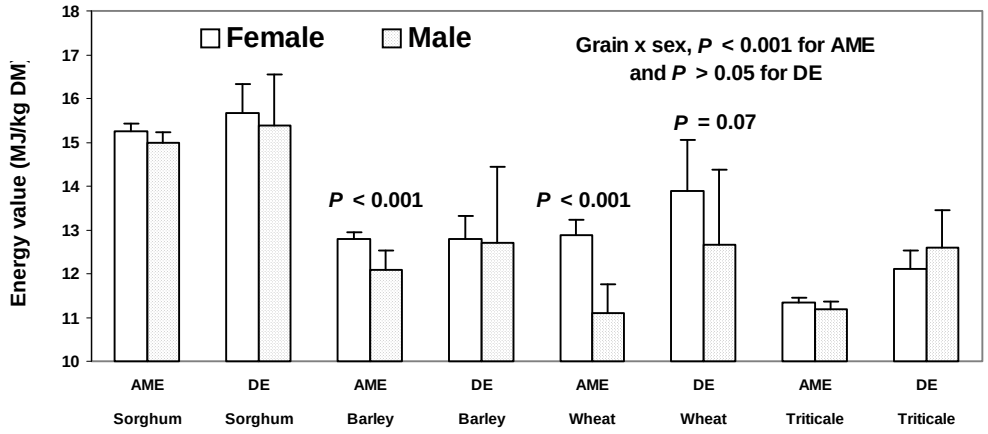

Figure 3. Effects of grain and sex of chicken on AME and ileal DE (means±SD).

Inclusion of antibiotics in the feed had no effect on AME or ileal DE values. The lack of a difference in the DE:GE ratio between males and females on sorghum, barley, wheat and triticale diets (Figure 3) implies that digestive and absorptive processes in the small intestine

were unaffected by the sex of the chicken. On the other hand, male chickens had significantly lower AME values than females when given barley and wheat diets. The differing effects of sex on DE and AME values shown in Figure 3 strongly imply that post-intestinal events associated with gut microflora were affected by the sex of the chicken. Likewise, variation in breath hydrogen concentrations (Figure 4) indicate that gender-of the host animal has a bearing on the metabolic activity of gut microflora, contrary to the expectation that antibiotics would significantly reduce the bacterial population, if not eliminate it.

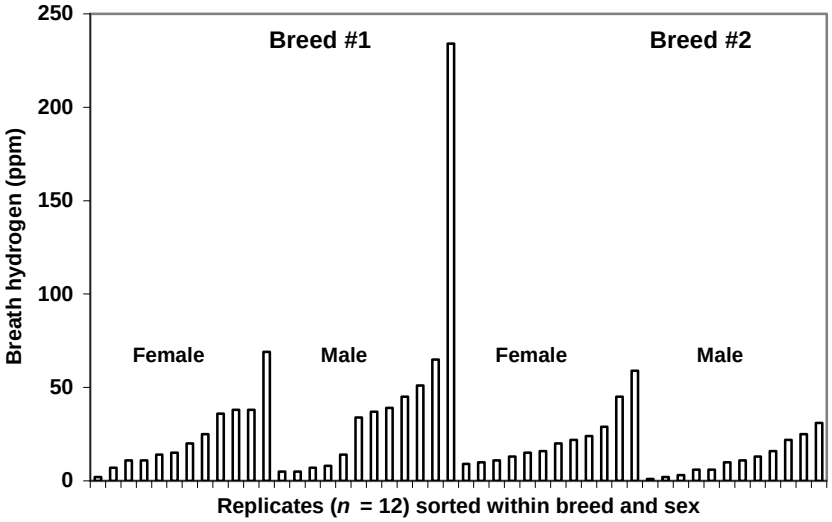

Figure 4. Hydrogen concentration in breath samples taken from chickens given a low AME wheat diet. Each bar in the figure is the result for one chicken.

VI. DISCUSSION

These observations lead to questions about processes at an organ or cellular level that result in marked changes in the numbers, species or activities of the gut microflora according to the nature of the feed consumed and the sex of the host animal. Kelly and King (2001) remarked that the molecular basis for how the gut distinguished between commensal and pathogenic bacteria was poorly understood but that there was "bi-directional communication" between epithelial cells, cells in the mucosal immune system, and gut bacteria. Similarly, Bedford and Apajalahti (2001) referred to a "two-way negotiated process" between host tissue and intestinal microflora.

VII. CONCLUSIONS

Alteration of the balance between the host and its resident microflora (by feeding different grains, enzymes, prebiotics, probiotics and other feed additives) is likely to result in outcomes that are difficult to predict, particularly when antibiotics are no longer added to feed to enhance growth. A fuller understanding of the role of the gut microflora is required.

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BROILER PERFORMANCE IN A SEMI-COMMERCIAL ENVIRONMENT USING DIETS CONTAINING UPPER LEVELS OF CANOLA OR COTTONSEED MEALS

R.A. PEREZ-MALDONADO and K.M. BARRAM

Previous work has shown that, high levels of selected cottonseed meal (CSM) and canola meal (CM) can support satisfactory broiler performance when diets are formulated on a digestible amino acid (AA) basis (Perez-Maldonado *et al.,* 2001). However, these trials were performed on chickens in cages and there is a need to evaluate broiler performance using diets containing practical upper levels of CM or CSM in a semi-commercial environment. The present trial was undertaken to provide the poultry industry with practical recommendations for CM and CSM for chicken meat production.

There were three treatments x 15 replicate pens x 40 birds (20 males and 20 females day old Cobb chicks) in a completely randomised block layout of the 45 pens. Crumbled diets during the starter period were fed from 1 to 21 d old including a control commercial diet, a CM diet (200 g/kg inclusion) and a CSM diet (200 g/kg inclusion). During the finisher period (21-43 d old), inclusion levels of each meal was increased to 300 g/kg and were offered as pelleted diets which were formulated on a digestible amino acid (AA) basis in both periods. The control diet contained sorghum (48%), wheat (20%), soybean meal (20%), meat and bone meal (7%) and poultry offal meal at 2% plus vitamins and minerals. Food, water, light and comfortable temperature were offered *ad libitum* to birds in each pen. Each meal was analysed for proximate and mineral composition, AAs, energy, antinutritional factors, AME and ileal digestible AA as described by Perez-Maldonado *et al.* (2001).

Dietary treatments	FI (g/bird)		LWG (g/bird)		Feed efficiency (g/g)	
	Starter	Finisher	Starter	Finisher	Starter	Finisher
Control	1150	3383[a]	825	1570	1.407	2.169[ab]
CSM Riverina	1134	3451[a]	813	1579	1.408	2.206[a]
CM Numurkah	1130	3263[b]	829	1538	1.372	2.134[b]
LSD (P=0.05)	28	119	19	68	0.038	0.041

[a-b] Means in a column with different superscripts differ significantly (P<0.05).

The results in the starter period indicated that feed intake (FI), liveweight gain (LWG) and feed efficiency were not influenced by inclusion of CSM or CM in the diet. During the finisher period, FI of birds fed on CM was lower (P<0.05) but this did not affect LWG or feed efficiency, which were not different (P>0.05) from the control diet. This semi-commercial broiler experiment indicated that bird production was not affected when fed diets with upper levels of either CSM or CM, confirming our earlier trials carried out in cages. It is concluded that up to 200 g/kg of either CSM (solvent extracted) or CM (solvent extracted or extruded) can be used during the starter phase, and up to 300 g/kg of either CSM (solvent extracted) or CM (solvent extracted or extruded) can be used during the finisher phase in diets formulated on a digestible AA basis. There were no detrimental effects on chickens during the course of this semi-commercial trial. Mortality and culled birds were not related to dietary treatments or leg problems.

Perez-Maldonado, R.A., Blight, G.W. and Pos, J. (2001). *Proc. Aust. Poult. Sci. Symp.*, Ed. D. Balnave.**13**: 156-159.

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COW PEA AND CHICKPEA AS FEED INGREDIENTS FOR LAYERS

D. SINGH, P. TRAPPETT and K.M. BARRAM

Many of the traditional ingredients used in poultry diets are forecast to be in short supply within ten years. The inevitable demand for protein feed is expected to be met largely by legumes. Some legume varieties, show promise as competitive sources of protein for livestock. Although most of the anti-nutritional factors (ANF) in grain legumes can be reduced by appropriate means such as heat treatment or enzyme additives, this is an extra cost that might be avoidable if varieties with low ANF levels can be obtained. The experiment reported here investigated the value for laying hens of two cowpea cultivars (Caloona and Red Caloona) (CP 22.4 & 23.0%) and Dooen chickpea either in entire form or decorticated (CP 17.3 and 19.7%). Each of the cowpea cultivars was included in mash diets (170g CP; 11.5 MJ ME) at 125, 250 and 375 g/kg and the chickpeas were included at 175 and 350 g/kg. A control treatment without grain legumes was included. Each of the eleven diets was fed *ad libitum* to 25 individually caged ISA Brown hens, aged 35 weeks in a 25-block randomised block design. The treatments were continued for a 15-week period.

Legume	Variety	Level (g/kg)	Rate of lay (%)	Egg wt (g)	Egg mass (g/d)	Feed intake (g/d)	FCR (g/g)	Bwt gain (g)	Egg spec gravity	Yolk colour score
Control	-	-	79.11^{b}	66.88	52.91^{abc}	126.7^{a}	2.563^{a}	136^{a}	1.0881^{ab}	12.21^{bc}
Cowpea	Red Caloona	125	83.74^{ab}	66.64	55.80^{ab}	125.4^{a}	2.314^{b}	38^{b}	1.0857^{c}	12.12^{cd}
Cowpea	Red Caloona	250	80.15^{ab}	68.22	54.68^{abc}	124.8^{a}	2.374^{ab}	-34^{bc}	1.0883^{ab}	12.49^{bc}
Cowpea	Red Caloona	375	78.24^{b}	66.78	52.25^{bc}	122.1^{ab}	2.489^{ab}	-112^{c}	1.0894^{a}	13.03^{a}
Cowpea	Caloona	125	82.57^{ab}	67.63	55.84^{ab}	127.7^{a}	2.392^{ab}	102^{ab}	1.0861^{bc}	12.38^{bc}
Cowpea	Caloona	250	84.55^{a}	66.59	56.30^{a}	126.0a	2.297^{b}	-28^{bc}	1.0902^{a}	12.59^{b}
Cowea	Caloona	375	77.66^{b}	67.03	52.06^{bc}	123.4^{a}	2.431^{ab}	-106^{c}	1.0887^{a}	12.57^{b}
Chickpea	Dooen	175	84.51^{a}	66.97	56.60^{a}	128.2^{a}	2.320^{b}	92^{ab}	1.0892^{a}	12.49^{bc}
Chickpea	Dooen	350	80.32^{ab}	66.12	53.11^{abc}	125.0^{a}	2.455^{ab}	-46^{bc}	1.0863^{bc}	12.51^{bc}
Chickpea	Dooen *(hulled)*	175	82.00^{ab}	66.98	54.92^{abc}	122.2^{ab}	2.289^{b}	76^{ab}	1.0890^{a}	11.82^{d}
Chickpea	Dooen *(hulled)*	350	78.28^{b}	65.96	51.63^{c}	116.5^{b}	2.281^{b}	-88^{c}	1.0896^{a}	12.13^{cd}
LSD (P<0.05)			*5.08*	*2.48*	*3.80*	*6.7*	*0.202*	*95*	*0.0023*	*0.41*

[1]Means in a column without a common superscript are significantly different (P<0.05).

There were no differences in average egg weight between treatments. Moderate levels of cowpea or chickpea tended to increase egg production: 250 g/kg Caloona cowpea or 175 g/kg Dooen chickpea resulted in significantly higher (P<0.05) egg numbers than the control diet. Diets containing low to moderate levels of cowpea or chickpea tended to result in higher egg mass output than the control diet or diets containing high levels of these legumes. Egg mass output was significantly lower (P<0.05) with 350 g/kg hulled Dooen chickpea than with several other treatments (excluding the control), and higher (P<0.05) with 175 g/kg Dooen chickpea or 250 g/kg Caloona cowpea than with 375 g/kg cowpea (of either type) or 350 g/kg hulled chick pea. Feed intake of birds given 350 g/kg hulled Dooen chickpea was lower (P<0.05) than that of most other groups (including the control). Significantly better feed efficiency than the control (P<0.05) was obtained with 125 g/kg Red Caloona, 250 g/kg Caloona, 175 g/kg entire or hulled Dooen and 350 g/kg hulled Dooen. Body weight gain consistently declined with increasing dietary levels of legumes. The results overall suggest that feeding levels of 250 g/kg cowpea and 175g/kg Dooen chickpea in the mash form supports good production in layers.

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EFFECT OF CHALLENGE WITH T-STRAIN INFECTIOUS BRONCHITIS VIRUS ON EARLY LAY PULLETS AFTER DIFFERENT VACCINATION HISTORIES

M.J. JOLLY, J.R. ROBERTS and W. BALL

Summary

The effect of T-strain infectious bronchitis virus on early lay pullets that had received different first vaccinations, and their third vaccination at either 14 or 18 weeks, was examined. For the whole flock there was an immediate decline in egg internal quality after challenge with the virus. Shell quality also declined as evidenced by both objective measurement and visual assessment. There were no major effects due to the strain of vaccine that the birds received at day old, although there were some effects of vaccine protocol. The revaccination of birds at 5% lay (18 weeks old) resulted in an increased decline in egg quality measurements when they were challenged with T-strain. This flock is continuing to be monitored to assess long-term effects.

I. INTRODUCTION

Infectious Bronchitis is a highly contagious disease, which occurs worldwide in commercial poultry flocks affecting bird health and production. Currently all commercial flocks in Australia are vaccinated against Infectious Bronchitis Virus (IBV) using a variety of live vaccine strains and vaccination methods. At present the majority of industry vaccinate birds at day old and twice more before lay, or as lay has commenced. However, there are still regular outbreaks of the disease, even in vaccinated flocks (Cavanagh and Naqi, 1997). IBV is capable of producing declines in egg production and quality within a flock, without any other clinical signs being evident (Cook and Huggins, 1986). If pullets are affected early in lay they often experience less of a reduction in quantity (Hofstad, 1972) and more of a reduction in quality of eggs. The present study aimed to quantify the decline in egg quality that occurs when early lay pullets are exposed to the nephropathogenic T-strain IBV. In addition, measurements were taken to estimate the changes in the physiological state of these young birds across the time period of an IBV infection. The birds challenged had received one of two vaccine strains at day old and two other vaccinations, with the third vaccination applied at one of two different stages in the development of the bird.

II. MATERIALS AND METHODS

Day-old commercial laying pullets were transferred to the isolation pens at the University of New England. On arrival the birds were divided into two groups, before being vaccinated by coarse spray against IBV, half (50 birds) received the A3 vaccine strain and the others VicS strain. The pullets were then raised on the floor of the isolation pens and vaccinated again at four weeks of age. All birds vaccinated with VicS at day old and half of those who received A3 were revaccinated with VicS; the remaining birds received A3. At 14 weeks of age the treatment groups were divided again with half being vaccinated at this time and the other half when they had reached 5% lay (18 weeks of age). At both times the vaccine strain used was the same as at 4 weeks.

At 25 weeks of age, the pullets were exposed to T-strain IBV. Blood samples were taken from the same 10 birds in each treatment group at two weeks prior to and then two, three, four and five weeks after exposure. The blood samples were analysed for plasma electrolytes (Na^+, K^+ and Ca^{++}) and haematocrit. Eighty eggs were collected (20 per treatment

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group) and analysed at one week before, then one, two, three, four, five and 10 weeks after challenge. Eggs were tested for both internal and external quality measures. At two weeks post challenge two birds per group were euthanased and both kidneys removed, weighed and calculated as a percentage of body weight. The kidneys, trachea and oviduct were removed for histological analysis at a later date. Daily egg production records were kept for all groups. External visual classification of shell defects was carried out daily recording all abnormalities.

Analysis of Variance was used to test the effect of challenge on each of the egg quality, blood analysis and kidney weight parameters measured. Claims of statistical significance were based on $P<0.05$. Fishers protected LSD was used to distinguish between means when significant effects were seen.

III. RESULTS

Egg Production and External egg classification

There were no observable effects of challenge on total egg production. Total egg abnormalities increased after exposure to T-strain IBV until 2 weeks post challenge. The highest daily percentage of eggs laid that had a visually obvious abnormality was 57%, recorded at 13 days after exposure to the virus. By the eighth week after IBV challenge, the percentage of abnormal eggs had returned to pre-virus levels. The incidence of one particular recorded abnormality, fine longitudinal wrinkles, increased from 0 to over 25% of the eggs produced (Figure 1).

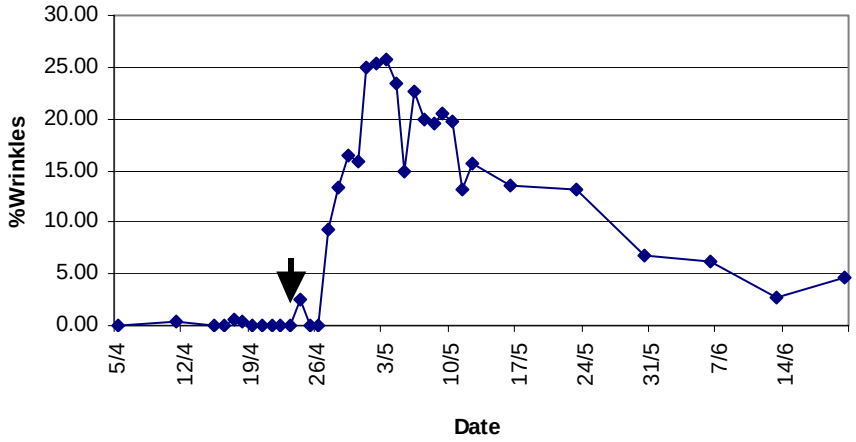

Figure 1. Percent of eggs produced that were wrinkled.

Egg Quality

Yolk colour and deformation were not affected by infection with T-strain IBV. Egg weight and shell weight increased significantly and steadily across the challenge period. Shell thickness remained constant during weeks 1-3 after challenge with T-strain, and then began to increase. At one-week post infection, breaking strength of the eggshell was significantly reduced, but had returned to pre exposure levels 10 weeks after challenge. Shell reflectivity was significantly increased by challenge with T-strain and it remained high. There was a significant drop in Haugh units measured at one week after challenge (Table 1). Haugh units for the group with the A3/VicS/VicS vaccination protocol were significantly higher than the A3/A3/A3 group ($P=0.0004$). Egg weight and Haugh units were significantly lower for the birds that were vaccinated late at 18 weeks of age. Shell reflectivity and percentage shell were higher for this treatment group (Table 2).

Kidney weights

At two weeks after exposure to T-strain IBV, kidney weight as a percentage of body weight was not significantly different among treatment groups.

Table 1. Effect of T-strain IBV challenge on egg quality parameters

	Before	After Exposure					
	1 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks	10 Weeks	P Value
Haugh Unit	[a]103.64 ± 4.21	[d]96.73 ± 4.12	[b]99.95 ± 4.85	[c]98.48 ± 3.91	[d]95.99 ± 4.25	[d]96.58 ± 5.29	[e]94.51 ± 5.80	<.0001
Reflect %	[c]23.46 ± 3.73	[b]28.73 ± 4.69	[b]28.71 ± 5.81	[a]30.53 ± 5.35	[ab]29.40 ± 4.75	[ab]29.03 ± 5.16	[a]30.42 ± 5.81	<.0001
Shell Wt. g	[d]5.56 ± .41	[c]5.76 ± .46	[c]5.86 ± .44	[c]5.84 ± .43	[b]6.02 ± .33	[ab]6.13 ± .47	[a]6.26 ± .47	<.0001
Shell thick. µm	[b]430.65 ± 22.42	[c]427.23 ±23.39	[bc]430.42 ± 23.82	[c]424.07 ± 19.50	[ab]436.89 ± 22.15	[b]435.91 ± 22.61	[a]443.33 ±25.95	<.0001
B.S. N	[ab]44.81 ± 7.38	[d]40.56 ± 6.90	[bc]43.24 ± 9.33	[cd]41.71 ± 7.79	[bcd]42.33 ± 7.75	[cd]41.45 ± 9.37	[a]45.87 ± 8.11	0.0002

Mean ± SE. Means in rows with no common superscript differ significantly (P<0.05)

Table 2. Effect of timing of third vaccination on egg quality parameters under the stress of challenge

	Early	Late	P Value
Haugh Units	[a]99.29± 5.48	[b]96.75± 5.14	<.0001
Reflect %	[a]28.00± 6.10	[b]29.17± 4.86	0.0086
Egg Wt g	[a]59.92± 4.63	[b]58.21± 4.47	<.0001
% Shell	[a]9.92± 0.64	[b]10.15± 0.66	<.0001

Mean ± SE. Means in rows with no common superscript differ significantly (P<0.05)

Blood

Blood analysis results across all vaccination groups over the duration of infection are shown in Table 3. Haematocrit increased at 2 weeks, declined, then increased steadily. Plasma sodium, calcium and potassium are significantly lower at 2 weeks post-challenge. Potassium levels in the blood were also lower at 4 weeks after infection. There were no significant differences resulting from vaccine strain used for first vaccination or timing of the third vaccination. Plasma sodium and calcium were significantly higher in the A3/A3/A3 group than the VicS/VicS/VicS group ([Na] P=0.003, [Ca] P=0.0003).

Table 3. Effect of challenge on blood analysis

	Before	After Exposure				
	2 weeks	2 weeks	3 weeks	4 weeks	5 weeks	P Value
Hct %	[c]20.95± 2.11	[b]27.89± 2.14	[bc]27.24± 1.94	[ab]28.01± 1.40	[a]28.81± 2.16	0.0006
[Ca] mM	[b]1.77± 0.09	[c]1.69± 0.10	[b]1.75± 0.10	[a]1.82± 0.12	[a]1.81± 0.11	<.0001
[Na] mM	[a]150.95± 2.40	[b]142.62± 3.21	[a]149.67± 2.25	[a]149.7± 3.15	[a]150.47± 4.11	<.0001
[K] mM	[a]5.71± .29	[c]5.16± .32	[a]5.70± .34	[d]5.00± .27	[b]5.43± .38	<.0001

Mean ± SE. Means in rows with no common superscript differ significantly (P<0.05)

IV. DISCUSSION AND CONCLUSIONS

The dramatic increase in the number of abnormal eggs was largely due to an increased incidence of eggs with wrinkled shells. These wrinkles were not the large undulations that are commonly referred to as an "IB egg" but ran the length of the egg and were no greater than 1mm in width. Wrinkled eggs are an indication that inflammation of the tissues of the oviduct, which occurs during T-strain infection, results in inadequate "plumping" of the egg and therefore a wrinkled appearance of the finished shell.

A steady increase in both egg weight and shell weight would be expected at this stage of lay. However, both quality parameters, particularly shell weight, appear to have experienced a delay in rate of increase across weeks one, two and three post-challenge. This slight, but significant ($P<0.0001$), effect corresponds to the leveling off of the normally expected increase in shell thickness during this period. Shell breaking strength decreased significantly at one week after infection and remained low but had returned to previous strength 10 weeks after challenge. Shell reflectivity was markedly increased (shell colour was paler) by exposure to T-strain and this effect was sustained. Paler shell colour may be due to extra calcium deposits laid down in the shell gland on the outside of an otherwise completed egg, another indication of a malfunctioning oviduct. Across the weeks after infection with T-strain IBV the Haugh units were seen to trend downward slowly, as would be expected at this stage of lay. However, at one week after challenge there was a significant drop in albumen height and therefore Haugh units. This drop in Haugh unit was expected, as IBV is known to cause a thinning of the albumen. However, the investigator noted that there was a decline in the quality of the thin albumen in the eggs for two weeks after infection with IBV. The birds that were vaccinated with A3 at day old and then VicS for the two revaccinations had higher Haugh Units than those that received three A3 vaccinations. This suggests that, as the birds reach point of lay, VicS has less effect on the oviduct than A3. The significant effects due to the timing of the birds' third IBV vaccination (Table 2) are decreased egg quality in the late vaccinated birds, compared to those vaccinated for the third time well before lay had commenced. Hence it can be concluded that vaccination with a live IBV vaccine virus at the onset of lay, when the oviduct is rapidly developing, may lead to damage of the oviduct and therefore a decrease in the quality of eggs produced. T-strain IBV is known as a nephropathogenic strain of the virus that causes a marked nephritis. While gross damage to the kidneys was not apparent in the kidneys of the euthanased birds, the changes in the plasma electrolyte levels are probably due to excess excretion of electrolytes. The differences recorded in the blood electrolytes among the different vaccination protocols may reflect different effects of the two vaccine strains on the kidneys of the birds.

In conclusion, exposure to T-strain IBV at 25 weeks of age had deleterious effects on egg quality and blood electrolytes, particularly if the birds had received their third IBV vaccination at the onset of lay.

V. ACKNOWLEDGEMENTS

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EFFICACY OF DIFFERENT DISINFECTANTS FOR THE CONTROL OF DISEASES

R.R. BRAGG

Summary

A number of different chemicals frequently used for disinfection in the poultry industry were compared to each other, using contact plates on hard surfaces. Of all the products tested, the newly developed boosted Didecyldimethylammonium chloride (DDAC) was found to be the most effective product. The efficacy of this boosted DDAC for the control of specific diseases under experimental or field conditions was also evaluated. The non-toxic boosted DDAC was tested as a means of limiting the impact of Infectious Coryza in experimentally infected chickens. It was demonstrated that the use of this disinfectant resulted in less severe clinical signs in chickens challenged with each of the different serovars of *Haemophilus paragallinarum.* It has been demonstrated that the use of this boosted DDAC on a commercial poultry farm prevented the spread of Newcastle Disease during a naturally occurring outbreak.

I. INTRODUCTION

Disease remains a limiting factor in poultry production in many parts of the world. Two of the more serious diseases are Newcastle Disease (ND) caused by Newcastle Disease virus and Infectious Coryza, caused by *H. paragallinarum.* Control of infectious diseases has mainly been through the use of antibiotics to control bacterial diseases and vaccines for both viral diseases and some bacterial diseases. There are, however, increasing problems with both of these approaches. There is concern about antibiotic resistance and many countries are investigating limiting the use of antibiotics in animals and animal feeds. There is also growing evidence that the selection of more virulent vaccines is selecting for more virulent field isolates of viral diseases. Consequently there is increasing emphasis on the use of disinfectants for the control of infectious diseases.

A novel formulation of a boosted DDAC based product has been developed and extensively tested, both *in vitro* and *in vivo* under experimental conditions and commercial conditions. Part of this development was the establishment and testing of a continuous disinfection program (Bragg & Plumbstead, 2003). This consisted of disinfection at clean out, continuous disinfection of the drinking water and daily spraying or misting with the boosted DDAC product. It was demonstrated that this program significantly reduced the incidence of disease in the experimental group of birds and consistently improved feed conversion ratios in the treated birds.

II. METHODS

a) Evaluation of different products on hard surfaces

Efficacy of different products used for disinfection in poultry houses was done in experimental poultry pens in which the floors were artificially inoculated with a combination of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Each pen was inoculated with the same volume of the combined bacterial solution. Once the floor had dried, washing and disinfection of the pens commenced, with each product being used according to

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label instructions One pen was used for each product under test. A total of 10 contact plates were collected from each of the pens before any treatment was undertaken.

These plates were incubated at 37^{O}C overnight and all colonies on plates with less than 300 colonies were counted. If there were more than 300 colonies on a plate, it was recorded as "Too many to count" or TMTC and a number of 300 was used for calculation purposes. Washing of the pens was performed with either detergent soap or diluted disinfectant. Once the floor was dry, another 10 contact plates were collected from each pen and were processed according to the methods described above after which each pen was disinfected with the different products. Once the floors were dry another 10 contact plates were collected from each pen and processed according to the methods described above.

b) Experimental infection of chickens with Infectious Coryza

Layers, which had been vaccinated with an inactivated IC vaccine, were obtained from a commercial poultry farm at 18 weeks of age. Another group of unvaccinated birds were obtained from the same source at 11 weeks of age, before vaccination against IC had taken place. Half of the vaccinated and unvaccinated birds was continuously treated with the Boosted DDAC product in the drinking water at 100 ppm dilution and were sprayed daily with a 1% dilution of the same product. All remaining birds from each challenge group were left as untreated controls. When the birds were 25 weeks old, each group (of 10 birds per group) was challenged with the different serovars of *H. paragallinarum*, according to the challenge model established by Bragg (2002). The clinical signs in each of the groups of birds were scored daily for 20 days according to the methods described by Bragg (2002) and a disease profile and mean disease score for each group was obtained. A comparison of the clinical disease in the treated and untreated groups of birds was made from the mean disease scores.

c) Newcastle Disease challenge on a commercial farm.

A large-scale experiment was undertaken to evaluate the continuous disinfection program on a commercial poultry farm. Three identical poultry houses, each housing 5000 broilers, were used in this experiment. One house was a control house which received only pre-placement disinfection with a gluteraldehyde based product. The two experimental houses were disinfected with the boosted DDAC product before placement of the birds. The drinking water in these houses was continually treated with a 100 ppm dilution of the boosted DDAC product. The air in these two houses was also disinfected with a 1% dilution of the same product on a daily basis. Daily mortalities and other production parameter readings were recorded daily. During this experiment, a severe outbreak of ND occurred on the farm and treatments were terminated on day 20.

III. RESULTS

a) Evaluation of different products on hard surfaces

The bacterial counts on the contact plates collected from the different pens can be seen in Table 1. These results are the mean bacterial counts from all 10 plates collected from each pen.

Table 1. Mean numbers of bacterial colonies on 10 contact plates collected from each of the different pens before treatment, after washing and after disinfection with different products. (Standard error in brackets)

Active of products used	Mean count before treatment	Methods of washing	Mean count after washing (Standard error)	Methods of disinfection	Mean count after disinfection (Standard error)
Boosted DDAC	300*	100 ppm of product	26.0 (2.1)	1: 100	11.2 (1.0)
Peroxygen Acid	300	1:1000 detergent soap	300	1: 100	25.8 (2.2)
20% Glut#.	300	1:1000 detergent soap	300	1: 200	80.8 (5.2)
12.5 % Glut.	300	1:1000 detergent soap	300	1: 128	172.3 (32.2)
Iodine based product	300	1:1000 detergent soap	300	1: 100	132.4 (7.1)
Mixture of Glut and QAC	300	1:1000 detergent soap	300	1:200	56.8 (6.1)
Phenol bases products	300	1:1000 detergent soap	300	1:100	95.0 (7.7)

* Plates marked as TMTC.

\# Glutaraldehyde

b) Experimental infection of chickens with Infectious Coryza

Table 2. Mean disease scores (calculated from the mean daily disease score over 20 days) obtained when vaccinated and unvaccinated layers were challenged with the different serovars of *H. paragallinarum* and treated with the full continuous disinfection program, or not treated. (Highest recorded daily disease score in brackets). Mean disease scores of 0.00 represent no clinical signs at all, with a mean disease score of 6 being the highest possible score.

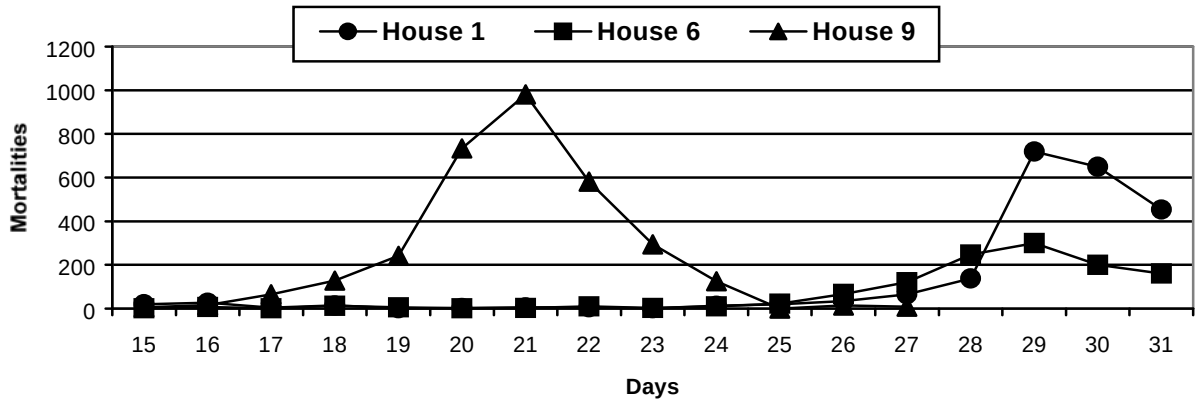
Serovar used for challenge	Vaccinated birds		Unvaccinated birds	
	Treated *	Untreated	Treated	Untreated
A-1	0.03 (0.2)	0.12 (0.6)	0.16 (0.8)	0.21 (1.2)
B-1	0.01 (0.2)	0.02 (0.2)	0.03 (0.4)	0.08 (0.4)
C-2	0.15 (2.0)	0.87 (2.4)	1.25 (3.2)	1.69 (4.8)
C-3	0.30 (0.8)	0.43 (1.2)	2.01 (5.2)	2.45 (6.2)

* Treated with full continuous disinfection program which consisted of continuous drinking water treatment with 100 ppm of the boosted DDAC product and daily spraying with a 1% dilution of the same product from placement date.

c) Newcastle Disease challenge on a commercial farm.

The objective of this project was the evaluation of the continuous disinfection program on a commercial farm under normal circumstances. The outbreak of Newcastle disease on this farm was not expected. Other houses on the site showed clinical signs of ND on Day 15 of this experiment. Graphical representations of the daily mortalities from Day 15 are presented in the Fig 1.

Fig 1. Graphic representation of the total number of mortalities in the three experimental houses after the first clinical signs of ND was recorded on the farm. House 1 and 6 were test houses while House 9 was the control house



IV. DISCUSSION

a) Evaluation of different products on hard surfaces

It can be seen from Table 1 that the lowest number of bacteria surviving after disinfection was found in a newly developed product containing a boosted DDAC as active ingredient. This product was consistently found to be the most effective in all subsequent similar experiments performed in different countries around the world.

b) Experimental infection of chickens with Infectious Coryza

It can be seen from Table 2 that the mean disease score was highest in the birds challenged with serovar C-3. In all cases, the mean disease score for the treated birds was lower than that of the untreated birds. It was also found (data not shown) that the duration of infection was significantly reduced in the treated group of birds. The results of this experiment demonstrates that the full continuous disinfection program with the boosted DDAC product reduced the impact of Infectious Coryza caused by all four serovars in vaccinated and unvaccinated birds.

c) Newcastle Disease challenge on a commercial farm.

It can be concluded from this data that a full continuous disinfection program with a novel boosted DDAC prevented the spread of Newcastle disease in the two experimental houses on a commercial farm. Clinical signs of Newcastle Disease was seen in these two houses four days after the full continuous disinfection program was stopped.

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IN VITRO EVALUATION OF BINDING ABILITY OF MODIFIED MANNANOLIGOSACCHARIDE, INACTIVATED YEAST AND UTPP ON AFLATOXIN B1 IN LIQUID MEDIA

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Summary

The *in vitro* binding efficiency of modified-mannanoligosaccharide (M-MOS) (0.1%) inactivated yeast (0.1%) and UTPP (aluminosilicate with organic acids) (0.5%) on aflatoxin B_1 in liquid media was evaluated at two pH levels (4.5 and 6.5) under simulated in situ conditions of Gl tarct of chicken. The aflatoxin B_1 levels were 100 and 200 ppb which were tested with binders at two pH levels on triplicate samples. Based on the aflatoxin B_1 recorded after the incubation and centrifugation, the supernatant of treated and control flasks were taken and the adsorption percentage was calculated. Modified-MOS showed significantly $P \leq 0.01$ higher binding ability (93.82%) compared to inactivated yeast and UTPP, (62.26 and 72.02%, respectively). Higher significant binding ability was recorded at higher level of aflatoxin and pH 6.5 for all the three binders tested.

I. INTRODUCTION

Several approaches have been tried to detoxify aflatoxins in feeds and feed ingredients. One promising approach is the use of aluminosilicates in feed (Phillips *et al.,* 1988; Doerr, 1989 Kubena, 1990; Sheila, 1993). Aluminosilicates have high adsorption properties with cationic exchange constituent without altering the structure, they possess active sites which can interact and immobilize certain molecules via electrostatic forces or by formation of covalent bonds.

Live yeast (*Saccharomyces cerevisiae*), Lactobacillus spp. and other bacterial and fungal spp. have been tried to reverse the adverse effects of aflatoxicosis (Ciegler *et al.,* 1966; Stanley *et al.,*1993; Trenholm *et al.,* 1994; Morton, 1996). Among these, live yeast has shown promising results. The aflatoxin counteracting ability of live yeast is attributed to the mannanoligosaccharide, present in its cell wall. The specific objective of this study was to compare the ability of modified mannanoligosaccharide (M-MOS), inactivated yeast and aluminosilicate with organic acids (UTPP) to bind aflatoxin B1 *in vitro*.

II. METHODS

The concentrations of the binding agents used in the study were; M-MOS (0.1%), inactivated yeast (0.1%) and UTPP (0.5%) in liquid media (buffer solution). The aflatoxin B1 standards (100 and 200 ppb) were dried in conical flasks and each binder was added separately to all the flasks containing both levels of aflatoxin, along with 50 ml of buffer solution of desired pH (4.5 or 6.5). The test was run on triplicate samples and controls were maintained for each toxin level. The samples (Toxin + Binder + Buffer) were mixed on a horizontal shaker for 30 minutes to facilitate contact of binder with toxin. Samples were then incubated for three hours at 37^0C. After incubation , the samples were centrifuged at 1500 rpm for 10 minutes to remove sediments which contained binder and bound toxin, the

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supernatant was extracted for its aflatoxin content using CB method (A.O.A.C., 1990). Aflatoxin concentration was estimated by TLC (Romer, 1975). The percentage values were changed to acsine values and the data subjected to ANOVA of the 3 × 2 × 2 factorial design using the General Linear Models procedures of the statisical analysis system (SAS lnstitute, 1987). The New Duncan Multiple Range test was employed for comparison of means (Duncan, 1955).

III. RESULTS

The results of aflatoxin binding by M-MOS, inactivated yeast and UTPP at two of aflatoxin and two pH levels are presented in Tables 1, 2, 3 and 4.

Modified-MOS showed significantly (P<0.01) higher aflatoxin binding (93.82%) in comparson to inactivated yeast (62.67%) and UTPP (73.02%) (Table 1). UTPP showed significantly, (P<0.01) higher binding than inactivated yeast.

Table 1. Per cent aflatoxin binding by M-MOS, Inactivated yeast and UTPP in liquid media

Binder	PH	Aflatoxin ppb	Binding %	Binders × pH Mean ±SE	Binder Mean ± SE
Modified-MOS (0.1%)	4.5	100	90.28	91.97±0.81	93.82[a]±0.69
		200	93.67		
	6.5	100	96.25	95.67±0.26	
		200	95.10		
Inactivated yeast (0.1%)	4.5	100	61.33	62.33±0.80	62.67[c]±0.94
		200	63.33		
	6.5	100	59.00	63.00±1.79	
		200	67.00		
UTPP (0.5%)	4.5	100	70.10	71.55±0.64	73.02[b]±0.82
		200	73.00		
	6.5	100	72.00	74.50±1.31	
		200	77.00		

abc: Means having same superscript do not differ significantly (P<0.01)

The levels of aflatoxin had a significant influence (P<0.01) on the amount of the toxin bound (Table 2). All three binders showed higher binding values at the higher level of aflatoxin (200ppb) than at the lower level (100 ppb).

Aflatoxin binding was significantly (P<0.01) influenced by the pH of the medium (Table 2). Higher binding of aflatoxin (77.72%) was seen at pH 6.5 than at pH 4.5 (75.28%).

Table 2. Mean values of binders at levels of aflatoxin bound at different aflatoxin concentrations (aflatoxin 100 and 200 ppb) and pH (6.5 and 4.5)

Aflatoxin (ppb)		pH	
100	200	4.5	6.5
74.82[b] ± 3.37	78.18[a] ± 2.98	75.28[b] ± 3.03	77.72[a] ± 3.35

ab: means having same superscript within each row and factor do not differ significantly (P<0.01)

Significant (P<0.01) interaction was noted between the binders and the level of aflatoxin (Table 3). Level of aflatoxin in media had significant influence on the binding abilities of inactivated yeast and UTPP, while no such trend was seen with M-MOS. On the other hand, percent binding of aflatoxin by inactivated yeast and UTPP was significantly (P<0.5) higher at 200 ppb level of aflatoxin than at 100 ppb level.

Table3. Interaction between the various binders and aflatoxin levels for per cent binding

Aflatoxin Ppb	Binders		
	Modified-MOS	Inactivated yeast	UTPP
100	93.3^{aA}	60.2^{bC}	71.1 ^{bB}
200	94.4 ^{aA}	65.2 ^{aC}	75.0 ^{aB}

ab: means having same superscript within each column do not differ significantly (P<0.01)
ABC: means having same superscript within each row do not differ significantly (P<0.01)

The liquid media pH and binders also showed significant interaction (P<0.01) for binding percentage (Table 4). Modifiled-MOS and UTPP showed significantly higher binding percentage (95.7 and 74.5, respectively) at pH 6.5 than at pH 4.5 (92.0 and 71.5%, respectively), while the binding percentage for inactivated yeast at both pH levels was statistically not different.

Table 4. Interaction between the various binders and pH level for percent aflatoxin binding

PH	Binders		
	Modified-MOS	Inactivated yeast	UTPP
4.5	92.0^{bA}	62.3^{aC}	71.6 ^{bB}
6.5	95.7 ^{aA}	63.0 ^{aC}	74.5 ^{aB}

ab: means having same superscript within each column do not differ significantly (P<0.01)
ABC: means having same superscript within each row do not differ significantly (P<0.01)

IV. DISCUSSION

The results of this study show that more aflatoxin is bound to binder at higher compared to lower concentrations of the toxin. This may be due to an increase in the availability of substrate for binding to binders as supported by the findings of the present studies and also the study conduted by Mahesh and Devegowda (1996a,b).

The results obtained in the trial with UTPP are in close agreement with those reported by Mahesh and Devegowda (1996b). However, the percent binding recorded with M-MOS in this trial (93.82%) is marginally higher than that observed by Mahesh and Devegowda with MOS (85.12%). This increase in binding with M-MOS over that of MOS might have been due to its modified chemical nature (esterified glucomannan).

Trenholm (unpublished as reported by Devegowda *et al*., 1998b) also recorded higher binding of aflatoxin with M-MOS than with aluminosilicate. However, the total binding percentages reported by him are higher those observed in this study. This may be due to the differences in the methodology applied in the determination of *in vitro* binding.

Morton (1996) while working with live yeast, recorded 56 percent degradation of aflatoxin at 48 hrs of incubation in Sabbaroud's broth cultures employing 250 ppb aflatoxin.

This value is close to the mean binding percentage (62.7) observed with inactivated yeast in the present trial. Higher binding percentage were observed at pH 6.5 than at 4.5 with modified-MOS. Further, Trenholm *et al.* (1994) also reported higher binding of zearalenone by modified oligosaccharide from yeast cell wall at pH 9 than at pH 4 in aqueous solutions.

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DIFFERENTIATION OF MAREK'S DISEASE VIRUS SEROTYPES USING PCR: RESEARCH AND FIELD EXPERIENCE

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Summary

PCR assays for serotypes 1, 2 and 3 Marek's disease virus (MDV) and their performance under experimental and field conditions, are described. The assays appear to be serotype-specific and are of broadly similar sensitivity as virus isolation and identification with immunostaining. They offer significant advantages over virus isolation in speed and cost of diagnosis, handling and storage of tissues for assay, and ability to discriminate between serotypes in mixed infections. Limitations include the ongoing requirement for invasive tissue sampling, the lack of quantification of viral load, inability to detect early MDV-1 infections in HVT-vaccinated birds and the inability to differentiate between oncogenic and attenuated vaccinal strains of MDV-1.

I. INTRODUCTION

Three serotypes of Marek's disease virus (MDV) are recognized. Serotype 1 MDV (MDV-1) comprises oncogenic MDV viruses of chickens and their attenuated vaccinal forms. Serotype 2 MDV (MDV-2) comprises non-oncogenic chicken strains and Serotype 3 MDV (MDV-3) comprises non-oncogenic turkey strains, also referred to as herpesvirus of turkeys (HVT). The latter two are widely used as Marek's disease (MD) vaccines alone, in combination with each other, or in combination with attenuated MDV-1 vaccines. Control of MD relies heavily on vaccination with live viral vaccines. In Australia, monovalent vaccines have been the rule to date, but it is likely that the use of bivalent and even trivalent vaccines will increase in the future, a practice already prevalent in the USA. Vaccination does not prevent infection with wild type MDV viruses, so birds may harbour admixtures of vaccinal and wild MDV.

Serotype-specific identification of MDV is of importance to poultry producers for both determining the efficacy of vaccination, and for the diagnosis of MDV infection. MDV can be isolated in cell culture but distinction between the different MDV serotypes on the basis of induced cytopathy is not reliable, so immunostaining of cultures with serotype-specific monoclonal antibodies is typically practiced to arrive at a serotype-specific diagnosis. The cell culture and immunostaining procedure is lengthy (typically 7-10 days), complex and costly. The requirement to maintain infectivity of the material to be tested also poses difficulties in the collection and transportation of samples, and precludes their easy storage for subsequent testing.

In this paper we report our experience with the use of serotype-specific PCR assays for MDV-1, MDV-2 and MDV-3 detection in both research and in industry.

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II. MATERIALS AND METHODS

The serotype 1 PCR assay, modified slightly from that described by Islam *et al*., (2001) utilised forward (5'-CAT GCA AGT CAT TAT GCG TGA-3') and reverse (5'-TGT TTC CAT TCT GTC TCC AAG A-3') primers to amplify a 199bp fragment of the glycoprotein A gene. The assay, utilising extracted DNA template from frozen peripheral blood lymphocytes (PBL) or whole spleen tissue, was run in a 50µl PCR reaction using 40.75µl milliQ water, 5µl 10xPCR buffer (QIAGEN, Pty. Ltd, Vic.), 1µl dNTPs (2mM), 1µl each of the two primers (10pmol/µl), 1µl Taq polymerase (QIAGEN, Pty. Ltd, Vic.) and 1µl template. The reaction mixture was overlaid with 30µL of mineral oil, started at 94˚C for 4 minutes followed by either 40 amplification cycles of 1 minute at 94˚C, 1 minute at 55˚C and 1 minute at 72˚C, followed by a final extension at 15˚C for 10 minutes and hold at 4˚C. The PCR products were analysed by electrophoresis on a 1.2% agarose gel with TBE buffer containing GelStar Nucleic Acid Gel Stain (FMC BioProducts) and bands were visualized under ultra-violet light.

The serotype 2 PCR assay utilised forward (5'-CCG TTT AGC GCG ATG CTG T-3') and reverse (5'-GAA CGC CAG TAC GAT CGC C-3') primers designed by Lee *et al.* (1999) to amplify a 210bp fragment of the glycoprotein B gene from the nucleotide sequence of the MDV-2 strain SB-1 reported by Yoshida *et al.* (1994). The assay was run in identical fashion to the serotype-1 PCR with the exception that reaction was started at 94˚C for 5 minutes and run for 36 cycles of 1 minute at 94˚C, 1 minute at 53˚C and 2 minutes at 72˚C. Final extension was at 72˚C for 3 minutes followed by hold at 4˚C.

The serotype 3 PCR assay utilised our own forward (5'-CGC CTA CGA CCG AAT TAT CTA C-3') and reverse (5-'GTA TTC GAT TGG ATT GAA CGT G-3') primers designed to amplify the glycoprotein C gene of HVT based on the published sequence of Kitazawa *et al.* (1993) The assay was run in identical fashion to the serotype-1 PCR with the exception that reaction was started for 3 minutes at 94˚C and run for 40 cycles of 1 minute at 94˚C, 1 minute at 58˚C and 1 minute at 72˚C followed by a final extension at 72˚C for 7 minutes and hold at 4˚C.

Samples from chickens came from a range of experiments or from the field, and generally comprised either PBL derived from 1-2ml of fresh blood in K_3EDTA, or derived from 10mg whole frozen (-20˚C) spleen tissue. PBL were separated using the Ficoll-paque method and treated with Proteinase K to extract the DNA as described by Islam *et al.* (2001). DNA was extracted from spleen samples using the DNeasy commercial kit (QIAGEN, Pty. Ltd, Vic.).

Reference strains of MDV were either commercial vaccines, kindly provided by the manufacturers, or field isolates kindly provided by Professor Greg Tannock, RMIT University, Melbourne. Isolation of HVT was by culture on CEF as described by Islam et al. (2001).

III. RESULTS AND DISCUSSION

The specificity (ability to detect only serotype-specific MDV) of the serotype-specific PCR assays described is summarised in Table 1. The assays appear capable of distinguishing between the 3 main serotypes of MDV, but not between virulent and attenuated MDV-1. The identification of MPF 147/13 as a serotype 2 virus is not inconsistent with the RMIT tests for this isolate (H19 mab negative). The other MDV-1 and MDV-3 positive results amongst the RMIT isolates are likely to be vaccinal contaminants.

The sensitivity of the MDV-1 PCR has been reported to be slightly, but not significantly, greater than that provided by virus isolation and immunostaining (Walkden-

Brown *et al.*, 2001). No direct comparison has been made between the MDV-2 PCR and virus isolation, but 3/4 broilers vaccinated with MDV-2 vaccine (Maravac, Fort Dodge) were positive to the MDV-2 PCR of spleen at 35d of age in one of our experiments while limited field data include 0/9 MDV-2 vaccinated birds positive at day 6 and 2/7 positive at day 38. For the MDV-3 assay, PCR detection from spleen in birds with a mixed MDV-3/MDV-1 infection was more sensitive than virus isolation from spleen, but comparable with isolation from PBL (Table 2).

Recent field experience with the PCR detection of MDV-3 and MDV-1 in broiler chickens using these assays is summarised in Tables 3 and 4 respectively. With regards to field prevalence of MDV-2 as determined by PCR, 0/30 unvaccinated sentinel broilers on three Tamworth, NSW farms (15 samples/farm) were positive for MDV-2 at day 35 of age.

Table 1. Specificity of the 3 serotype-specific PCR assays when tested against Australian vaccinal and field strains of MDV (+ positive; - negative).

Strain	Source	Supplier	Serotype	PCR assay			Comments
				Sero 1	Sero 2	Sero 3	
MPF 145/1	Field isolate	RMIT	MDV-1	-	-	-	Unvaccinated
MPF 145/3	Field isolate	RMIT	MDV-1	+	-	-	Unvaccinated
MPF 145/9	Field isolate	RMIT	MDV-1	+	-	-	Unvaccinated
MPF 146/9	Field isolate	RMIT	MDV-1	+	-	+	HVT-vaccinated
MPF 146/14	Field isolate	RMIT	MDV-1	+	-	-	HVT-vaccinated
MPF 147/13	Field isolate	RMIT	MDV-1	-	+	-	HVT-vaccinated
MPF57	Field isolate	RMIT	MDV-1	+	-	-	
MPF57	Infected birds	UNE	MDV-1	+	-	-	Many samples
Woodlands P 14	Field isolate	RMIT	MDV-1	+	+	-	
Woodlands 60/2 P78	Vaccine	RMIT	MDV-1	+	-	-	
Rispens CVI988	Vaccine	Intervet	MDV-1	+	-	-	
Rispens CVI988	Vaccinated birds	Baiada	MDV-1	+	-	-	
BH-16	Vaccine	Intervet	MDV-1	+	-	-	
Rispens CVI988	Vaccine	The Mareks Co.	MDV-1	+	-	-	
MD 19 (Maravac)	Vaccine	Fort Dodge	MDV-2	-	+	-	
MD 19 (Maravac)	Vaccinated birds	UNE	MDV-2	-	+	-	
NBSL S.AR	Vaccine	Intervet	MDV-3	-	-	+	
HVT FC126	Vaccine	The Mareks Co.	MDV-3	-	-	+	
HVT FC126	Vaccinated birds	UNE/Baiada	MDV-3	-	-	+	Many samples
SPF-Spleen	SPF chickens	Steggles	Nil	-	-	-	

Table 2. Comparison of virus isolation (without immunostaining) and PCR for early detection of HVT in chickens vaccinated with HVT *in ovo*, by a variety of methods, on days 17.5-18.5 of incubation. Chickens were challenged with MDV-1 on day 3 of age.

Age of chickens (Days)	Method of detection		
	Isolation. PBL on CEF	Isolation. Spleen on CEF	PCR. Spleen
4		15/30 (50%)	30/47 (63.8%)
10	21/41 (51.2%)	15/45 (33.3%)	27/47 (57.4%)
17	32/44 (72.7%)	3/37 (8.1%)	36/48 (75%)
24	29/48 (60.4%)		

Table 3. Detection of HVT in broiler chickens by PCR of spleen tissue for HVT. Chickens were vaccinated *in ovo* with HVT at days 17.5-18.5 of incubation.

Week of Age	Farms tested (n)	Birds tested (n)	HVT positive (n)	% Positive
2	11	162	89	54.9
3	10	111	86	77.5
5	7	71	38	53.5
6	3	30	16	53.3
7	3	30	20	66.7

Table 4. Detection of MDV-1 in broiler chickens by PCR of spleen tissue for MDV-1.

Age(weeks)	HVT vacc.	Farms tested	Birds tested	MDV positive	% Positive
1	Yes	1	12	0	0
2	Yes	1	10	0	0
3	Yes	5	62	0	0
4	Yes	2	22	0	0
4	No	1	10	0	0
5	Yes	8	83	0	0
5	No	3	30	4	13.3
6	Yes	4	42	5	11.9
7	Yes	3	30	3	10.0
7	No	3	30	29	96.7

These assays do not provide a great increase in sensitivity relative to virus isolation, as has been found by others (eg. Handberg *et al.*, 2001). However they do provide significant advantages in the speed and specificity of detection and the ability to work from samples stored at –20˚C. They also enable detection of MDV-1 infection in HVT-vaccinated birds. The assays are robust and have been successfully transferred to a commercial laboratory where they have proved useful in monitoring post-vaccinal viraemia and detecting MDV-1 infections in broiler chickens. Increases in sensitivity can be achieved with the use of nested or semi-nested PCR (Layton *et al.*, 2001) but this increases the complexity of the assay and risk of contamination.

Key challenges that remain for the PCR diagnosis of MDV infection include unequivocal differentiation between vaccinal and pathogenic MDV-1, development of non-invasive sampling methods (eg blood spot, feather tip, poultry dust), increased sensitivity, quantification of viral load and establishing predictive relationships between MDV load and disease outcomes. Progress is occurring on each of these fronts. Real-time PCR technology (Mackay *et al.*, 2002) will improve the sensitivity of detection of MDV and provide quantification of MDV copy number and is likely to displace normal PCR as the costs associated with real-time PCR decrease. All Australian groups working on MDV are now using this technology and it is likely to lead to significant improvements in our understanding of MD over the next few years.

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COMPARISON OF PROTECTIVE EFFICACY OF MANUAL AND AUTOMATED *IN OVO* VACCINATION AGAINST MAREK'S DISEASE IN BROILER CHICKENS

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Summary

Automated *in ovo* vaccination (IOJ) against Marek's disease in broiler chickens is now a routine practice in the poultry industry. This method deposits vaccine mostly into the extra-embryonic (EE) spaces around the embryo, principally the amnion. In earlier experiments of ours, manual EE (MEE) vaccine deposition has not produced good protection in contrast to the apparent efficacy of the automated *in ovo* method. Two experiments are reported, one confirming this difference in protection and the other demonstrating that there are significant differences in the precise site of vaccine deposition between the two methods, with a higher incidence of allantoic deposition in the case of MEE (25.5%) compared to OIJ (5.7%). However, this finding alone cannot explain the difference in protection between the two methods. We suggest that different methods of vaccinal virus uptake from the amnion may be involved.

I. INTRODUCTION

Marek's disease (MD), a ubiquitous viral disease of chickens, is primarily controlled by vaccination. Traditionally, vaccination was performed on the day of hatch by subcutaneous injection. However, over the last decade, vaccination of broiler chickens against MD has increasingly been done via the *in ovo* (in the egg) route using the automated INOVOJECT®**,** vaccinator (Embrex Inc NC USA). Currently more than 80% of North American and Australian broiler chickens are vaccinated against MD using this method.

In ovo vaccination is performed between days 17.5 and 18.5 of embryonation in the hatchery. Vaccine is deposited either extra-embryonically (EE) into the structures surrounding the embryo or intra-embryonically (IE) into the body of the embryo. During EE deposition, there are three probable deposition sites, namely the allantoic sac, the amniotic sac and the yolk sac (Sharma and Burmester, 1982; Gildersleeve *et al.*, 1993). Earlier studies by our group using HVT vaccine showed that manual EE (MEE) deposition resulted in delayed vaccinal viraemia and very low levels of protection following challenge with very virulent MD virus (MDV), whereas manual IE (MIE) deposition produced earlier vaccinal viraemia and consistently high levels of protection (Islam *et al.*, 2001). As the vaccine deposition site using the automated INOVOJECT® method (IOJ) has a higher frequency of EE than IE deposition (Islam *et al.*, 2001) and EE deposition produced very low level of protection, we hypothesised that the timing of post-vaccinal viraemia and the level of protection provided by IOJ vaccination will be intermediate between that provided by MEE and MIE deposition. This paper reports the results of an experiment to test this hypothesis and the results of a separate study to determine the precise site of vaccine deposition by the IOJ and MEE methods.

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II. MATERIALS AND METHODS

Experiment 1 (comparison of vaccination efficacy) utilized a completely randomized design with 8 treatments as shown in Table 2. Vaccination was performed as described (Islam *et al.* 2001) using HVT vaccine (FC 126). Chickens (Cobb broiler) of each treatment were identified by toe-web marking and placed together in 16 replicates in floor pens. All the chickens were challenged with 50pfu of MDV (strain MPF 57) intra-peritoneally at day 3.

On each of days 4, 10 and 17 post-hatch, spleen samples were collected from 40 chickens (10 from each of the 4000pfu MEE, MIE and IOJ vaccinated groups) after euthanasia. The presence of vaccinal virus (HVT) in spleen was determined by serotype 3-specific PCR assay as described by Islam *et al.*, (2001). Live weight (LW) of individual chickens was determined on day 49 and birds were sexed on the basis of external signs. Post-mortem examinations were carried out on all chickens that died during the experiment or were euthanased at its termination on day 66. All were examined for gross MD lesions. Protective indices for each treatment group were calculated as described by Sharma and Burmester (1982).

Experiment 2 (determination of vaccine deposition sites) utilised a 2 x 2 factorial design with two types of vaccine delivery (MEE or IOJ) and two embryo ages of (17.5 and 18.5 days). The same two operators as those used in our earlier experiments performed manual *in ovo* vaccination into the EE compartment attempting to reproduce, as accurately as possible, the method used in earlier experiments. Determination of deposition sites was performed using the method kindly provided by Drs Bruce Singbeil and Chris Williams (Embrex Inc. NC, USA) and involved injection of a protein dye into the embryo with subsequent euthanasia and the dissection to determine the deposition site. Eggs were weighed immediately prior to this.

In experiment 1, LW data were analysed by analysis of variance (AOV) using Statview® (SAS Institute Inc.) to test the main effects of Sex and Treatment and their interaction. The LW data of sham-vaccinated groups were pooled during final analysis. Virus isolation data and MD lesion data were coded as 0 and 1 for the absence and presence of virus and/or lesions respectively and were analysed by AOV using generalised linear model for binomial data of S-Plus (Mathsoft Inc). In experiment 2, differences in the ratios of the frequency of deposition sites were tested by χ^2 tests. A significant level of $P<0.05$ is used throughout.

III. RESULTS

Experiment 1

Analysis of HVT detection data showed that there was a significant effect of Treatment ($P<0.03$) but not Day ($P=0.07$)) with no significant interaction. Overall, the highest rate of HVT detection was in the MIE vaccinated group followed by IOJ and MEE groups (Table 1).

Table 1. Experiment 1. Post-vaccinal HVT detection in spleen following manual MEE, MIE or IOJ vaccination

Treatment	Day 4		Day 10		Day 17		Overall	
	HVT+ /total	% HVT+	HVT+/ total	% HVT+	HVT+/ total	% HVT+	HVT+/ total	% HVT+
MEE4K17.5	$5/9^{A}$	56	$7/10^{A}$	70	$6/10^{A}$	60	$18/29^{A}$	62
MIE4K17.5	$10/10^{B}$	100	$6/10^{A}$	60	$7/10^{A}$	70	$23/30^{A}$	77
IOJ4K17.5	$8/10^{B}$	80	$6/10^{A}$	60	$8/10^{A}$	80	$22/30^{A}$	73
IOJ4K18.5	$6/10^{AB}$	60	$4/10^{A}$	40	$9/10^{A}$	90	$19/30^{A}$	63

^{AB}Figures differ significantly if superscripts do not share a common letter ($P<0.05$).

Analysis of LW data revealed a significant effect of Treatment ($P<0.04$) and Sex ($P<0.001$) with no significant interaction between these effects. Manual EE and sham vaccinated groups had significantly lower LW than that of the other vaccinated groups (Table 2).

Table 2. Experiment 1. Mean (±sem) live weight (LW,g)of experimental chickens at 49 days of age

Treatment (Vaccination method & dose)	n	Treatment Abbreviation	LW Female	LW Male	Overall LW
MEE at day 17.5 (4000pfu)	49	MEE4K17.5	2909±76	3492±87	$3183±71^{B}$
MIE at day 17.5 (4000pfu)	57	MIE4K17.5	2958±91	3598±63	$3328±67^{A}$
IOJ at day 17.5 (4000pfu)	49	IOJ4K17.5	3046±52	3669±74	$3326±62^{A}$
IOJ at day 18.5 (4000pfu)	43	IOJ4K18.5	2920±91	3688±59	$3295±80^{A}$
Subcutaneous (4000pfu)	93	SC4K	2964±45	3596±71	$3263±52^{A}$
IOJ at day 18.5 (0pfu)	49	IOJ0K18.5	2828±39	3535±44	$3112±38^{B}$
IOJ at day 17.5 (0pfu)	56	IOJ0K17.5			
Subcutaneous at hatch (0pfu)	89	SC0K			

^{AB}Figures within columns differ significantly if superscripts do not share a common letter ($P<0.05$).

The first chicken to die exhibiting MD lesions died on day 33. Analysis of all post mortem data revealed a significant effect of Treatment ($P<0.0001$) and Sex ($P<0.01$), but not their interaction, on the incidence of MD lesions. The percentage of MD positive chickens and the protective indices of different vaccination group are shown in Table 3.

Table 3. Experiment 1. Protective index provided by the different vaccination treatments

Treatment	Total Chickens	MD positive	% MD	PI
All sham vaccinated groups	219	98	44.75^{A}	-
MEE4K17.5	52	23	44.23^{A}	1.2
MIE4K17.5	59	9	15.25^{B}	65.9
IOJ4K17.5	56	7	12.50^{B}	72.1
IOJ4K18.5	46	5	10.87^{B}	75.7
SC4K	92	19	20.65^{B}	53.8

^{AB}Figures differ significantly if superscripts do not share a common letter in the %MD column.

Experiment 2

Analysis of vaccine deposition site data revealed that there were significant effects of the vaccine delivery system ($P<0.01$) and embryo age ($P<0.01$) on the deposition site. The effect of operator was not significant and was excluded from the analysis. The frequency of amnionic and MIE deposition was higher with the IOJ delivery system than MEE delivery (Table 4). The main effect of embryo age with IOJ was due to a significantly higher proportion of MIE depositions at day 18.5 and for both delivery systems, a higher proportion of allantoic deposition at day 17.5.

Table 4. Experiment 2. Comparison of vaccine deposition sites following automated *in ovo* vaccination by INOVOJECT® machine and manual EE *in ovo* vaccination

Embryo age (day)	Vaccine delivery system	N	Mean egg weight ±se (g)	Deposition sites		
				Amnion	Intra-embryonic	Allantois
17.5	IOJ	51	58.3 ± 0.64^{A}	46 (90%)	0 (0%)	5 (10%)^{A}
17.5	MEE	49	57.8 ± 0.74^{A}	29 (59%)	0 (0%)	20 (41%)^{B}
18.5	IOJ	55	59.4 ± 0.71^{A}	40 (72%)	14 (26%)	1 (2%)^{C}
18.5	MEE	49	60.3 ± 0.64^{A}	44 (90%)	0 (0%)	5 (10%)^{A}

^{abc}Ratios of deposition sites differ significantly if superscripts do not share a common letter.

IV. DISCUSSION

The findings of experiment 1 did not support our main hypothesis as there was no significant difference in the protection provided by the manual intra-embryonic, automated *in ovo* and day old vaccination methods, all of which were superior to manual EE vaccination.

Although IOJ vaccination deposits vaccine predominantly in the EE compartments (Islam *et al.*, 2001), it produced very good protection against challenge in contrast with MEE vaccination. The large difference in the protection level provided by MEE and IOJ vaccination suggests that the two methods were not depositing vaccine in the same extra-embryonic compartments. Recently, Wakenell *et al.* (2002) showed that amniotic vaccine deposition produced very good protection (>80%) whereas allantoic deposition produced almost no protection (<20%). Experiment 2 showed that our manual EE method resulted in a higher frequency of allantoic deposition, especially at day 17.5. Nevertheless, even at this age close to 60% of EE deposition was into the amnion which should have produced protection levels well above those observed. This suggests that the lower protection obtained following MEE vaccination cannot be due solely to increased frequency of deposition into the allantois.

We consider that there are two possible explanations for these findings. Firstly, it may be that the operators in Experiment 2 did not exactly reproduce the manual injection method used in the protection studies in Experiment 1. However, both the operators were extremely careful to avoid bias and we consider this explanation to be highly unlikely. Secondly, the mode of vaccine uptake by the embryo might differ between the two methods even when vaccine is deposited in the amniotic sac. It is possible that the needle of the automated INOVOJECT® vaccinator may scratch the skin of the embryo when depositing into the amniotic sac and thus provide an alternative entry path for the vaccine virus. The INOVOJECT® method uses a semi-sharp bevelled needle that is driven straight down into the egg and is very likely to encounter the embryo before sliding off into the amniotic sac which completely encloses the embryo. The chance of scratching the embryo with manual EE vaccination is very much less as the needle used has the sharp tip completely removed and

the operators are deliberately trying to avoid the embryo. Having said this, a cursory examination of several embryos vaccinated by the IOJ method for evidence of scratch marks failed to reveal any, suggesting that such scratches, if they occur, may be difficult to detect under the feather coat.

Unfortunately we do not have a data set combining exact vaccine deposition sites and levels of protection in the same experiment. Such an experiment would be useful in resolving these issues. Detailed mapping of vaccinal viral entry into the embryo in the first 24 hours following *in ovo* vaccination into different embryonic sacs would also provide useful information.

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INTERLEUKIN-6 CYTOKINE ENHANCES INTESTINAL IMMUNITY

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Cytokines are molecules that regulate the character and duration of an immune response. In this regard they can drive a particular type of immune response to enhance protection from pathogens (Fresno *et al*., 1997). Interest in enhancing intestinal immunity, and in particular, intestinal IgA antibody production in chickens (Muir *et al*., 2000), has led to early investigations into cytokine regulation of avian mucosal immunity. Ramsay *et al.* (1994) observed significant reductions in IgA producing plasma cells in the intestine of IL-6 gene knockout mice, demonstrating the regulatory role of IL-6 in intestinal IgA antibody production. Production of IgA antibody was restored following the supply of exogenous IL-6. The present study was designed to investigate the potential of IL-6 to enhance intestinal IgA antibody production in chickens. As chicken IL-6 had not been identified at the time these studies were undertaken, porcine IL-6 (pIL-6), which has shown cross-species reactivity, was used.

At 14 days of age all chickens received an intraperitoneal immunisation of whole killed *Salmonella typhimurium* in adjuvant, followed with an oral booster of whole killed *S. typhimurium* at 28 days of age. All birds were then randomly allocated to four cytokine treatment groups. On each of the next four days, pIL-6 treated birds received 0.5mL by gavage containing either 1 or 10 μg pIL-6 or 10 μg porcine IL-3 cytokine control per bird per day. A third group of birds were treated with 10 μg of pIL-6 only on the second and fourth day after the oral booster. Control birds received 0.5mL phosphate buffered saline on the four days following the oral booster. At 35 days of age serum and intestinal scrapings (IS) were collected for ELISA determination of anti-*S. typhimurium* IgA antibody titres.

In a second experiment groups of eight birds were unvaccinated, vaccinated or vaccinated with four daily oral pIL-6 treatments. From 35 days of age all birds were challenged with live *S. typhimurium* via cohabitation on litter with *S. typhimurium* infected seeder birds. At days 7 and 14 post challenge (pc) isolation of *S. typhimurium* from cloacal swabs, and enumeration of *S. typhimurium* in the spleen and liver was undertaken.

Repeated oral delivery of 10μg pIL-6 following an oral booster immunisation significantly ($P<0.05$) increased anti-*S. typhimurium* IgA antibody titres in the serum and IS. Oral delivery of 10μg pIL-6 on days 2 and 4 following the oral booster significantly ($P<0.05$) increased anti-*S. typhimurium* IgA in the IS. On day 7 pc fewer *S. typhimurium,* (not statistically significant) were isolated from the spleen and liver in pIL-6 treated birds. Similarly, at 14 days pc, pIL-6 treated birds were less frequently infected (not statistically significant) with *S. typhimurium* than control birds. Further, fewer *S. typhimurium* were isolated from the spleen and liver of these chickens.

These studies demonstrate the potential for orally administered pIL-6 to significantly enhance local IgA antibody production at the intestinal surface in chickens. Increased IgA antibody production resulted in reduced levels of *S. typhimurium* infection.

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Hruby, M.	127	milan.hruby@danisco.com
Huang, K.	70, 71, 113	khjhuang@yahoo.com
Hughes, R.J.	172	Hughes.Bob@saugov.sa.gov.au
Husband, A.J.	202	
Hyde, M.L.	71	
Irish, G.G.	58, 62, 69	
Islam, A.F.M.F.	192, 197	
Jin, G.	127	
Jolly, M.	179	
Kharole, M.U.	77	
Kocher, A.	95, 112	akocher@metz.une.edu.au
Kumar, A.	76, 83	akumar@hau.nic.in
Kumar, S.	79	skgcari@yahoo.co.uk
Langhout, D.J.	58	
Lemme, A.	58, 62	andreas.lemme@degussa.com
Li, X.	67, 70, 71, 113	
Liu, K.	123	Kevin.Liu@adisseo.com
Lunam, C.A.	159, 160	
Mack, S.	58, 62	
Mahajan, N.K.	76	
Maisonnier, S.	123	
Majumdar, S.	83	
Martin-Venegas, R.	66	
Mascord, L.	192	
McBurney, W.	131	
McGoldrick, S.	145	
Molloy, J.B.	161	
Moran, C.A.	119	
Muir, W.I.	71, 99, 113, 202	wmuir@camden.usyd.edu.au
Murray, S-A.	161	
Muthukumar, S.P.	79, 83	
Newman, R.E.	153	ronaldn@camden.usyd.edu.au
Ng, M.	87	Mariko.Ng@nre.vic.gov.au
Nicholas, F.W.	42	frankn@vetsci.usyd.edu.au
Nolan, J.V.	135	jnolan@metz.une.edu.au
Okere, C.	164	
Parkinson, G.B.	87	
Perez-Maldonado, R.A.	177	PerezR@prose.dpi.qld.gov.au
Petri, A.	58, 62	
Pettersson, D.	95	
Pierce, J.	114, 119	

Name	Page(s)	Email Address
Pittolo, P.H.	99	
Plummer, J.L.	159, 160	
Pruthi, A.K.	77, 78	akpruthi@hau.nic.in
Pym, R.A.E.	i-v	
Ravindran, V.	1, 68, 70, 103, 114, 131	V.Ravindran@massey.ac.nz
Roberts, J.R.	72, 91, 108, 179	jrobert2@metz.une.edu.au
Ross, G.	95, 112	
Ruiz, J.	159	
Saxena, V.K.	79, 83	
Schat, K.A.	51	
Sefton, A.E.	119	
Selle, P.H.	99, 113	sellep@camden.usyd.edu.au
Serrao, E.A.	161	
Sharma, K.L.	164	ksharma_fj@yahoo.com
Sheoran, V.	76	
Shivakumar, B.M.	79, 83	
Sim, J.S.	154	
Singh, D.	178	danny.singh@dpi.qld.gov.au
Singh, N.S.	79, 83	
Singh, R.V.	83	
Sivaraman, G.K.	79, 83	
Soni, S.K.	77	
Strom, A.D.G.	202	
Suawa, E.	108	
Sulaiman, A.	72	
Sun, Y.	71	
Sunwoo, H.	154	
Svihus, B.	17	
Tannock, G.W.	131	
Taylor, R.	139	ndrdt@alinga.newcastle.edu.au
Thakral, N.	78	
Thomas, D.V.	68	D.V.Thomas@massey.ac.nz
Trappett, P.C.	178	
Vakabua, J.N.	164	
Van Wichen, J.	153	
Viera, D.B.	162	
Walkden-Brown, S.	192	swalkden@metz.une.edu.au
Wang, Y.	154	
Waters, D.L.E.	95	
Weir, K.A.	159, 160	Kristy.Weir@flinders.edu.au
Wells, B.	99	
Wijtten, D.J.	58	

Name	Page(s)	Email Address
Wilkinson, S.J.	153	stuartw@camden.usyd.edu.au
Wu, Y.B.	103, 114	
Young, P.L.	192	

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Syd Wilkins received his tertiary education at Hawkesbury Agricultural College and developed a career as a Poultry Officer in the NSW Department of Agriculture, becoming its Senior Poultry Officer by the late 1950's. In the mid to late 1960's he transferred to Allied Feeds, where he remained as a Senior Executive of the Allied Mills Group until his untimely death in 1982. During all this time he was very active in the affairs of the WPSA Australian Branch, including a period as President. He was also elected as one of the Vice Presidents of WPSA world body in the 1970's, a position he still held on this death. He was actively involved in the conduct of the 1974 and 1978 World's Poultry Congresses in New Orleans and Rio de Janeiro.

Syd Wilkins was also involved with the PHRF virtually from its beginning, and served many years as its Vice-President. He was the recipient of the Australian Poultry Award in 1974. Syd's career was contemporary with the application of the 20th century poultry technology revolution in Australia. In his own low-key and unassuming way he contributed very significantly to its progress.

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CONTENTS

AUTHOR INDEX

Name	Page(s)	Email Address
Hocking, P.M.	34, 168	
Hruby, M.	127	milan.hruby@danisco.com
Huang, K.	70, 71, 113	khjhuang@yahoo.com
Hughes, R.J.	172	Hughes.Bob@saugov.sa.gov.au
Husband, A.J.	202	
Hyde, M.L.	71	
Irish, G.G.	58, 62, 69	
Islam, A.F.M.F.	192, 197	
Jin, G.	127	
Jolly, M.	179	
Kharole, M.U.	77	
Kocher, A.	95, 112	akocher@metz.une.edu.au
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Lemme, A.	58, 62	andreas.lemme@degussa.com
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Lunam, C.A.	159, 160	
Mack, S.	58, 62	
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Maisonnier, S.	123	
Majumdar, S.	83	
Martin-Venegas, R.	66	
Mascord, L.	192	
McBurney, W.	131	
McGoldrick, S.	145	
Molloy, J.B.	161	
Moran, C.A.	119	
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Muthukumar, S.P.	79, 83	
Newman, R.E.	153	ronaldn@camden.usyd.edu.au
Ng, M.	87	Mariko.Ng@nre.vic.gov.au
Nicholas, F.W.	42	frankn@vetsci.usyd.edu.au
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Okere, C.	164	
Parkinson, G.B.	87	
Perez-Maldonado, R.A.	177	PerezR@prose.dpi.qld.gov.au
Petri, A.	58, 62	
Pettersson, D.	95	
Pierce, J.	114, 119	

Name	Page(s)	Email Address
Pittolo, P.H.	99	
Plummer, J.L.	159, 160	
Pruthi, A.K.	77, 78	akpruthi@hau.nic.in
Pym, R.A.E.	i-v	
Ravindran, V.	1, 68, 70, 103, 114, 131	V.Ravindran@massey.ac.nz
Roberts, J.R.	72, 91, 108, 179	jrobert2@metz.une.edu.au
Ross, G.	95, 112	
Ruiz, J.	159	
Saxena, V.K.	79, 83	
Schat, K.A.	51	
Sefton, A.E.	119	
Selle, P.H.	99, 113	sellep@camden.usyd.edu.au
Serrao, E.A.	161	
Sharma, K.L.	164	ksharma_fj@yahoo.com
Sheoran, V.	76	
Shivakumar, B.M.	79, 83	
Sim, J.S.	154	
Singh, D.	178	danny.singh@dpi.qld.gov.au
Singh, N.S.	79, 83	
Singh, R.V.	83	
Sivaraman, G.K.	79, 83	
Soni, S.K.	77	
Strom, A.D.G.	202	
Suawa, E.	108	
Sulaiman, A.	72	
Sun, Y.	71	
Sunwoo, H.	154	
Svihus, B.	17	
Tannock, G.W.	131	
Taylor, R.	139	ndrdt@alinga.newcastle.edu.au
Thakral, N.	78	
Thomas, D.V.	68	D.V.Thomas@massey.ac.nz
Trappett, P.C.	178	
Vakabua, J.N.	164	
Van Wichen, J.	153	
Viera, D.B.	162	
Walkden-Brown, S.	192	swalkden@metz.une.edu.au
Wang, Y.	154	
Waters, D.L.E.	95	
Weir, K.A.	159, 160	Kristy.Weir@flinders.edu.au
Wells, B.	99	
Wijtten, D.J.	58	

Name	Page(s)	Email Address
Wilkinson, S.J.	153	stuartw@camden.usyd.edu.au
Wu, Y.B.	103, 114	
Young, P.L.	192	