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Enquiries regarding the Proceedings should be addressed to:

The Director, Poultry Research Foundation
Faculty of Veterinary Science, University of Sydney
Camden NSW 2570

Tel: 02 46 550 656; 9351 1656
Fax: 02 46 550 693; 9351 1693

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CLIMATE CHANGE: IMPLICATIONS FOR WATER UTILISATION IN ANIMAL AGRICULTURE AND POULTRY, IN PARTICULAR

N.D. COSTA[1]

Summary

Climate change has raised the public awareness of the environmental impacts of a particular foods and diet choices. The Australia poultry industry is through its structure and efficiency is uniquely positioned to market itself as an environmentally-responsible and prudent industry. In the light of the likely consequences of climate change, the poultry industry has a natural advantage over other livestock industries because of its low global warming potential. Given the relatively low water requirements of poultry and its capacity to calculate its water use, the Australia poultry industry should aim towards a water neutral footprint. Being able to market these claims about its low global warming potential as well as moving towards water neutrality would place the Australian poultry industry at an advantage to other livestock industries in animal agriculture, especially amongst those persons who place an emphasis on sustainability in deciding their dietary preferences.

I. THE AUSTRALIAN POULTRY INDUSTRY AND GLOBAL WARMING

The Australia chicken meat or broiler industry is almost on a par with the beef and veal industry as the major meat industry in terms of consumption in Australia and is almost double pork, and treble lamb and mutton consumption (ABARE, 2007). Unlike the red meat industries that rely in varying degrees on exports for their viability, the broiler industry's sales are 95% domestic. Moreover, the consumer demand for chicken meat has been growing steadily over the last decade. Other attributes that delineate the chicken meat industry from red meat industries are the fact that the industry is intensively-based and vertically integrated, with more than 80% of chicken meat market supplied by three producers: Inghams Enterprises, Bartter Enterprises and Baiada (Australian Chicken Meat Federation, 2007). By these, and many other measures, the Australian poultry industries, primarily broiler and egg, are major industries and consequently identified by the public as flag carriers for the animal industries. Thus the Australian chicken meat industry, in particular, is uniquely positioned in terms of both industry structure and production systems to enact practices and policies to reduce their environmental impact.

The environmental impact of livestock industries has become a prominent public and industry concern (Costa, 2007). The IPCC Reports (2007a and 2007b) have documented the underlying atmospheric science and the measurable effects of global warming, and stated that anthroprogenic climate change in now more than 95% possible. The Stern Report (2007) and the Garnaut Report (2008) have both highlighted the role of livestock production, particularly the red meat industries, in increasing anthropogenic greenhouse gases linked to global warming and climate change. Through the Meat Livestock Australia (MLA), the red meat industries have been pro-active in addressing environmental concerns. This action was driven in large part by contribution of methane produced during digestion in ruminants that accounts for approximately 11% of Australia's greenhouse gases under Kyoto accounting conventions (OCC 2008). Importantly, the proportion of greenhouse gases derived from ruminants in Australia has declined from 1990 to 2005 mainly as a result of a 42% reduction in sheep

[1] School of Environmental Science, Murdoch University, Murdoch, WA 6150

numbers. Poultry are not significant producers of methane during digestion which is a major advantage during periods of concern about global warming potential. Using a full life-cycle analysis of UK agricultural systems, Williams et al. (2006) proposed that poultry meat production appeared to be the most environmentally efficient form of meat production, followed by pig meat and sheep meat (primarily lamb) with beef the least efficient. The factors underlying poultry's advantage included: the very low overheads of poultry breeding stock (*c*. 250 progeny per hen each year *vs* one calf per cow); very efficient feed conversion; high daily weight gain of poultry (made possible by genetic selection and improved dietary understanding). In the Garnaut Report on Climate Change (pp592, 2008), emissions from the Australian poultry industry were estimated at 0.8 kg of CO_2-e per kg of produce compared with 4.1 for pork, 16.8 for lamb and mutton and 24.0 for beef and veal. Moreover, sheep and cattle production is highly vulnerable to the biophysical impacts of climate change, such as water scarcity (Garnaut 2008). Noting this, combined with the potential increased costs for methane emissions, Garnaut (2008) postulated a transition toward greater production and consumption of lower-emissions forms of meat, such as chicken. Thus the Australian poultry industry should be at a relative advantage if environmental concerns about global warming and climate change become strong drivers of consumer demand.

However, poultry and pigs consume high value feeds and effectively live on arable land, as their nutritional needs are overwhelmingly met by arable crops such as wheat, sorghum and soybeans (produced both in Australia and overseas). Ruminants can digest cellulose and so make good use of land in the Australia that is not suitable for arable crops, but is highly suited to red meat production from ruminants. The major environmental disadvantage for ruminants will continue to be emission of enteric methane which has a 100-year global warming potential (GWP) that is 21 times greater than carbon dioxide. This contributes to the ratios of global warming potential (GWP) produced to primary energy consumed, being about 50% higher for ruminant than pig or poultry meats. Unlike most of industry and domestic activity that are carbon-based, the global warming potential from the non-livestock sector of agriculture is dominated by nitrous oxide, not by methane from ruminants or carbon dioxide from fuel use. Importantly, nitrous oxide has a 100-year GWP of 310 times greater than carbon dioxide (USA EPA 2002). Nitrous oxide contributes about 80% to global warming potential in wheat production (both organic and non-organic). Moreover, since the underlying driver is the nitrogen cycle, the global warming potential of crop production is relatively similar across contrasting productions systems, including organic (Williams et al., 2006). The balance of global warming gas emissions and fossil fuel consumption is thus quite different from most industries. In crop-based agriculture, nitrous oxide dominates, compared with the predominant contributions from methane for ruminant livestock. Consequently, a carbon footprint does not adequately describe agriculture; as Williams et al. (2006) proposed, agriculture has a carbon-nitrogen footprint. Indeed, the nitrogen fluxes in agriculture (and other types of land use) also contribute to eutrophication and acidification. The majority of environmental burdens arising from the production of agricultural food commodities arise either directly or indirectly from the nitrogen cycle and its modification, in organic and non-organic systems. In fact, 27% less energy was used for organic wheat production compared with non-organic (Williams et al. 2006). However, the large reduction in energy used by avoiding synthetic N production is offset by lower organic yields and higher inputs into field work. Therefore, GWP was only 2-7% less for organic than non-organic field crops, reflecting the need for N supply to equal N take-off and the consequent emissions to the environment as nitrous oxide to air and nitrate to water under EU environmental guidelines and legislation (Williams et al., 2006).

In the UK, most organic animal production reduced primary energy use by 15% to 40%, but organic poultry meat and egg production actually increased energy use by 30% and

15% respectively (Williams et al., 2006). The benefits of the lower energy needs of organic feeds were over-ridden by lower bird performance. Besides energy use, more of the other environmental burdens were larger from organic production, but abiotic resource use was mostly lower for all agricultural commodities except for poultry meat and eggs. The GWP from organic production ranged from 42% less for sheep meat to 45% more for poultry meat. Land use was always higher in organic cropping systems for equivalent yield outputs due to lower yields per ha and higher overheads for fertility building and cover crops (Williams et al., 2006).

Thus the basic digestive physiology of domestic poultry confers an advantage over other meats because poultry do not emit significant amounts of methane. However, nitrous oxide emissions produced indirectly through production of feed such as wheat, sorghum and soy for poultry are the major contributor to the greenhouse impact of the Australian poultry industry. So to reduce its greenhouse impact, the Australian poultry industry would need to work with feed-millers and grain producers. Garnaut (2008) suggested that 'nitrous oxide emissions that result from soil management can be reduced through currently feasible activities—fertiliser management, soil and water management, and fertiliser additives (de Klein & Eckhard, 2008). These mitigation activities can significantly reduce costs. Organic additives are low-emissions alternatives to conventional fertiliser that are already available.' On the other hand, Williams et al. (2006) calculated that the organic systems of poultry production in the UK, in comparison with the other animal industries such as pork or beef, do not seem to confer any advantage in terms of GWP. If this calculation holds for Australia, then the Australian poultry industry, which is predominantly not organic, does not need to change its current production systems to reduce its direct greenhouse impact. Nevertheless, the industry should consider research and development on the optimum approach to reduce its GWP further, possibly through appropriate offsets against the impact of nitrous oxide from feed production.

II. WATER REQUIREMENTS OF POULTRY

Water is the most important nutrient consumed by animals, and poultry are no exception. Nevertheless, by strict definition, water is not a nutrient, even though it is often called the 'forgotten nutrient'. The poultry industry, both meat and egg production, has a natural advantage through the intrinsic physiology and reproduction of the domestic fowl since the basic renal anatomy and physiology of birds is aimed at conserving water and lowering water turnover. This potential advantage stems from the particular renal physiology and pathway of nitrogen excretion as uric acid both of which reduce water requirements in birds. On the other hand, poultry are unable to sweat as a means of regulating body temperature, so their method of heat control involves increasing the respiratory rate (panting) to expel surplus heat, which results in the release of large amounts of moisture from the bird that must be replaced or the bird will become dehydrated.

The feed requirements of growing poultry are directly related to bird weight. Water requirements are related to feed consumption, with over half of the water intake of poultry is obtained from the feed, and to the air temperature. Feed and water consumption are very closely correlated with a correlation coefficient of 0.98 (Lott et al., 2003). Monitoring water consumption on a daily basis has been shown to be a reliable measure of broiler performance (Halder, 2008). Automatic watering equipment ensures poultry have free access to water at all times. In fact, broilers can drink a great deal of water. It has been calculated that during its lifetime, a 2.3-kg broiler will consume about 8.2 litres of water, compared to approximately 4.55 kg of feed (Lacy, 2002). Pesti et al. (1985) estimated the daily water consumption of broilers by multiplying the age of the bird in days by 6 ml. For example, a 10-day old bird

will drink about 60 ml of water during a 24-hr period, while a 35-day old bird will drink about 210 ml per day. It is also important to be sure that water is delivered to birds. Lott et al. (2003) found that nipples with low flow rates can decrease flock performance and proposed that adequate flow rates (in ml/ minute) could be estimated by multiplying 7 ml x bird age in weeks and adding 20. So, adequate nipple flow rates for 4-week old birds would be 7 x 4= 28 + 20 = 48 ml/min. Delivering more water than the birds need is not a problem, but delivering less reduced performance. Under Australian conditions, once air temperatures exceed 30°C, the expected water consumption can increase by 50% above normal consumption rates. Canadian estimates of the daily water consumption of 1,000 broiler chickens at different stages of growth are shown in Table 1 which also illustrates the effect air temperature can have on water consumption rates.

Table 1 Water consumption of broiler chickens by age [Source: Water requirements for livestock (2007)[a]]

Broiler chicken age (weeks)	Water requirement (litres per 1000 birds per day)	
	21°C	32°C
1-4	50-260	50-415
5-8	345-470	550-770

[a]Typical consumption over a year on a daily basis under average agricultural conditions in Ontario.

Estimates of daily water consumption by other common classes of chickens are 180–320 ml/bird per day for laying hens of 1.6–1.9 kg and broiler breeders of 3.0–3.5kg (Water requirements of livestock 2007). Again, temperatures have a major influence on the water consumption rate expected from these other poultry classes. Egg production level obviously affects the water consumption of laying hens, with estimates that laying hens will drink about 4 kg of water per dozen eggs produced (Water requirements of livestock 2007). Poultry have a daily water requirement of approximately 250ml per $kg^{0.75}$ or per kg metabolic body weight calculated from tables of water requirements (Water requirements of livestock 2007) which is comparable to other species in terms of their requirement per unit of metabolic body size. In 2006/2007, 453.9 million chickens were slaughtered in Australia (Australian Chicken Meat Federation 2008). If we assume that each bird drank approximately 8 litres of water during its lifetime, then chickens in Australia drank between 3,500 million to 4000 million litres of water during 2006/2007. This volume represents the minimum water requirements to allow for the daily needs of the birds themselves. The additional volume requirements are many and varied: those of the producer ie water requirements for feedstuff crops; temperature control and ventilation of sheds; cleaning and hygiene of the sheds; those of the processor ie processing of the birds; those of the retailer ie packaging of meat and eggs; and ultimately those of the consumer.

For the poultry industry, the real impact of climate change is likely to be felt through the projected increased temperature and evaporation, and reduced rainfall. The scenario that is most likely is drying of southern Australia and possibly wetter regions in the north of Australia. Since the feedstuffs such as wheat for poultry are grown on the arable land of southern Australia, the impacts of a drying climate are likely to be complex. Generating more information about likely scenarios and impacts will allow farmers to make timely decisions on whether new money should continue to be invested in locations that seem to be severely damaged by climate change, or whether it is better to find new livelihoods in less challenging locations. Investment in plant and animal genetics may be able to diminish the loss of productivity associated with higher temperatures and changing rainfall patterns. Investment in

water retention or storage will sometimes be an economically sensible response to more variable rainfall. Garnaut (2008) has stated that while hardest of all, the most effective adaptive responses in agriculture to climate change will sometimes require fundamental changes in attitudes, policies and institutions. This is the challenge for the Australian poultry industry, not only to ensure supply of feed but also to provide fro sustainable water supply in general.

III. VIRTUAL WATER, WATER NEUTRALITY AND THE AUSTRALIAN POULTRY INDUSTRY

As part of the impacts of climate change, Australia faces projected increased temperature and evaporation and reduced rainfall. For Australia such a scenario presents a particularly acute problem in sustaining water supplies. Australia's urban water supply infrastructure is old, inadequate for current population levels, and not designed to cope with the projected climate conditions. By 2100 the Garnaut–Treasury reference case points towards an Australian population of 47 million people (Garnaut 2008). Population growth alone will place significant additional stress on urban water supply infrastructure. Garnaut (2008) proposes the expansion and opening of water markets to allow the emergence of the lowest-cost supply options and the optimal balance between reduction of use and expansion of supply. In addition to improved use of existing water sources, it is likely that new forms of supply such as desalination of sea water that are not climate dependent will be required for coastal cities and towns. For Australia's major inland cities and towns, recycled water and purchase of irrigation entitlements may be among the few alternative water supplies available.

The Australian poultry industry, unlike the other animal industries, is reasonably dependent on urban water supply due to the location of the majority of the industry. Given the 95% probability of climate change, especially on the current trajectory of greenhouse gases concentrations, the consequences of water scarcity and competition for drinking water presents unique difficulties for a poultry industry that peri-urban. Thus the poultry industry should explore the concepts of 'virtual water', 'water footprint' and 'water neutrality' with the overall aims of ensuring a sustainable supply of water for the industry itself, and marketing its credentials as a prudent and environmentally-responsible corporate entity. It must be noted that these concepts are controversial. Allan (1998) first proposed the concept of 'virtual water' (as opposed to real water) as a partial solution to problems of water scarcity in the Middle East. Allan elaborated the idea of using virtual-water import (coming along with food imports) as a tool to release the pressure on the scarcely available domestic water resources. Virtual-water import thus becomes an alternative water source, alongside endogenous water sources. Since that time, the utility and applicability of the 'virtual water' has been criticised and debated (Hoekstra 2008, Frontier Economics 2008).

Virtual water is the amount of water that is embedded in food or other products needed for its production. For example, to produce one kilogram of wheat requires about 1,000 litres of water, i.e. the virtual water of this kilogram of wheat is 1,000 litres.

Allan (1998) proposed that along with the trade of food crops or any commodity, there is a virtual flow of water from producing and exporting countries to countries that consume and import those commodities. Consequently, water-scarce country could import products that required a lot of water for their production rather than producing them domestically. By doing so, it allowed real water savings, relieving the pressure on that country's water resources or making water available for other purposes. At the global level, virtual water trade has geo-political implications: it induces dependencies between countries. Therefore, it can be regarded either as a stimulant for co-operation and peace or a reason for potential conflict.

Chapagain and Hoekstra (2003) calculated the international virtual water flows related to the trade in livestock and livestock products as shown in Table 2.

Table 2 Virtual water content of different live animals at slaughter age for selected countries in 1000L per animal. [From Chapagain and Hoekstra, (2003)]

	Beef	Dairy Cows	Sheep	Pigs	Broilers	Layers
Australia	6393	58250	337	723	5	33
N.Z.	4985	54391	263	391	8	45
USA	5484	39443	303	398	3	18
Brazil	4553	31383	264	341	3	18
France	4220	35350	279	228	2	11

While these virtual water flows are significant for the beef and dairy industries, not least because of their prominence in export and therefore nominal transfer of water out of Australia, the poultry industries are almost entirely domestic meaning that this virtual water remains in Australia. So the concept of virtual water is not as threatening to the poultry industries but is it useful in their operations and strategies? To develop this argument, we will explore the concept of 'water footprint'

A water footprint is quite simply the volume of water used. The total water footprint can be broken down into three components: the blue, green and grey water footprint. The blue water footprint is the volume of freshwater that evaporated from the global blue water resources (surface water and ground water) to produce the goods and services consumed by the individual or community. It excludes the part of the water withdrawn from the ground or surface water system that returns to that system directly after use or through leakage before it was used. The green water footprint is the volume of water evaporated from the global green water resources (rainwater stored in the soil). The grey water footprint is the volume of polluted water that associates with the production of all goods and services for the individual. A water footprint is expressed in litres. The water footprint of a nation is equal to the use of domestic water resources, minus the virtual water export flows, plus the virtual water import flows. The total 'water footprint' of a nation is a useful indicator of a nation's call on the global water resources. The water footprint of a nation is related to dietary habits of people. High consumption of meat brings along a large water footprint. Also the more food originates from irrigated land; the larger is the water footprint. The four major direct factors determining the water footprint of a country are: volume of consumption (related to the gross national income); consumption pattern (e.g. high versus low meat consumption); climate (growth conditions); and agricultural practice (water use efficiency).

The water footprint is an indicator of water use that looks at both direct and indirect water use. The water footprint of a product such as chicken meat or eggs is the volume of fresh water used to produce that product, summed over the various steps of the production chain. The water footprint of consumers is the sum of their direct water use, i.e. the water used at home or in the garden, and their indirect water use, i.e. the water used in the production and supply chains of the goods and services consumed. The water footprint of the Australian poultry industry consists of its direct water use, for producing, manufacturing and supporting activities, plus its indirect water use, i.e. the water used in the business' supply chain. 'Water use' is measured in terms of water volumes consumed (evaporated) and/or polluted. As stated earlier, the 'water footprint' includes three components: consumptive use of rainwater (green water), consumptive use of water withdrawn from groundwater or surface water (blue water) and pollution of water (grey water). A water footprint is more than a figure for the total water volume used; it refers specifically to the type of water use, and where and when the water was used.

The increased interest in the water-footprint concept has prompted the question about what businesses like the poultry industry can do to reduce its water footprint. 'Water neutrality' is a concept that can be instrumental in this context. 'Water neutral' means that an individual or business attempts to reduce the water footprint of an activity as much as reasonably possible and offsets the negative externalities of the remaining water footprint. In some particular cases, when interference with the water cycle can be completely avoided – e.g. by full water recycling and zero waste – 'water neutral' means that the water footprint is nullified. However in many other cases like crop growth for feed, water use cannot be nullified since it is an essential blue and green water requirement. Therefore 'water neutral' generally does not mean that water use is brought down to zero, but that the negative economic, social and environmental externalities are reduced as much as possible and that the remaining impacts are fully compensated.

The fundamental basis of the water-neutral concept is to stimulate individuals and corporations that undertake water consuming or polluting activities to make their activity 'water neutral' by reducing water consumption and pollution and by compensating for the negative impacts of remaining water consumption and pollution through investing in projects that promote the sustainable and equitable use of water within the environment and community that is affected. Water consumption and pollution can be reduced for example by investing in water saving technology, water conservation measures and wastewater treatment. Compensation for negative impacts can be done for example by investing in improved watershed management or by supporting communities, such as remote indigenous communities or those overseas that do not have access to clean water to set up and maintain their own water supply system.

The 'water neutral' concept is analogous to the carbon-neutral or carbon-offset concepts developed in response to the challenge of taking climate change counter-measures. The principle of the concept is that an industry reduces its water footprint as much as possible and then pays a justified amount of money for the residual water footprint that it presses on water resources that will be increasingly scarce as a result of decreasing rainfall and higher evapo-transpiration due to climate change. Such action on the part of the poultry industry in Australia can be an instrument to raise awareness, stimulate measures that reduce water footprints and generate funds for the sustainable and fair use of freshwater resources.

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CCRPSI – MEETING THE CLIMATE CHANGE CHALLENGE THROUGH COORDINATION OF PRIMARY INDUSRTY RESEARCH

O. CAMERON[1]

Summary

This paper considers four aspects in relation to meeting the challenge of climate change: (i) the challenges of climate change – risk and uncertainty, planning and investing, (ii) the value of coordinating research – the climate change context, (iii) the example of the Climate Change Research Strategy for Primary Industry [CCRSPI] and (iv) specific considerations for the poultry industry.

I. THE CHALLENGES OF CLIMATE CHANGE: RISK AND UNCERTAINTY, PLANNING AND INVESTING

Climate change poses specific challenges for policy makers and business planners, including: (i) increased natural hazard volatility affecting markets and health, (ii) increasing legal and regulatory pressures – both domestically and in export markets, (iii) uncertain impacts on insurance markets, business resources, efficiency and (iv) increasing public concern, media scrutiny, and shareholder activism.

The impacts of climate change cross sectors, regions, regulatory boundaries and natural biophysical boundaries. Mitigating these impacts of climate change, and adapting to climate change, necessitates putting in place measures that must operate across different social, economic and political cycles. The changes in the Australian climate predicated for the next few decades are substantial, and the effects will vary widely across the country. For example, some regions are predicted to experience average warming of up to 1.8 degrees Celsius by 2030, and in other areas average rainfall (compared to 1990 levels) is predicted to decrease as much as 40% by 2070. Meeting the "diabolical" policy challenges of climate change therefore requires truly integrated, long term planning – an approach that can challenge many institutional interests.

The scientific complexity of climate change can also be a challenge for policy and business planning- simple sound bites and easy answers, whilst appealing, rarely lead to much except more "smoke and mirrors" posturing. Rainfall patterns, temperature, the incidence of frosts and extreme weather events, the availability of soil moisture, and sea levels, are all expected to be affected by climate change. Debates over the uncertainty inherent in climate change can also lead to "climate fatigue", as individuals, communities, and organisations struggle to come to terms with the reality of limits to our knowledge, and also debate what any one person can do given the scale of the issue.

Agricultural producers and associated business have to manage business risk, and plan investments, facing regulatory uncertainty and a high potential for rapid changes in market share, customer demand, and distribution networks. The cost of undertaking and communicating climate change research can be very high, given the complexity of the topic. Australia's climate and ecological realities give a real imperative to identifying and

[1]Program Manager, Land & Water Australia, Braddon, ACT.

realising innovations that support mitigating and adaptation to the impacts of climate change. It is also important to note that there will be winners and losers, as happens with any rapid changes in environment. Jurisdictions will need to plan to address the social equity and social justice issues that will emerge when some sectors cannot adapt to the new carbon economy.

However, there will also be winners and, as with any uncertainty, one can see risks and threats or opportunities. Communicating a slightly different perspective can encourage a more positive perspective for primary producers, businesses, and consumers.

Firstly, uncertainty is a core part of planning and investments. Financial Analysis (terminal values, discount rates), Portfolio Planning and Management, Scenario Analysis, Strategic Planning (future landscapes and emerging priorities), Pricing Analysis, and many other commonly used tools and techniques, all have inherent uncertainty and are subject to qualitative judgements as core drivers of subsequent quantifications. The recent Global Credit Crisis has helped expose the fallacy that econometric models can predict, with accuracy, future economic directions. All future planning, both with climate science and international financial markets, involves managing uncertainty.

Secondly, the "Good News" is that business, producers, innovators and consumers have all clearly demonstrated they can deal with and create value out of uncertainty. Ecologists and Biologists recognise that systems and species evolve both through linear and saltation (rapid jumps in state) based change. Farmers have been dealing with seasonal and climate variability for centuries. Businesses invest in research and development as they recognise that new products and services can rapidly morph the economics of a market place.

Climate change offers exciting opportunities for businesses and producers:

- Many mitigation measures represent good business management (resource efficiency).
- Valuations and risks weightings will change (theme based research) reflecting how well businesses disclose their strategies.
- Significant regional market opportunities may emerge during adaptation.
- Early movers can realise significant competitive advantage (market share, grants) and corner available public funds to support restructuring.
- The market is already moving to provide climate related products and services.
- Changes in export markets will offer opportunities for Australian companies.

Moreover, there are a number of strategic and operational measures that companies can immediately commence to help build capacity and address the challenges of climate change:

- Analyse and disclose financial risks and opportunities related to climate change.
- Develop company-wide plan to address climate change risks and opportunities.
- Educate CEOs and board members.
- Educate customers.
- Require major suppliers to adopt principles for engagement on climate change.
- Engage in policy dialogue at the state, regional and national levels.
- Have relevant disclosure material prepared for the investment community and insurance companies.

Perhaps most pertinently climate change mitigation and adaptation represent a great opportunity for innovation and investment, an opportunity that can and should be supported by the public sector – President Obama's vision for the creation of green jobs is a great example of how public investment can be used to address market failure issues whilst also creating a positive climate supporting innovation.

II. THE VALUE OF COORDINATING RESEARCH

There is already a wide body of literature that considers the need for the public sector to support research and development in light of public good considerations and market failures. From an economic perspective, pooling investment funds can generate economies of scale that make a potential investment viable, whereas no one firm could justify the investment in isolation.

The involvement of an organisation operating as an "honest broker" can help resolve investment barriers around ownership and returns, as well as potentially providing a valuable channel between industry and policy interests. Publicly owned infrastructure can generate a platform within which initial research and development activity can be used to identify derivative projects that have positive returns (as opposed to the investment required to set up the initial facilities).

In the current economic climate, with accompanying pressures on available capital, it is even more important to make wise strategic investment decisions. There is clear driver, in both the public and private sectors, for efficiency in investment allocation. It is equally important to maximise efficiency and avoid any duplication of investment.

The uncertainties inherent in climate change can make it very costly to undertake research and develop mitigation and adaptation measures. Pooling capital earmarked for climate change research can lead to savings from economies of scale and scope. In addition, through sharing information on service providers it is possible to realise further savings. Lastly, given the complexity of climate change research, establishing ongoing relationships with other organisations can reduce the overheads associated with 'coming up to speed' and also avoid 'reinventing the wheel'.

In summary, coordination of research activity can support the efficient use of scare investment capital both through reducing duplication of RDE activity and also through addressing market failure issues. Moreover, there are also many non-economic benefits associated with coordinating research activity in general, and climate change research activity in specific. Coordination can support the exchange of material information (data, research results, and price data):

- Across jurisdictional and geophysical boundaries.
- Along the RDE value chain, linking researchers to end users.
- Amongst the purchasers of research, enhancing Value for Money.
- Enabling cross sector and cross region issues to be addressed.

Coordination also supports the development of informal networks that can enhance consultation for evidence based policy, can be mobilized to rapidly respond to funding or investment opportunities, and can make it easier for organisations to interact under formal setting. Indeed, coordination can introduce and support a culture of collaboration and partnership, and the economic returns that can be realized through such

a culture have been explicitly recognized in the private sector with the emergence of alliance contracting, strategic alliance, and partnership arrangements.

Coordination of research investment programs is a well established practice in the private, multilateral and public sectors (for example, in Australia one can refer to the Council of Australian Governments [COAG] water related initiatives, the Agricultural Research Western Australia [ARWA] Alliance, or the Land and Water Australia's Managing Climate Variability [MCV] program).

The remainder of this paper shall briefly discuss the specific example of the Climate Change Research Strategy for Primary Industry [CCRSPI] initiative, before concluding with a consideration of specific mitigation and adaptation measures that may be material to the Poultry sector.

III. THE CLIMATE CHANGE RESEARCH STRATEGY FOR PRIMARY INDUSTRY

Recognising that changes to Australia's already variable climate will present great challenges and opportunities for the nation's primary industries, a collaborative partnership - CCRSPI - was established in 2007. The organizations involved in CCRSPI represent Australia's key researchers and research providers in the primary industries, with responsibility for investing in climate change research and for representing the end users of research.

The CCRSPI partnership initially involved co-investment to develop and implement a strategy identifying collaborative research opportunities that can help primary industries better understand, and build capacity to meet, climate change mitigation and adaptation needs. This strategy was released in 2008 as the CCRSPI Stage 1 report, along with two other reports from specific CCRSPI supported projects (all the documents are available from the CCRSPI website: www.lwa.gov.au/ccrspi)

Current activities involve coordination and communication of partner research initiatives, targeted research projects on behalf of the partners, completion of an audit of climate change projects across Australia, and development of a national RDE cross sectoral framework for climate change in the primary industries.

As illustrated in the diagram on the next page ("The CCRSPI Network), the key organizations involved in the CCRSPI partnership include:

- all the Rural Research and Development Corporations;
- the Australian Government and State Agencies through the Primary Industries Standing Committee [PISC][2];

[2] The Primary Industries Standing Committee (PISC) is a national committee supporting the Primary Industries Ministerial Council [PIMC]. PIMC consists of the Australian/State/Territory and New Zealand government ministers responsible for agriculture, food, fibre, forestry, fisheries and aquaculture industries/production and rural adjustment policy. Primary Industries Standing Committee agencies hold significant research capacity, provide regional extension services and also are responsible for national and regional policy relating to the primary industries. The PISC agencies include: NSW Department of Primary Industries, Victorian Department of Primary Industries, Queensland Department of Primary Industries & Fisheries, Department of Primary Industries & Resources SA, Department of Agriculture and Food WA, Department of Primary Industries and Water Tasmania, NT Department of Primary Industry, Fisheries and Mines, ACT Department of Territory and Municipal Services, Australian Government Department of Agriculture, Fisheries and Forestry [DAFF].

- the CSIRO Climate Adaptation Flagship[3]; and
  the Australian Council of Deans of Agriculture [ACDA][4].

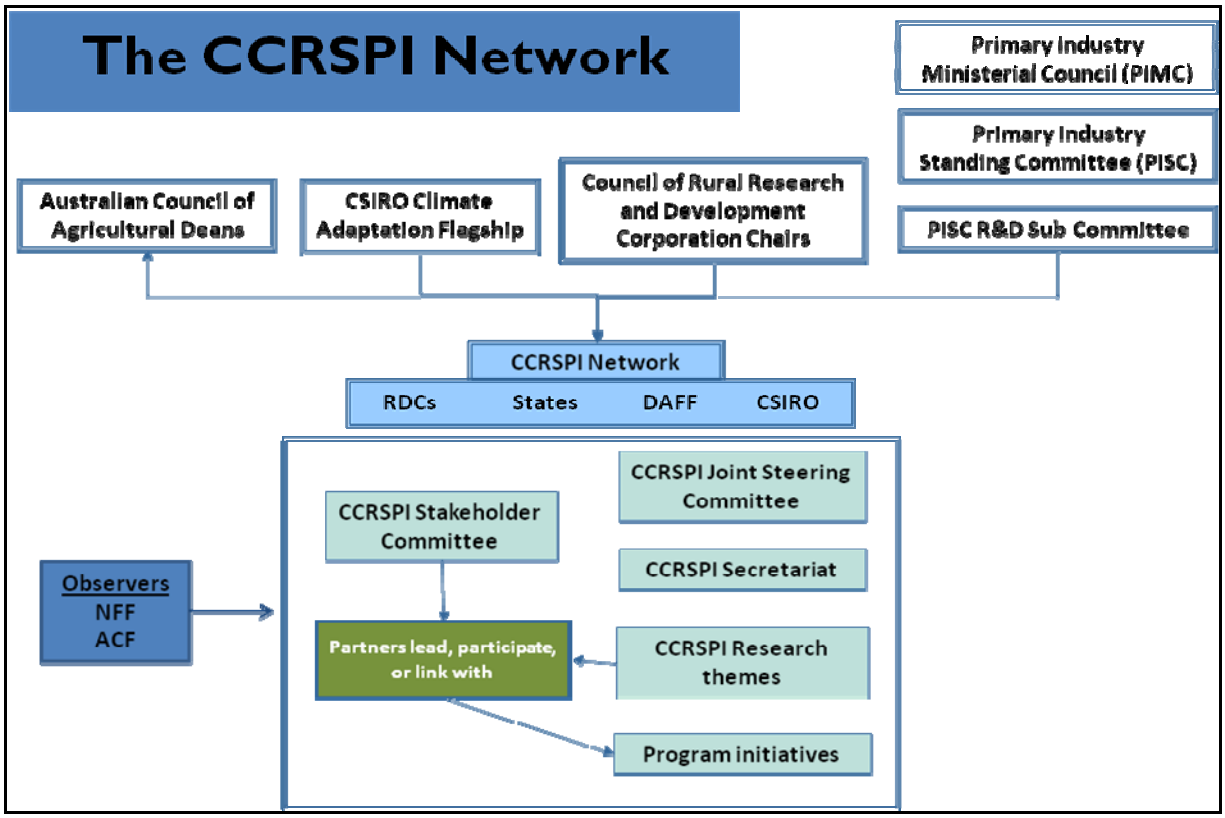
CCRSPI is governed, on behalf of the partners, through a Steering Committee, and with reference to a wider Stakeholder Committee. The CCRSPI Steering Committee is a national committee that was established by, and reports to, the PISC R&D Sub Committee and the Council of Rural Research and Development Corporations Chairs [CRRDCC][5].

Universities and research leaders from across Australia have also been involved in the CCRSPI network, both during the consultation process for development of the Stage 1 CCRSPI report and also through CCRSPI's ongoing activities. The CCRSPI unincorporated partnership operates with a focus on delivering value to partners through a 'lean' administrative Secretariat hosted within an organization – Land & Water Australia [LWA] – very familiar with the Research Development and Extension [RDE] environment. In essence, the CCRSPI Secretariat takes on the 'honest broker' role representing all the investing partners. Financial contributions were based on pre agreed formula, endorsed through PISC, and unused funds will be returned to partners using the same formula. Partners share all outputs and Intellectual Property (which is placed in the public domain).

[3] The CSIRO Climate Adaptation Flagship was announced in April 2007. The flagship aims to deliver the best available scientific information and expertise to support Australia's efforts to adapt to climate change today, and includes the development of adaptation options for Australia's agriculture, forestry and marine industries to reduce the vulnerabilities and enhance strategic opportunities as part of the theme "Adaptive primary industries, enterprises and communities". In 2008/09 the CSIRO Climate Adaptation Flagship will include 25-30 FTEs dedicated to primary industries research.

[4] The Australian Council of Deans of Agriculture [ACDA] was established by the Deans and Heads of Schools of Agriculture in Australian universities in July 2007. The Universities represented include Adelaide, Charles Sturt, James Cook, La Trobe, Melbourne, New England, Queensland, Sydney, Tasmania and Western Australia and Western Sydney. The ACDA was established in part to champion human capital development in agriculture and agricultural research, and ensure agricultural graduates meet the needs from the workforce. The ACDA represents a key network for accessing postgraduates, early career scientists and research groups that deliver approx 35% of Australia's agricultural research.

[5] The Council of Rural Research and Development Corporations' Chairs (CRRDCC) is the forum for coordination and discussion of cross-rural industry research and development corporation issues.



CCRSPI focuses on co-ordination and communication, not control of the research agenda. The CCRSPI framework operates by consensus and transparency, with regular reporting to CCRSPI Steering Committee, and is flexible to different partner needs / processes (partners are free to pursue their own research interests as desired). CCRSPI partners 'lead', 'participate' or 'link' research activity on agreed research themes, and work collaboratively on Submissions or projects as needed and as fits with the internal approvals processes and guidelines of the individual partners.

There are other opportunities to coordinate research, outside of the CCRSPI partnership. The National Climate Change Adaptation Research Facility [NCCARF], managed by Griffith University and funded by the Australian Government Department of Climate Change [DCC], is establishing eight Climate Change Adaptation Research Networks to support coordination and communication of research and the development of national adaptation research plans.

One of these networks will focus on primary industry. A proposal for a Primary Industry Climate Change Adaptation Research Network [PICCARN] is currently awaiting Ministerial Decision. Whenever the primary industry network is announced, it will provide further opportunities to link researchers will the users of research, and develop a truly national response to the challenges of climate change. The CCRSPI partners, through Land & Water Australia, have already made a financial commitment to support PICCARN in addition to the proposed funds from NCCARF.

Further opportunities for collaboration exist, for example under the overall PISC framework, during development of the DCC lead National Climate Change Science Framework, and as part of the Council of Australian Governments [COAG] programs.

IV. CONSIDERATIONS FOR THE POULTRY INDUSTRY

So, climate change is complex and uncertain, coordinating research can be a cost effective means of addressing some of the associated challenges, and the example of CCRSPI shows that this can be effectively be applied to Australian primary industry. But what does this all mean for the Poultry sector?

Well, firstly it is worth highlighting that the Poultry sector is already involved with CCRSPI through the involvement of the Australian Egg Corporation, other RDCs, and the relevant sections of the State Governments and DAFF. Moreover, the Poultry industry provided valuable input during the extensive consultation process that lead to development of the CCRSPI Phase 1 report. Further consultations will be held to ensure the research needs of poultry producers are considered, both through CCRSPI and within the proposed PICCARN network.

In addition to the measures that all business can adopt – highlighted in section 2 of this paper – it is also possible to identify some specific mitigation and adaptation issues and options that are of immediate relevance to the Poultry sector.

In a 2008 report produced for the CCRSPI partners, CSIRO noted that the Australian poultry industry is primarily focused on chicken meat (broiler) production and egg productions, with a small number of turkey and other fowl producers. Chicken meat production has become increasingly regionalised, following initial development near the major capital cities. Most commercial grow-out farms are intensive and highly mechanised. Chickens tend to be raised in large open sheds, with 3 to 10 sheds per farm, holding 40,000 to 60,000 chickens per shed. Egg production and supply is largely met by just over 400 companies (ABS 2005) mainly located around major metropolitan or regional centres with easy access to feed stock. Farms range in size, with the largest having between 100,000 and 500,000 hens contained in multiple level sheds (although many will not have more than 20,000 birds). For both chicken meat and egg production, shed temperature, humidity and air quality are carefully controlled and regulated, and a guaranteed supply of water is required.

Poultry flocks are particularly vulnerable to climate change because birds can tolerate narrow temperature ranges. More dramatic events such as storms increase stress and may affect productivity. Farmers have clearly stated that they need much greater information on climate variations within decades and regions, as opposed to the current focus of policy and media dialogue on longer term climate projections.

The Future Farming initiative has highlighted (2008) other specific opportunities and challenges for Poultry farmers, as set out in the table below:

OPPORTUNITIES	CHALLENGES	
Savings	Productivity	Costs
Winter energy costs may reduce as warmer winters reduce the need to heat buildings and flocks can be acclimatised outside.	Housing systems need to be managed to maintain optimal seasonal temperatures and reduce the risk of heat stress	Increased energy costs to cool buildings in summer Building infrastructure and maintenance to cope with more intense weather events and increased rainfall.
Locally grown produce reduces feed costs and reduce poultry food miles.	Increased investment in ventilation and cooling systems	Stocking density may need to be reduced in extreme temperatures, potentially increasing costs
Meat products may increase in price and with feed prices possibly decreasing (yield to increase as a result of rising CO2 levels) poultry farming may become more profitable	Decreased reproductive capacity due to increased temperature	Actively controlled ventilation could become essential in transportation Increasing need for ventilation to reduce housing humidity

Consideration of these challenges and opportunities helps one identify mitigation measures that can be used by poultry farmers, such as using biomass boilers or anaerobic digestion of poultry litter, or installing renewable energy power for poultry sheds. Poultry specific adaptation measures could include timing building renewals, and updating buildings design, so to investment in structures that can more effectively cope with new climate and weather extremes, and to base the design of these structures on ongoing studies that assess heat stress on animals.

Other asset and investment decision making can consider installing special equipment – such as ventilation and cooling systems – that will helps poultry farmers to adapt to new climate extremes affecting their stock.

THE POULTRY NURITIONISTS CHALLENGE

J. RATCLIFF[1]

Summary

Higher feed costs have resulted in the search for alternative feed ingredients and suppliers. At face value, alternative sources of ingredients may appear more economic, however, there are inherent risks associated with extending the global supply chain. Contamination with undesirable substances or analytical variation can often override any economic advantage. An extended supply chain also places greater reliance on robust traceability and supplier assurance. Evaluation of new ingredients and their optimisation within a formulation are the nutritionist's responsibility to avoid compromising gut integrity or performance.

I. INTRODUCTION

The feed industry is facing unprecedented challenges and the spotlight is very much on the nutritionists to maintain cost effective solutions without compromising performance. The challenge arises not just because of escalating costs but from associated problems of supply, reliability and optimisation.

II. INGREDIENT SUPPLY

a) Availability

The most immediate problem for certain raw materials and particular areas of the world is availability. The task of sourcing raw materials has become more of a challenge as highlighted by the supply problems in 2008 associated with phosphorous and methionine. Any future restriction on the supply of essential nutrients, minerals and vitamins could have a devastating effect on the feed industry leading to the prospect of rationing of total feed production and hence reduced livestock numbers. In many regions of Asia, a reliable supply of both protein and energy sources can be a problem due to weather, harvest failures, transport problems and border issues. In extreme cases, formulations can be changed almost on a daily basis due to uncertainty over supply. Frequent changes in the raw material composition of formulations are something nutritionists should try to avoid. However, many by-products are only available on an infrequent or limited volume basis depending on other associated industries, and this can lead to large swings in raw material content which in turn can be associated with gut integrity problems.

b) Traceability

Once availability is secured the next significant challenge related to supply is traceability – where has the material originated and how has it been handled and stored between production and supply to the feedmill or premix plant? Traceability has become THE challenge for the global feed industry. Melamine contamination in China and dioxin contamination in Ireland have renewed a global awareness of the need for due diligence and traceability for all raw materials, ingredients and feed additives. A major part of any feed companies purchase procedures should now be invested in supplier approval. In Europe the feed industry has collaborated with the supply industry to produce independently audited standards of assurance based upon Hazard Analysis and Critical Control Point (HACCP) and Good

[1] F.A.C.S Ltd - UK

Manufacturing Practice (GMP). In other areas of the world where no such audit standard is available, the due diligence lies with the purchasing feed company to inspect and audit all suppliers and thereby create a list of approved suppliers and ingredients based on risk assessment. Failure to do so will result in an unacceptable level of risk within the feed and food supply chain.

c) Consumer Acceptance

The feed industry is also increasingly prone to the issue of which ingredients or additives may or may not be consumer acceptable. Obvious examples are genetically modified raw materials, antibiotic growth promoters and the use of mammalian by-products. However, NGOs are now highlighting issues such as deforestation (soya and palm oil), sustainability (fishmeal) and antibiotic residues (fermentation by-products) all of which places further pressure on the supply chain.

III. RELIABILITY

a) Nutritional consistency

Assuring consistency of the quality of raw materials is nothing new for the feed industry. Comprehensive raw material testing and matrix evaluation procedures already exist, many of which are based upon rapid NIR technology. However, the scale in variation of by-products from load to load places additional pressures on the nutritional challenge.

b) Undesirable Substances

Of greater concern can be the unseen variability in terms of contamination with undesirable substances such as mycotoxins, heavy metals, pesticides and dioxins/PCBS. Many companies do not have the resources to regularly test for such chemicals and even where facilities exist, often the test results are too late to prevent problems at farm level. It is essential that a testing plan based on raw material risk assessment is implemented in association with a robust supplier approval process. Reliance on certificates of analysis is not an effective or acceptable risk assessment procedure.

c) Micro ingredient residues

Special mention should be made about micro ingredients including premix, minerals, vitamins, medicines and feed additives. Often this sector receives least awareness in terms of suppliers risk assessment and yet potentially can represent a greater risk in terms of not only undesirable contamination but other chemical contamination such as antibiotics. Manufacturers in this sector need to apply rigid HACCP and GMP procedures to ensure absence of contamination risk and feed companies should avoid suppliers that cannot meet these quality standards.

IV. CONSTRAINT OPTIMISATION

The final challenge for the nutritionist is to decide what level of ingredient or additive to use in the formulation. The decision is complex and involves cost, nutrient availability and the presence or level of anti nutrient factors and undesirable substances. The sudden rise in raw material costs magnifies the complexity of the problem and places the nutritionist under even more pressure to justify constraint levels balanced against performance criteria such as wet litter or palatability. In many cases however, the decision is too conservative and ends up incurring unnecessary cost to the company. In other cases, the true nutrient availability is not reflected or fails to make use of existing technologies.

The nutritionist should look to embrace products and technologies that are acceptable to the consumer and which enhance nutritional contribution (e.g. latest generation enzymes, novel proteins and organic minerals) or help limit the impact of constraint limitations imposed by undesirable substances or anti nutrient factors (e.g. mycotoxin binders and products that help maintain gut integrity).

The most cost effective policy is to invest in the early feeding phase and ensure animals get off to the right start by optimising nutrient availability and ingredient palatability. Failure to optimise nutrition at this stage will often lead to impaired gut integrity at a later stage of life, resulting in performance deterioration, wet litter and the need to resort to medication.

CLIMATE CHANGE AND RAW MATERIAL SUPPLY FOR POULTRY

R. A. SWICK[1]

Summary

Agricultural commodities used as raw materials by the poultry industry are under threat from climate change. Government mandated use of renewable biofuels to mitigate global warming has created new competition in commodity markets. Production of these commodities also significantly contributes to greenhouse gas emissions from oxidation of soil carbon and nitrogen during tillage operations. Greenhouse gas affects variability in weather patterns causing droughts and floods to be more severe thus increasing the probability of crop failure. To date, production and supply of wheat, maize and soy has easily kept up with human population growth and meat consumption over the past decades. Over the past 2 to 3 years however, the industry has experienced short ending stocks and sharply increased prices. In addition to mandated increases in biofuel production has been a large increase in the world's meat eating population and several years of drought in Australia that has had a major negative impact on wheat yields. Record high petroleum prices and interest by hedge funds in agricultural commodities has further driven up prices during this period. With a continued growing human population, increasingly severe weather patterns and greater demand for a finite amount of petroleum, sporadic scarcity and price spikes of raw materials will likely continue well into the future. As this happens, sustainable production of raw materials will become a major issue. Biotechnology enhanced (GMO) crops such at Bt corn and glyphosate tolerant soybeans have been effective in reducing greenhouse gas emissions. These crops are suited for zero tillage production systems that reduce oxidation and release of soil carbon and nitrogen. In addition, these improvements have steadily increased yields and total output. It is unclear when the general public may consider biotechnology enhanced crops more sustainable than traditional crop varieties. Consumer understanding of the sustainability issue may help mitigate climate change. The future may hold opportunity to those poultry producers who are first to market their products on the basis of sustainability.

I. INTRODUCTION

It is well accepted that changes in global climate are occurring and the planet is becoming warmer due to higher atmospheric concentrations of greenhouse gases including CO_2, CH_3, NO_2. Figure 1 shows the relationship between average global temperature and CO_2 levels in ice core samples (Keeling and Whorf, 2004). About two-thirds of greenhouse gas emissions are human induced and include burning of fossil fuel, methane release from ruminant production and release from soil during tillage. About one-third of current greenhouse gas emissions are caused by natural events such as volcanic eruptions, release of CH_3 from the oceans and swamps, forest fires and other natural causes. Greenhouse gas levels from ice core samples representing atmospheric conditions over thousands of years suggest that levels are higher now than ever before (Petit *et al*., 1999). There is strong evidence from ice core samples that average levels of atmospheric greenhouse gases have risen since preindustrial times. Since 1750 for example, carbon dioxide levels have risen 37% from 280 ppm to 384 ppm; methane has risen 250% from 70 ppb to 1745 ppb, nitrogen dioxide has increased 16% from 270 to 314 ppm and non-degradable freon (CFC-12) has risen from zero to 553 ppt.

[1]Linden Nutrition Pte Ltd, My SingPost Box 88120, Singapore 919191

Human burning of fossil fuels, crop production and ruminant production have been the major contributors of the recent increases.

Large scale conversion of crop resources to renewable biofuels has occurred in recent years as a way to limit burning of fossil fuels, mitigate greenhouse gas caused warming and decrease dependence on the OPEC petroleum cartel. During this period, the poultry industry has experienced record high raw material prices. Crops high in sugar, starch and fat have been in great demand. Governments all over the world have mandated addition of biofuel to gasoline and diesel fuel. Politicians and lending institutions have become involved in this frenzy, causing speculation in grain and oilseed markets that has amplified the situation. In 2006, petroleum prices for the first time in history became highly correlated to grain and oilseed prices (Campiche *et al*., 2007). Superimposed on this has been a relentless demand increase for poultry products from the burgeoning broiler and egg eating human population. While the poorer general economic situation of recent months has reduced prices and unwound speculative positions of both petroleum and crop based energy sources, it is likely such relief will only be temporary. The population base is still increasing and demand for lower carbon footprint energy sources will continue. Once there are better economic times, demand and higher prices will resume. This paper deals with climate change and its affects the poultry raw material supply, sustainability and use of biotechnology enhanced crops.

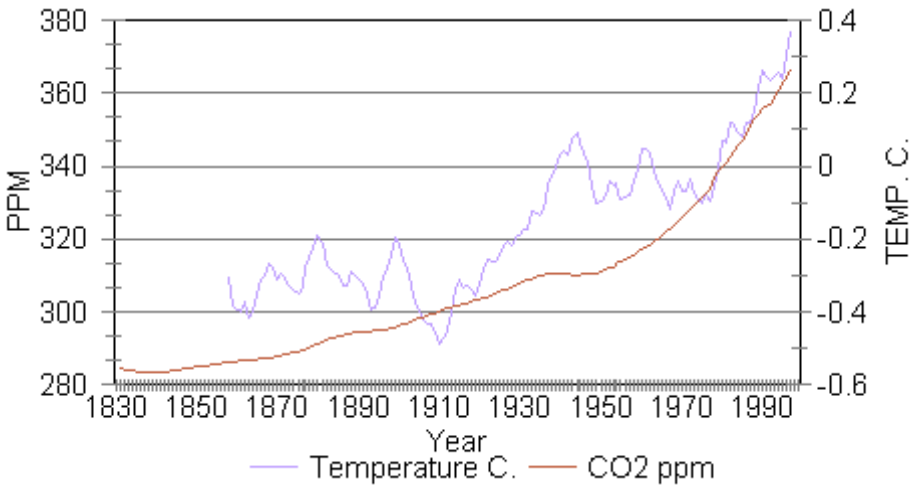


Figure 1 CO_2 levels in ice core samples (Law Dome, Antartica and Mauna Loa, Hawaii) and global average temperature variation from 1830 to 1998. (Adapted from Keeling and Whorf, 2004)

II. POPULATION AND DEMAND FOR POULTRY PRODUCTS

The world's population is projected to increase from six billion in 2000 to over nine billion by 2050. Countries with large population bases and high growth rates are Indonesia- 240 m, 1.5%, India - 1,065 m, 1.4%, Pakistan - 161 m, 2.0%, Bangladesh -143 m , 2.1% and Brazil - 185 m 1.1% (Anon, 2007). Figure 2 shows global demand for meat over the past 19 years. Pork has led the way. China now leads the world in total pork production at around 44.5 mmt of carcass weight equivalent. This was about the same as 2005. In 2006 pork production was reduced in China to 42.9 mmt because of problems associated with highly pathogenic PRRS disease. According to Chinese Government sources, the disease is now under control. The U.S. leads the world in poultry production with a yearly production of 19.5 mmt ready to cook equivalents (Anon, 2008). Total demand for beef remained flat in the 1990's indicating

beef consumption has lost significant market share. Broiler meat production is increasing at a more rapid pace than pork and has the potential to outpace pork in coming years due to large increases in the world's Muslim population. In many countries, per capita income levels have more than doubled over the past two decades. Although purchasing power has increased for almost everyone in the world over the past decade, patterns of household spending on food differ dramatically between high and low income countries. High-income consumers spend more on meat and dairy products than do low income consumers. Low-income consumers will alter their food choices more readily when prices or income levels change. A 10% increase in income would result in a 1% increase in food expenditure in the U.S., a 6.5% increase in the Philippines and 18% in Tanzania (Seale and Bernstein, 2003). Above about U.S. $1,000 per capita income, food expenditures change greatly with significantly more money spent on meat. Countries growing from the $500 to $5000 per capita income range thus can be expected to have rapidly growing poultry and feed industries if there is economic growth and a stable political system. Changes in population and income levels have had much more of an impact on demand for commodities than demand for biofuel production.

According to the FAO, the world-wide total production and use of animal feed exceeded 4,000 mmt in 2002 of which some 550 mmt were milled feeds (Bruinsma *et al.,* 2002). The largest proportion of the 4,000 mmt of feed was used by small farmers in developing countries. There is a continuing rise in the demand for animal products and particularly those from poultry and pigs. FAO and other institutions suggest that global production of animal products will rise impressively over the next 20 years. Industrial feed production will gain at the expense of on-the-farm or rural feed production due to increased public concern about contaminants and health, and demand for safety, regulation, traceability and sustainability. The use of high quality feed ingredients from known sources will increase in the future as subsistence animal agriculture gives way to a more efficient, business minded, low carbon footprint, integrated approach.

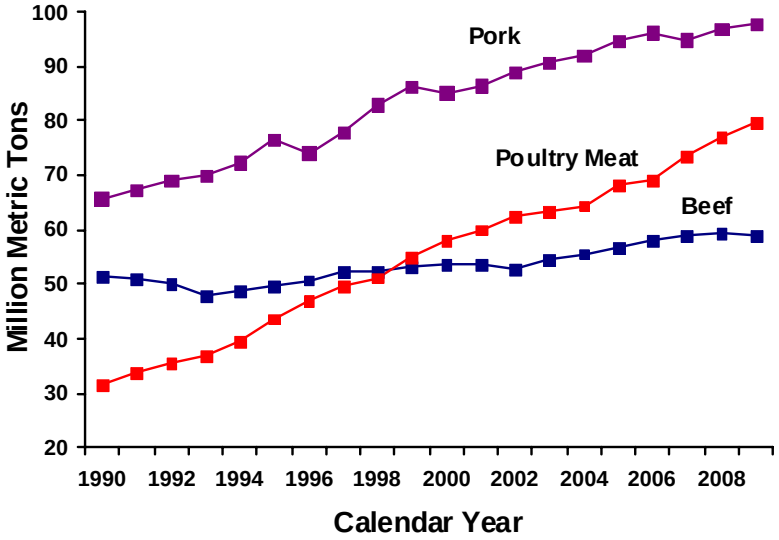


Figure 2 Increases in global meat production (Anon, 2008)

III. RAW MATERIAL SUPPLY AND CLIMATE CHANGE

The U.S. dominates world trade in maize and is projected to produce 310 mmt in the 2008/2009 crop year. This represents 39% of the world's production of 785 mmt Figure 3. Domestic U.S. demand for corn will increase in the coming years. Although China's corn production is projected to increase, China will become a net corn importer in 2010/2011 as

demand for livestock feed overtakes China's internal supplies of corn and more acreage goes to horticultural production. World coarse grain trade is expected to expand nearly 21 mmt (18 percent) from 2007 to 2017. About two-thirds of global coarse grain production is used as animal feed. Industrial uses, such as starch, ethanol, and malt production, are smaller but growing. Food use of coarse grains, concentrated in parts of Latin America, Africa, and Asia, is projected to continue declining. World prices for grains have risen during the last several years as global stocks of grain declined sharply. Although the higher prices are projected to stimulate grain production, neither stocks-to-use ratios nor prices will likely to be as low as average levels seen over the past three decades. A steady and persistent population driven growth in the livestock sectors of developing countries in Asia, Latin America, North Africa, and the Middle East is projected to account for most of the growth in world coarse grain imports during the next decade.

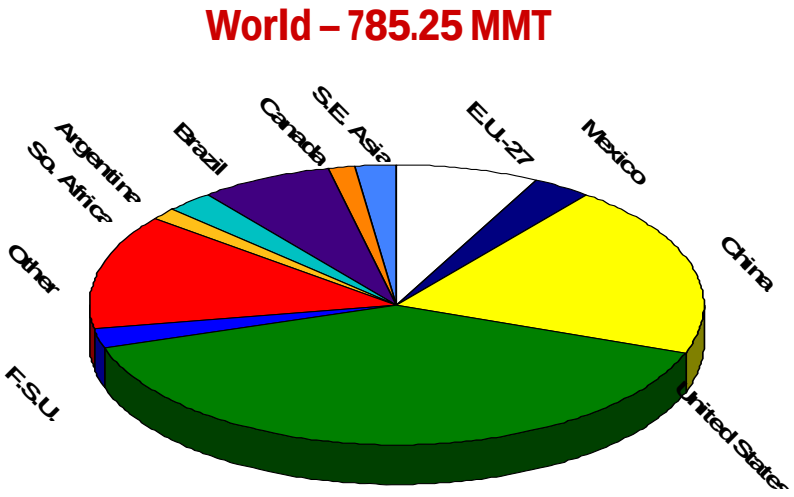


Figure 3 World maize production in 2008/2009

Global oilseed production for 2008/2009 is projected to be 420 mmt with soybeans accounting for 239 mmt (Wescott *et al.,* 2008). Soybean meal consumption has increased more rapidly than corn, wheat or rice over the past 14 years. Soybean meal consumption grew by 110%, while corn consumption grew by 40% and wheat by only 7% over that period. The soybean production increase has been driven by demand for soy oil and meat consumption. The 2008/2009 U.S. soybean crop has been recently projected to be 81 mmt. Figure 4 shows that soybean production in South America is increasing at a much faster rate than in North America. Figure 5 shows the projected global soybean exports to 2016. Brazil will expand its production by opening vast tracts of land to soybean production. Brazil has the equivalent of new land available as all of the cultivated land in the U.S. (Sato, 2004).

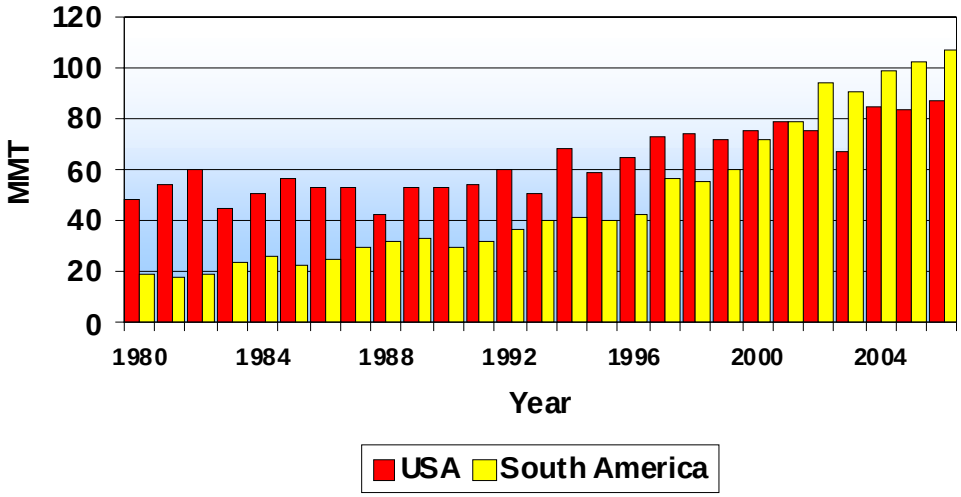


Figure 4 U.S. and South American soybean production from 1980 to 2007. (Anon, 2008)

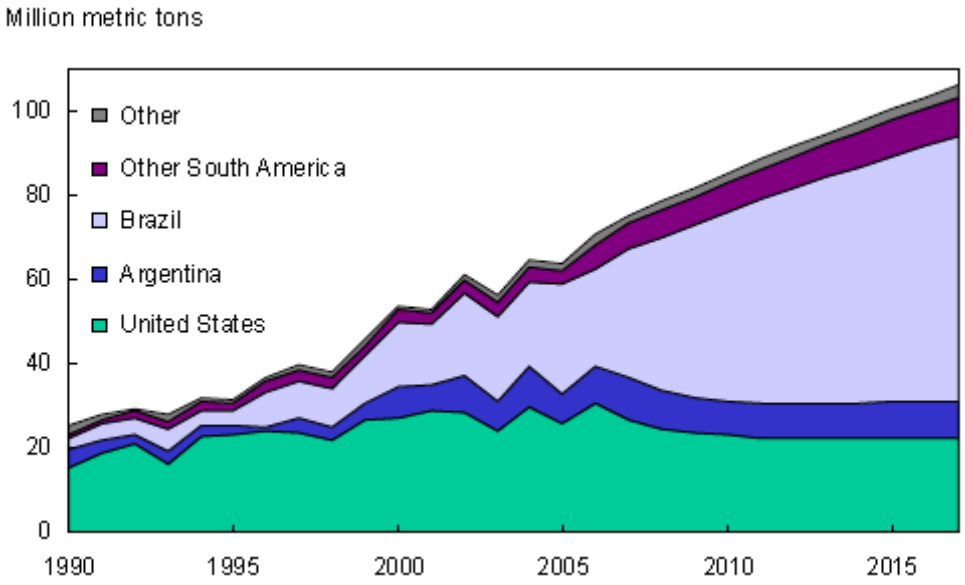


Figure 5 Global exports of soybeans to 2016 (Wescott *et al*, 2008).

Of concern is the potential disruptive impact of climate change on crop production from climate variability and occurrence of extreme events. Agricultural systems are vulnerable to climate extremes, with effects varying from place to place because of differences in soils, production systems, and other factors. Changes in precipitation type (rain, snow, or hail), timing, frequency, and intensity, along with changes in wind (windstorms, hurricanes, and tornadoes), are likely to have significant consequences. Heavy precipitation events cause erosion, water-logging, and leaching of animal wastes, pesticides, fertilizers, and other chemicals into surface and groundwater. A major source of weather variability is the El Niño Southern Oscillation (ENSO). ENSO effects vary widely across countries and growing areas. Better prediction of these events would likely allow farmers to plan ahead, altering their choices of which crops to plant and when to plant them. The value of improved forecasts of ENSO events under their current intensity and frequency has been estimated at approximately

$500 million per year in the U.S. alone. Mitigation of climate change will be the responsibility of many industries. Capable and strong leadership will be required to change destructive habits.

IV. SUSTAINABILITY AND TECHNOLOGY

The term "sustainable agriculture" as described by the U.S. Code of Federal Regulations (Title 7, Section 3101) is an integrated system of plant and animal production practices having a site-specific application that will over the long-term:

* Satisfy human food and fiber needs.
* Enhance environmental quality and the natural resource base upon which the agriculture economy depends.
* Make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls.
* Sustain the economic viability of farm operations.
* Enhance the quality of life for farmers and society as a whole.

Crop production can be either more or less sustainable depending upon energy consumption, land use and deforestation, emissions, material consumption and risks to the environment such as soil conservation and runoff of fertilizers and pesticides. It may be in the best interest of the poultry industry to examine and consider what makes one raw material more sustainable than another. The BASF Company has developed a system to index the eco-efficiency of various alternative products within a given industry. This concept might be applied to the raw materials used by the poultry industry. The system is depicted in Figure 6.

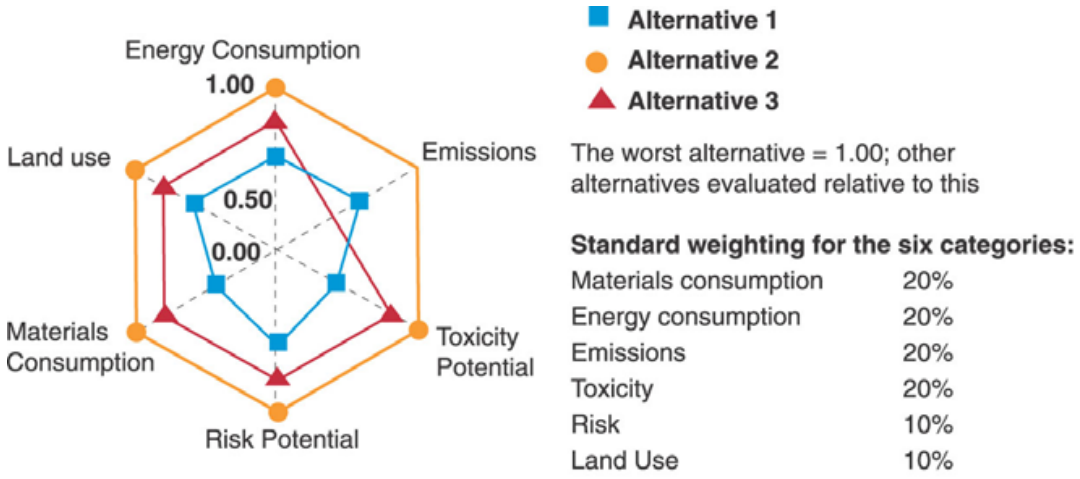


Figure 6 Eco-efficiency analysis index (Kicherer, 2005)

Ingredients from different suppliers such as different origins of soybean meal or fishmeal could be evaluated and indexed. Those requiring higher fertilizer and pesticide input and long shipping distances would get a poorer index value. Those alternatives requiring less tillage, less insect damage and lower potential for mycotoxin contamination would get a more favorable index value. Index values could then be incorporated into the feed formulation program along with price and available nutrient content to formulate a a least cost, more highly sustainable feeds that meets nutrient requirements. Biotechnology enhanced crops such as Bt corn and glyphosate tolerant (RoundUp Ready) soybeans might be considered

more sustainable using this system as these crops are suited to zero till production systems. Less tillage results in a more positive soil carbon balance. Less fertilizer and pesticides are used and there is lower risk of infestation with insects and mycotoxins. This concept of sustainability of raw materials could be used as a marketing tool to promote certain brands of poultry products.

V. CONCLUSION

As the climate changes and the biofuels industry grows, agricultural producers will experience direct impacts on their income levels as the demand for feedstocks expands. Crop producers will benefit from an increased demand and price for their products, while livestock and poultry producers will face higher production costs as they compete for raw materials at least until such time as cellulosic production of ethanol or importation of ethanol from Brazil become a reality. Weather related events are likely to increase in intensity and may cause shortages when they occur. As the finite supply of petroleum is consumed, prices will soar, and the production of renewable fuels will become increasingly important. Growth of this industry has tremendous implications for agriculture and opens up many new opportunities and risks. Market prices of agricultural commodities have already become dependent on petroleum prices. An understanding of the relationship between agricultural commodity and fossil fuel price will become essential for poultry feed producers to make informed decisions. The poultry industry is an integral and growing segment of the food supply chain. Poultry products are a vital and important food source for the world's 6.3 billion people who are multiplying at a rate of an additional 72 million per year. Increasing broad-based income growth and urbanization are changing eating patterns leading to increased meat consumption. Industrial feed production is growing at a rate of at least two to three percent per annum globally. The world will likely experience more tightness and price spikes in coarse grains and vegetable oils as renewable biofuel production increases and China becomes a net importer of corn. Production of soy will increase dramatically in South American especially Brazil. Raw material sustainability will be come an important issue and poultry feed may one day be formulated on an index of sustainability. Biotechnology enhanced crops will become more globally accepted when the general public realizes their environmental benefit in terms of soil conservation, carbon sequestration, lower pesticide use and better overall quality from reduced insect and mold infestation. This technology also holds promise to provide higher available nutrients and lower levels of anti-nutritional factors. The poultry industry will continue to be a dynamic place punctuated by major technological developments and more variation in raw material production due to climate change.

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NATIONAL ADOPTION AND EVOLUTION OF THE WPSA 'HIGH SCHOOLS POULTRY EDUCATION PROJECT'

P. KENT[1]

I. INTRODUCTION

The World's Poultry Industry has a high media profile resulting in constant public scrutiny. The acceptance of animal products is largely dependent upon community perception of how the industry produces its products. Misleading information about the poultry industry appears regularly in Australia's national media and it is often reinforced by people with a high standing in the community. There is a public perception that poultry are responsible for many human health problems and other issues, with this incorrect information including: (i) hormones are fed to poultry, (ii) antibiotics are used indiscriminately, (iii) alternative productions provide a more wholesome product, (iv) free range production is environmentally sounder and (v) eggs are linked to an increase in coronary heart disease.

These perceptions can impact on the industry through loss of consumer support i.e. sale of product. However, this image appears to be gradually changing as a result of community education. In 2000 the World's Poultry Science Association (WPSA) developed the High School Poultry Industry Education project to promote awareness and knowledge to the school community on all aspects of the poultry industry and disseminate correct information to the consumer. Over the last nine years, the project has been conducted in 85% of Queensland's agricultural schools. Participation has increased by 5% each year and now 54% of Queensland schools with agricultural studies take part in the annual project.

II. PROJECT OBJECTIVES

The objectives of the project are:

- To provide accurate and balanced information to the community on the poultry industry
- Strengthen community and consumer confidence in poultry products and practices
- Promote the poultry industry as a sound career opportunity
- Strengthen the way industry members feel about their role in the industry and the contribution their industry makes as a provider of green clean food to the nation.

III. PROJECT OPERATION

At the beginning of each year, schools are invited to take part and participating schools are provided with a resource pack consisting of:

- Guidelines and rules
- A schedule of operational time lines
- Industry information and contacts
- Poultry information such as web sites and reference material suitable as teaching aids.
- Participating schools conduct a simple poultry trial during second term (6-8 weeks).

[1] Extension Officer, Department of Primary Industries & Fisheries, WPSA Education Project Co-ordinator

As part of this, students are required to undertake a literature review of the Australian poultry industry in relation to their trial. This not only supports their trial but also provides an opportunity to learn about the poultry industry, their products and practices.

At the end of term three, the school teams submit a report (100 marks) on their trial and the project's activities culminate in fourth term when representatives from each school support their findings by presenting posters (50 marks) at an Open Day held at a Department of Primary Industries and Fisheries' facility. At this day, students, teachers and guests are given short informative presentations on career opportunities and roles within the industry. Certificates and prizes are presented to the students based on marks received for their reports and posters.

IV. PROJECT EVOLUTION

The project was started in 2000 to combat misinformation about the use of hormones in poultry production and although it is now over 50 years since the poultry industry ceased this practice, it still lives with the stigma of hormone use e.g. in 2004, a fast food outlet was advertising a product as being made from hormone free chicken breast meat. To further assist in providing correct information, in 2003/2004 the Australian Egg Corporation Limited (AECL) sponsored the development of a project reference manual containing 40 relevant Australian poultry information web sites.

Responding to media of the day *(Choice Survey 2003: 84% of Australians are concerned about eating genetically modified food; World Poultry 2003: cholesterol and egg intake led to the 1972 Cholesterol phobia),* the project team produced a second manual containing information on genetically modified foods (GMO), quality assurance and antibiotics.

In 2005 in the Australian Poultry Co-operative Research Centre's (CRC) "Education and Training Requirements for the Australian Poultry Industry" report, the industry described itself as having a very poor image and stated:
"Better education and training would encourage more skilled and quality people to the industry and in turn make the industry more professional and *68% of respondents thought that there should be more information about the poultry industry and career opportunities delivered in schools and to the media.*

In response to this, in 2006 the project co-ordinator changed some of the rules, the most significant being to remove the requirement of only grade 10 or higher eligible to participate in the competition, and increased the focus on poultry training/career opportunities and global warming (carbon footprint) while still fulfilling the project's original role of counteracting wrong or misleading information.

In 2008, the Poultry CRC provided funds to expand the competition nationally and through discussion it was decided to concentrate initially on New South Wales, Victoria and South Australia and invite a small number of schools from these states to take part in the 2008 competition.

Due to different teaching curriculums and time frames between states, it is imperative that the competition has the support of state Agricultural teachers. To achieve this, the competition team have been working with state Agriculture co-ordinators and will continue to use their educational expertise to make sure the poultry education competition expands and remains relevant.

The best outcome for this competition would be to have a coordinator/s working under the Poultry CRC, AECL, Australian Chicken Meat Federation (ACMF), or other leading groups such as WPSA. These co-ordinators in turn would liaise with state agriculture committees while the annual operational requirements such as birds, poultry products,

donations and venues would be sponsored by state WPSA sub-branches and the Australian poultry industry.

In the 2008 project, 26 Queensland schools participated, with poultry trials relating to product quality and feed comparisons. The winning Queensland projects examined:

- Egg production comparison between Barn and Free-range
- The effects of green feed on yolk colour and freshness
- Dietary effects on point of lay performance and
- Does the addition of green feed improve growth?

The winning entry for New South Wales was from New England Girls School, which studied "Chooks and their therapeutic benefit for prep to grade 10 boarding school students".

The winning South Australia entrant was the Tintinara Area School in which their year 6/7students researched the advantages and disadvantages of showing poultry and produced a poultry showing manual.

The industries' poultry competition has evolved to keep pace with the communities' changing needs and continues to provide:

- A teaching aid that develops skills including team building, report writing, poster design, experimental design and operation, probabilities and statistics.
- Experience in animal contact/handling and husbandry, with the animals' size and temperament making it suitable for all ages.
- Career information and industry contacts for students and teachers.
- A unique opportunity for students to meet with livestock professionals, nutritionists, veterinarians, agricultural scientists and poultry academics.
- Communication towards increasing awareness of correct production information and healthy choices for good quality and nutritious foods.

For the Australian Poultry industry it provides some unique opportunities in a communication channel:

- for delivering unbiased information at a time when young Australians are at their learning peak
- As a vehicle for new industry practices/information to be communicated to the community. e.g. Avian Influenza.
- In which information can be easily kept up to date and channelled into each year's competition.
- To Combat negative publicity and misconceptions.

Due to the national skills shortage, similar school based programmes are now being implemented in other states by other animal industries. e.g. the dairy programme in Victoria.

The WPSA School Education Competition has now been developed to a point where its continued growth and national adoption depends upon the whole Australian Industry for their sponsorship and promotion of this unique national program and to take advantage of the opportunities it provides to inspire and educate Australia's most important asset, our younger generation. Today's students will be tomorrow's parents, consumers, politicians and legislators. The knowledge they gain will flow on for several generations and help cement industry sustainability both socially and ecologically.

ACKNOWLEDGEMENTS

The competition team of Paul Kent, Julie Roberts, Tanya Nagle, Alison Spencer and Judy Boge would like to thank the Queensland sub-branch of WPSA, Australian Poultry CRC, industry members and groups, Australian Egg Corporation Limited (AECL) and the Department of Primary Industries and Fisheries, Queensland for their continued support of the project.

NEAR INFRARED REFLECTANCE ANALYSIS OF GRAINS TO ESTIMATE NUTRITIONAL VALUE FOR CHICKENS

J.L. BLACK[1], R.J. HUGHES[2], S.G. NIELSEN[3], A.M. TREDREA[4] and P.C. FLINN[5]

Summary

Maximising bird growth rate, feed efficiency and profitability of broiler production depends, among other factors, on accurate measurements of the apparent metabolisable energy (AME) content (MJ/kg) and AME intake (MJ/d) of cereal grain based diets. Near infrared (NIR) calibrations have been developed to measure these characteristics for cereal grains. Further research is being conducted to strengthen the calibrations so they can become the primary basis for trading grains for livestock in Australia.

I. INTRODUCTION

Apparent metabolisable energy values for ingredients are used by the broiler industry to formulate diets that meet predetermined energy density (MJ/kg) specifications. A single mean estimate for the AME value for each grain species is used commonly during diet formulation. However, research from the Premium Grains for Livestock Program (PGLP) showed that there was a large range in AME values (MJ/kg DM) for Australian sourced grains; being 11.9-15.3 for wheat, 10.9-13.6 for barley, 12.1-14.5 for triticale and 15.3-16.7 for sorghum (Black et al., 2005). Traditionally, glucanase and/or xylanase enzymes are added to broiler diets to reduce the range and increase the absolute values for AME in broiler diets. Recent analysis of the effects of addition of xylanase and phytase enzymes to diets formulated from 38 wheat, 8 triticale and 3 sorghum samples from PGLP showed that AME values were actually depressed by the enzymes for 12 wheat, 3 triticale and all 3 sorghum based diets (Black, 2008). Although the addition of enzymes raised the mean AME content of the wheat based diets by approximately 0.5 MJ/kg, the variation in AME values across the diets remained almost constant at 4.13 MJ/kg for diets without enzymes and 4.22 MJ/kg for diets with enzymes.

Several other important results for the broiler industry were obtained from PGLP (Black, 2008). First, there was no relationship between the AME content of a diet (MJ/kg) and the amount of the diet consumed by broiler chickens ($R^2 = 0.003$). This result suggests that different characteristics of the grain determine digestibility compared with intake. Secondly, there were significant differences ($P < 0.05$) within grain types in the intake (g/d) by broilers when grain samples were incorporated into diets. For example, the intake of wheat based diets by broilers varied by 20% depending on the particular wheat sample incorporated at a constant proportion into the diet. The daily intake of AME (MJ/d) by broilers varied by approximately 34% across the wheat based diets. Thirdly, broiler growth rate was more closely related to AME intake (MJ/d) than to the AME content of the diet (MJ/kg).

Cereal grains with their high starch content are the major energy source for broilers and represent from 60-70% of the diet. The results from PGLP and supported by the work from Scott (2005) indicate that values must be obtained for both the AME content (MJ/kg) and AME intake (MJ/d) to fully describe the energy value of any batch of cereal grain for

[1] John L Black Consulting, Warrimoo NSW 2774
[2] SARDI, Pig and Poultry Production Institute (PPPI), Roseworthy, SA 5371
[3] DPI Agriculture, Orange, NSW 2800
[4] University of Sydney, Plant Breeding Institute, Narrabri, NSW 2390
[5] Kelspec Services Pty. Ltd, Dunkeld, VIC 3294

broilers. Estimates of the money value of a 1 MJ/kg difference in the AME content of grain range from $11.50/t to $27/t depending on the base cost of the grain relative to other high and low energy ingredients (Black, 2008). Similarly, an increase in AME intake (MJ/d) that simulates growth rate and results in chickens reaching sale weight one day earlier has been estimated to be worth $2m/year for a 1 million bird per week broiler operation (Black, 2008). Rapid methods for measuring both the AME content of a grain and the relative AME intake of that grain compared with other grains when incorporated into a diet would be of great value to the broiler industry. In addition, a rapid method for estimating the effect of enzyme addition on the AME content and AME intake of grains would help identify when adding enzymes may be disadvantageous.

II. USE OF NEAR INFRARED TECHNOLOGIES

Near infrared spectroscopy (NIR) technology is now used widely to predict many chemical components of cereal grains. NIR has been applied to the results from PGLP to predict the AME content and AME intake index for any batch of grain for broiler chickens. The AME intake index was calculated by dividing the AME intake value (MJ/d) for every grain fed to broilers in PGLP by the highest value and multiplying by 100 to give values potentially from 1 to over 100. The AME intake index was used to provide a relative estimate of the likely intake for a broiler diet based on a particular grain rather than an absolute value in MJ/d which changes as chickens grow. The NIR calibrations were established across grain species and include all grains fed to broilers in PGLP. The NIR scans were on samples of whole grain rather than milled grain to reduce the cost and time taken for the analysis.

The relationship between NIR predicted values and observed AME (MJ/kg as fed) for broilers is presented in Figure 1. The dashed lines represent ± 1 standard deviation (SD) from the observed mean values with individual grains predicted to be outside this range identified.

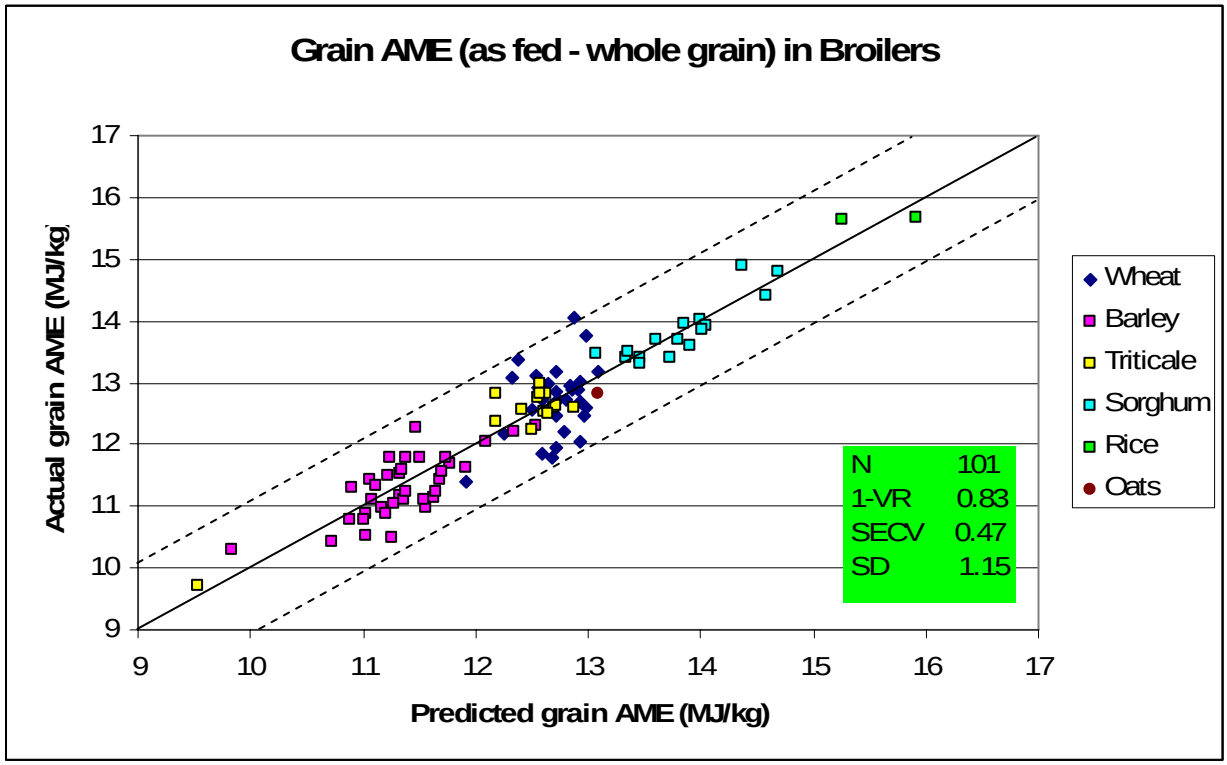

Figure 1 Relationship between observed and NIR predicted AME for grain based diets fed to broilers.

1-VR (1-Variance Ratio) is the fraction of the variance in observations accounted for when some of the observations are used for 'cross validation' as determined by the calibration software. A value of 0.83 indicates acceptable robustness of the calibration. The value of the calibration for predicting unknown samples is assessed by (RPD) the Ratio of Prediction to experimental Deviation (SD/SECV) = 2.4. The calibration is rated as 'quantitative' with predictions being within ± 0.47 MJ/kg DM in 95% of samples measured.

The relationship between NIR predicted and observed AME intake index values for broilers is presented in Figure 2. The value of the calibration as assessed by RPD was 1.8. The calibration is rated as 'useful' distinguishing between high and low values with predictions being within ± 4.96 index units in 95% of samples measured.

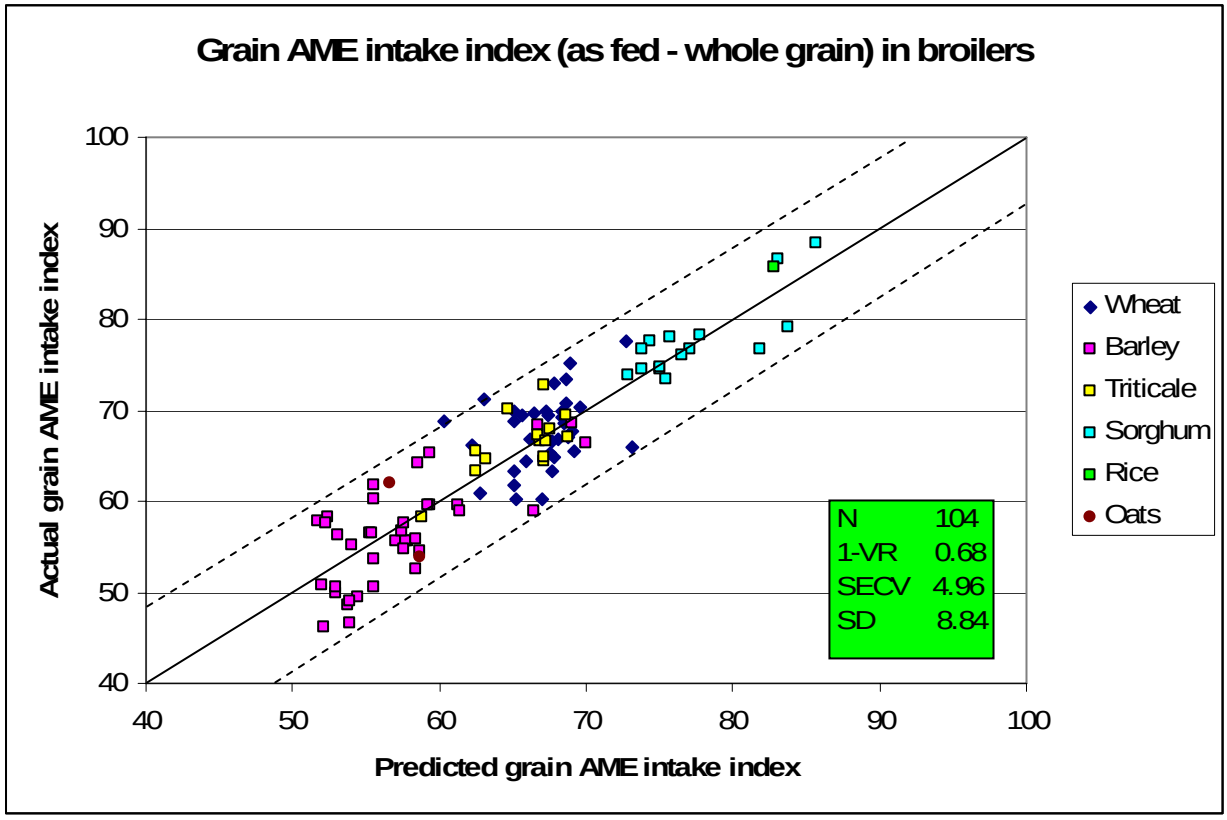

Figure 2 Relationship between observed and NIR predicted AME intake index values for grain based diets fed to broilers.

III. VALUE OF THE CALIBRATIONS FOR INDUSTRY

Figures 1 and 2 show that grain samples differing in energy value can be identified using the NIR technology both within and across grain species. A case study was conducted where samples of wheat from 37 locations in southern Australia were collected in 2005 and their energy value determined using NIR technology (Spragg, 2007). Two samples were selected, one with high energy (AME 13.1 MJ/kg as fed; AME intake index 69.3) and one with low energy (AME 12.5 MJ/kg as fed; AME intake index 64.2). These grains were compared, with and without enzymes, and with a wheat provided by a broiler company (AME 12.8 MJ/kg as fed; AME intake index 67.4) with enzymes in a full broiler growth study. The average number of days for male and female birds to reach 2.45 kg was 38.0, 37.8, 36.0, 35.8 and 37.1, respectively for the low energy wheat without and with enzymes, the high energy wheat

without and with enzymes and the company wheat with enzymes. The study showed that NIR selected high-energy wheat reduced the time to sale by 2 days compared with the low energy wheat and by 1.3 days compared to the company selected wheat. These results suggest there is value in improving the accuracy and reliability of the calibrations for commercial use.

IV. RESEARCH TO IMPROVE CALIBRATIONS

The Rural Industries R&D Corporation is currently funding two projects to expand the number and type of grain samples included in the calibrations. In one study, 90 cereal grains will be selected from samples provided from broiler companies and the Pork Cooperative Research Centre (CRC). These grains will include high screening samples, grains grown under irrigation, red wheat varieties, experimentally sprouted and water stressed grains. The second study will examine a range of new triticale cultivars. AME results and associated NIR scans of grains will be added to the existing PGLP database to improve the calibrations.

V. DELIVERY OF CALIBRATIONS TO INDUSTRY

The Pork CRC is also enhancing the NIR calibrations for measuring the energy value of cereal grains for pigs. The Grains R&D Corporation has licensed the Pork CRC to make the PGLP and enhanced calibrations for assessing the energy value of cereal grains for broilers, pigs and ruminants commercially available across the feed grain value chain. Sub-licences are being provided to the major feed testing laboratories, livestock integrator companies, stockfeed manufacturers and grain handling and broking companies across Australia. The NIR calibrations will provide a more suitable method for assessing the energy value of grain for livestock than the current methods based on test weight (kg/hl) and screenings percentage. The NIR calibrations are proposed to be used as the primary basis for trading grains for livestock in Australia.

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INFLUENCE OF MAIZE PARTICLE SIZE AND PHYTASE SUPPLEMENTATION ON THE PERFORMANCE OF BROILER STARTERS

A.M. AMERAH[1] and V. RAVINDRAN[1]

The degree of grain grinding may influence the efficacy of exogenous enzymes in poultry diets, but published data on this aspect are limited. Amerah et al. (2008) reported that the effectiveness of exogenous xylanase in broiler diets is influenced by the particle size (PS) of wheat. The aim of the present experiment was to examine the interaction between maize PS and phytase supplementation on the performance and toe ash contents of broilers. The experimental design was a 2 x 2 factorial arrangement of treatments evaluating two maize PS (medium and coarse) and phytase supplementation (without or with 500 FTU/kg diet). The two PS were achieved by grinding the whole maize in a hammer mill to pass through 3 and 7 mm sieves, respectively. The geometric mean diameter (GMD) of the coarse and medium maize were determined to be 0.611 and 0.849 mm, respectively. Broiler starter diets, based on maize and soybean meal, were formulated to meet or exceed the requirements for major nutrients for broiler starters, except calcium and phosphorus (P). Each diet was fed to six pens of eight male broilers (Ross 308) each from day 1 to 21 post-hatching.

Particle size	Phytase	Weight gain (g/bird)	Feed intake (g/bird)	Feed/ gain (g/g)	Toe ash (%)
Medium	-	598	826	1.399	10.41[a]
	+	687	917	1.320	11.65[b]
Coarse	-	647	875	1.401	11.42[b]
	+	706	929	1.357	11.78[b]
SEM		15	15	0.020	0.13

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

Phytase supplementation increased ($P < 0.001$) the feed intake and, improved ($P < 0.05$ to 0.001) weight gain and feed per gain in both medium and coarse PS diets. Coarse grinding improved ($P < 0.05$) weight gain, but had no effect ($P > 0.05$) on feed intake and feed per gain. No interactions ($P > 0.05$) between phytase supplementation and PS were observed for the performance parameters. However, a phytase x PS interaction ($P < 0.01$) was observed for the toe ash contents. Phytase supplementation increased the toe ash contents of birds fed the coarse PS diet but had no effect in those fed the medium PS diet. Interestingly, the toe ash content of birds fed the coarse PS diet was higher ($P < 0.01$) than those fed the medium PS diet, suggesting that grinding the maize coarsely has a beneficial effect on P bioavailability. Similar positive effects of coarse grinding of maize have been reported by Kasim and Edwards (2000). The present data suggest that, unlike xylanase supplementation in wheat-based diets, the response of broilers to phytase supplementation in maize-based diets is not influenced by maize PS.

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[1] Institute of Food, Nutrition and Human Health, Massey University Palmerston North, New Zealand

COMBINING NSP-ENZYMES AND PHYTASE: THE FORMULATION CHALLENGE IN BROILER NUTRITION!

M. FRANCESCH[1], K. LIU[2], P. DALIBARD[3] and P-A. GERAERT[3]

Summary

In order to investigate the benefits of a multi-enzyme complex (Rovabio^{TM} Max) containing carbohydrolases and phytase activities on the performance and bone mineralization of broilers fed corn-soybean meal based diets, 2268 male Ross broiler chicks were allocated to 9 dietary treatments: a positive control diet formulated to be adequate in nutrients and four reduced nutrient diets (NC), with gradual decrease on AME, CP and digestible AA, available phosphorus (avP) and calcium contents, with or without supplementation of the enzyme-complex. Supplementation of the NC diets with the enzyme-complex improved feed intake, weight gain and feed conversion. The magnitude of the enzyme effect was stronger for the most reduced avP/Ca diets. The first limiting nutrient factor appeared to be the avP. With enzyme, there were no significant differences in growth of birds fed on NC or PC diets. Enzyme increased feed intake of birds fed on NC diets nearby the level of feed consumption of the positive control and resulted in better FCR. Enzyme restored bone mineralisation to that of the PC, with the low reduction on AME and CP and digestible AA. The results indicate that dietary supplementation with a multi-enzyme complex containing NSP-enzymes and phytase is efficient in reducing the phosphorus, energy, protein and amino acid specifications of a corn-soybean meal diet without performance losses, but not always fully restoring bone mineralisation.

I. INTRODUCTION

In commercial broiler production, combinations of different enzymes or multi-enzyme complexes are used to increase nutrient and energy availability from feed ingredients, especially combinations of different carbohydrolases and phytase. The use of carbohydrolases, targeted to hydrolyse soluble NSP, is widely and successfully implemented when viscous-cereals (wheat, barley, rye, oats or triticale) are used. Although both corn and soybean meal are considered highly digestible ingredients, there is some room for the improvement of their nutritional value supporting the use of NSP-enzymes in such diets (Maisonnier et al., 2004). The use of phytase has become standard practice to reduce P levels in the environment and to compensate the drastic increase of the cost of inorganic phosphates. The effects of phytase in poultry have been extensively reviewed by Selle and Ravindran (2007). They reported that phytase increases P retention and tibia ash, but it has also positive effects on growth performance and digestibility parameters.

There are two strategies to benefit from enzyme supplementation: reducing the nutrient specifications of the complete feed appropriately or assigning specific nutritional values to the individual ingredients (Dalibard and Geraert, 2004). Both approaches require accurate knowledge of how much nutrients will be made available by adding the enzyme to the diet. Many factors can influence the response to an enzyme complex, since it depends on the enzyme specificity and concentration of the substrate, doses of enzymes and interactions between enzymes, ingredient quality and type, level of nutrients in the diet, age of animals.

[1] IRTA, Mas de Bover, Ctra. Reus-El Morell Km. 3,8, E-43120 Constantí (Tarragona), Spain.
[2] Adisseo Asia Pacific Pte Ltd, 179803 Singapore.
[3] Adisseo France SAS, 42 Avenue Aristide Briand, 92160 Antony, France.

The current study was designed to assess the reformulation matrix with the application of a multi-enzyme complex, combining carbohydrolase and phytase, in corn-soybean meal fed broilers, and to revise the different limiting factors supporting the performance benefits.

II. MATERIALS AND METHODS

A multi-enzyme complex in liquid form was tested (Rovabio^{TM} Max, Adisseo, France), containing carbohydrolases produced from the fermentation of *Penicillium funiculosum* and bacterial 6-phytase (EC 3.1.3.2.6), derived from *E. coli*. The enzyme was applied after pelleting at a dose rate of 200 mL/tonne of feed to provide a minimum of 1100 visco-units of endo-β-1,4-xylanase, 100 AGL units of endo-1,3(4)-β-glucanase and 500 FTU (phytase units)/kg of feed, and pectinase, protease and mannanases side activities.

Five basal diets were tested: one positive control (PC) diet formulated to be adequate or to exceed all nutrients requirements, and four negative control (NC1 to NC4) diets with a combined reduction of AME, CP and digestible essential amino acids (CP-dAA), available phosphorus (avP) and calcium (Ca). The applied nutrients reduction was: NC1 (-0.27 MJ/kg, -1.5 % CP-dAA, -0.15 pcent point avP, -0.12 pcent point Ca); NC2 (-0.36 MJ/kg, -3.0 % CP-dAA, -0.15 pcent point avP, -0.12 pcent point Ca); NC3 (-0.27 MJ /kg, -1.5 % CP-dAA, -0.20 pcent point avP, -0.16 pcent point Ca); NC4 (-0.36 MJ/kg, -3.0 % CP-dAA, -0.20 pcent point avP, -0.16 pcent point Ca). PC diet provided 4.3 and 3.9 g/kg, NC1 and NC2 2.8 and 2.4 g/kg and NC3 and NC4 2.3 and 1.9 g/kg of avP from 0-21 d and 22-43 d, respectively. There were a total of nine dietary experimental treatments replicated six times each and allocated at random by blocks.

2268 one-day Ross 380 male broiler chickens were distributed into 54 floor pens, 42 chickens per pen. Feed, in pellets, and water were provided ad-libitum throughout the experiment. Average daily weight gain (WG), average daily feed intake (FI) and feed conversion ratio (FCR) were calculated for the periods 0-21, 22-42 and 0-42 d. Mortality was also recorded. At 43 d, twelve chickens per treatment were randomly selected and euthanized to determine the percentage of tibia ash as well as total phosphorus and calcium concentrations.

Data were analyzed as a randomized complete block design with a two-way analysis of variance (block and treatment) using the GLM procedure of SAS. Differences between PC and NC diets, with and without enzyme, were compared by a set of contrasts. Moreover, data without the PC group were analyzed by a 4 x 2 factorial analysis of the variance to determine the main effects of nutrient reduction and enzyme and their interaction.

III. RESULTS AND DISCUSSION

The effect of diets and enzyme-complex on performance from 0 to 42 d is presented in Table 1. Growth of birds fed NC1 and NC2 diets was not significantly different from growth of birds fed PC diet. Further avP and Ca reduction resulted in a lower ($P < 0.001$) WG compared with the PC, by 13% for NC3 and 16% for NC4 diet. With enzyme, there were no significant differences in growth of birds fed on NC with the PC diets. Feed intake of birds fed on NC diets were lower ($P < 0.05$) compared with PC, and in all cases, enzyme supplementation increased it nearby the level of feed consumption of the PC. Enzyme supplementation to NC diets resulted in all cases in better ($P < 0.05$) FCR than the PC. Mortality of birds fed NC3 and NC4 diets was higher ($P < 0.05$) compared with the PC and it was reduced by the enzyme. The magnitude of the enzyme effect in increasing WG and FI was stronger for the most reduced avP and Ca diets (NC3 and NC4). No significant differences were detected between the two levels of energy and CP/dAA reduction, only significant differences

between the two levels of avP/Ca could be detected. This might indicate that without enzyme supplementation, avP/Ca reduction affected more greatly the growth performance than the energy and CP/dAA reduction.

Tibia ash of birds fed NC diets was lower ($P < 0.05$) compared to birds fed PC (Table 1). Enzyme supplementation to NC1 and NC3 diets returned bone mineralisation to that of the PC, whereas tibia ash in birds fed NC2 and NC4, the most energy and CP-dAA reduced diets, remained lower ($P < 0.05$).

Overall and with the multi-enzyme-complex, performance of birds fed reduced nutrient diets was not different than that of broilers fed PC diet, even better with NC1 and NC2 diets. Enzyme-complex increased feed intake and weight gain and improved FCR, and the strongest effects were observed with the lowest avP and Ca levels. This might indicate that the first limiting nutrient was P and one time the P deficiency was overcome by phytase and feed intake was restored, the carbohydrase enzymes could increase the nutritive value of diet compensating the reduction in AME (by 2.8%) and CP/dAA (by 1.5-3.0%). Cowieson et al. (2006) suggested the feasibility of reducing -0.61 MJ/kg AME, 0.13% P, 0.12% Ca and 1 to 2% amino acids of a corn-soybean meal diet without significant impairment of performance at 42 d, by the use of a combination of xylanase, amylase, protease and phytase. The mode of action of the enzymes in corn-soybean meal diets has been linked to the disruption of the cell wall matrix, facilitating the release of encapsulated nutrients and the digestive enzymes access, and also to the modification of the intestinal microbiota communities (Bedford, 1996; Cowieson, 2005, Classen, 2006). Some works also suggested that carbohydrolases, that are able to breakdown the cell wall NSP-matrix, can facilitate the access of phytase to phytate molecule (Olukosi et al., 2007), supporting the thought that the use of a combination of enzyme can fully strengthen their effects.

IV. CONCLUSION

In summary, results of the present experiment indicate that the use of a multi-enzyme complex containing xylanase, β-glucanase and phytase as main activities, and pectinase, protease and mannanases side activities, allows the reduction of the AME, CP-dAA, avP and Ca contents of a corn-soybean meal diet without penalising performance, but not always restoring fully the bone mineralisation.

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Table 1 Effect of dietary nutrient reduction and enzyme on performance and bone mineralization of chickens (0 to 42 days)

Basal Diet[1]	Enzyme	Weight gain[2] (g/day)	Feed intake[2] (g/day)	Feed/ gain[2] (g/g)	Mortality[2] (%)	Bone ash[3] (43 days) (%)
PC	-	70.3	121.8	1.733	1.84	44.2
NC1	-	68.4^{b}	116.9^{a}	1.709	1.47^{bc}	42.0^{bc}
NC1	200 mL/t	70.2^{ab}	118.8^{a}	1.694	5.32^{ab}	43.4^{ab}
NC2	-	68.7^{b}	116.9^{a}	1.704	3.34^{ab}	42.2^{b}
NC2	200 mL/t	71.0^{ab}	120.0^{a}	1.692	2.22^{ab}	42.4^{ab}
NC3	-	61.3^{c}	103.8^{b}	1.693	5.25^{a}	38.5^{d}
NC3	200 mL/t	70.4^{ab}	118.4^{a}	1.681	3.27^{ab}	44.1^{a}
NC4	-	59.1^{c}	102.3^{b}	1.731	8.08^{a}	40.4^{c}
NC4	200 mL/t	72.0^{a}	120.9^{a}	1.679	0.36^{c}	42.1^{bc}
Pooled SME		0.98	1.30	0.0115	1.359	0.61
Main effect						
Diets						
NC1		69.3	117.9	1.701	3.39	42.7
NC2		69.8	118.5	1.698	2.78	42.3
NC3		65.9	111.1	1.687	4.26	41.3
NC4		65.6	111.6	1.705	4.22	41.3
Enzyme						
No		64.4	110.0	1.709^{a}	4.53	40.8
Yes		70.9	119.5	1.686^{b}	2.79	43.0
P for contrast						
PC vs. NC1 no enz		NS	*	NS	NS	*
PC vs. NC2 no enz		NS	*	†	NS	*
PC vs. NC3 no enz		***	***	*	*	***
PC vs. NC4 no enz		***	***	NS	*	***
PC vs. NC1 enzyme		NS	NS	*	†	NS
PC vs. NC2 enzyme		NS	NS	*	NS	*
PC vs. NC3 enzyme		NS	†	**	NS	NS
PC vs. NC4 enzyme		NS	NS	**	NS	*
P for factorial analysis[4]						
Diets		***	***	NS	NS	*
Enzyme		***	***	**	NS	***
Diet * enzyme		***	***	NS	*	***

[1]NC1 (-0.27 MJ/kg, -1.5 % CP-dAA, -0.15 pcent point avP, -0.12 pcent point Ca);
NC2 (-0.36 MJ/kg, -3.0 % CP-dAA, -0.15 pcent point avP, -0.12 pcent point Ca);
NC3 (-0.27 MJ /kg, -1.5 % CP-dAA, -0.20 pcent point avP, -0.16 pcent point Ca);
NC4 (-0.36 MJ/kg, -3.0 % CP-dAA, -0.20 pcent point avP, -0.16 pcent point Ca)

[2]Values are means of six replicates of 42 (0-21 days) and 40 chickens (22-42 days).

[3]Values are least square means of twelve birds per treatment.

[4]Using treatments T-2 through T-9. NS P > 0.1; † P < 0.1; * P < 0.05; ** P < 0.01; *** P < 0.001.

[a,b,c,d]Means within a column not sharing a common superscript differ (P<0.05).

SUPPLEMENTATION OF WHEAT-BASED BROILER DIETS WITH XYLANASE AND PHYTASE, INDIVIDUALLY AND IN COMBINATION

D.J. CADOGAN[1], P.H. SELLE[2], G.G. PARTRIDGE[3] and V. RAVINDRAN[4]

Summary

The combined inclusion of xylanase plus phytase in a wheat-based diet increased the ileal digestibility coefficients of 17 amino acids by an average of 8.6% (0.830 versus 0.764), which exceeded the average increases individually generated by xylanase (4.8%) or phytase (5.5%). Of probable relevance is that the combination of xylanase plus phytase markedly increased ileal digestibility of sodium from -0.516 to 0.043.

I. INTRODUCTION

The simultaneous inclusion of exogenous xylanases and phytases in wheat-based broiler diets is an increasingly common practice. Despite initial indications that this approach held promise (Zyla et al., 1999), few relevant studies have been reported, particularly where the effects of enzymes on ileal amino acid digestibility have been determined. The purpose of this study was to determine the effects of xylanase and phytase, individually and in combination, on digestibility of amino acids and electrolytes (sodium, potassium, chloride) in diets based on a selected wheat.

II. MATERIALS AND METHODS

A wheat with a pre-determined AME of 12.3 MJ/kg, which contained 9.2 g/kg soluble NSP and 1.90 g/kg phytate-P was used to formulate positive (3.8 g/kg nonphytate-P) and negative (2.6 g/kg) control diets. Xylanase (2000 XU/kg; Avizyme 1310) and phytase (500 FTU/kg; Phyzyme XP), individually and in combination, were added to the negative control diet. The practical diets were based on wheat, soyabean meal and canola meal and contained 3.0 g/kg titanium oxide as an inert marker. Each of the five dietary treatments was offered to six cages of eight male chicks (Ross 308) from day 1 to 21 post-hatch. On Day 21, all birds were euthanased with sodium pentobarbitone and contents of the lower ileum were expressed and digesta from birds within a pen were pooled. Concentrations of amino acids, sodium (Na), potassium, chloride and titanium oxide in diets and digesta were analysed to determine apparent ileal digestibility (AID) coefficients.

III. RESULTS

The influence of enzyme inclusions to the negative control diet on ileal amino acid digestibility is shown in Table 1. Xylanase significantly increased the ileal digestibility of 13 amino acids and increased AID coefficient of 17 amino acids by an average of 4.8%. Phytase significantly increased the digestibility of the same 13 amino acids with an overall, average increase of 5.5%. In combination, xylanase plus phytase significantly increased the digestibility of 15 amino acids: arginine (6.0%),

[1] Feedworks. Romsey Vic 3434.
[2] University of Sydney. Camden NSW 2570
[3] Danisco Animal Nutrition. Marlborough UK.
[4] Massey University. Palmerston North NZ.

Table 1 Influence of xylanase and phytase addition, individually and in combination, on apparent ileal digestibility coefficients of essential and non-essential amino acids for broilers

Treatment	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl-alanine	Threonine	Valine
Negative control	0.832[a]	0.704[a]	0.760[a]	0.781[a]	0.825[a]	0.881[a]	0.767[a]	0.709[a]	0.748[a]
Xylanase	0.853[b]	0.798[b]	0.792[b]	0.824[b]	0.865[bc]	0.906[bc]	0.816[b]	0.767[b]	0.748[a]
Phytase	0.860[b]	0.794[b]	0.824[c]	0.830[bc]	0.856[b]	0.903[b]	0.836[b]	0.769[bc]	0.770[a]
Xylanase plus phytase	0.882[c]	0.809[b]	0.836[c]	0.847[c]	0.875[c]	0.916[c]	0.839[b]	0.798[b]	0.819[b]
Pooled SEM	0.004	0.007	0.008	0.007	0.006	0.003	0.009	0.010	0.009

Treatment	Alanine	Aspartic acid	Cystine	Glutamic acid	Glycine	Proline	Serine	Tyrosine
Negative control	0.743	0.714[a]	0.667[a]	0.849[a]	0.718[a]	0.816[a]	0.743[a]	0.737
Xylanase	0.746	0.780[b]	0.668[a]	0.881[b]	0.765[b]	0.845[b]	0.802[b]	0.733
Phytase	0.751	0.775[b]	0.693[a]	0.890[b]	0.777[bc]	0.853[b]	0.806[b]	0.721
Xylanase plus phytase	0.774	0.819[c]	0.776[b]	0.909[c]	0.802[c]	0.866[b]	0.827[b]	0.743
Pooled SEM	0.013	0.009	0.012	0.006	0.010	0.007	0.010	0.012

[a,b,c] Means within columns without common superscripts are significantly different ($P < 0.05$).

histidine (14.9%), isoleucine (10.0%), leucine (8.5%), lysine (6.1%), methionine (4.0%), phenylalanine (9.4%), threonine (12.6%), valine (9.5%), aspartic acid (14.7%), cystine (16.3%), glutamic acid (7.1%), glycine (11.7%), proline (6.1%) and serine (11.3%). The overall, average increase was 8.6%. Responses to xylanase plus phytase were generally of a greater magnitude and the percentage increases generated by the combination exceeded the sum of the increases of the individual enzymes for five amino acids: arginine, valine, alanine, cystine and tyrosine. The ileal digestibilities of potassium and chloride were not influenced by treatment. However, the AID coefficient of sodium in the low-P diet of -0.516 was significantly increased to 0.043 by xylanase plus phytase and to -0.038 by phytase alone (Table 2).

Table 2 Influence of xylanase and phytase addition, individually and in combination, to negative control diets on apparent ileal digestibility of sodium, potassium and chloride

Treatment	Sodium	Potassium	Chloride
Positive control	-0.672[a]	0.868	0.163
Negative control	-0.516[ab]	0.879	0.149
Xylanase	-0.459[b]	0.883	0.222
Phytase	-0.038[c]	0.894	0.160
Xylanase plus phytase	0.043[c]	0.897	0.276
Pooled SEM	0.054	0.007	0.053

[a,b,c]Means within columns without common superscripts are significantly different ($P < 0.05$).

IV. DISCUSSION

The wheat used in the present study contained moderate concentrations of soluble NSP and phytate; nevertheless, xylanase plus phytase increased amino acid digestibilities by an average of 8.6%. Assessments of xylanase plus phytase on ileal amino acid digestibility in broilers appear to be confined to two studies. Ravindran et al. (1999) reported that xylanase plus phytase increased AID coefficients of 14 amino acids by an average of 8.7% (0.868 versus 0.800) in wheat-casein diets and Selle et al. (2003) found that the combination increased AID coefficients of 16 amino acids by 4.6% (0.816 versus 0.781) in diets based on a wheat-sorghum blend. In both studies, the percentage increases generated by the combination exceeded the sum of the increases of the individual enzymes for the majority of amino acids assessed. Therefore, in the present and previous studies, more robust increases in amino acid digestibility were observed with the combination in comparison to individual enzymes and, at times, these responses were of a synergistic nature. The quantities of ileal digestible amino acids generated by xylanase and phytase, particularly in combination, are indicative of the magnitude of matrix values that could be applied to the least-cost formulation of wheat-based broiler diets with added phytase plus xylanase in practice.

The simultaneous inclusion of xylanase and phytase in wheat-based broiler diets generates more robust responses than the individual components. Importantly, Parkkonen et al. (1997) found that xylanase increased access of proteolytic enzymes to their substrates in the aleurone layer, presumably by degrading the cell wall matrix under *in vitro* conditions. Phytate is concentrated in the aleurone of wheat so the likelihood is that xylanase similarly facilitates phytase activity by increasing substrate access so it is possible that phytate degradation by phytase is enhanced by the presence of xylanase. Additionally, the absorption

of nutrients liberated by phytase may be increased by xylanase-induced reductions in gut viscosity.

In this study xylanase did not influence ileal Na digestibility; however, the impact of phytase and the enzyme combination on Na was quite profound as AID coefficients were restored to 'parity'. This is consistent with the findings of Ravindran et al. (2006) who demonstrated that phytate has the capacity to drag Na into the small intestinal lumen and that this shift is ameliorated by phytase and similar findings have been recorded by Cowieson et al. (2004) and Ravindran et al. (2008). The situation is not clear but the phytate-induced movement of Na into the gut lumen may be as sodium bicarbonate to buffer the polyanionic molecule (Cowieson et al., 2004) and/or counter gastric hyper-secretion of hydrochloric acid (HCl) and pepsin. Given that protein-phytate complexes are refractory to pepsin digestion (Vaintraub and Bulmaga, 1991) it seems possible that this would trigger additional outputs of pepsin and HCl as a compensatory mechanism. Irrespective of the underlying reason, the capacity of phytate to drag Na in the small intestinal lumen has prompted speculation that this may be compromising Na^+-dependent transport systems and/or the activity of the 'sodium pump' (Na^+-K^+-ATPase) and, in turn, intestinal uptakes of amino acids and other nutrients (Selle and Ravindran, 2007). Some support for this possibility was provided by Dilworth et al. (2005) as these workers reported that phytate reduced Na^+-K^+-ATPase activity by approximately 80% in the jejunum and ileum of rats. It is also relevant that phytase is more likely to enhance ileal amino acid digestibility in broiler diets with low Na levels; whereas, at relatively high Na levels responses were diminished (Ravindran et al., 2008). This is consistent with the concept that phytase has a 'Na-sparing' effect.

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INTESTINAL NUTRIENT UPTAKE IN RESPONSE TO MANNO-OLIGOSACCHARIDES AND DIETARY THREONINE IN BROILER CHICKENS

S.H.CHEE[1], P.A. IJI[1], M.CHOCT[1], L.L. MIKKELSEN[1] and A. KOCHER[2]

Previous studies showed that dietary threonine and manno-oligosaccharides (MOS) modulate intestinal mucin dynamics and mucosal development (Faure *et al.*, 2005; Uni and Smirnov, 2006). The effects of MOS and threonine on intestinal nutrient uptake were further examined.

A 3 x 2 factorial experimental design was used to investigate the interaction between threonine levels at 70, 100 and 130% of NRC (1994) recommendations and MOS (Bio-MOS®, Alltech Inc.) at 0 and 2 g/kg on ileal uptake of L-threonine and D-glucose. One hundred and fifty day-old male Cobb broiler chicks were randomly assigned to 6 treatments each consisting of 5 replicate cages of 5 birds. At 3 weeks of age, one bird per cage and 3 replicates per treatment were randomly selected for the intestinal nutrient uptake assay using the *in situ*-perfusion technique. Two 10 cm loops, each at 1 cm apart, were made on the ileum of the anaesthetised bird. Blank avian Krebs-Ringer-Bicarbonate and Krebs-Ringer-Bicarbonate containing pre-determined concentration of 50 mM of D-glucose and L-threonine solutions were injected into loop A and B, respectively. After 10 min of incubation, the bird was euthanised and the loop contents were recovered for the determination of D-glucose and L-threonine using Megazyme D-Glucose Assay Kit (Megazyme International Ireland Ltd., Wicklow, Ireland) and AccQTaq chemistry method (Waters, Milford, MA, USA), respectively. The length of the respective loops were measured and recorded. The true ileal mucosal uptake of D-glucose or L-threonine (mmol/cm/h) was calculated based on the following equation:

$$\text{True nutrient uptake} = \left[\frac{\left(\text{Vol I}_B \text{ x Nutrient I}_B\right) - \left(\text{Vol R}_B \text{ x Nutrient R}_B\right)}{\text{Length}_B \text{ x } 0.167}\right] - \left[\frac{\left(\text{Vol R}_A \text{ x Nutrient R}_A\right)}{\text{Length}_A \text{ x } 0.167}\right]$$

where Vol I_B is the volume (l) injected into loop B, Vol R_A or R_B is the volume (l) recovered from loop A or B, Nutrient I_B is the concentration (mM) of D-glucose or L-threonine injected into loop B, Nutrient R_A or R_B is the concentration (mM) of D-glucose or L-threonine recovered from loop A or B, $length_{A \text{ or } B}$ is the length (cm) of loop A or B and 0.167 is the duration of incubation in hour.

Ileal uptake of D-glucose and L-threonine averaged at 0.045 and 0.031 mmol/cm/h, respectively. Dietary MOS tended to interact with threonine to increase the ileal uptake of D-glucose ($P = 0.084$) and L-threonine ($P = 0.077$), especially in MOS-treated birds fed the excess threonine. The main effects of MOS ($P < 0.05$) or adequate feeding of threonine ($P < 0.05$) also increased ileal uptake of D-glucose and L-threonine. Results from current study indicate the possible link between mucosal nutrient uptake and the modulating effects of these supplements on intestinal mucosal development and mucin dynamics. This may be associated with a relatively higher proportion of mature enterocytes and absorptive area, which would be expected to improve the capacity for mucosal nutrient absorption.

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[1] School of Environmental and Rural Science, University of New England, Armidale NSW 2351

[2] Alltech Biotechnology P/L 64-70 Nissan Drive, Dandenong South, Vic 3175

PECTINASES BREAK DOWN CELL WALLS IN LEGUMES

A. ALI[1], I.H. WILLIAMS[1], G.B. MARTIN[1] and S. SIPSAS[2]

A complete breakdown of pectin in plant cell walls requires a combination of two pectinases, polygalacturonase (PG) and pectin methyl esterase (PME). PME removes the methyl ester radicals attached to carbon six atom in the galacturonic acid units of the pectin chain (Kester *et al*., 2000; Ali *et al*., 2005). After these radicals are removed, PG can attack the glycosidic bonds and break down the main pectin chain. However, a high dose of PME has undesirable effects, increasing the viscosity and water-holding capacity of pectin. We therefore tested the hypothesis that there is an optimum combination of PME and PG that will break down the cell walls sufficiently so that more than 5% legumes can be used in poultry diets. A dose-response experiment was conducted to test the hypothesis. Six levels of PME (0, 200, 400, 600, 800 and 1000 units) in combination with PG (1400 units) were incubated *in vitro* with four legumes (dehulled lupins, lathyrus, field peas and faba beans) in an acid medium for 1 hour. We followed the analytical procedures and measurements described for previous *in vitro* experiment (Ali *et al*., 2005).

For dehulled lupins, the lowest dose of PME (200 units) in combination with 1400 units PG induced a 27% breakdown of cell walls, and reduced viscosity by 18% and water-holding capacity by 14%, compared to the control (no PME + PG). In the other legumes (lathyrus, faba bean, field pea), enzyme treatment had a smaller effect. Higher doses of PME (400, 600, 800 and 1000 units), in combination with PG, had no extra effect (data not shown). We concluded that the combination of the lowest level of PME (200 units) + PG (1400 units) was the most appropriate treatment for reducing the viscosity and water-holding capacity of feed legumes. This optimal treatment should allow feed manufacturers to increase substantially (up to 30%) their use of legumes in broiler and egg layer diets without compromising the productivity of birds or increasing the incidence of wet droppings.

Legumes	Lupins		Lathyrus		Field pea		Faba bean	
PME + PG (200 + 1400 units)	–	+	–	+	–	+	–	+
Viscosity (m.Pas/sec)	1.98^{a}	1.63^{b}	1.72^{a}	1.60^{b}	1.62^{a}	1.49^{b}	1.57^{a}	1.46^{b}
Water-holding capacity (g:g)	3.81^{a}	3.28^{b}	3.37^{a}	3.00^{b}	2.78^{a}	2.55^{b}	2.66^{a}	2.43^{b}
Cell-wall polysaccharide (%)	25.6^{a}	18.7^{b}	23.4^{a}	19.5^{b}	16.5^{a}	14.1^{b}	16.2^{a}	13.7^{b}
Pectin (%)	11.8^{a}	7.44^{b}	5.03^{a}	4.38^{b}	4.55^{a}	4.23^{b}	5.37^{a}	5.02^{b}
Methyl ester of pectin (%)	24.2^{a}	11.6^{b}	14.4^{a}	11.3^{b}	21.6^{a}	13.7^{b}	18.5^{a}	13.3^{b}
Length of pectin chain	65.1^{a}	24.4^{b}	37.2^{a}	30.5^{b}	30.3^{a}	25.5^{b}	35.0^{a}	29.1^{b}

Means within rows with different superscripts differ (P<0.05).

In the future studies we will investigate whether, for broilers and egg layers, the optimum combination of PME and PG improves feed conversion efficiency and increases the metabolisable energy of diets based on dehulled lupins and, at the same time, reduces wet droppings. If *in vivo* work provides positive results, a commercial pectinase preparation of PME + PG needs to be developed.

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[1] Institute of Agriculture, The University of Western Australia, Crawley WA 6009
[2] Crop Improvement Institute, Agriculture WA, South Perth WA 6151

PECTINASES ALLOW BROILERS TO PERFORM WELL ON DIETS WITH 20% DEHULLED LUPINS

A. ALI[1], I.H. WILLIAMS[1], G.B. MARTIN[1] and S. SIPSAS[2]

Our recent *in vitro* experiment showed that a combination of pectinases, pectin methyl esterase (PME) and polygalacturonase (PG), substantially broke down cell walls and pectins and reduced the water-holding capacity and viscosity of legumes, in particular dehulled lupins (Ali et al., 2009). As a result, we tested the hypothesis that a combination of PME and PG would reduce the viscosity of digesta and the incidence of wet droppings associated with dehulled lupins by breaking down the pectins of cell walls in dehulled lupins *in vivo*. In addition, this should improve the nutritive value of dehulled lupins, increase the growth of broilers and allow dehulled lupins to be incorporated into the diet to a level of 30%. We therefore conducted a 4x2 factorial experiment with four levels of dietary inclusion of dehulled lupins (0, 10, 20 and 30%) and two levels of PG+PME enzyme (minus and plus) with 3-week-old broilers (Ross 308) for 14 days. PME was added at 200 units/kg and PG at 1,400 units/kg of diet. All diets were cold-pelleted.

The table below summarises the effects of PG and PME on broiler performance. The combination of PME and PG broke down pectin by 6-fold and cell walls by 2.5-fold, and reduced digesta viscosity by 29%, water intake and wet droppings by 11%. As expected, the PG + PME treatment also increased feed conversion efficiency and apparent metabolisable energy (AME) of the diet by 6% for birds fed 10 and 20% dehulled lupins in the diet. In contrast, at 30% dehulled lupins in the diet, PG+PME broke down cell wall pectins and reduced viscosity, but these reductions were not reflected in bird performance.

Dehulled lupins	0%		10%		20%		30%	
PG + PME	–	+	–	+	–	+	–	+
Feed intake (g/bird/day)	139	140	141	141	133	134	124	126
Weight gain (g/bird/day)	63.5	63.4	62.7^{a}	66.7^{b}	58.4^{a}	61.4^{b}	53.6	55.4
Feed conversion ratio (g:g)	2.28	2.27	2.33^{a}	2.20^{b}	2.34^{a}	2.23^{b}	2.39	2.34
AME (MJ/kg diet)	12.3	12.4	11.8^{a}	12.5^{b}	11.1^{a}	11.6^{b}	10.6	10.8
Viscosity (m.Pas/sec.)	5.11	5.06	6.38^{a}	4.54^{b}	7.78^{a}	6.82^{b}	9.61^{a}	8.99^{b}
Water intake (ml/bird/day)	296	294	326^{a}	294^{b}	353^{a}	332^{b}	388	379
Faecal moisture (%)	62.6	63.0	67.9^{a}	60.4^{b}	70.1^{a}	64.4^{b}	72.8	71.0
Breakdown of cell wall (%)	4.2	4.2	4.8^{a}	11.3^{b}	3.5^{a}	6.3^{b}	3.1^{a}	4.2^{b}
Breakdown of pectin (%)	2.3	2.3	2.5^{a}	14.9^{b}	2.1^{a}	6.7^{b}	1.9^{a}	3.8^{b}

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

We concluded that PG + PME significantly improved the nutritive value of dehulled lupins for broilers and should allow feed manufacturers to include at least 20% of dehulled lupins in diets for broilers whilst eliminating wet droppings completely. Broiler producers looking for lower feed costs can save about $34 per tonne of diet if they replace imported soybean meal supplement with 20% dehulled lupins plus PME + PG. In view of the achievable improvements, a commercial preparation of pectinases should be developed for future application to broiler diets.

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[1] Institute of Agriculture, The University of Western Australia, Crawley WA 6009
[2] Crop Improvement Institute, Agriculture WA, South Perth WA 6151

EFFECT OF PHYTASE SUPPLEMENTATION OF A LOW PHOSPHORUS SORGHUM-SOYBEAN DIET ON LAYER PERFORMANCE

A. KUMAR[1], J.G. DINGLE[1] and J. SANDS[2]

Summary

This study was conducted to demonstrate the effects of phytase in a low phosphorus sorghum and soybean meal based layer diet. Two basal diets, a standard P and a low P diet were fed as is and a low P diet was also supplemented with two levels of commercial phytase enzyme Phyzyme XP. The diets were offered to 26 weeks old ISA brown layers for a period of 22 weeks. Hen housed egg production and egg mass were significantly less for hens fed the low P diet than for those fed the standard P diet. However, egg production and egg mass of hens fed the low P diet were significantly improved by phytase supplementation. Feed intake and feed conversion ratio were not significantly influenced by phytase supplementation.

I. INTRODUCTION

Only 30-40 per cent of the phosphorus from plant sources is freely available for digestion and absorption by poultry. The rest is in the form of phytate phosphorus, which is not efficiently utilised by chickens (Perney et al., 1993). The phytate not only binds phosphorus but also exhibits other antinutritional properties in the feed (Caldwell, 1992; Carnovale et al., 1998). Due to the lack of an endogenous phytase enzyme in chickens to hydrolyse the phytate bound phosphorus (Nelson, 1976), the current practice is to make up the phosphorus needs of chickens is by including either meat and bone meal or inorganic phosphorus in the feed. Undigested and unutilised inorganic phosphorus and phytate phosphorus residues are excreted. High phosphorus levels in the faeces and urine may cause a major environmental problem when animal waste is used as a manure for growing crops (Ravindran et al., 1998). It has been shown that phytase supplementation of the feed improves the utilisation of phytate phosphorus in laying hens (Van der kils et al., 1997; Um and Paik,1999; Jalal and Scheideler, 2001; Scott et al., 2001; Keshavarz, 2003). This study was undertaken to compare the effects on the production of layers of two levels of phytase when added to a low phosphorus sorghum-soybean meal diet compared with their performance when fed the diet without phytase supplementation.

II. METHODOLOGY

A total of 360, twenty six weeks old, ISA brown layers, were randomly allocated three hens to a cage with minimal cage floor area of 450 square cent meters per hen in an extended block design. Prior to feeding the experimental diets, egg production was recorded for one week to allocate the birds to achieve uniform egg production among all the treatment groups.

There were two basal diets in this study, a standard phosphorus and a low phosphorus diet. The basal diets contained sorghum and soybean meal as major ingredients and were formulated to provide 11.5 MJ ME, 160g CP and 6.6 g lysine/kg.

The major difference between these two basal diets was in their total and digestible phosphorus content. By adding dicalcium phosphate (DCP) the standard P diet had a total P

[1] School of Animal Studies, University of Queensland, Gatton, Qld-4343
[2] Danisco Animal Nutrition, Marlborough, Wiltshire, UK

content of 5.3 g/kg and a digestible P content of 2.7 g/kg, whereas the low P diet (without any DCP) had a total P content of 3.2g/kg and a digestible P content 1.0 g/kg diet.

To test the efficacy of the phytase enzyme, the low P diets was supplemented with two levels of Phytase. The dietary treatments in this study were as follows: 1. Standard P diet, no phytase, 2. Low P diet, no phytase, 3. Low P diet + 60g phytase /tonne, 4. Low P diet + 90g phytase /tonne of feed. Each diet was offered to 90 hens. Each diet had 10 replications (a block of three cages of three hens per cage per replicate) for egg production, egg weight, egg mass, feed consumption, feed conversion ratio (FCR) and mortality.

Experimental mash diets without or with 60 or 90 g Phyzyme XP per tonne were offered ad libitum to the laying hens from 26 to 48 weeks of age. Hens in the trial were on sixteen and a half hours lighting programme with a minimum light intensity of 10 lux. Daily egg production was recorded for the twenty two week trial and all eggs laid in one day were weighed once every four weeks. Feed intake was measured at the end of the trial.

Data were subjected to analysis of variance to test for the probability of significant differences among the means and least significant differences (LSD) were used to test for significant difference between means (SAS 2005).

III. RESULTS

Mean egg production performance of the hens fed phytase supplemented and unsupplemented sorghum soybean meal based layer diets are presented in Table 1. The overall production performance of the hens in the trial was very good and on parity with the production performance published in the ISA Brown Production Manual (2002).

The hen housed egg production of hens fed the low-P diet with no phytase supplement was significantly ($P < 0.05$) less (4.4 %) than those fed the standard-P diet. However, hens fed the low-P diet supplemented with phytase at 60 or 90 g per tonne of feed significantly improved their hen housed egg production over those fed the unsupplemented diet. Adding phytase to a low P layer diet hydrolyses phytate bound phosphorus resulting in increase in available phosphorus from feed ingredients. Similar results were reported in phytase supplemented groups by Um and Paik (1999); Jalal and Scheideler (2001); Lim et al., (2003); Onyango et al., (2005) and Wu et al., (2006). Increasing the dose of phytase supplementation of low P layer diets from 60 to 90 g per tonne did not significantly improve the hen housed egg production. Egg weight was not significantly influenced either by phosphorus level in the diet or by enzyme supplementation.

Egg mass was significantly higher in groups fed the standard-P diet than for those fed the low-P diet with no phytase supplement. Phytase supplementation of the low-P diet at 60 or 90 g per tonne significantly improved the egg mass of layers.

Table 1 Effects of Phyzyme XP supplementation on egg production performance in layers fed diets containing low phosphorus

Measurements	Standard-P diet	Low-P diet	Low-P diet + 60 g phytase	Low-P diet + 90 g phytase	LSD	P
Egg production (HH) (%)	94.14[a]	89.98[b]	96.47[a]	95.97[a]	2.77	0.001
Egg weight (g/egg)	67.21	66.71	66.61	66.87	1.23	0.77
Egg mass (g/hen/day)	63.25[a]	60.07[b]	64.26[a]	64.20[a]	2.26	0.008
Feed intake (g/hen/day)	118.50	119.20	119.90	119.5	2.55	0.73
FCR (g feed/g egg)	1.868	1.901	1.865	1.858	0.048	0.29
Mortality (%)	1.11[b]	12.22[a]	2.22[b]	3.33[b]	7.50	0.015

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

There was no significant difference in egg mass between the hens fed the standard-P diet and those fed the phytase supplemented low-P diets. Feed intake and feed conversion ratio were not significantly different between the birds fed the standard-P diet, the low P diet or the phytase supplemented low-P diets. Mortality was significantly greater in the low P diet fed groups than those fed the standard P diet or the enzyme supplemented low P diets.

IV. CONCLUSION

Hen housed egg production and egg mass of hens fed a low phosphorus diet can be significantly improved by supplementing the diet with phytase at 60g/tonne of feed. Phytase can totally replace dicalcium phosphate in sorghum soybean meal layer diets without significantly affecting layer performance.

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APPLICATIONS OF SAPONINS AS FEED ADDITIVES IN POULTRY PRODUCTION

P. R. CHEEKE[1]

Summary

Saponins are natural detergents or surfactants found in a wide variety of plants. The major commercial saponin-containing products are those derived from *Yucca schidigera* and *Quillaja saponaria*. Yucca is harvested from the wild in northern Mexico, while quillaja is a tree native to the Andes region of South America. It is harvested from the wild in Chile. Plantation production of quillaja also has begun. Saponins have detergent properties because they contain both water-soluble and lipid-soluble moities. They consist of a lipophilic nucleus with one or more side chains of carbohydrate, which confer water solubility. In yucca saponins, the nucleus (sapogenin) is a steroidal structure (Oleszek et al., 2001), while in quillaja saponins the sapogenin is a triterpenoid.

Yucca and quillaja products are available to the feed industry as extracts and whole plant powders. The extracts contain water-soluble components, while the whole plant powders contain all phytochemicals present in the plants. Although yucca and quillaja have traditionally been viewed mainly as sources of saponins, they also contain other phytochemicals, including oligosaccharides and polyphenolics (Oleszek et al., 2001). These constituents may contribute to the beneficial properties of these products as feed additives. Some of the positive effects of yucca and quillaja as feed additives are the following: (i) reduction of environmental ammonia and odor, (ii) hypocholesterolemic activity, (iii) anti-inflammatory activity (iv) anti-protozoal activity, (v) nematocidal activity and (vi) growth promotion and improved feed conversion efficiency.

I. INTRODUCTION

Mechanisms of action to account for these effects will be briefly described here. Yucca contains polyphenols which bind ammonia and hydrogen sulfide, thus improving air quality in poultry houses. Saponins in both yucca and quillaja bind cholesterol, accounting for hypocholesterolemic activity and reduction of egg cholesterol contents. The cell membranes of all animals contain cholesterol. Binding of saponins to membrane cholesterol can modify membrane function and structure. Cholesterol binding accounts for the anti-protozoal activity of saponins (McAllister et al., 2001). Quillaja saponins are used commercially as nematocidal agents in crop production. Their nematocidal activity is probably a result of membrane cholesterol binding. Effects of saponins on nematode parasites of livestock and poultry have yet to be investigated. Anti-inflammatory effects of yucca have been attributed to polyphenolics such as resveratrol and yuccaols (Marzocco et al., 2004). Anti-inflammatory agents reduce formation of cytokines, reducing the diversion of nutrients from growth to immune responses. Anti-inflammatory activity has been advanced as the explanation of the growth-promoting effects of antibiotics (Niewold, 2007).

Nutrafito Plus is a proprietary feed additive (Desert King International) containing whole plant powders of yucca and quillaja, enriched with quillaja polyphenols. A series of broiler trials with Nutrafito Plus were conducted in the United States and Mexico, under the sponsorship of Desert King International.

[1]Department of Animal Sciences, Oregon State University, and Scientific Advisor, Desert King International, 7024 Manya Circle, San Diego, CA 92154 USA

II. TRIAL 1

Trial 1 was conducted at the International Institute for Animal Research at Queretaro, Mexico. The Institute is located 1800 meters above sea level. Ross-Ross 308 chicks were used, with 20 males and 20 females per cage. There were three floor cages per treatment, for a total of 120 birds per treatment. The floor cages were four square meters. The experimental period was 49 days. The negative control diet was based on sorghum, supplemented with soybean meal, and containing no growth promotants. The positive control diet contained 100 ppm Flavomycin (4%). Treatments were negative control plus 100 ppm and 150 ppm Nutrafito Plus, and positive control plus 100 ppm and 150 ppm Nutrafito Plus. All treatments contained 0.550 kg monensin per ton.

The feeding program was as follows: starter phase, 0-21 days; grower phase, 21-35 days; finisher phase, 35-49 days. Feed and water were available ad libitum. The ventilation program was by use of side windows and interior fans. Lighting was natural day light. Results were statistically analyzed using a totally randomized distribution model.

Growth rate, feed intake and feed conversion data are shown in Table 1. Weight gain and feed conversion were improved ($P < 0.05$) in the positive control versus the negative control. All treatments with Nutrafito Plus had higher gain ($P < 0.05$) and improved feed conversion ($P < 0.05$) compared with the positive control. There were no statistically significant differences between the groups fed Nutrafito Plus alone or with the antibiotic.

Table 1 Performance parameters of broilers fed Nutrafito Plus with or without an antibiotic growth promoter at 49 days post-hatch (Mexico).

Treatment	Body Wt. (kg)	ADG (g/day)	Feed Intake (g/day)	Feed Conversion (g/g)
Negative Control	2.411[a]	48.3[a]	102.3	2.118[a]
Positive Control (PC)	2.462[b]	49.3[b]	102.4	2.077[b]
PC + 100 ppm Nutrafito Plus (NP)	2.520[c]	50.5[c]	102.5	2.029[c]
PC + 150 ppm NP	2.513[c]	50.4[c]	102.3	2.029[c]
PC + 100 ppm NP + 100 ppm Flav. (F)	2.518[c]	50.5[c]	102.8	2.035[c]
PC + 150 ppm NP + 100 ppm F	2.515[c]	50.4[c]	102.0	2.023[c]

a vs b vs c $P < 0.05$.

Data for % breast meat and % abdominal fat are shown in Table 2. These parameters were not affected by dietary treatment. There were no differences in mortality, which was low. The only group with no disease-related mortality was the 100 ppm Nutrafito Plus group.

Table 2 Carcass parameters of broilers fed Nutrafito Plus with or without an antibiotic growth promoter (Mexico)

Treatment	Males		Females	
(Same as in Table 1)	% Breast meat	% Abdominal fat	% Breast meat	% Abdominal fat
Negative Control	21.0	1.8	22.3	2.3
Positive Control (PC)	21.2	2.0	22.1	2.2
PC + 100 ppm Nutrafito Plus (NP)	21.3	1.8	23.6	2.3
PC + 150 ppm NP	22.2	2.3	23.0	2.7
PC + 100 ppm NP + 100 ppm Flav. (F)	23.1	2.1	22.9	2.4
PC + 150 ppm NP + 100 ppm F	22.8	1.9	22.1	2.7

The main conclusion of this trial is that Nutrafito Plus gave results in terms of growth and feed conversion that were superior to those obtained with an antibiotic growth promoter. This indicates that Nutrafito Plus is a potential replacement for antibiotic growth promotants in broiler diets.

III. TRIAL 2

The second trial was conducted at Mississippi State University. Male Ross 708 broiler chicks were used. There were 6 pens per treatment, with 13 birds per pen, for a total of 312 birds. Corn-soy diets were used: pre-starter, 0-7 days; starter, 7-21 days; and grower, 21-42 days. Feed and water were ad libitum, and lighting was 23 light:2 hour dark. Treatments were negative control (NC), NC + bacitracin, NC + 100 ppm Nutrafito Plus, and NC + 150 ppm Nutrafito Plus. All diets contained monensin at 90 g per ton.

There were no significant differences in growth or feed conversion compared to the negative control or to the positive control containing Bacitracin (Table 3). However, the reduction in mortality with Nutrafito Plus was statistically significant. There was slight evidence of anti-inflammatory activity (Table 4). There were no effects on carcass parameters (Table 5). In contrast to Brazilian studies with quillaja powder (Cheeke and otero, 2005), no effects on intestinal villi were noted (Table 6). Finally, litter parameters were not affected (Table 7).

Table 3 Effect of Nutrafito Plus on performance parameters of broilers (Mississippi State)

Treatment	Bodyweight at 42 days (g)	Feed Conversion	Mortality (%)
Negative Control (NC)	2579	1.79	10.2[a]
NC + Bacitracin	2625	1.74	2.6[b]
NC + 100 ppm Nutrafito Plus (NP)	2615	1.74	1.3[b]
NC + 150 ppm NP	2606	1.75	1.3[b]

a different than b ($P < 0.05$)

Table 4 Immune responses and lymphoid organ relative weights of broilers fed bacitracin or Nutrafito Plus (Mississipii State)

Treatment (Same as in Table 3)	PHAP[1]	SRBC log[2]	Bursa[3]	Spleen[3]
Negative Control (NC)	23.7	5.4	0.16	0.11
NC + Bacitracin	20.6	6.2	0.21	0.14
NC + 100 ppm Nutrafito Plus (NP)	18.8	4.8	0.20	0.13
NC + 150 ppm NP	19.8	5.3	0.19	0.13

[1]% inflammation response relative to the untouched toe-web.
[2]log value of titer in primary response to sheep red blood cells.
[3]weight as % of body weight.

Table 5 Carcass parameters of broilers fed bacitracin or Nutrafito Plus (Mississippi State)

Treatment (Same as in Table 3)	Carcass yield (%)	Abdominal fat (%)	Breast meat (%)
Negative Control (NC)	70.9	1.59	21.32
NC + Bacitracin	71.1	1.54	21.33
NC + 100 ppm Nutrafito Plus (NP)	71.8	1.66	21.46
NC + 150 ppm NP	70.9	1.66	21.48

Table 6 Jejunal-villi length and width (microns) of broilers fed bacitracin or Nutrafito Plus (Mississippi State)

Treatment (Same as in Table 3)	Length	Width
Negative Control (NC)	1210	78
NC + Bacitracin	1158	91
NC + 100 ppm Nutrafito Plus (NP)	1261	109
NC + 150 ppm NP	1210	83

Table 7 Litter characteristics at day 42 of broilers fed bacitracin or Nutrafito Plus (Mississippi State)

Treatment (Same as in Table 3)	Moisture (%)	pH	N (%)	NH_3[1.]
Negative Control (NC)	28.4	7.78	4.03	617.4
NC + Bacitracin	29.7	7.91	3.85	703.0
NC + 100 ppm Nutrafito Plus (NP)	31.5	7.90	4.08	783.6
NC + 150 ppm NP	30.8	7.87	4.24	721.2

[1]mg ammonia per m^2 per hour

In this trial, bacitracin and Nutrafito Plus gave similar results. The most interesting finding was a sharp reduction in mortality of broilers fed Nutrafito Plus and a tendency of lower feed conversion (not statistically significant).

IV. TRIAL 3

The third trial with Nutrafito Plus was conducted at Texas A&M University. Cobb x Ross straight run broiler chicks were used. There were 20 chicks per pen with 10 replications per treatment. The starter and grower diets contained an anticoccidial (Coban 60) at 454 g per ton. The starter phase was 0-21 days; grower, 21-35 days; and finisher, 35-42 days. Corn-soy diets were used. Treatments were Negative Control (NC), NC + BMD 50 (454 g/ton), NC + 100 ppm Nutrafito Plus, NC + 150 ppm Nutrafito Plus, and NC + 100 ppm Nutrafito Plus + BMD 50 (454 g/ton).

Both Nutrafito Plus and BMD increased rate of gain and final body weights (Table 8). Feed conversions were also improved. Mortality averaged 3.6% and was not affected by treatment.

Table 8 Performance of broilers fed Nutrafito Plus (Texas A&M)

Treatment	Total gain (kg)	Final Body Weight (kg)	Feed/ Gain
Negative Control (NC)	2.37[a]	2.41[a]	1.79[a]
NC + BMD 50	2.52[b]	2.57[b]	1.76[a,b]
NC + 100 ppm Nutrafito Plus	2.58[b]	2.62[b]	1.77[a,b]
NC + 150 ppm Nutrafito Plus	2.57[b]	2.61[b]	1.76[b]
NC + 100 ppm Nutrafito Plus + BMD 50	2.58[b]	2.62[b]	1.75[b]

a different than b ($p < 0.05$)

All treatments with Nutrafito Plus (2 through 5) resulted in significantly lower litter ammonia ($P < 0.05$) at week four of the study (Table 9) but no treatment was significantly different from the control ($P < 0.05$) at week six.

Table 9 Environmental ammonia readings of broilers supplemented with BMD50 or Nutrafito Plus (Texas A&M)

	Week 4	
Treatments	Ammonia (ppm)	Litter Temp (^oF)
Control	6.9 $\pm$ .6[a]	65.5
BMD50	5.3 $\pm$ .7[bc]	65.4
Nutrafito Plus™ @ 100 ppm	4.3 $\pm$ .5[c]	65.6
Nutrafito Plus™ @ 150 ppm	4.3 $\pm$ .8[c]	66.0
Nutrafito Plus™ + BMD50	4.9 + .6[c]	65.6

V. CONCLUSIONS

In three trials, Nutrafito Plus produced improvements in growth, feed conversion and other performance data that were similar to those seen with antibiotic growth promoters. Based on these findings, it is apparent that Nutrafito Plus is a valuable feed supplement for broilers, and potentially can serve as a replacement for antibiotic growth promoters.

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THE POTENTIAL OF HIGH-YIELDING TRITICALE VARIETIES FOR POULTRY

A.V ELANGOVAN[1], P.A. IJI[1], M. BHUIYAN[1] and R. JESSOP[1]

Triticale has remained a minor cereal grain in Australia mainly due to the low volume of production. Recent breeding at the University of New England has created new varieties, which are high-yielding and contain more protein (around 20%) than most other cereal grains. These varieties are being released for cultivation and there is a need to investigate their feeding value for poultry.

A total of 350-day-old Cobb broiler chicks obtained from local hatchery was randomly allocated to 10 treatments of 5 replicates each (7 birds per replicate). The chicks were reared in brooder cages with wire floor in environmentally controlled experimental room. The 10 diets consisted of 5 cereal diets viz., one wheat and four triticale varieties (H55, H128, H431 and H261) incorporated at 400g/kg diet and 5 corresponding diets supplemented with an enzyme (Avizyme 1302). Celite (acid insoluble ash, 5 g/kg) was added to the diets as a marker for digestibility measurement. The diets were fed for 22 days, followed by sampling for measurement of organ weights, digestive enzyme activities and nutrient digestibility.

The body weight gain of chicks up to 7 days of age was not significantly affected by treatment. However, body weight gain up to 14 days was higher ($P < 0.01$) in the wheat fed group than only triticale variety H261. Weight gain to 22 days of age on the wheat-fed group was higher ($P < 01$) than on H128 and H261 but not the groups raised on H55 and H418. Feed intake was not significantly different up to 7 days of age but to 14 days, feed intake in the wheat-based diet was higher ($P < 0.01$) than on all the triticale varieties except H128. Feed intake up to 22d was higher ($P < 0.01$) on the wheat based diet than on all triticale groups. The performance difference was probably due to the protein levels in the test ingredients (108 and 116 g/kg in H128 m and H261) and diets (188 and 194 g/kg in H128 and H261). FCR was not affected by cereal type, enzyme supplementation or interaction during the entire phase except at 7 days, wherein chicks on the wheat-based diet were less efficient ($P<0.01$) than those on triticale H261. The weight of the pancreas in non-supplemented groups was higher ($P<0.05$) than in the enzyme supplemented groups. The weight of other visceral organs assessed was not affected by cereal type or enzyme supplementation.

Table Growth performance of broiler chicks

	Gain (g/bird)			Feed intake (g/bird)			FCR (g/g)		
	0-7d	0-14d	0-22d	0-7d	0-14d	0-22d	0-7d	0-14d	0-22d
Wheat	85.4	327.2^{a}	773.0^{a}	109.8	446.0^{a}	1181.3^{a}	1.30^{b}	1.39	1.56
Triticale H418	75.4	281.8^{ab}	710.1^{ab}	104.9	389.5^{b}	1040.2^{b}	1.40^{ab}	1.41	1.49
Triticale H55	75.1	286.6^{ab}	733.8^{ab}	104.5	394.3^{b}	1041.5^{b}	1.40^{ab}	1.45	1.47
Triticale H128	78.8	288.2^{ab}	679.7^{b}	110.2	416.6^{ab}	1059.2^{b}	1.41^{ab}	1.48	1.58
Triticale H261	73.3	276.9^{b}	691.3^{b}	107.1	402.7^{b}	1073.2^{b}	1.48^{a}	1.51	1.58
SEM	1.61	5.36*	8.85**	1.29	5.28***	12.23**	0.018*	0.018	0.017

* P<0.05, ** P<0.01, *** P<0.001.

Jejunal mucosal protein was lowest ($P<0.055$) in all enzyme-supplemented groups. The activity of alkaline phosphatase was also significantly higher ($P<0.05$) in chickens on H55 and H128 than on the wheat-based or H418 diets. Ileal protein digestibility on the wheat-based diet was higher ($P<0.01$) than on H128 and H261, with a similar trend as that of 22d live weight gain. It is most probable that these triticale varieties would have similar feeding values as wheat for broiler chickens, provided the diets are iso-caloric and iso-nitrogenous.

[1] School of Environmental and Rural Science, University of New England, Armidale NSW 2351 Australia

EFFECTS OF A SIX-HOUR-THERMAL CONDITIONING ON PERFORMANCE AND THERMO-TOLERANCE ACQUISITION IN TWO BROILER CHICKEN STRAINS

M.A.M. SAYED[1] and J. DOWNING[1]

Summary

The purpose of present work was to evaluate the effect of thermal conditioning (TC) on performance and thermo-tolerance in two broiler chicken strains (Cobb and Ross) when exposed to moderately high temperatures. Newly hatched broiler chickens (n=252) of each strain were used in this trial. Chicks were exposed to normal brooding temperatures or to high thermal conditioning temperatures of (36-37°C) or (38-39°C) for six hours at one, three or five days of age. Birds were exposed to high ambient temperature (32±1°C for 9 hours daily) during week 6 of age. Body weight gain, feed intake, mortality rate, Plasma T_3, glucose and cholesterol levels, H/L ratio and rectal body temperatures were determined. Cobb birds had better performance and appeared to exhibit better heat tolerance than Ross birds. Although TC had no influence on performance, it significantly decreased circulating T3, body temperature at 36 day of age and H/L ratio only in Ross birds. Thermal conditioning for six hours failed to induce thermo-tolerance in Cobb birds.

I. INTRODUCTION

Research is ongoing, especially in countries with hot humid climates, to develop new methods to lessen the adverse effects of heat stress on broiler performance. Broiler chickens are more susceptible to heat stress prior to marketing because they have high metabolic rates and consequently produce more metabolic heat. It had been reported that exposing broiler chickens to high temperature early in life through thermal conditioning (TC) improves their ability to tolerate heat stress later in life (Arjona et al., 1990; Yahav and McMurtry, 2001; Moraes et al., 2003; Yahav et al., 2004). It has been suggested that TC influences thyroid function, decreasing the secretion of thyroxine (T_3) during heat stress. A decrease in circulating T_3 inturn reduces the metabolic rate and eventually affects body heat production. This enables birds to expend less energy to regulate their body temperature during thermal challenge. The lower energy expenditure is related to improved feed efficiency and performance during heat stress. Previous evidence indicates that TC is successful in reducing the number of mortalities and improves thermo-tolerance when broilers were subjected to extreme temperatures (35-40°C). However, its effects have not been determined under moderately elevated temperatures (30-32°C). In the present study the effect of TC on birds' performance and thermo-tolerance under moderately high temperature is evaluated.

II. MATERIALS AND METHODS

Six hundred fertile eggs of two broiler chicken strains (Cobb and Ross) were incubated under the same conditions. Two hundred and fifty two day old male chicks of each strain were used in the study. At hatch, birds were divided into seven treatments, with six replicates per treatment, and six birds per replicate. All chicks were wing banded, individually weighed, and housed in metabolism cages. Broilers were offered starter (d 1-14), grower (d 14-28) and finisher (d 28-42) diets formulated according to NRC 1994 recommendations. Birds were either reared under normal brooding temperature of 32°C during the first three days, then,

[1] Faculty of Veterinary Science, The University of Sydney. Camden NSW 2570

temperature was reduced 2°C every week (control) or subjected to a temperature of 36-37°C or 38-39°C for six hours at one, three or five days of age (TC1, TC3 and TC5, respectively) and reared similarly to the controls at other times. At 5 weeks of age, broilers were exposed to high temperature (32 ± 1 °C from 8:30 am to 5:30 pm then 24 ± 1°C from 5:30 pm to 8:30 am) for six days. The temperature was increased to 34 ± 1°C on day seven of the high temperature period. Rectal temperatures were taken from randomly selected sample of birds (4 birds per treatment) each day during the period of high temperature. At the end of the study, blood samples (from one bird in each cage) were collected from the jugular vein. Blood smears were prepared on glass slides and treated using Geimsa and MayGrunwald stains. The H/L ratio was calculated by counting 100 cells and dividing the number of heterophils by the number of lymphocytes. Blood was centrifuged at (1000 × g for 20 minutes at 4°C) and plasma was stored at (-80°C) until assayed for T_3, glucose and cholesterol using commercially available kits.

III. RESULTS AND DISCUSSION

The effect of thermal conditioning on Cobb and Ross broiler chicken performance is shown in (Table 1). The Cobb birds were significantly heavier ($P < 0.001$) and consumed more feed ($P < 0.05$) than Ross birds from hatch through to market age. In addition, Cobb birds had significantly lower FCR ($P < 0.01$) than that of the Ross birds during weeks five and six.

Exposing broiler chickens to high ambient temperatures early in life resulted in a significant reduction in body weight gain during the first week. However, no significant differences were observed in body weight gain from two to six weeks of age between thermally conditioned and control birds. These results are in agreement with those of (Yahav and McMurtry, 2001; Basilio et al., 2002). There were no significant differences in feed intakes among thermally conditioned and control groups from two to five weeks of age. During week one, it was observed that TC5 birds consumed significantly less feed than did those of TC1, TC3 and control groups. In addition, TC3 birds consumed significantly less feed than did their counterparts in TC1 group. At week six, it was found that TC5 birds consumed significantly less feed than did those of TC3 and control groups. During the first week of age, all thermally conditioned birds had significantly higher FCR when compared with their controls. However, these effects disappeared as the birds aged. The temperature used for TC had no significant effect on performance traits during the trial period. Although, not statistically different, the Cobb broilers had lower mortality percentage than that of the Ross birds (4.3% versus 7.9%), which might indicate a better capacity to handle the heat stress.

A significant strain × thermal treatment interaction was observed in plasma T_3 concentrations. All Ross thermally conditioned birds, except those exposed to 38-39°C at five days, had significantly lower circulating T_3 in plasma when compared with controls. These results are consistent with those of (Yahav and Hurwitz 1996; Yahav 2000; Yahav and McMurtry 2001; Moraes et al., 2003). It is well known that T_3 plays a major role in controlling metabolic rate (and consequently heat production) in mammals and birds (Yahav et al., 2004). Therefore, it is thought that broilers with lower circulating T_3 during thermal challenge are more heat tolerant. In Cobb birds, thermal conditioning failed to influence T_3 concentrations in plasma. These results indicate that either thermal conditioning for six hours is not sufficient to induce heat adaptation or that the acquired thermo-tolerance did not last for six weeks in the Cobb strain. Furthermore, it was observed that Ross control birds had significantly higher plasma T_3 concentrations than that of Cobb control birds. This suggests that Ross birds might have a higher basal metabolic rate and are potentially more susceptible

to heat stress than Cobb birds. Altan et al. (2003) reported that Ross broiler chickens were more susceptible to lipid peroxidation induced by heat stress than Cobb birds.

Table 1 Effect of strain and thermal conditioning on performance of Cobb and Ross broiler chickens

Strain/Treatment	Hatching BW (g)	Weight Gain (g)	Feed intake (g)	Cumulative FCR
Ross (Control + TC)	43[b]	2071[b]	3772[b]	1.82[a]
Cobb (Control + TC)	45[a]	2315[a]	4064[a]	1.76[b]
Control (Ross + Cobb)	-	2221	3923	1.77
TC (Ross + Cobb)	-	2188	3918	1.79
P value				
Strain	0.0001	0.0001	0.0001	0.013
Treatment	-	0.4651	0.9495	0.2619
Strain × Treatment	-	0.7971	0.6137	0.6930
SEM	0.23	25.66	46.7	0.01

[a,b] means within columns with no common superscript differ significantly.

Similarly, on the first day of heat stress, there was a significant strain × TC interaction for rectal temperature. Thermal conditioning did not affect rectal temperature in Cobb birds. However, Ross thermally conditioned birds had significantly lower rectal temperatures than their controls. The TC1 and TC3 (exposed to 38-39°C) tended to have lower rectal temperatures than control birds. This reduction in body temperature could be associated with the decrease in T_3 of thermally conditioned birds during heat stress, with the decrease reducing their metabolic rate and hence heat production (Yahav and McMurtry, 2001; Moraes et al., 2003). The differences in rectal temperature were not observed after the first day of heat stress. This might be attributed to the ability of birds to acclimatize with repeated exposures to high temperatures. On the last day of heat stress, Ross birds had significantly higher rectal temperatures than Cobb birds. This increase occurred after raising the ambient temperature from 32 to 34 ± 1°C.

Under stress conditions, the number of heterophils increase, however the number of lymphocytes decrease. The H/L ratio is considered a reliable measure of chronic stress (Siegel, 1995). The Ross birds had significantly higher H/L ratios than those of Cobb birds. These results suggest that the Cobb birds were more tolerant to the thermal challenge than Ross birds. All thermally conditioned Ross groups had significantly lower H/L ratio than their controls. These results are consistent with those of Arjona et al. (1990) who reported lower H/L ratios in thermally conditioned broilers when exposed to eight hours of high temperature (35-37°C) at 43 and 44 days of age.

In general, stress results in hyperglycaemia and hypercholesterolemia in humans and animals (Pare et al., 1972; Zardooz et al., 2006). The secretion of corticosterone stimulates glycogen breakdown in liver and fat deposition in abdominal and adipose tissues. These events result in increased glucose and cholesterol concentrations in blood. In present study, the glucose and cholesterol concentrations in plasma were not affected by thermal conditioning. The different TC temperatures had no significant effects on plasma glucose, cholesterol and T_3 concentrations, rectal temperatures and H/L ratio.

IV.CONCLUSION

The two broiler chicken strains responded in a different manner to thermal conditioning. Cobb broilers appeared to be more heat tolerant than Ross birds. Thermal conditioning provided no advantage to birds under moderately high ambient temperatures (cyclic heat stress) used in this study.

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DOES ANTIOXIDANT SUPPLEMENTATION BENEFICIALLY AFFECT REDOX HOMEOSTASIS AND PERFORMANCE IN BROILER CHICKENS EXPOSED TO SHORT TERM HEAT STRESS?

M.A. M. SAYED[1] and J. DOWNING[1]

Summary

The effect of supplementing different concentrations of vitamin E and selenium in broiler chickens diets on performance and redox status under short-term heat stress was investigated. One day old Cobb broiler chickens (n = 576) were assigned to 16 treatment groups with 6 replicates per treatment and 4 birds per replicate. Birds were kept under thermo-neutral temperatures and fed basal starter and grower diets from 1 to 28 days of age. On day 29, birds were fed either basal finisher or basal diet supplemented with different levels of an organic source of selenium and/or vitamin E. all birds were exposed to cyclic heat stress (22-30-22°C) during week six. It was found that antioxidant supplementation had no effect on performance, corticosterone, malondialdehyde, and cholesterol levels in plasma, H/L ratio and glutathione proteins in livers.

I. INTRODUCTION

Antioxidant supplementation (i.e. tocopherol, retinol, selenium, ascorbate, etc) in broiler diets is commonly used as a practice to alleviate the negative effects of heat stress. The relationship between heat stress and the increased production of free radicals (FR) and reactive oxygen species (ROS) is well established (Altan et al., 2000; Lin et al., 2000). These agents react destructively with cellular molecules (e.g. proteins, DNA, lipids) to cause cell damage. The use of antioxidants at low concentrations can act to neutralize FR and ROS without harming cellular components. Antioxidants are present in sufficient amounts in the body to account for the normal rate of FR generation during nutrient oxidation and metabolism (redox homeostasis). However, in a stressful environment in which inherent antioxidant defences increase, there may be the need for the addition of antioxidants to the diet. While there are reports illustrating the beneficial effects of antioxidant supplementation on improving performance of heat stressed broiler chickens, it is important to keep in mind that many of these studies exposed birds to temperatures as high as 32-35°C for six weeks (long-term heat stress). Furthermore, some studies have used a constantly high temperature during the day and night. These patterns of heat stress seldom occur normally. Therefore, the objective of current study was to elucidate the efficacy of antioxidants supplementation (vitamin E and/or selenium) on broiler chicken's performance during short-term cyclic heat stress.

[1] Faculty of Veterinary Science, University of Sydney, Camden NSW 2570

II. MATERIALS AND METHODS

Five hundred and seventy six-one day old Cobb broiler chicks were individually weighed and housed in metabolism cages. Birds were reared under normal brooding temperatures and offered starter (d 1-14), grower (d 15-28) and finisher (d 29-42) diets formulated according to NRC 1994 recommendations. At the start of week 5, broiler chickens were distributed into treatments (n=16) according to body weight. Birds were fed either a basal diet supplemented with 0.00, 150, 300 or 500 mg vitamin E/kg feed, basal diet supplemented with 0.00, 0.15, 0.3 or 0.45 mg selenium/kg feed or all combinations of both antioxidants. Each treatment group consisted of 6 replicates with 6 birds per replicate. At 5 weeks of age, broilers were exposed to heat stress of 30 ± 1°C from 0830 : 1730 h and 22 ± 1°C from 1730 : 0830 h for a period of one week. One bird from each cage was bled prior to and at the and at the end of heat stress period. Blood samples (from one bird in each cage) were collected from the jugular vein and blood smears were prepared and stained using Geimsa and MayGrunwald stains for the determination of the H/L ratio. The H/L ratio was calculated by counting 100 cells and dividing the number of heterophils by the number of lymphocytes. Blood samples were certificated (1000 × g for 15 minutes) and plasma was stored at (- 80°C) until assayed for corticosterone, glucose and malondialdehyde (MDA; the major product of lipid peroxidation) using commercially available kits. Liver samples were collected from one bird in each cage at the end of trial for the determination of total, reduced and oxidized glutathione as described by (Mahmoud and Edens, 2003).

III. RESULTS AND DISCUSSION

Separately or in combination, supplemental vitamin E and selenium did not affect body weight gain, feed intake, FCR, or mortality rate. These results are not in agreement with other studies showing significant improvements in performance of heat stressed broilers and quails when fed diets supplemented with antioxidants (Sahin et al., 2001; Sahin et al., 2002; Sahin et al., 2003).

Although exposure to high temperature significantly increased malondialdehyde (MDA concentration µM/dl mean ± SE; 0.94 ± 0.1 in thermo-neutral vs. 1.3 ± 0.2 in heat stress) concentrations, there were no significant differences between antioxidant fed treatments and control birds. These results are not in agreement with those of Sahin *et al*., (2001; 2003) who reported lower plasma MDA concentrations in heat stressed broilers fed diets supplemented with different antioxidants. However, it is important to bear in mind that in their trials, broilers were exposed to severe heat stress (constant temperature of 32°C from 1 to 42 days of age). In addition, exposing broiler chickens to repeated episodes of heat stress may help the birds to adapt to the environment. Elevated body temperature during heat stress accelerates biochemical reactions and metabolic rates in cells and is responsible for increasing FR and ROS concentrations (Lin et al., 2006). Because acclimated broiler chickens have the ability to reduce their body temperatures compared to non-acclimated birds under heat stress (Wiernusz and Teeter, 1996), it is possible that broilers in the current study were acclimatized to the heat and were able to reduce body temperature to some extend and reduce the severity of the oxidative stress.

It was observed that total, reduced and oxidized glutathione (TGSH, RGSH and GSSG, respectively) concentrations and RGSH/GSSG ratio in liver were not affected by antioxidants supplementation. These results suggest that the activity of the enzymes glutathione peroxidase (GPX) and glutathione reductase were not influenced by antioxidant supplementation. Similarly, Payne and Southern (2005) reported that sodium selenite and selenium enriched yeast supplementation in broiler chicken diets (0.3 ppm) did not alter GPX activity in plasma when compared with non-supplemented birds. However, Balogh et al., (2004) observed increased glutathione concentrations and higher GPX activity in liver tissues from broiler chickens supplemented with sodium selenite in drinking water. Supplementing basal diets with 200 mg/kg vitamin E or 0.3 mg/kg organic selenium increased glutathione concentrations and enhanced GPX activity in liver tissues when broilers were exposed to cold stress, but not under optimum conditions (Özkan et al., 2007). However, Mahmoud and Edens (2003) reported enhanced GPX activity in both blood and livers of broiler chickens supplemented with selenium under optimum and heat stress conditions. These discrepancies may be related to the differences in selenium concentrations of the basal diets used and the degree of stress imposed. In addition, the nutritional status of the parent flocks in terms of antioxidant deficiency or adequacy can affect the antioxidant defense system of the offspring during the embryonic and early postnatal stages (Surai, 2000).

The use of heterophil to lymphocyte (H/L) ratio and plasma concentrations of corticosterone as indicators of stress in animals and birds is well established (Siegel, 1995). In the present study, corticosterone concentrations in plasma and the H/L were not affected by antioxidant supplementation during thermo-neutral and heat stress periods (week 5 and 6, respectively). The corticosterone concentrations in plasma obtained before the start of heat stress were not significantly different to the concentrations at the end of the heat stress period. This could suggest some degree of acclimatization by the birds. Corticosterone concentrations in plasma increase rapidly (within minutes) after exposure to heat stress and peak within an hour of exposure. However, concentrations decline as the stress continues (Siegel, 1995; Zardooz et al., 2006). In the present study, exposure to heat stress significantly increased H/L ratio in all groups. These results are in agreement with those of Siegel (1995), who reported that under stress conditions, the number of heterophils increase; however the numbers of lymphocytes decrease. Although heat stress significantly increased H/L ratio, the values obtained are not reflective of severe stress (mean H/L ratio ± SE; 0.30±0.02 in heat stress vs 0.21±0.03 in thermoneutral).

Glucose concentrations in plasma were not affected by the high temperature. These results are not in agreement with the other reports identifying a positive correlation between increased plasma glucose concentrations when birds are exposed to different stressors (Zardooz et al., 2006). The stress parameters used in the current study may indicate that broiler chickens were capable of adapting to repeated episodes of high temperature. The glucose concentrations in plasma were not affected by vitamin E supplementation. These results also contradict those of others who have reported a linear reduction in serum glucose concentrations in heat stressed broiler chickens fed diets supplemented with vitamin E (Sahin et al., 2001; Sahin et al., 2002). In the present study, selenium supplementation significantly decreased glucose ($P<0.01$) concentrations in plasma during week 6. The effect on plasma glucose concentrations agree with previous

reports showing several insulin-like effects of selenium in isolated rat adipocytes (Ezaki, 1990; Hwang et al., 2007).

IV. CONCLUSION

In conclusion, exposing broiler chickens to cyclic heat stress for a short period did not induce harmful oxidative stress. It is possible that exposing broilers to repeated episodes of high temperature induces acclimatization and adding antioxidants to broilers diets under such conditions had no influence on production performance.

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FATTY LIVER HAEMORRHAGIC SYNDROME (FLHS) IN LAYING HENS: AN UPDATE

S. SHINI[1], A. SHINI[1] and W.L. BRYDEN[1]

Fatty liver haemorrhagic syndrome is a non-infectious disease of concern to the egg industry, worldwide. It is a metabolic disorder characterized by excessive accumulation of fat in the liver and abdominal cavity, massive hepatic haemorrhage and sudden death of laying hens (Julian, 2005). The condition occurs mainly in commercial caged layers and in affected flocks it is associated with decreased egg production and increased mortality. The aetiology of FLHS has been an enigma for the past 50 years and it is still an unresolved metabolic disease of laying hens (Leeson, 2007). A fundamental issue that is not well understood is why only some hens develop FLHS, as all laying hens develop fatty livers. We commenced our studies on FLHS with an epidemiological survey of commercial layers in Queensland, which showed that FLHS is present in layer flocks. It was confirmed that 36% of all birds necropsied during 3 months had FLHS (Shini et al., 2008). It should be noted that death from FLHS occurs only in extreme cases following massive liver haemorrhage. Significant number of birds within a flock may also suffer from "chronic FLHS," which does not result in mortality, but may cause impairment of production. It was also shown that most deaths due to FLHS occurred in heavier hens over 40 wks of age. Plasma levels of triglycerides were significantly increased in affected flocks. We demonstrated for the first time that birds in thermo-neutral conditions (controlled environment shed) developed FLHS at a similar rate to hens in naturally ventilated sheds.

Our current investigations are focused on understanding the aetiology and delineating the pathogenesis of FLHS in commercial layers. However, the study of FLHS is different in naturally occurring cases, as it happens sporadically and it is very difficult to diagnose before hens die. Therefore, we have reproduced FLHS using an oestrogen-induced laying hen model and assessed hormonal, metabolic and physio-pathological responses of hens as they develop FLHS. Interestingly, our blood test results show that there is a significant increase in total leukocyte counts, predominately total lymphocyte counts in oestrogen-treated birds, which demonstrates that inflammation might be a feature of FLHS. This could be a potential window through which to explore the link between metabolically triggered inflammation and occurrence of FLHS as it has been recently demonstrated in humans (Hotamisligil, 2006). Along with experimental studies, monitoring of the layer flock in the Gatton Poultry Controlled Environment Facility from start of lay until 60 wks of age will provide detailed data on hen performance, metabolism and health status during different stages of the disease as it develops in hens under commercial conditions. These observations will provide insights on how to manipulate the interface of metabolic and inflammatory response pathways for preventing FLHS in commercial layers.

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[1] School of Animal Studies, University of Queensland, Gatton QLD 4343, Australia

BACTERIA VERSUS PARASITES: *LACTOBACILLUS RHAMNOSUS* CELL FREE SUPERNATANT INHIBITS THE SPORULATION OF *EIMERIA* OOCYSTS *IN VITRO*

A.L. MOLAN[1], S. DE[1], D.V.THOMAS[1] and V.RAVINDRAN[1]

Avian coccidiosis, caused by infection with *Eimeria* species, is one of the most important diseases of domestic poultry worldwide. The purpose of this study was to determine if the probiotic bacterium, *Lactobacillus rhamnosus*, generates compounds that affect the sporulation of the oocysts of three species of *Eimeria* using an *in vitro* assay. *L. rhamnosus* was grown in Mann-Rogosa-Sharpe broth for 24 h at 37 ^{o}C anaerobically and the cells were removed from the broth by centrifugation to obtain the cell-free supernatant (CFS). The oocysts were incubated with undiluted or diluted (up to 32-fold dilution in water) CFS for 48 h at 25-29^{o}C. Following incubations, sporulated and non-sporulated oocysts were counted and percent sporulation determined.

In control incubations containing oocysts of *E. acervulina*, *E. tenella* and *E. maxima*, 85-89% of the oocysts sporulated. In incubations containing 8-fold diluted CFS, 75, 76 and 51% of the oocysts, respectively, of these three species sporulated. Relative to the control incubation, these sporulations corresponded to 12, 15 and 41% inhibition ($P<0.05$ to 0.0001) in sporulation. Exposure of the oocysts of the three species of *Eimeria* to 4-fold diluted CFS resulted in 37, 33 and 46% inhibition of sporulation, respectively, while exposure to undiluted CFS resulted in 100% inhibition of sporulation in all species. These results show, for the first time, that CFS from *L. rhamnosus* cultures have anticoccidial activity and suggest the possibility of a natural biological control mechanism for avian coccidiosis. The nature of the anti-coocidial components secreted by *L. rhamnosus* into the supernatant in our study remains to be determined.

[1] Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

EFFECTS OF FEED RESTRICTION AND STRAINS ON MODERN BROILER PERFORMANCE

K. HUANG[1], T. ACAMOVIC[3], J. OFFER[3] and C. KEMP[2]

Broiler feed intake is one of the principle determinants of broiler growth and performance. Together with broiler weight gain, it determines the feed conversion ratio (feed intake/gain, g/g), which is an important parameter of broiler production. Some practical circumstances in the field may involve a degree of feed restriction; eg. feeder management, poor feed form. This study investigated the effects of feed restriction on male broiler performance in two modern broiler strain crosses. During the experimental period, broilers were housed in individual cages and fed one of four feed regimes: ad lib, 90%, 80%, or 70% of ad lib intake from 24 to 31 days of age with 15 replicates. The feed restriction was based on individual ad lib intake during the adaptation period (18–21 days) and their respective bodyweight, subsequent feed intake was increased according to the primary breeding company's broiler performance objectives during the experimental period (24–31 days). All birds received a low nutrient density broiler grower maize-based diet (12.66 MJ/kg, 9.2 g/kg digestible lysine). Feed intake and body weight were recorded, and FCR was calculated. Excreta samples were collected every 24 hour during the trial period to determine AME and N retention.

The effects of feed restriction on body weight gain were similar for both strain crosses. Birds fed ad lib gained significantly more weight and had a significantly better FCR than those fed on a restricted basis. Weight gain was similar for both strain crosses with starting weight used as covariate; similarly there was no difference in FCR. AME and nitrogen retention were not significantly affected by feeding regime and there were no significant differences between strains.
This study clearly shows that feed restriction has a significant effect on modern broiler performance during the age period studied. This was similar for both strain crosses. Field conditions limiting feed intake may compromise performance of commercial broiler strain crosses. In this study, relatively large reductions in feed intake during a short time period were investigated. Further studies are warranted to examine the effects of broiler feed restriction during the entire growing cycle while varying nutrient density.

[1] Aviagen Inc. Email: Khuang@aviagen.com.
[2] Aviagen Ltd, Newbridge, Scotland
[3] SAC,ASRC, Auchincruive Estate, Ayr, Scoltand

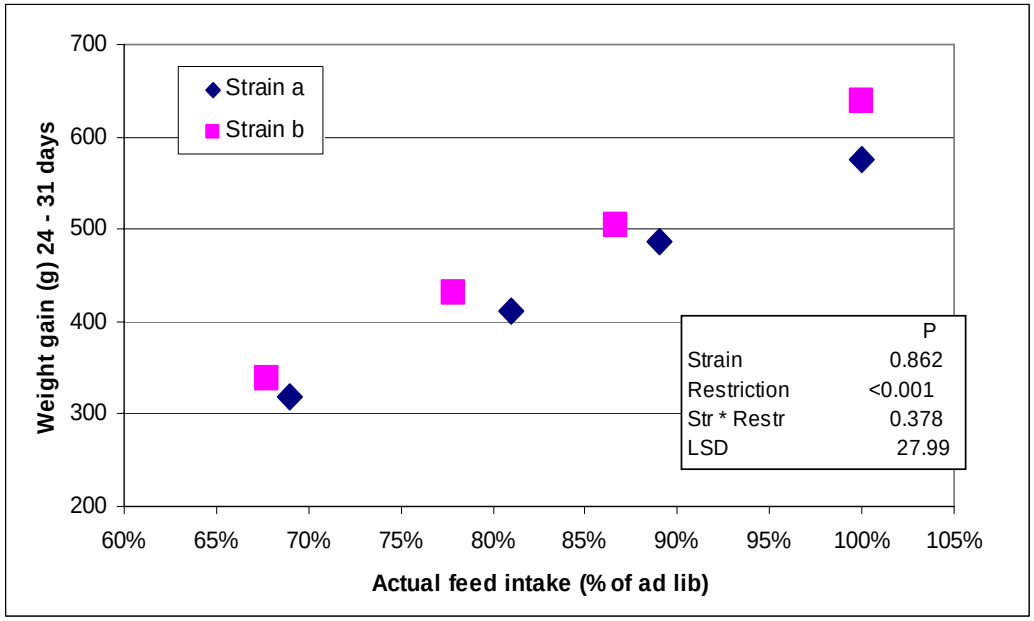

Figure 1 Weight gain between 24 -31days post-hatched

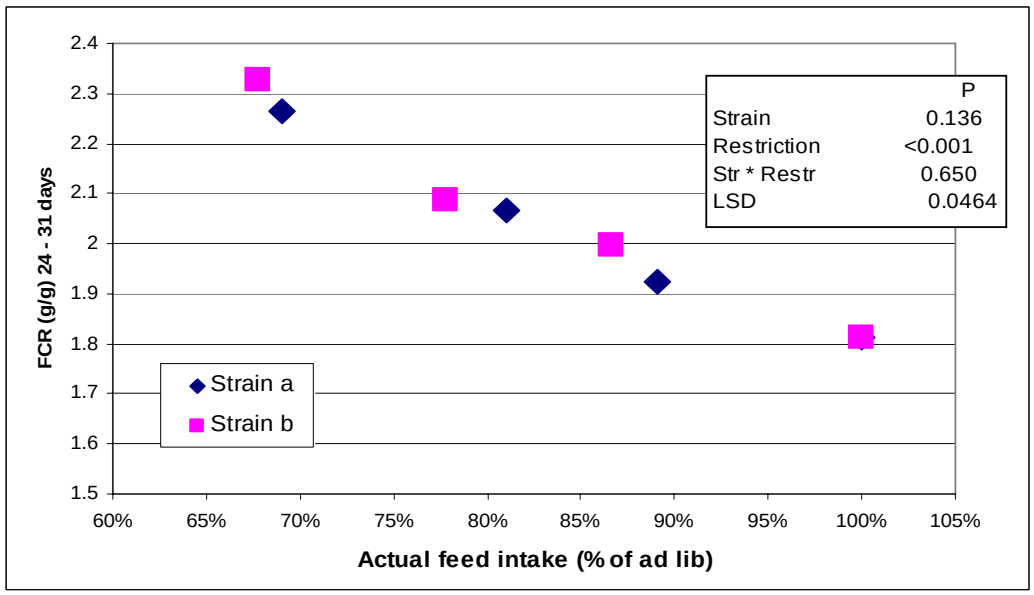

Figure 2 Feed Conversion ratio 24-31days post-hatched

THE FINE STRUCTURE OF RATITE SKIN

C.A. LUNAM[1] and K.A. WEIR[2]

Knowledge of the structure and composition of ratite skin, and how processing and storage alters skin structure is fundamental to the production of first grade tanned skins. We investigated the structures that comprise the skin of the ostrich and emu and discuss how these structures give the skin its unique properties. Light microscopy and scanning electron microscopy (Siemens Autoscan SEM) revealed that the skin of the ostrich and emu are structurally very similar and are comprised of the same fundamental layers as described for volant birds (Lucas and Stettenheim, 1972).

The surface of the skins consisted of a thin epidermis comprised of two to three layers of cells covered by keratin, the *stratum corneum.* As the epidermis is removed during liming it is not a component of tanned skin. However, in raw skins it may act as a barrier to physical damage of the underlying dermis during handling and also to serve to inhibit colonisation of the connective tissue by microbial flora during storage.

The dense connective tissue was comprised predominantly of collagen which was organised into two distinct layers, a thin superficial grain layer and an extensive corium layer. Both these layers consisted of three-dimensional arrays of collagen fibres orientated perpendicularly to one another and predominantly aligned parallel to the surface of the skin. The collagen fibres within the grain layer were more compact and thinner compared to those in the adjacent corium. In addition, the grain and corium layers were separated by a narrow band of loose connective tissue. This band of tissue is likely to provide little resistance to shearing forces during processing of the skins and would account for the lamination and loose grain defects prevalent in tanned skin of the ostrich and the emu.

Verhoeff and van Gieson staining revealed very few elastic fibres scattered between the collagen bundles. These fibres were predominantly found either in small bundles deep within the dermis or associated with the attachment of smooth muscles to the feather follicles. The paucity of elastic fibres suggests that their contribution to the elasticity of the skin is minimal. This suggests that the strength and flexibility of skin in the ostrich and the emu is derived almost exclusively from the three-dimensional cross-weave arrangement of collagen fibres. Both the band of loose connective tissue and the overlying grain layer contained numerous blood vessels. The high vascularity near the surface of the skin would explain why the skin is readily bruised during transport and handling prior to slaughter.

In the ostrich filoplumes formed a semi-circular pattern at the base of the feather follicles in every skin. Their density and distribution between the feather follicles was highly variable among individual birds. In the current study skins were examined from different flocks separated by a period of two years. Our finding of these miniature feathers at the base of the follicles of all skins, raises the possibility that all ostriches may have filoplumes at the base of the follicles and that it is only the extent of their distribution between the follicles that is subject to genetic variation. After tanning the pattern of tiny discrete pinholes paralleled the distribution of filoplumes in individual skins. As discrete plumping of the skin was observed immediately surrounding all pinholes, we suggest that the plumping of the follicles of the contour feathers is augmented by the presence of the filoplumes at their base.

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[1] Department of Anatomy and Histology, Flinders University, Bedford Park, SA 5042
[2] School of Biomedical Sciences, The University of Queensland, St. Lucia, QLD 4072

SELENIUM FORM AND FUNCTION: IMPACT OF SEL-PLEX® ON BROILER EFFICIENCY AND MEAT QUALITY

A. NAYLOR[2], V. RAVINDRAN[1], G. RAVINDRAN[1], D.V. THOMAS[1], A. KOCHER[2], A. SACRANIE[2]

Summary

The aim of this study was to compare the effects of organic selenium (Sel-Plex®, Alltech Inc.) versus inorganic selenium (sodium selenite) on the performance and meat quality of broilers. The results showed that organic selenium supplementation had a marked positive influence on the feed efficiency of broilers and is superior to sodium selenite as a source of selenium in poultry feed formulations. Feed efficiency was progressively increased with increased rate of selenium addition as Sel-Plex from 0.2 to 0.4 mg/kg. Organic selenium was also better retained in poultry meat and had beneficial effects on meat quality.

I. INTRODUCTION

Selenium (Se) is an essential micronutrient required for normal growth and metabolism in animals (Surai, 2002). It is routine to supplement poultry diets with inorganic forms of Se, but there is growing interest in the use of organic Se in recent years. The present study was conducted to compare the effects of organic Se (Sel-Plex®) versus inorganic Se (Na selenite) on the performance and meat quality of broilers.

II. MATERIALS AND METHODS

A total of 900 day-old male broilers (Ross 308) were assigned on a weight basis to 45 floor pens of 20 chicks each. Nine pens were then randomly assigned to each of the following five dietary treatments: (i) control diet (C) with no added Se, (ii) C + 0.3 mg/kg Se from Na selenite, (iii) C + 0.2 mg/kg Se from Sel-Plex, (iv) C + 0.3 mg/kg Se from Sel-Plex and (v) C + 0.4 mg/kg Se from Sel-Plex. The control diet, based on wheat, soybean meal, canola meal and, meat and bone meal, was formulated to meet commercial specifications for all nutrients, except Se. The trace mineral-vitamin premix was prepared without Se and Se was added in the form of selenite or Sel-Plex.

A 3-phase feeding programme (starter, grower and finisher), as per standard commercial practice in New Zealand, was used. The analysed Se contents of the starter, grower and finisher control diets were 0.050, 0.046 and 0.043 mg/kg, respectively.

Body weights and feed intake were recorded at weekly intervals throughout the 42-d trial period. On d 42, two birds per replicate were weighed and processed, and weights of eviscerated carcass, breast meat and abdominal fat were recorded. Requisite samples of breast meat were subjected to the following measurements: tissue Se levels, drip loss, cooking loss and thiobarbituric acid reactive substances (TBARS).

[1] Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, NZ.
[2] Alltech Biotechnology, Dandenong South, VIC, Australia
[3] Alltech UK, Stamford, United Kingdom

III. RESULTS AND DISCUSSION

Weight gain of broilers tended (P = 0.06) to be affected by dietary treatments, with gains of birds fed diets with 0.3 and 0.4 mg/kg Se as Sel-Plex tending to be higher (Table 1). Feed intake and feed per gain of broilers fed the Se-deficient control diet was higher (P < 0.05) than those fed diets containing Na selenite and Sel-Plex. Feed intake and feed per gain of birds fed diets with 0.3 mg/kg Se as selenite and 0.2 mg/kg Se as Sel-Plex were similar (P > 0.05), and these were higher (P < 0.05) than those of birds fed diets with 0.3 and 0.4 mg/kg Se as Sel-Plex. Increasing additions of Se from 0.2 to 0.4 mg/kg as Sel-Plex resulted in progressive reductions in feed per gain, with birds fed the diet with 0.4 mg/kg Se having the lowest (P < 0.05) value. Compared with the treatment with 0.3 mg/kg Se from selenite, feed conversion was lowered by 8 and 14 points, respectively, in treatments with 0.3 and 0.4 mg/kg Se from Sel-Plex. Carcass yield and relative weight of abdominal fat were unaffected (P > 0.05) by the source and levels of Se. However, breast meat yield was affected by Se (Table 1), with yield from broilers fed the Se-deficient control diet being lower (P < 0.05) than those fed diets containing sodium selenite and Sel-Plex.

Table 1 Influence of Se source on the performance (1-42 days post-hatch) and breast meat yield of male broilers

Treatment	Weight gain (g/bird)[1]	Feed intake (g/bird)	Feed per gain (g/g)	Breast meat, % body weight
Control (C)	3115	5348[a]	1.743[a]	19.5[b]
0.3 mg/kg Se from selenite	3084	5046[b]	1.638[b]	20.7[a]
0.2 mg/kg from Sel-Plex	3019	4938[b]	1.638[b]	20.2[a]
0.3 mg/kg from Sel-Plex	3159	4770[c]	1.555[c]	21.0[a]
0.4 mg/kg from Sel-Plex	3138	4679[c]	1.499[d]	20.9[a]
Pooled SEM	34.9	50.4	0.012	0.30

[a,b,c,d] Means in the same column with different superscripts are significantly different (P < 0.05).[1]P = 0.06.

The effects of dietary treatments on selected breast meat quality parameters are summarized in Table 2. Both Se sources increased (P < 0.05) tissue Se concentrations. However, the increments with 0.3 mg/kg inorganic Se diets were lower (P < 0.05) than those with 0.2 and 0.3 mg/kg organic Se diets. Increasing dietary Se concentrations from organic Se source increased (P < 0.05) tissue Se concentrations in a dose-dependent manner. The drip loss from breast meat was not influenced (P > 0.05) by Se treatments, but losses during the cooking of the meat were affected. Cooking losses in frozen meat from birds fed diets with 0.3 and 0.4 mg/kg organic were lower (P < 0.05) than those from birds fed the diet with 0.3 mg/kg inorganic Se. The degree of oxidation, as measured by TBARS, was influenced by Se treatments. In meat samples chilled for 7 days, the degree of oxidation in the negative control and the inorganic Se treatment was similar (P > 0.05), but significant differences were observed between the sources of Se, with samples from organic Se treatments having lower (P < 0.05) oxidation that those from the inorganic Se treatment.

Table 2 Influence of Se source on selected breast meat quality parameters.

Treatment	Se content (mg/kg, dry basis)	Drip loss at 48 h (% weight)	Cooking losses, (% weight)	TBARS (µg malondialdehyde /g fat)
Control (C)	0.034[a]	1.38	23.4[ab]	7.42[a]
0.3 mg/kg Se from selenite	0.080[b]	1.07	23.9[a]	7.78[a]
0.2 mg/kg from Sel-Plex	0.181[c]	1.04	23.5[a]	6.06[b]
0.3 mg/kg from Sel-Plex	0.225[d]	0.99	22.1[bc]	5.22[b]
0.4 mg/kg from Sel-Plex	0.270[e]	1.06	21.8[c]	5.85[b]
Pooled SEM	0.003	0.15	0.55	0.35

[a,b,c,d,e] Means in the same column with different superscripts are significantly different (P<0.05).

VI. CONCLUSION

The present data showed that Sel-Plex supplementation has marked positive influence on the feed efficiency of broilers and is superior to Na selenite as a source of Se in poultry feed formulations for fast growing broilers. This improvement was a result of the reduction in feed intake, rather than improved weight gain. These findings are in agreement with previous work by Choct et al. (2004). The reduction in feed intake was related to the actual level of selenium in the diet as well as the source of selenium supplied to the animals. The current study showed that birds given organic Selenium had reduced feed intake and improved FCR in comparison to birds fed inorganic selenium at the same inclusion level. Preliminary work using gene expression studies demonstrated that the form of selenium has a marked effect on intestinal gene expression profiles related to key metabolic functions (Power, personal communication). Of particular interest are the progressive improvements in feed efficiency with increased rate of Se addition as Sel-Plex from 0.2 to 0.4 mg/kg. The data also demonstrated that organic Se is better retained in poultry meat and has beneficial effects on meat quality. Increasing the selenium content of the diet improved the FCR of broilers.

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THE POST-HATCH GUT MICROBIOTA DEVELOPMENT IN BROILER CHICKENS

V.A. TOROK[1,2], G.E. ALLISON[3], K. OPHEL-KELLER[1] and R.J. HUGHES[2,4]

Summary

The development and succession of the overall ileal microbiota, *Lactobacillus* and related genera were investigated in male broilers (Cobb 500) from day 3 to 17 of age. Diets were based on a commercial starter diet which were either supplemented with one of three in-feed antimicrobials or remained supplement free. Both diet and age were found to influence significantly the ileal microbiota development and succession. However, regardless of the dietary treatment all groups exhibited similar temporal shifts in ileal microbial communities from 3-5 days, 5-12 days and 12-17 days. In-feed antimicrobials had no influence on the presence of *Lactobacillus.* Age of birds did, however, significantly alter *Lactobacillus* profiles. As birds grew older the prevalence of *L. johnsonii* and *L. reuteri* increased. *Pediococcus acidilactici* was only detectable in chicks at three days of age.

I. INTRODUCTION

Gut microbiota can influence the host's gastrointestinal development, biochemistry, immunology, physiology, and non-specific resistance to infection (Gordon and Pesti, 1971). The role of commensal gut microbiota in animal production is now receiving much interest, particularly since the withdrawal of in-feed antimicrobials in the European Union in 2006. In-feed antimicrobials have been used as a means to prevent the colonization of pathogenic bacteria, and ensure optimal bird performance.

A favourable gut microbiota is important for the optimal growth and performance of chickens. Recently, a direct correlation has been demonstrated between overall changes in gut microbiota associated with non-starch polysaccharide degrading enzyme supplementation and improved bird performance (Torok et al., 2008). Alternatively, an unfavourable microbiota may promote enteric infections, leading to decreased growth rates and increased mortality. However, gut bacteria need not be pathogenic to impact negatively on bird performance and production (Rehman et al., 2007).

The search for natural alternatives to in-feed antimicrobials for the poultry industry will require understanding the process of optimal intestinal microbiota establishment and development post-hatch. In this report we investigated the post-hatch development of ileal microbiota, *Lactobacillus* and related genera in broiler chickens fed diets either free of in-feed additives or containing one of three in-feed antimicrobials currently used within Australia.

II. MATERIALS AND METHODS

A chicken study was done with 640 male birds (Cobb 500) aged 1-17 days. Chickens were raised in floor pens in a temperature-controlled room. The experiment was approved by the Animal Ethics Committees of the University of Adelaide and the Department of Primary Industries and Resources South Australia.

[1]SARDI, Plant and Soil Health, GPO Box 397, Adelaide, SA 5001
[2]Australian Poultry CRC, University of New England, Armidale, NSW 2351
[3]School of Biochemistry and Molecular Biology, and ANU Medical School, The Australian National University, Canberra, ACT 0200
[4]SARDI, Pig and Poultry Production Institute, Roseworthy, SA 5371

The four experimental diets (n=160/group) were based on a standard commercial starter diet without any coccidiostats. The diets were: Control (basal diet with no additives); ZnB (basal diet + 50 ppm zinc bacitracin); Flavophospholipol (basal diet + 2 ppm flavophospholipol); and Avilomycin (basal diet + 15 ppm avilomycin)

At 3, 5, 7, 12, 14 and 17 days post-hatch 12 birds per treatment were killed. A 3 cm section of tissue and associated digesta from the midpoint of the ileum was collected from each chicken. Samples were freeze-dried and total nucleic acids extracted for analysis of total gut bacterial community composition by terminal-restriction fragment length polymorphism (T-RFLP; Torok et al., 2008), or *Lactobacillus* and related genera community composition by denaturing gradient gel electrophoresis (Lac PCR-DGGE; Walter *et al.,* 2001). Identification ladders for DGGE were prepared by combining the Lac PCR products from DNA extracted from the reference strains: *Lactobacillus avarius, L. acidophilus*, *L. crispatus*, *L. gasseri*, *L. johnsonii*, *L. reuteri*, *L. salivarius* subsp. *salivarius*, *Pediococcus acidilactici* and *P. pentosaceus*. Multivariate statistical methods (PRIMER-6; PRIMER-E Ltd., Plymouth, UK) were used to analyze T-RFLP and Lac PCR-DGGE generated data.

III. RESULTS

Significant differences ($P < 0.05$) were found in overall ileal microbial community composition associated with in-feed antimicrobials (data not shown) and with age regardless of dietary treatment. Figure 1 shows the clustering of ileal microbial communities associated with age for birds raised on the control diet. Three main clusters were observed separating ileal microbial communities for birds aged 3-5 days, 5-12 days and 12-17 days. The cluster for birds aged 3-5 days was comprised of three sub-clusters and similarity in ileal microbial communities between these birds were generally lower than for the older birds.

Significant shifts in bacterial community composition were detected between various age groups which have allowed indicator bacteria to be identified. Figure 2 compares the composite T-RFLP profiles from all birds on the control diet at three days of age to birds at 17 days of age. Some bacterial species or operational taxonomic units (OTU) were present/absent in one age group but not the other. Furthermore, some OTUs which were detected in both age groups contributed differently to the overall bacterial population. For example, at 17 days of age OTUs 86, 492, 518 and 560 became less predominant, while OTU 894 became more predominant. OTU 60 which was detected in birds at three days of age was not detected at 17 days of age, while OTU 286 became a dominant species at 17 days of age. OTU 180 and 186 were dominant species regardless of age.

In-feed antimicrobials did not influence the presence of *Lactobacillus* communities, however, the species that were evident varied with age of the birds. As birds grew older *L. johnsonii* and *L. reuteri* increased in prevalence. *P. acidilactici* was only detectable in chicks at three days of age.

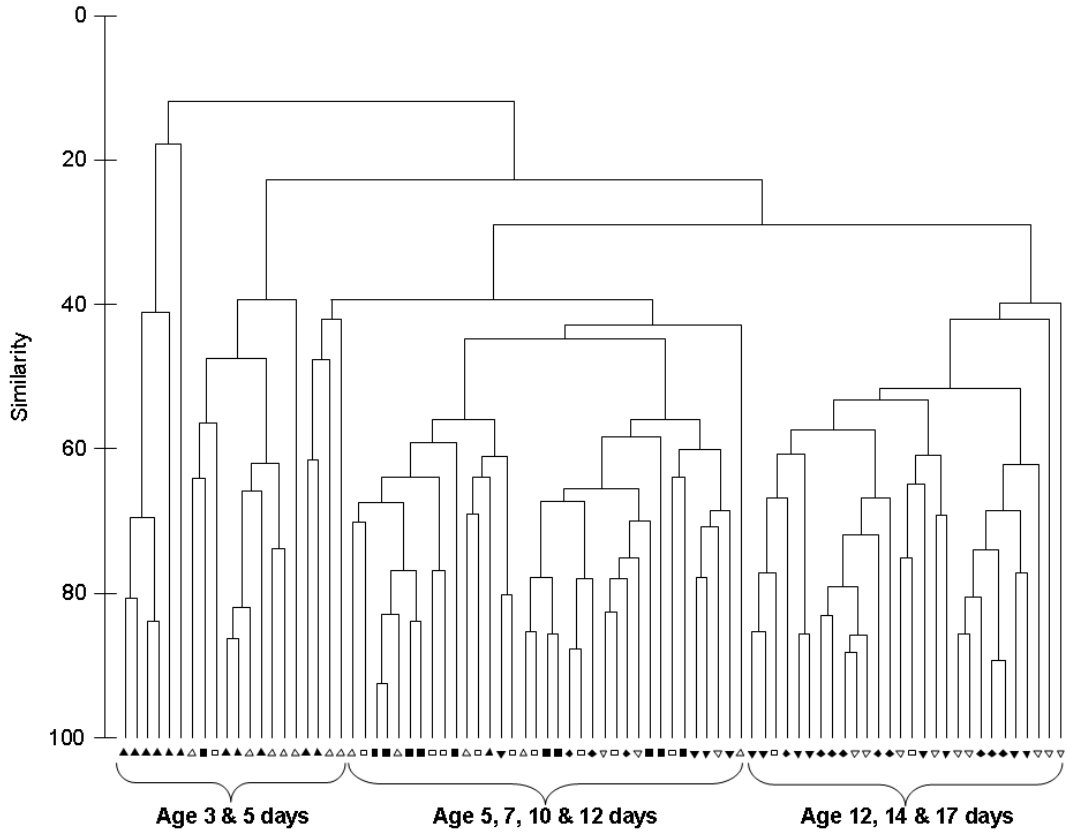

Figure 1 Dendogram representing relationships between T-RFLP profile of ileal bacterial communities from individual birds at 3, 5, 7, 10, 12, 14 and 17 days post-hatch. All birds were raised on the control diet. ▲ = 3 d, △ = 5 d, ■ = 7 d, □ = 10 d, ▼ = 12 d, ▽ =14 d and ◆ = 17 d.

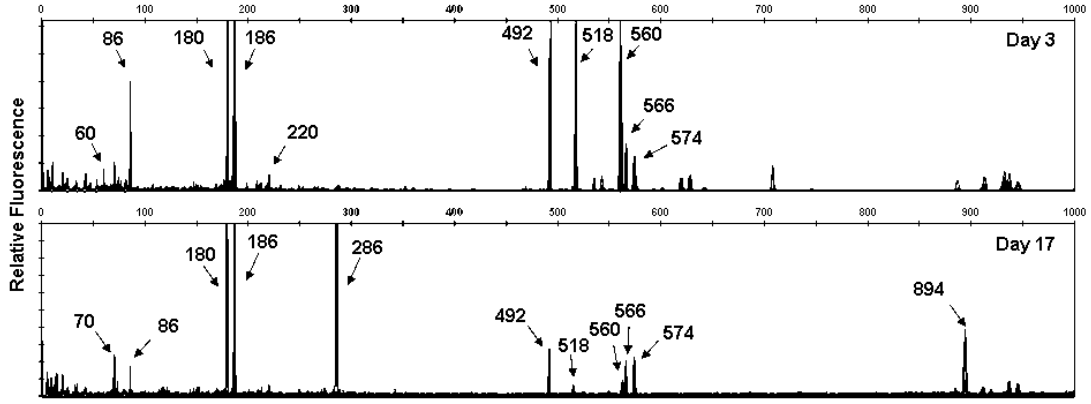

Figure 2 Comparison of composite T-RFLP profiles representing the overall ileal bacterial communities from birds raised on the control diet at 3 and 17 days of age (n=12/treatment). Peaks represent bacterial species or taxonomically related groups of bacteria and are identified as operational taxonomic units (OTU). Size of OTUs is indicated in base pairs.

IV. DISCUSSION

Our results show that the composition of the post-hatch ileal microbiota changes regardless of dietary treatment during the period 3-17 days of age. Three major shifts in ileal microbial community composition were observed at 3-5 days, 5-12 days and 12-17 days. Differences observed related both to the contribution apparently ubiquitous bacteria made to the community structure, as well as, presence/absence of unique bacterial species. Bacterial species (OTU 180 and 186) which appeared to be present in high numbers for all birds aged 3-17 days appear to represent species belonging to *Lactobacillus* (data not shown). This is supported by evidence that *Lactobacillus* were indeed detected throughout this study using Lac PCR-DGGE. Most lactic acid bacteria reference strains were detectable in chicks 3 to 17 days of age. *P. acidilactici* was only detectable in birds aged 3 days and *L. johnsonii* and *L. reuteri* were more prevalent in the older chicks. These data support the autochthonous nature of these species in the chicken gastrointestinal tract, which have been reported to be present in birds of various ages (Knarreborg et al. 2002; Lu et al., 2003; Gong et al., 2008; Guan et al. 2003).

In addition to the influence that age had on the post-hatch ileal microbiota development, significant dietary differences were detected in response to the various in-feed antimicrobials used in this study. We are currently isolating and sequencing key OTUs driving these differences in order to gain a better understanding of the optimal gut microbiota development under a variety of dietary conditions. By evaluating the effects of in-feed antimicrobials on post-hatch gut microbiota development and identifying key bacterial species this information may assist in the formulation of diets which facilitate beneficial microbial colonisation of the gastrointestinal tract. Such knowledge will aid in the development of natural alternatives to current in-feed antimicrobials in sustainable poultry production.

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THE EFFECT OF LITTER MATERIAL ON PRODUCTIVITY AND HEALTH OF BROILER CHICKENS

M.A. ALI[1], P.A. IJI[2], R. MACALPINE[1] and L.L. MIKKELSEN[2]

Summary

Many different litter materials are used for broiler production in Australia. In this study the effect of litter material on productivity and health of broilers was investigated. Broilers were reared on the following litter types; rice hulls, softwood sawdust, pine shavings, reused single batch litter (originally based on pine shavings), hardwood sawdust, shredded paper and chopped straw. Litter type significantly affected feed intake and growth to 21 days of age. However, the performance of broilers on all the litter types was comparable at 42 days of age. Although some litter materials stimulated gizzard development, this did not lead to differences in the microbial population in the digesta samples of the gizzard, ileum or caeca. Short chain fatty acids and lactic acid in the caecal contents showed variation caused by the different litter materials used.

I. INTRODUCTION

Broilers are in constant contact with litter used as bedding. Studies have shown that broilers consume litter materials used (Kubena *et al.*, 1974, Malone and Chaloupka, 1983; Deaton *et al.*, 1985). However, numerous studies comparing the performance of broilers on different litter types concluded that litter material did not have a significant effect on broiler performance (Lien *et al.*, 1992; Brake *et al.*, 1993; Martinez and Gernat, 1995; Anisuzzaman and Chowdhury, 1996; Swain and Sundaram, 2000; Biswas *et al.*, 2001). In addition to this, Kubena *et al.* (1974) and Deaton *et al.* (1983) had reported that litter consumption stimulated gizzard development and function in birds. Rogel *et al.* (1987) observed that stimulation of gizzard development improved digestion. This contrast with lack of differences in gross performance observed in birds reared on different litters despite litter ingestion which may stimulate organ development. The primary objective of the present study was to investigate if broilers ingested different types of litter and if litter ingestion resulted in gizzard stimulation and differences in growth performance as a consequence.

II. MATERIALS AND METHOD

Broilers were reared on seven different litter types (rice hulls, softwood sawdust, pine shavings, reused single batch litter, hardwood sawdust, shredded paper and chopped straw) and fed *ad libitum* on a commercial broiler feed regime (Inghams Enterprises, Australia Pty Ltd.). The birds were weighed at 7, 14, 21, 28 and 42 days and the amount of feed consumed was determined. Efficiency of feed conversion (g feed/g gain) was calculated for each litter treatment. At 14 and 28 days three birds per pen were dissected, the gizzard was weighed and a visual score for the amount of litter present in the gizzard was conducted. Litter content observed in the gizzard was ranked from 0 (nil) to 3 (high). The digesta samples collected at 28 days from the gizzard, ileum and caeca were analysed for microbial profiles. The plate culture method was used to determine number of lactobacilli, coliform, lactase-negative enterobacteria, *Clostridium perfringens* and total anaerobic bacteria present in the digesta.

[1] Inghams Enterprises Pty. Limited, Leppington, NSW 2179
[2] School of Environmental and Rural Science, UNE, Armidale, NSW 2351

The digesta samples were also analysed for short-chain fatty acids (formic acid, acetic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid), lactic acid and succinic acid using gas chromatography.

III. RESULTS

Visual gizzard litter scores and gizzard weight varied with litter treatment (Table 1). There was no evidence of litter present in the gizzard of birds on paper. More litter was present in the gizzard of birds reared on fresh pine shavings than birds reared on reused pine shavings. Significant differences were observed in gizzard development due to differences in the litter type. Hardwood sawdust resulted in the highest gizzard weight as percentage of live weight.

Litter material significantly affected feed intake and growth at 14 days (Table 2). However, mean differences in the final production parameters (weight gain, feed consumption and feed conversion) of broilers at 42 days of age were comparable for the birds reared on the different litter types.

Lactobacilli, coliform, lactase-negative enterobacteria, and total anaerobic bacterial counts in the digesta of the gizzard, ileum and caeca were not affected by the different litter materials used. *C. perfringens* was not detected in any of the digesta samples from the various sections of the gut. The total microbial population was highest in the caeca, then in the ileum and lowest in the gizzard ($P < 0.01$).

The different litter types had no effect on short chain fatty acid (SCFA) and lactic acid concentrations in the gizzard. However, there were significantly higher concentrations of formic acid ($P < 0.01$) and total SCFA ($P < 0.01$) in the ileum of birds raised on the reused litter. The valeric acid concentration in caeca of birds reared on hardwood sawdust was significantly lower ($P < 0.01$) than in birds from all other litter types. Birds on hardwood sawdust also had the lowest average SCFA levels in the caeca. Within treatment variation in performance (live weight and feed consumption) at 42 days was lower for birds on hardwood sawdust than on the other treatments.

Table 1 Gizzard litter score and average gizzard weights of birds on different litter at 1 4 and 28 days.

Litter type	14 days of age		28 days of age	
	Gizzard score	Gizzard wt (% of live weight)	Gizzard score	Gizzard wt (% of live weight)
Rice hulls	1.00	2.97	1.42	1.76
Softwood sawdust	0.58	2.79	0.33	1.42
Pine shavings	1.50	2.93	1.25	1.73
Reused litter	0.92	2.89	0.63	1.58
Hardwood sawdust	1.33	3.20	1.42	1.78
Shredded paper	0.00	2.63	0.00	1.40
Chopped straw	1.75	2.74	1.46	1.55
S.E.M.		0.046*		0.06**

*P < 0.05, **P < 0.01

Statistical analysis was not performed on gizzard score.

Table 2 Feed consumption, live weight and feed conversion efficiency for birds in the period 0 to 14 days of age on different litter types.

Litter type	Feed intake (g/bird)	Live weight (g/bird)	Feed conversion (g of feed/g gain)
Rice hull	447	389	1.28
Softwood sawdust	453	394	1.28
Pine shavings	454	393	1.29
Reused litter	442	380	1.30
Hardwood sawdust	462	400	1.28
Shredded paper	446	385	1.30
Chopped straw	461	395	1.30
S.E.M.	2.2*	2.1**	0.004

*P < 0.05, **P < 0.01

IV. DISCUSSION

Some differences in feed consumption and weight gain were observed at an early age. However, the results of this experiment suggest that litter material does not cause any major difference in the final production parameters of broilers at 42 days of age. The differences in visual score for presence of litter in the gizzard indicated that birds prefer to consume some litter materials over others and that degradation rate once ingested may differ. Low visual scores may be due to the litter materials being broken down by the gizzard. Since the amount of litter ingested was not measured, it is difficult to conclude which of the litter materials were preferred. However, larger gizzard sizes were observed in birds which had higher visual scores. This indicates that the observed gizzard stimulation may be caused by the litter retained in the gizzard. However, gizzard stimulation did not cause any difference in the microbial profile of the gut. One of the possible reasons for this could be the use of zinc bacitracin in the feed. Zinc bacitracin is effective in controlling *C. perfringens* and *Lactobacillus salivarius* (Engberg *et al.*, 2000). *C. perfringens* was not detected in any of the digesta samples from the various sections of the gut in this experiment. This confirms that in-feed zinc bacitracin was effective and altered the gut microflora. The predicted differences in the microbial population counts due to gizzard stimulation may not have been observed due to effective microflora control by the antibiotic.

The high concentrations of formic acid and total SCFA in the ileum of birds raised on the reused litter could be due to the pathogen load in the litter. The low levels of SCFA in the caeca of birds on hardwood sawdust could be indicative of a lower rate of fermentation due to a better developed gizzard. These birds had the largest gizzard to live weight ratio at day 14. Also at 42 days bird performance within treatment was uniform for hardwood sawdust when compared to other treatments. The organ development stimulated at an early age for birds reared on hardwood sawdust, may have given birds the opportunity to achieve early stability in gut functions, leading to a decrease in variation in performance within the treatment.

V. CONCLUSION

The final performance of broiler chickens reared on rice hulls, softwood sawdust, pine shaving, reused litter, hardwood sawdust, shredded paper and chopped straw was comparable. Birds ingested litter used as bedding material. This caused differences in gizzard development but failed to translate to an improvement in performance. The gut microflora profile was not affected by gizzard development or by the differences in the litter type. This

could be due to the presence of zinc bacitracin in the diet. Indigestible litter retained in the gizzard stimulated gizzard size. Early gizzard stimulation could improve broiler uniformity, even if less impact on growth and feed efficiency.

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THE EFFECTS OF LACTOFERRIN ON BROILER PERFORMANCE, GUT MICROBIAL COMMUNITIES AND INTESTINAL MUCOSAL IMMUNE SYSTEM

M.S. GEIER[1,2], V.A. TOROK[2,3], M. BOULIANNE[4], G.E. ALLISON[5], V. JANARDHANA[2,6], P. GUO[2,6], A.G.D BEAN[2,6], R.J. HUGHES[1,2]

Summary

The influence of in-feed lactoferrin on bird production, the intestinal microbiota, and mucosal immune system was assessed in male Cobb 500 broilers. Performance parameters were not significantly different in birds fed 250 or 500 mg/kg lactoferrin compared to birds fed diets supplemented with zinc bacitracin, or without additives. The profiles of caecal microbial communities were significantly different in birds fed zinc bacitracin compared to birds fed a diet with no additives, or supplemented with 250 mg/kg lactoferrin. Birds fed 250 mg/kg lactoferrin also had a different microbial profile compared to birds fed 500 mg/kg lactoferrin. No differences in ileal villus height, crypt depth or goblet cell proportions were observed amongst dietary treatments. In-feed inclusion of lactoferrin can influence the intestinal microbial profiles and lymphocyte subsets of broilers, but not affect performance.

I. INTRODUCTION

The search for natural antibiotic alternatives for the poultry industry is crucial in light of recent bans on the use of in-feed antibiotics in the European Union, and pressure for further withdrawal in other regions. Compounds such as probiotics, prebiotics, organic acids, and plant extracts are candidates for incorporation into poultry feed to prevent pathogenic infection, facilitate the development of an optimal intestinal microbiota and mucosal immune system, and promote growth in broiler chickens (Patterson and Burkholder, 2003). Whilst these compounds have demonstrated efficacy in broiler chickens, the results are often inconsistent and further research is needed to identify the most suitable compounds.

Broiler performance can be influenced by many factors, including; the composition and activity of the intestinal microbiota (Yegani and Korver, 2008), bird immune status (Klasing, 2007), and intestinal structure and function (Iji et al., 2001). However, Geier and colleagues have also demonstrated that diet can influence these parameters without affecting bird performance either positively or negatively (Geier et al., 2008; Janardhana et al., 2008). Therefore, a greater understanding of how these parameters can be influenced by diet, and inturn, how these changes impact upon bird health and performance, is required.

In the current study we aimed to investigate the influence that in-feed lactoferrin had on bird health and performance, compared to diets containing antibiotics, or no additives. Lactoferrin is an 80kD iron-binding glycoprotein, derived from exocrine secretions, neutrophils and blood (Pan et al., 2007). Lactoferrin has been investigated as a treatment for a range of infectious and inflammatory conditions in both humans and animals, largely due to its immunoregulatory, antibacterial, antifungal and antiviral activities (Pan et al., 2007).

[1] SARDI, Pig and Poultry Production Institute, Roseworthy, SA 5371
[2] Australian Poultry CRC, University of New England, Armidale, NSW 2351
[3] SARDI, Plant and Soil Health, GPO 397, Adelaide, SA 5001
[4] Faculté de médicine vétérinaire, Université de Montréal, Québec, J2S 7C6, Canada
[5] School of Biochemistry and Molecular Biology, and ANU Medical School, The Australian National University, Canberra, ACT 0200
[6] CSIRO Australian Animal Health Laboratory, Geelong, Vic 3219

II. MATERIALS AND METHODS

An apparent metabolisable energy study was performed with male Cobb 500 birds aged 25-32 days. Chickens were raised in floor pens in a temperature-controlled room. The experiment was approved by the Animal Ethics Committees of the University of Adelaide and the Department of Primary Industries and Resources South Australia.

The four experimental diets (n=24/group) were based on a standard commercial starter diet without any added antibiotics or coccidiostats. The diets were: Control (basal diet with no additives); ZnB (basal diet + 50 ppm zinc bacitracin); Lf 250 (basal diet + 250 mg/kg lactoferrin); and Lf 500 (basal diet + 500 mg/kg). Bovine lactoferrin (Australia's Own Pty Ltd, New South Wales, Australia) was used.

At 36 days post-hatch all birds were killed. One caecum and 3 cm sections of tissue and associated digesta from the midpoint of the ileum were collected. Samples were freeze-dried and total nucleic acids extracted for profiling of the intestinal microbiota by terminal-restriction fragment length polymorphism (T-RFLP; Torok et al., 2008). Spleen and caecal tonsil (CT) immune cell parameters were assessed by flow cytometry. Ileal sections were stained with periodic Schiff/alcian blue. Volatile fatty acid (VFA) concentrations in caecal contents were determined by gas chromatography.

III. RESULTS

Lactoferrin supplementation did not influence any performance parameters ($P > 0.05$; Table 1). Supplementation with ZnB did not influence bird performance. Similarly, VFA were unaffected by dietary treatments VFA (data not shown).

Overall bacterial communities were profiled using T-RFLP. In the ileum no significant differences were observed in microbial community composition among birds fed the various diets (global $R=-0.009$ $P=0.674$). However, in the caecum, the microbial communities present in birds fed ZnB were significantly different compared to Control-fed birds and birds fed 250 mg/kg lactoferrin ($P < 0.05$; Table 2). Interestingly, birds fed 250 mg/kg also had a significantly different microbial profile compared to those fed 500 mg/kg lactoferrin. No differences were observed in caecal volatile fatty acid profiles amongst groups ($P > 0.05$; data not shown).

No differences were observed in ileal villus height and crypt depth amongst treatment groups ($P > 0.05$; Table 3). The proportions of goblet cells producing acidic, intermediate or neutral mucins were also not significantly different amongst treatments ($P > 0.05$; Table 3); however there was an observable trend toward an increase in acidic mucin producing goblet cells in lactoferrin-treated birds ($P < 0.1$).

Phenotypic analysis of CT lymphocytes indicated that birds fed lactoferrin had a higher proportion of $CD4^+$ cells ($P > 0.05$; Table 4). In the spleen, birds fed lactoferrin and ZnB had a higher proportion of $CD8^+$ lymphocytes (data not shown).

Table 1 Performance parameters from chickens following the seven day apparent metabolisable energy (AME) study period

	Body weight start (g)	Body weight gain (g)	Feed intake (g/bird/day)	FCR	AME (MJ/kg)
Control	1110 ± 21	643 ± 15	153 ± 2.4	1.68 ± 0.03	13.99 ± 0.10
ZnB	1104 ± 23	641 ± 12	153 ± 2.3	1.68 ± 0.02	14.02 ± 0.10
Lf 250	1092 ± 25	645 ± 13	157 ± 2.9	1.70 ± 0.02	14.33 ± 0.13
Lf 500	1137 ± 21	655 ± 12	157 ± 2.4	1.69 ± 0.02	14.03 ± 0.11

Body weight is expressed as mean (g) ± SEM. Feed intake is expressed as mean (g/bird/day) ± SEM. Feed conversion ratio is expressed as feed intake/body weight gain ± SEM. Apparent metabolisable energy (AME) is expressed as mean (MJ energy/kg) ± SEM (n=24/group).

Table 2 One-way analysis of similarities (ANOSIM) of caecal microbial communities

	Control	ZnB	Lf 250	Lf 500
Control	-	0.069	0.001	0.005
ZnB	*0.030*	-	0.099	0.027
Lf 250	*0.424*	*0.006*	-	0.058
Lf 500	*0.331*	*0.170*	*0.048*	-

Data are expressed as the R-statistic (bold), with significance level in italics. For all analyses a significance level of 0.05 was considered significant. The global R-value was 0.043 at a significance level of 0.02 which is considered significant (n=24/group).

Table 3 Ileum morphometry and goblet cell analysis

	Morphometry		Goblet Cell Type		
	Villus Height (μm)	Crypt Depth (μm)	Acidic	Intermediate	Neutral
Control	773 ± 22	171 ± 4	252 ± 24	821 ± 46	207 ± 25
ZnB	796 ± 32	174 ± 5	266 ± 30	881 ± 88	177 ± 25
Lf 250	795 ± 33	172 ± 9	329 ± 44	922 ± 109	171 ± 34
Lf 500	814 ± 39	170 ± 8	392 ± 57	919 ± 56	135 ± 29

Villus height and crypt depth are expressed as mean (μm) ± SEM. Goblet cell numbers are expressed as mean cells/epithelial area (mm^2) ± SEM (n=12/group).

Table 4 Cell phenotypes in caecal tonsils

	MHC I	MHC II	CD3	CD4	CD8
Control	64.9 ± 3.1	33.9 ± 1.5	32.1 ± 1.7	15.7 ± 0.7^{a}	16.5 ± 1.2
ZnB	63.6 ± 1.7	32.8 ± 2.0	36.7 ± 0.9	17.5 ± 0.6^{ab}	19.7 ± 1.2
Lf 250	62.5 ± 2.2	31.2 ± 1.8	37.0 ± 2.2	18.6 ± 1.1^{b}	20.1 ± 1.4
Lf 500	64.7 ± 1.4	30.8 ± 1.4	36.3 ± 1.2	19.6 ± 1.2^{b}	19.6 ± 1.1

Cell marker data are expressed as mean ± SEM (n=12/group).

IV. DISCUSSION

The current study indicated that whilst dietary inclusion of lactoferrin influenced the intestinal microbiota and influenced some lymphocyte subsets it did not affect bird performance, intestinal microarchitecture or the proportions of goblet cells producing

different mucin sub-types. Previously, lactoferrin has been reported to increase body weight gain and intestinal villus height in piglets (Wang et al., 2006). Additionally, lactoferrin, combined with lysozyme, has been demonstrated to improve feed efficiency and increase villus height in broilers (Humphrey et al., 2002).

Previously, Geier et al., (2008) have demonstrated that diet-induced shifts in overall microbial communities may not necessarily be associated with altered bird performance; however, it has also been reported that microbial shifts may indeed be linked with changes in bird energy metabolism (Torok et al., 2008). Together, these data indicate that a high level of performance may be sustained by an array of microbial compositions and that the nature and extent of a diet-induced shift may determine whether an effect on bird performance is observed. Further research into the microbial species associated with high and low bird performance is required. This may lead to the identification of indicator organisms for broiler performance, and the formulation of diets to facilitate the colonisation of beneficial microbial species. In addition to the profile of microbial species present in the intestine, it is important to consider the metabolic activity of the microbiota. Significant diet-induced changes in microbial activity have been reported in studies where the overall microbial composition has not altered (Rehman et al., 2008). In the current study we did not observe major shifts in volatile fatty acid production, indicating that the observed shifts in microbial species did not alter this aspect of microbial activity.

In summary, lactoferrin supplementation did not improve nor impair broiler growth performance; however, significant microbial shifts were observed. The need for natural alternatives to in-feed antibiotics will continue to heighten as pressure for antibiotic withdrawal increases outside of the European Union. Further research into natural antibiotic alternatives is required in order to identify compounds which can consistently promote optimal bird performance. It may be the case that a combination of compounds are required that target different aspects of bird heath and energy metabolism, including the intestinal microbiota, intestinal structure and function, and the mucosal immune system.

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EFFECTS OF NOVEL PROBIOTIC ON GROWTH PERFORMANCE AND MICROBIAL COMPOSITION OF BROILER CHICKENS WITH *SALMONELLA SOFIA* CHALLENGE

C.G. OLNOOD[1], M. CHOCT[2], L.L. MIKKELSEN[1] and P.A. IJI[1]

Summary

The effects of *L. johnsonii* on gut microflora and bird performance were assessed using 288 day-old Cobb broilers challenged with *Salmonella sofia* (*S. sofia*). A 3 x 2 factorial design which consisted of three treatments, *i.e*., a negative control (NC) containing no additives, a positive control (PC) containing antimicrobials (Zinc-bacitracin, 50 ppm; monensin 100ppm) and a probiotic group (Pro) receiving a probiotic via oral gavage; and two factors, *i.e.*, with or without *S. sofia* challenge. *L. johnsonii* (10^9 cfu/chick) was gavaged on days 1, 3, 7 and 12. Chicks were gavaged with *S. sofia* (10^7 cfu/chick) on days 2, 8 and 13. Results showed that the challenge itself markedly reduced ($P<0.05$) bird performance and feed intake, and transient clinical symptoms of the infection with *S. sofia* were observed from the second time they were challenged with *S. sofia* in the negative challenge groups. The novel probiotic candidate *L. johnsonii* reduced the number of *S. sofia* and *C. perfringens* in the gut environment, and improved the birds' resistance to *S. sofia*.

I. INTRODUCTION

The use of competitive exclusion (CE) microflora against *Salmonella* contamination on poultry is proven to be effective (Jin et al., 1998). The most important advantage is that CE products ensure the establishment of a complex intestinal microflora that resists colonization by poultry pathogens, and they are produced as a consortium of bacteria that can coexist as a stable community in the enteric ecosystem (Wagner, 2006). In young chicks, administration of gut microflora has been shown to be effective against several *Salmonella* spp., such as *S. typhimurium* (Mead, 2000), *S. kedougou* (Ferreira et al., 2003). It is suggest that the importance of early establishment of beneficial bacterial populations in preventing *Salmonella* colonization using animal models. Based on these principles, a novel probiotic of chicken origin, *L. johnsonii*, was selected for this experiment because of its production of bacteriocin-like inhibitory activities effective (Olnood et al., 2007) that may be in controlling *S. sofia* infection in broilers.

II. MATERIALS AND METHODS

a) The probiotic

A pure *L. Johnsonii* isolate was grown in MRS broth overnight (at 39 ˚C) and harvested by centrifugation at 4420 *g* for 15 minutes. It was re-suspended in PBS (pH 7.4) and this pre-mixture of PBS solution was used for oral gavage of chicks in the probiotic treatment group on d 1, 3, 7 and 12. Each administration dose rate of *L. johnsonii* was > 10^9 cfu/mL.

b) Infectious strain of *Salmonella sofia*

The strain of *S. sofia* was obtained from the Biotechnology Laboratory, RMIT University and maintained in Luria Bertani (LB) broth with 30% (v/v) glycerol at -20°C. The strain was made rifampicin resistant as described by Eisenstadt et al. (1994). The mutant strain was amplified by growth in LB broth overnight at 39°C, harvested by centrifugation at 5000 *g* for 15 minutes,

[1] School of Rural Science and Agriculture, University of New England, Armidale, NSW Australia 2351
[2] Australian Poultry CRC, PO Box U242, University of New England, Armidale, NSW Australia 2351

re-suspended in PBS (pH 7.4), the challenge models as described by Bjerrum et al. (2003), the infection dose rate of *S. sofia* was 10^7 cfu/mL, administered by oral gavage on d 2, 8, and 13.

c) Analyses and performance measurements

The basal diets (starter and finisher) were based on corn, wheat and soybean meal and provided as pellets (compassion of diet not shown). Six treatments were provided as two diet batches as follows: 1) the negative control (NC-), negative challenge(NC+), unchallenged probiotic (Pro-) and challenged probiotic (Pro+) groups were provided with the basal diet; 2) the positive control (PC-) and positive challenge (PC+) treatments were provided with the basal diet, but with the antibiotic, Zinc-bacitracin (ZnB, 50 ppm) added.

The 288 one-day-old male Cobb broiler chickens were obtained from Baiada hatchery, Kootingal (Tamworth, NSW) and allocated to 36 cages divided into two groups, unchallenged and challenged, and randomly assigned to 6 cages for each treatment. Feed and water were provided *ad libitum* and bird performance was measured on a weekly basis by recording the group weight and feed intake for each cage. Mortalities were recorded daily and feed per gain values were corrected for mortality. The bacterial numeration technique was according to Miller and Wolin (1974), the counts were transformed to $\log_{10}$ values before analysis.

Statistical analysis was performed using multifactor analysis of variance with treatment and challenge as the factors (Statgraphics, Manugistics Inc., Maryland, USA). The Animal Ethics Committee of the University of New England approved the experiments in this study (authority number: AEC07/148).

III.RESULTS AND DISCUSSION

The *S. Sofia* started to grow after the first streak on the side of mutant gradient plate where the rifampicin concentration was low (80 μg/mL). After the sixth streak, however, the strain grew strongly, showing resistant to 120 μg/mL of rifampicin in the agar. Indeed, results proved that the mutant strain grew normally in LB broth, reaching concentrations of *S. sofia* $>2.5 \times 10^7$ cfu/mL in BPS solution (data not shown).

Clinical symptoms were observed in the birds after the second time challenged with *S. sofia* in the NC- group. Within a few hours of the second inoculation chicks were showing obvious clinical symptoms; they huddled in the corners of the cage, showing somnolence, loss of appetite and inhibition in drinking. They were generally depressed and reluctant to move, A thin, yellowish diarrhoea appeared with some chicks. These behavioural changes were pronounced for about 8 hours, recovery being complete within 24 hours. None of the chicks died during the 48 hours after inoculation. The mortality rate for these chickens was less than 8.3% (4/48) compared with the NC- group where it reached 6.25% (3/48) during 5 weeks.

Growth, FI and FCR were all depressed during the second week in NC+ groups. However, this trend was not evident in the following weeks. By the end, there was no difference in performance between the challenged and unchallenged groups (Table 1).

Older birds inoculated with salmonella parenterally were less easily infected than when younger. In this experiment, we used an established 1-day-old chick model to assess the effects of *L. johnsonii* upon colonization and persistence of *S. sofia*, the result indicated that *L. johnsonii* acted against *S. sofia* infection and reduced the clinical symptoms affecting bird performance.

The number of *Enterobacteria* found in the ileum and caecum on d 14 was higher in the all challenged groups than in the all unchallenged groups (Table 2). Furthermore, the number of *C. perfringens* in the caecal contents of unchallenged groups (NC-, 6.29; PC-, 6.14; Pro-,

5.99) was lower (P<0.05) than those in the challenged groups (NC+, 7.86; PC+, 7.38; Pro+, 8.15) on d 14. This trend was also found on d 35.

Table 1 Performance[1] of broilers challenged with *S. sofia* (n=6)

	Treatments[1]						*P* value		
	NC-	NC+	PC-	PC+	Pro-	Pro+	T[2]	C[3]	TxC[4]
				Day 1-7					
BWG(g/Bird)	169.2	167.6	174.0	169.2	175.7	168.5	0.54	0.67	0.87
FI(g/Bird)	187.9	189.1	190.1	187.6	196.8	186.5	0.82	0.12	0.51
FCR(g/g)	1.11	1.13	1.09	1.11	1.12	1.11	0.24	0.76	0.44
				Day 1-14					
BWG(g/Bird)	385.1[a]	334.2[b]	401.9[a]	380.8[a]	390.2[a]	377.0[a]	0.03	0.01	0.01
FI(g/Bird)	462.1[a]	310.0[b]	478.2[a]	453.1[a]	464.4[a]	456.1[a]	0.02	0.01	0.02
FCR(g/g)	1.20[a]	0.93[b]	1.19[a]	1.19[a]	1.19[a]	1.21[a]	0.03	0.02	0.04
				Day 1-35					
BWG(g/Bird)	1806.8	1813.5	1834.6	1799.7	1824.5	1811.7	0.31	0.27	0.17
FI(g/Bird)	3112.3	3234.5	3129.8	3079.9	3154.4	3189.1	0.94	0.68	0.55
FCR(g/g)	1.72	1.78	1.71	171	1.73	1.76	0.59	0.18	0.38
Mortality (%)	6.25	8.33	4.17	4.17	6.25	4.17		-	

1 Treatments: NC-, unchallenge negative control; NC+, challenged negative control; PC-, unchallenge positive control; PC+, challenged positive control; Pro-, unchallenge probiotic control; Pro+, challenged probiotic control. 2 T: treatments; 3 C: challenge; 4 TxC: variance interaction between treatment and challenge; [a, b]: Means within the same row with no common superscripts differ significantly (P<0.05)

Bjerrum et al. (2003) indicated that dose levels of around 10^7 cfu/g yielded stable infections in 14-day-old chickens. In the current study the spleen and liver of chicks became positive for salmonella on d 14, although only few remained positive at end of the experiment. In addition, the ileum had the lowest level of salmonella present in most chickens at d 14. The inoculation established a high level of *S. sofia* infection in current study, which was detectable from d 14 reaching 6.11 cfu/g in the ileum and 8.97 cfu/g in the caeca. No *S. sofia* was detected in the digesta from the ileum and caeca on d 35. The control chickens were free of *Salmonella* throughout the experiments, verified by LB agar both with or without rifampicin and by enrichments from spleen, liver, ileal digesta and caecal digesta (Table 3). The result supported by Bjerrum et al. (2003) who demonstrated that the passage time through the ileum is very fast compared with that of the caeca where the bacteria have more time to establish. Pascual et al. (1999) also found rifampicin-resistant *L. salivarius* reduced *S. enteritidis in vivo* together with its ability to colonize the gastrointestinal tract of chickens after a single inoculation. Newly hatched chicks are highly susceptible to salmonella infection (Desmidt et al., 1997). Barrow et al. (1988) found long-term infection in the birds inoculated at d 1, whereas no *Salmonella* could be detected in the ileum inoculated at d 21. This observation was confirmed in the current study which found no *S. sofia* in the ileum at d 35.

The results also shown that the number of lactobacilli was higher (P<0.05) in the Pro- and Pro+ groups on d35. La Ragione et al. (2004) documented that a single oral dose of 1×10^9 cfu *L. johnsonii* inhibited the growth of S. *enteritidis* and *C. perfringens* and reduced the extent of colonization and persistence in 1-day-old and 20-day–old chick models. The

probiotic strain *L. johnsonii* may increase the VFAs concentration after inoculation. The CE culture was administered to broilers a day before salmonella was administered, resulting in a dramatic reduction in the number of salmonella observed (van der Weilen et al., 2002). Results obtained in the current study are in agreement with the findings of these on CE cultures *in vivo*.

The infection model for *S. sofia* resulted in stable colonization of the ileum and caeca for chickens receiving three successive inoculations starting from d 2. This study demonstrated that oral inoculation with the novel probiotic *L. johnsonii* was able, through the CE, to reduce *S. sofia* and *C. perfringens* in GIT, and provide resistance to *S. sofia* in broiler chickens.

Table 2 Effects of experimental treatment on bacterial counts (Lg CFU/g) in digesta of birds on d 14 and 35 challenged with *S. sofia* (n=6)

	Treatments						*P* value		
	NC-	NC+	PC-	PC+	Pro-	Pro+	T	C	TxC
				Day 14					
Ileum									
Lactobacilli	7.24	6.88	6.61	7.97	7.94	7.23	0.52	0.16	0.21
Enterobacteria[1]	5.07^{c}	6.17^{a}	5.19^{c}	6.32^{a}	5.51^{b}	6.45^{a}	0.03	0.01	0.04
S. sofia[2]	0.00^{c}	6.11^{a}	0.00^{c}	4.78^{b}	0.00^{c}	5.09^{b}	0.01	0.01	0.01
Caeca									
Lactobacilli	8.48	8.58	8.72	8.94	9.12	8.85	0.26	0.33	0.19
Enterobacteria	8.19^{b}	9.07^{a}	8.45^{b}	8.87^{a}	8.55^{b}	8.91^{a}	0.03	0.01	0.03
C. perfringens	6.29^{b}	7.86^{a}	6.14^{b}	7.38^{a}	5.99^{b}	8.15^{a}	0.01	0.01	0.01
S. sofia	0.00^{c}	8.97^{a}	0.00^{c}	5.57^{b}	0.00^{c}	5.70^{b}	0.01	0.01	0.01
				Day 35					
Ileum									
Lactobacilli	7.05^{b}	7.48^{b}	7.35^{b}	7.38^{b}	8.16^{a}	8.60^{a}	0.04	0.01	0.02
Enterobacteria	5.78	6.74	5.83	6.37	5.93	5.72	0.57	0.28	0.10
Caeca									
Lactobacilli	7.96^{c}	7.63^{c}	8.50^{b}	8.51^{b}	9.03^{a}	9.30^{a}	0.01	0.01	0.01
Enterobacteria	7.91	7.66	7.13	7.92	7.72	7.27	0.30	0.55	0.29
C. perfringens	5.13^{b}	6.55^{a}	4.17^{c}	6.29^{a}	4.44^{c}	6.27^{a}	0.04	0.01	0.05

1 Enterobacteria are coliform and lactose negative enterobacteria. 2 The detection limit of the cfu was 10^{2}, samples registered as zero could still contain small amounts of *S. sofia*. $^{a, b, c}$: Means within the same row with no common superscripts differ significantly (P<0.05).

Table 3 Results of enrichments from different organs[1] on day 14 and 35

	Day 14				Day 35			
Treatments	Spleen	Liver	Ileum	Caecum	Spleen	Liver	Ileum	Caecum
Cntrol	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12
NC+	12/12	12/12	11/12	12/12	3/12	2/12	0/12	2/12
PC+	12/12	11/12	7/12	12/12	1/12	0/12	0/12	0/12
Pro+	12/12	12/12	6/12	12/12	0/12	1/12	0/12	0/12

[1]The total of 12 birds from each treatment and numbers of positive birds showed as in table.

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APPLICATION OF A VACCINE CARIER DIET DESIGNED FOR THE ORAL DELIVERY OF ANTIGEN

W.I. MUIR[1], G. VANDENBERG[2] and T.A. SCOTT[3,4]

Many pathogens initially challenge the host via the mucosal surfaces of the gastrointestinal tract (GIT). Despite the presence of gut-associated lymphoid tissues (GALT) the oral administration of non-replicating antigen (Ag) typically initiates a weak immune response. Several factors contribute to this including the damaging effect of the digestive processes of the GIT on the Ag and poor Ag uptake by GALT (Walker, 1994; Muir et al., 2000). PerOs Systems Technologies Ltd (Canada) has developed a carrier diet [Oralject™ (OJ)] for the oral delivery of Ag in aquaculture. OJ is formulated to alter the GIT environment, favouring Ag sampling and uptake by GALT. This study investigated the application of OJ as a carrier diet for oral delivery of Ag in chickens, as determined by the immune response following immunisation.

OJ was evaluated with the oral delivery of three Ag, bovine serum albumin (BSA), heat inactivated epizootic haematopoietic necrosis virus and killed *Salmonella typhimurium*, phage type 12 (*St*), in four week old broiler chickens. Two preparations of OJ were tested, OJ dietary ingredients which were delivered in either a wet form via gavage (OJG) or a dry form directly in the feeder (OJF), or the liquid retrieved on extraction of bioactives from ingredients used in OJ, identified as OJ extraction solution (OJES), delivered via gavage. OJG and OJF were assessed with all three Ag and OJES was assessed with BSA and *St* only. Ag and OJ preparations were generally delivered together, however OJ was also assessed when delivered 30 or 120 min. prior to BSA and *St.* A primary immunisation was followed two weeks later by an identical secondary immunisation. The ensuing Ag-specific IgG and IgA antibody titres were assessed in serum, from the secondary immunisation, each week for four weeks. These titres were compared to titres in birds receiving oral Ag administered alone, and to the positive control group where Ag, emulsified in Montanide ISA 50V oil adjuvant (Tall Bennett Group, Sydney), was delivered via intramuscular injection.

In these studies the OJ carrier diets OJG and OJF did not demonstrate any benefits in terms of the resultant serum anti-IgG and IgA titres, compared with the delivery of Ag alone. From one week after secondary immunisation, birds receiving BSA with OJES had anti-BSA IgG levels that were significantly higher ($P < 0.01$) than birds administered BSA alone. Notably 3 weeks after the primary immunisation the anti-BSA IgG and IgA titres in birds receiving BSA with OJES were comparable to the responses seen in the positive control group where BSA was injected with adjuvant. However, this was an Ag-specific response observed with BSA only; it was not observed when killed *St* was delivered with OJES.

In conclusion OJES shows potential as an Ag carrier for oral Ag immunisation in chickens. However, further research is required to optimize the formulation for delivery in poultry and to understand the interactions between Ag, OJES and GALT.

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[1]Faculty of Veterinary Science, University of Sydney, Camden, NSW, Australia, 2570.
[2]PerOs Systems Technologies Inc., 788 Boul.Methot St Nicholas, Quebec, Canada.
[3]Provimi Research and Innovation Centre, Lenneke Marelaan, B1932 Sint-Stevens-Woluwe, Belgium.
[4]Supported by the CRC for the Australian Poultry Industry, Armidale, NSW, Australia, 2315

EFFECTS OF VACCINE STRAINS OF INFECTIOUS BRONCHITIS VIRUS ON THE OVIDUCT OF HENS

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

Summary

In Australia, currently, all pullets reared for egg production are vaccinated by live attenuated strains of infectious bronchitis virus. Various vaccines and protocols to control this viral disease have been developed, although the severity of the disease varies from place to place and flock to flock. In the present trial, effects of vaccine strains on the oviduct of laying hens were assessed by determining the presence and persistence of viral load following experimental infection. There was no drop in egg production in any of the groups. Both A3 and Vic S vaccine strains were detected in the oviduct of vaccinated and unvaccinated hens, mainly on the 12th day p.i. Both the vaccines appeared to be safe for the oviduct.

I. INTRODUCTION

Infectious bronchitis virus (IBV) is a major respiratory virus of chickens. In Australia, currently, all pullets reared for egg production are vaccinated by live attenuated strains of infectious bronchitis virus. It is usual practice to administer three doses in the rearing phase, before point of lay. However, Australian commercial egg producers still have problems with egg quality. This raises the question as to whether it is necessary and advisable to revaccinate the flock during lay for oviduct protection. It has been reported previously that IBV revaccination during the laying phase can cause deterioration of egg shell quality (Sulaiman, 2004). The majority of the work on Australian vaccine strains has been conducted to evaluate efficacy against the nephropathogenicity of IBV. In our previous study, we showed that field Australian IBV strains do not cause a drop in egg production in unvaccinated Isa brown laying hens at full lay (Chousalkar and Roberts, 2007). However, both these strains of IBV cause a drop in egg quality parameters such as Haugh units and egg shell colour (Chousalkar and Roberts, 2008). During the present trial, IBV vaccine load in the oviduct was studied by employing LNA probe based quantitative PCR. There are two vaccines commercially available in Australia. Vaccine strain A3 was developed from a field strain isolated in Armidale, New South Wales, in 1962 which was passaged 25 times. The Vic S vaccine was developed after the 20th passage in chicken embryos of a strain isolated in Victoria (Ratanasethakul and Cumming 1983).

II. MATERIALS AND METHODS

Day old chickens were obtained from the Baiada Hatchery at Marsden Park, NSW. At day-old, all the chickens received Rispens vaccine against Marek's disease but no other vaccinations at the hatchery. The chickens were raised in isolation sheds at the University of New England. The birds were divided into two groups and placed in separate isolation sheds. Half of the birds were vaccinated with Vic S on day 1 by the intraocular route at the dose rate of $10^{4.5}$ embryo infective dose ($E.I.D._{50}$). At 4 weeks of age, one in four birds was vaccinated with A3 at the dose rate of $10^{3.9}$ $E.I.D._{50}$. At 13 weeks of age, one in four birds was again vaccinated with Vic S at the dose rate of $10^{4.5}$ $E.I.D._{50}$. Vaccines were obtained from Fort Dodge, Australia. All birds were reared on the floor in contact with one another. The other

[1] University of New England, NSW, Australia

half of the birds remained unvaccinated. At 25 weeks of age, the unvaccinated and vaccinated birds were divided into two control groups, unvaccinated unchallenged (UC), vaccinated unchallenged (VC) and four treatment groups, unvaccinated challenged with A3 (UA), vaccinated challenged with A3 (VA), unvaccinated challenged with Vic S (UV) and vaccinated challenged with Vic S (VV) (Table 1). All the birds were moved into cages at the age of 25 weeks. At 30 weeks of age, each bird from groups UA, VA, UV and VV received A3 strain of virus at the dose rate of $10^{4.9}$ embryo infective dose ($E.I.D._{50}$) or Vic S strain at the dose rate of $10^{5.5}$ embryo infective dose ($E.I.D._{50}$) and the control birds were sham inoculated with normal saline. Daily egg production was recorded. Two hens from each challenge group and one hen from each control group were euthanized at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 days post infection (p.i.). Oviduct samples were collected aseptically for virus detection and quatitation by real time PCR. LNA probe based quantitative PCR was developed and optimised as described earlier (Chousalkar *et al*., 2008).

Table 1 Experimental design

Group	Age at vaccination			Challenge strain
	1 day old	4 weeks	12 weeks	30 weeks
UC	None	None	None	None
VC	Vic S	A3	Vic S	None
UA	None	None	None	A3
VA	Vic S	A3	Vic S	A3
UV	None	None	None	Vic S
VV	Vic S	A3	Vic S	Vic S

Oviduct scrapings were weighed and used for RNA extraction using commercial kits (RNAeasy, Qiagen). Extracted RNA was quantified and stored at -70^{o}C until used for real time PCR. 50 ng of extracted oviduct RNA was used in each reaction. The LNA probe-based real time reverse transcriptase polymerase chain reaction (RT-PCR) was developed and performed using a Rotor Gene 3000 real time PCR machine (Corbett Research, Sydney, Australia) and a one-step RT-PCR kit (Invitrogen Australia Pty Limited). Raw data were analysed using the default settings of the software for determination of baseline and threshold of the reaction. The test sensitivity was determined by running 10 fold serial dilutions of the plasmid DNA (recombinant plasmid DNA /clone) with known copy numbers. In each assay, a standard curve was generated and used to derive the infectious bronchitis viral copy number from the oviduct samples. Each sample was analysed in duplicate.

III. RESULTS

a) Clinical findings

One hen from UV group showed respiratory signs like sneezing and rales on day 6 p.i. and respiratory signs were also noticed in one hen each from group VA on days 8 and 10. All respiratory signs had disappeared after day 10 p.i. No hens died after infection with either strain of IBV and there was no drop in egg production in any group. Respiratory signs were not recorded in unvaccinated or vaccinated control group. Watery whites or runny albumen was not recorded in any of the infected group.

b) Real time PCR findings

Virus was not detected from the oviduct of hens from the unvaccinated control (UC) or vaccinated control group (VC). Virus was detected in the oviduct of one hen from the UV group on day12 p.i. In group VA, virus was also detected from the oviduct of one hen killed

on day 6 p.i and both the hens killed on the 12th day p.i. In the UV group, virus was detectable in the oviduct of both the hens killed on the 8th and 12th days p.i. Also virus was detectable in the oviduct of one hen killed on the 10th and 18th day p.i. In group VV, virus was detectable on the oviduct of both the hens on the 12th day p.i. and in one hen on the 14th day p.i. The detailed results are presented in Table 2. The real time PCR test designed earlier and used to test the samples during this experiment could detect a minimum of 10 viral copies, with threshold cycle (Ct) value of 41.1, from the infected samples (Chousalkar *et al*., 2008). One sample from UA, three samples from VA, five samples from UV and one sample from VV group showed very high Ct values, which were below our limit of detection, hence considered to be negative.

Table 2 QRT-PCR analysis of the individual oviduct samples from vaccinated and unvaccinated IBV infected hens killed at different days post infection.

		Oviduct			
Days post-infection	Hen	Group UA	Group VA	Group UV	Group VV
		mean VCN*	mean VCN	mean VCN	mean VCN
2	1	0	0	0	0
	2	0	0	0	0
4	1	0	0	0	0
	2	0	0	0	0
6	1	0	12	0	0
	2	0	0	0	0
8	1	0	0	25	0
	2	0	0	14	0
10	1	0	0	21	0
	2	0	0	0	0
12	1	0	20	0	199
	2	133	139	152	167
14	1	0	0	0	13
	2	0	0	0	0
16	1	0	0	0	0
	2	0	0	0	0
18	1	0	0	16	0
	2	0	0	0	0
20	1	0	0	0	0
	2	0	0	0	0
22	1	0	0	0	0
	2	0	0	0	0
24	1	0	0	0	0
	2	0	0	0	0

* VCN- Viral copy number

IV. DISCUSSION

There is a dearth of literature regarding *in-vivo* studies on the effects of vaccine strains on the oviduct of mature laying hens. This could be due to the amount of labour involved and the need to maintain birds free from unplanned IBV exposure.. In this trial, clinical respiratory symptoms were short-lived in both the groups (between 6-10 days p.i.) a finding which is in

accordance with Ratnasethakul and Cumming (1983). In the present study, there was no direct relationship between respiratory and reproductive tropism of vaccine strains of IBV. Despite respiratory signs, the reproductive performance of hens remained unaffected and virus was not detected from the oviduct of such hens. Also, it was interesting that the hens which had virus detected in their oviduct did not demonstrate any respiratory signs during virus infection. Earlier studies have shown that British vaccine strains can induce permanent cellular damage to the oviduct of young chickens (Duff *et al*., 1971) and can replicate in oviduct organ cultures (Peters *et al*., 1979). The present experiment was designed to check the safety of Australian vaccine strains of IBV *in-vivo*. It should also be noted that all the hens vaccinated during rearing in the present study were laying normally and virus was not detectable in any of the oviducts of control vaccinated unchallenged hens. Hence it could be assumed that vaccination during the rearing phase does not induce permanent damage to the oviduct of young chickens although further studies are essential to prove this. The viral load of vaccine strains of IBV in the oviduct was very low compared to the load of wild strains (Chousalkar *et al*., 2008). The detection of virus at regular intervals from the oviduct in lower quantity suggests that the virus can replicate in the oviduct without causing a drop in egg internal quality or production. Earlier, Dhinakar Raj and Jones (1997) observed that vaccine strains replicated in oviduct organ culture without causing ciliostasis. Further studies regarding the extent of cellular damage caused by vaccine strains in the oviduct would be instructive and are in progress in our laboratory.

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EFFECTS OF VACCINE STRAINS OF INFECTIOUS BRONCHITIS VIRUS ON EGG QUALITY IN UNVACCINATED AND VACCINATED LAYING HENS

J.R. ROBERTS[1], K.K. CHOUSALKAR[2] and R. TURNER[2]

Summary

The effect of two vaccine strains of infectious bronchitis virus (IBV - VicS and A3 strains) on internal and external quality of eggs was studied in Isa Brown hens in full lay. Birds were either unvaccinated for IBV or had been vaccinated during rearing. The main effects of exposure to the vaccine viruses were that VicS resulted in paler coloured shells, mainly in the unvaccinated birds and the eggs from the hens challenged with VicS were more elongate than the other groups. These findings are consistent with our earlier findings with field strains of IBV and differ from those reported in the literature for different strains of IBV.

I. INTRODUCTION

Infectious bronchitis virus (IBV) is a viral disease of poultry which affects epithelia in a number of parts of the body including the respiratory system, kidneys and oviduct. IBV is reported to cause drops in egg production and reduced egg quality. Australian field strains of IBV have been shown to have negative affects on the oviduct (Chousalkar and Roberts, 2007 a,b; Chousalkar *et al.*, 2007 a,b) and thereby on egg quality (Chousalkar and Roberts, 2008). However, little is known of the effects of vaccine strains of IBV on the oviduct and therefore on egg quality. The present study was undertaken to study the effects of IBV on egg and egg shell quality in unvaccinated laying hens and hens which were vaccinated during rearing.

II. MATERIALS AND METHODS

Day-old Isa Brown hens (250) were obtained directly from a commercial hatchery and transferred to isolation sheds at the University of New England. Half of the birds were vaccinated according to the standard UNE protocol of VicS at day-old, A3 at 4 weeks and VicS at 13 weeks. Vaccine was administered by eyedrop. All birds were reared under strict isolation and biosecurity with vaccinated and unvaccinated birds being maintained completely separate. The birds were divided into six groups: unvaccinated control (UC), vaccinated control (VC), unvaccinated and exposed to VicS (UV), vaccinated and exposed to VicS (VV), unvaccinated and exposed to A3 (UA) and vaccinated and exposed to A3 (VA). At 30 weeks of age, birds were exposed to one of two vaccine strains of IBV at a dose 10 times the usual vaccination dose: VicS strain (at the dose rate of $10^{5.5}$ embryo infective dose ($E.I.D._{50}$) or A3 strain (at the dose rate of $10^{4.9}$ embryo infective dose ($E.I.D._{50}$), or left unchallenged as a control. Vaccines were obtained from Fort Dodge, Australia. All eggs were collected at 3 and 2 weeks prior to challenge and then daily during the week immediately before infection to determine any inherent differences among the groups. Eggs were collected and analysed daily up to 5 weeks post infection (p.i.) and again at weekly intervals 6, 7, 8, 9 and 10 weeks p.i. All eggs were analysed for the internal quality parameters albumen height, Haugh units and yolk colour score.

[1] Australian Poultry CRC, PO Box U242, UNE, Armidale, NSW 2351
[2] School of Environmental and Rural Science, UNE, Armidale, NSW 2351

Egg shell quality was measured as shell reflectivity, egg weight, deformation, breaking strength, shell weight (from which percentage shell was calculated), shell thickness, egg length and breadth (from which shape index was calculated as breadthx100/length). Data were analysed by ANOVA and Fisher's protected LSD was used to distinguish differences between means. Significance was assumed at $P<0.05$.

III. RESULTS

Egg production was not significantly different among the treatment groups. In addition, some measures of egg quality were not significantly different among the treatment groups. There was a significant effect of time on all variables measured except percentage shell and these effects were generally consistent with the increasing age of the flock. There were no main effects of the treatments on percentage shell, and no main effect of vaccination treatment on any indicator of egg shell quality except shell colour. In addition, there was no main effect of challenge treatment group on shell breaking strength, deformation, shell breadth, shell thickness or percentage shell.

Egg weight was highest in the control groups (62.0 g), lowest in the A3 groups (61.1 g) with the VicS groups intermediate (60.5 g). The main effects on egg shell quality were changes in shell colour and egg shape. For shell reflectivity, a measure of shell colour lightness, there were significant main effects of time in relation to challenge, challenge treatment and vaccination treatment. Over the entire experimental period, shell colour was lightest in the VicS challenged birds, due to the increased shell reflectivity in the unvaccinated birds exposed to VicS (Figure 1). The control and A3 challenged groups had darker shells than the VicS group and were not significantly different from each other. Shell colour was significantly lighter for the unvaccinated treatment groups than for the birds which had been vaccinated during rearing. There were also significant interactions between time postchallenge and challenge treatment, time and vaccination treatment, and challenge treatment and vaccination treatment. There was a significant main effect of time in relation to challenge, and challenge treatment, on shape index with eggs being more elongated in the VicS challenged groups (Shape Index 77.9%) than in the Control groups (77.5%) and A3 groups (77.6%). Over the entire experimental period, there was no significant effect of vaccination treatment although the eggs in the unvaccinated birds tended to be more elongate (Shape Index 77.8%) than in the vaccinated groups (77.5%). Differences in shape index were due primarily to differences in length, rather than breadth. Shell weight was highest in the control groups (6.21 g), lowest in the VicS groups (6.07 g), with the A3 groups intermediate (6.15 g).

For egg internal quality, Haugh Units were higher in the vaccinated groups (94.0%) than in the unvaccinated groups (93.1%). However, Haugh Units were highest in the VicS groups (94.3%), followed by the A3 groups (93.5%) with the control birds having lower Haugh Units (92.6%). Yolk colour score was significantly higher for the unvaccinated groups (12.11) than for the vaccinated groups (11.85) and highest for the A3 groups (12.12), lowest for the VicS groups (11.86) with the control intermediate (11.94).

IV. DISCUSSION

The results presented here are consistent with those reported earlier from our laboratory concerning the effects of field strains of IBV on egg quality (Chousalkar and Roberts, 2008). Effects of IBV on egg quality are also consistent with our previously reported findings of histopathological effects on parts of the oviduct and virus localisation in the oviduct (Chousalkar and Roberts, 2007a,b; Chousalkar et al., 2007a,b; Chousalkar and

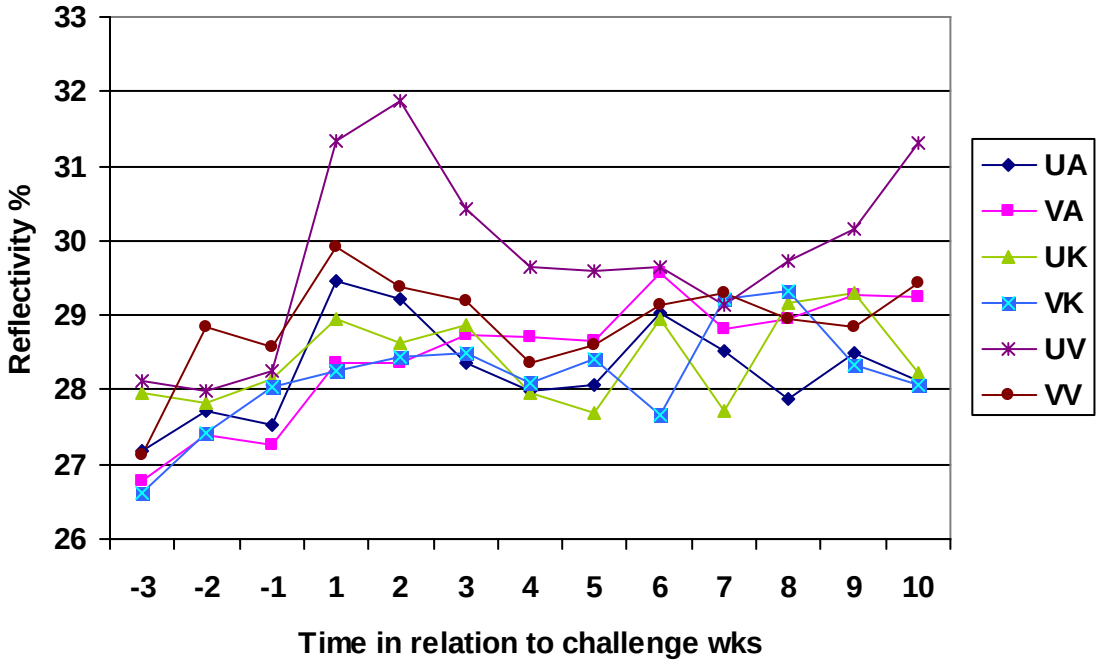

Figure 1 Shell reflectivity (%) in eggs collected during the experimental period

Roberts, 2008 in press; Roberts, 2005). It is important that these findings are documented as they are at odds with the published literature on effects of IBV on egg quality in other countries (Sevoian and Levine, 1957; Cook, 1971).

The mechanisms whereby exposure to IBV results in paler coloured egg shells and more elongate eggs are not understood. The factors affecting deposition of the porphyrin pigment in the cuticle of the egg shell are poorly defined.

Reductions in albumen quality following exposure to field strains of IBV might have contributed to the production of more elongate eggs (Chousalkar and Roberts, 2008, in press). However, this is unlikely to have been the case in the present study where albumen height and Haugh Units were higher for the vaccine challenged groups than for the control. The reasons for this are not clear. In addition, the cause of the effects of vaccination during rearing and challenge with vaccine strains of IBV on yolk colour is not known but may be the result of effects on lipid metabolism. Further work is needed to clarify this.

It is important to understand how the effects of Australian strains of IBV differ in their effects on egg quality from IBV strains which are common in the U.S. and Europe. Textbooks on disease describe production drops and the appearance of thin-shelled, shell-less and rough-shelled eggs, in addition to reductions in shell colour (Cook, 2008; Cavanagh and Gelb, 2008) and earlier textbooks talked about the classic “IB egg” which was wrinkled and corrugated. The differences between the effects of IBV on egg quality reported in the literature and those found in our laboratory may be due to differences in the strain of IBV used. It is also possible that the strain of bird has an influence. Even within our own laboratory, we have observed that unvaccinated or vaccinated White Leghorns exposed to IBV show more severe histopathology in the oviduct than do Isa Brown hens.

Reduced shell colour and decreased albumen height result from exposure to both Australian and overseas strains of IBV. However, the gross changes in egg shell quality reported for overseas strains have not been demonstrated experimentally in Australia. In addition, the changes in egg shape which have resulted from exposure to Australian field strains of IBV (Chousalkar and Roberts, 2008) have not been reported for overseas strains.

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EMBRYO METABOLISM

G. M. FASENKO[1], E.E. O'DEA CHRISTOPHER[1] and J.A. HAMIDU[1]

Summary

Many factors influence embryonic metabolism including parent flock age, genetic strain, duration of cold egg storage and incubation conditions. By measuring embryo metabolism, we can gain an understanding of how these factors affect the embryo's growth and development. During incubation, the embryo uses nutrients in the yolk and albumen and oxygen from outside the egg, resulting in the production of carbon dioxide, water vapour, and energy. This process is called embryonic metabolism. Various methods have been used over the past several decades to measure embryonic metabolism. In the authors lab, an indirect calorimetry system for measuring embryonic metabolism in domestic avian embryos has been developed and refined. This system has enabled precise measures of embryonic metabolism to be recorded on up to 22 eggs with relatively little labour required. Using an early version of the current system to measure embryonic CO_2 production, it was established that embryos in broiler hatching eggs exposed to cold storage for 15 days had a lower metabolic rate than embryos in eggs stored for only four days. The same method was used to assess embryonic metabolism in embryos from three genetic strains at two flock ages. The results showed that differences in total embryonic CO_2 production between the strains over the entire incubation period were dependant on parent flock age. Recently, upgrades to the system used in the previous experiments have allowed for the direct measurement of both embryonic CO_2 production and O_2 consumption. This upgrade has enabled collection on the the actual daily respiratory quotient (ratio of CO_2 production/O_2 consumption) of embryos throughout most of incubation. This proceedings includes a discussion of the most recent research conducted on the effect of genetic strain and parent flock age on the metabolism of both turkey and broiler chicken embryos.

I. INTRODUCTION

There are many factors which have the capacity to impact embryonic metabolism, including genetics, breeder flock age, cold egg storage, and incubator and hatcher conditions. Measuring embryonic metabolism provides one indication of how these factors are influencing the growth and development of the embryo. Since the incubation period of domestic chickens and turkeys is only 21 and 28 days, respectively, it could be argued that this pre-hatch phase of growth is not as important as the much longer post-hatch growth period. However, genetic selection for fast and efficient broiler growth over the past 60 years has decreased the post-hatch lifespan of the modern broiler. Havenstein *et al.* (2003) demonstrated this by comparing the 42 day body weights of a 1957 and a 2001 broiler strain. When both strains were fed a 2001 diet, the 1957 strain only reached a body weight of 578g which was 4.2 times less than the 2001 strain at 2441g. Similar results have also been reported for 1966 versus 2003 turkey strains (Havenstein et al., 2007). Thus, as genetic selection for growth decreases the number of days to market, the time spent in the incubator makes up a larger percentage of a bird's total lifespan. For broilers, the 3 week incubation period comprises 33% of the bird's total pre- and post hatch lifespan. For this reason,

[1] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

research that examines how particular factors influence embryonic development, growth, and survival, has increased importance in modern strains.

II. METHODS TO MEASURE AVIAN EMBRYONIC METABOLISM

During avian embryonic metabolism, nutrients from the yolk and albumen are oxidized and carbon dioxide (CO_2), water vapour and energy are produced. The ratio of CO_2 produced to oxygen (O_2) consumed is called the respiratory quotient (RQ); this value provides an indication of the proportion of nutrients being metabolized (Krogh and Lindhard, 1920) during a particular developmental stage. For example, the oxidation of pure carbohydrate would produce an expected RQ of 1, while a value of 0.7 would be the expected RQ if pure fat was being oxidized. From the RQ, embryonic metabolism (measured as heat production) can be mathematically calculated (Kleiber, 1987). Methods that measure metabolism in this manner are referred to as indirect calorimetry.

Over the years, researchers have developed and used various methods to measure the metabolism of avian embryos. Some researchers used labour intensive methods on small numbers of eggs (embryos) in order to obtain actual values of both embryonic CO_2 production and O_2 consumption (Vleck and Kenagy, 1980). Others opted to develop a calorimetry system that could obtain numerous embryonic CO_2 production values on each day of incubation without removal of eggs from the incubator (Segura et al., 2006). The disadvantage of this calorimetry system was that O_2 consumption was not able to be measured directly. O'Dea et al. (2004) originally used the method published by Segura and colleagues (2006) to obtain actual embryonic CO_2 production values, then using a previously reported average RQ for the entire incubation period (0.84 - Romanoff, 1967) O_2 consumption was mathematically estimated. What neither of these two above methods could efficiently or accurately determine was whether factors that may influence embryonic metabolism affect metabolism throughout the entire incubation period, or just at particular and crucial stages of embryonic development. Thus, a more precise and less labour intensive indirect calorimetry system has been developed (Hamidu et al., 2007) based on the previous method reported by Segura et al. (2006).

The refined indirect calorimetry system (Hamidu et al., 2007) has the capability of measuring O_2 and CO_2 concentrations in the same air sample from each of 24 metabolic chambers housed within a small incubator. The metabolic chambers contain a single egg. The design enables numerous, daily gas concentrations to be recorded from embryos of individual eggs without removing the eggs from the chambers or the incubator. With this system, data on O_2 and CO_2 concentrations can be collected from the first day of incubation through to hatching. Embryonic metabolism data has been collected from both broiler (Hamidu et al., 2007) and turkey species (Hamidu et al., unpublished). One existing limitation of this equipment is that because the differential between embryonic O_2 consumed and CO_2 produced is very low during the first three days of incubation, the precision of data collection prior to four days of incubation needs to be further refined.

III. THE BASIS BEHIND WHY IT IS IMPORTANT TO MEASURE AVIAN EMBRYONIC METABOLISM

Even though published research has documented the drastic changes in the growth of broiler chickens (Havenstein et al., 2003) and turkeys (Havenstein et al., 2007) due to genetic selection, there is a lack of research focusing on how genetic selection has altered embryonic growth and metabolism. The hatching egg industry has made improvements to the efficiency, precision, and monitoring capability of artificial incubation equipment. However, relatively

few changes to incubation conditions have been made over the past 50 years to accommodate changes in embryo metabolism that may have occurred due to genetic selection. Also, because of a lack of scientific based evidence examining embryonic metabolism in modern versus historic strains, hatchery managers have no concrete information upon which to make incubation changes. Anecdotal evidence from the industry has indicated that embryonic metabolism and heat output, especially in the final 4-5 days of broiler incubation, has increased. Hulet et al. (2007) provided evidence that embryonic temperatures (estimated using egg shell temperature) during the last five days of incubation significantly affect post-hatch neonate growth. There is also some evidence to suggest that embryonic metabolism increases with breeder parent flock age (O'Dea et al., 2004).

In addition to changes in overall metabolic rate, newly published comprehensive research has reported the effects of genetic selection on embryonic cardiac growth and metabolic function in turkeys. Christensen et al. (2008) demonstrated that long term selection for 16 week body weight in turkeys has altered the embryo's myocardium metabolism towards relying on gluconeogenesis during the final stages of development and hatching; this likely contributes to higher late incubation embryonic mortality.

The function of an artificial incubator is to meet the temperature, humidity and oxygen needs of the embryo. Currently, most modern hatcheries incubate eggs based on the assumption that embryos from different genetic strains, parent flock ages, or egg weights have basically the same requirements. The focus of embryonic metabolism research in my lab is to examine how different factors may influence embryonic metabolism so that incubation parameters may be altered to better meet the physiological needs of the embryo.

IV. THE RELATIONSHIP OF EGG SHELL CONDUCTANCE TO EMBRYONIC METABOLISM

Embryonic metabolism can also be affected by the capacity for gasses and moisture to diffuse across the eggshell; this is called eggshell conductance (G) (Ar et al., 1974). In turkeys, G has been found to increase with parent flock age as a result of increasing egg size, but G was not affected by genetic strain (Christensen et al., 2001a). In broiler hatching eggs, O'Dea et al. (2004) found the opposite to be true, with eggs from a young broiler breeder flock having a higher G compared to eggs collected at older flock ages even though egg size increased as the flock aged. Adjusting humidity within the incubator according to the G of the eggs improves hatchability (French, 2000). It is also known that the effective removal of CO_2 from the incubator is critical to successful incubation (Ernst et al., 2007). For this reason, it is important that O_2 and CO_2 can easily diffuse across the eggshell, being exchanged between the egg and its environment. The concentrations of O_2 and CO_2 exchanged by avian embryos during incubation are related both to embryonic heat production (metabolism) (Kleiber, 1987) and the respiratory quotient (RQ) (Cathcart and Markowitz, 1927).

V. EFFECTS OF EGG STORAGE ON EMBRYONIC METABOLISM

Research in the Fasenko laboratory has measured the metabolism of broiler embryos in eggs stored for either four or 15 days using the indirect calorimetry methods described by Segural et al., (2006). Through measuring embryonic CO_2 production and then calculating O_2 consumption and heat production based on an RQ of 0.84 (Romanoff, 1967) it was found that embryos from 15 day stored eggs have a slower metabolic rate than embryos in eggs stored for four days (Fasenko et al., 2002). This data provided evidence that pre-incubation, long-term cold storage somehow changes the metabolism of the broiler embryo. In previously conducted research on turkey embryos, Fasenko (1996) found that embryos from eggs stored

for 14 days depended more on gluconeogenesis during pipping and hatching when compared to embryos from eggs stored for four days. Related results have been obtained from a broiler breeder strain whose embryos are resistant to mortality due to cool storage; these embryos had more glycogen reserves in the heart and muscles than embryos from a line susceptible to storage induced mortality (Christensen et al., 2001b). This body of research shows that long term cold storage changes the metabolism of an embryo in a negative manner which ultimately puts embryonic survival at risk.

VI. HOW GENETIC STRAIN AND FLOCK AGE INFLUENCE BROILER EMBRYONIC METABOLISM

a) Modern versus unselected genetic strains

A study was undertaken to compare G of eggs and embryonic metabolism between three different broiler strains at different flock ages. A modern strain selected for high breast yield, a modern strain selected for the whole bird market, and a strain unselected since 1978 were the three strains examined (O'Dea et al., 2004). Embryonic metabolism was determined according to the methods of Segura et al. (2006) that have been summarized previously in these proceedings.

Strain did not influence G, or total embryonic CO_2 production over the entire 21 day incubation period, but as parent flock age increased, G rose significantly. In comparing embryos produced by 32 week old parents, the unselected strain had higher total embryonic CO_2 production over the entire 21 days of incubation (4.04 L) than embryos from the whole bird market strain (3.53 L). The embryos from the high breast yield strain (3.74 L) did not differ from either of the other strains. However, by 38 weeks of parent flock age, the pattern was reversed, and the embryos from the whole bird market strain had higher total CO_2 production (4.98 L) than the embryos from the unselected strain (4.38 L). Again, the embryos from the high breast yield strain (4.83 L) did not differ from either of the other strains examined (O'Dea et al., 2004). This study showed that the differences between the strains in embryonic CO_2 production over the entire incubation period were dependant on parent flock age.

Daily differences in CO_2 production were reported between genetic strains on days eight, nine, and 21 of incubation (O'Dea et al., 2004). It was speculated that the metabolic differences between strains on days eight and nine were due to disparities in the stage of embryonic development between the strains. However, this cannot be confirmed since data on embryonic developmental stages was not collected. The differences observed between strains on day 21 was most likely a reflection of differences in time of hatching between the strains, as CO_2 production spiked once the chick had fully exited the shell. Beginning at three days of incubation and ending at 21 days of incubation, daily CO_2 production varied between embryos from the 32 and 38 week old parent flocks with the 38 week old flock consistently having higher CO_2 production. This result indicates that embryos from different parent flock ages have different metabolic rates throughout most of the incubation period. This may add an additional perspective to the problem of poor hatchling performance in broilers hatched from young breeder flocks. Future research should investigate whether embryonic survival and chick quality can be improved in eggs produced by different breeder flock ages through providing incubation conditions tailored for the various flock ages.

b) Comparing two modern genetic strains

Following the previously described trial, improvements were made to the equipment used to measure embryonic metabolism. This included the addition of an O_2 analyzer, which subsequently allowed for both embryonic CO_2 production and O_2 consumption to be directly measured. From this data, the actual daily RQ's during incubation could be established (CO_2 produced/O_2 consumed). This has enabled obtaining a more precise measure of embryonic heat production (Kleiber, 1987). Following the calorimetry equipment upgrades, an experiment was conducted to compare G and embryonic metabolism between two modern strains (Hamidu et al., 2007). One strain examined (Ross 308) was genetically selected to meet broad market requirements while the other strain (Cobb 500) was selected for high breast meat yield. For each of the two strains, the effect of various flock ages was also examined. (Young flock (29 wk, both Ross and Cobb); Peak flock (34 wk for Ross and 36 wk for Cobb); Post-peak flock (40 wk, both Ross and Cobb); Mature flock (45 wk, both Ross and Cobb); Old flock (55 wk, both Ross and Cobb); and Very old (59 wk, both Ross and Cobb).

Neither strain nor parent flock age were found to influence G (Hamidu et al., 2007). Average embryonic metabolism over the entire 21 day incubation period was not different between the two genetic strains. However, there were daily differences in embryonic metabolism between the strains on specific days during early and late incubation. Strain differences in embryonic O_2 consumption occurred on days one, seven, 16, 17, 19 and 20 of incubation, while CO_2 production differences were reported on each of days one to four and 16 to 20 of incubation; calculated heat production differed between the strains on days four, seven, and 16 to 19 of incubation (Hamidu et al., 2007). This result was very interesting since the differences in embryonic metabolism between the strains occurred on days of incubation when most embryonic mortality occurs. Examination of this relationship between embryonic metabolism and mortality may lead to future improvements in embryo survival. With regard to the effects of parent flock age, embryonic metabolism was consistently highest in embryos from old and very old parents. This supports the findings of O'Dea et al. (2004) that embryonic metabolism increases with increasing parent flock age.

In their research Hamidu et al. (2007) determined that genetic strain and parent flock age affected embryonic metabolism on a daily basis throughout incubation. In this research, the experimental eggs were selected to be the same weight (i.e. even though the eggs were from different parent flock ages and genetic strains, the egg weights were selected to be the same). It is acknowledged that by selecting all eggs to be within a narrow weight range, normal average egg weights for each strain and flock age were not being compared. Thus it was decided that in future studies, eggs would be collected based on average egg weights for that particular strain and flock age. In order to account for any effects of egg weight on metabolism, embryonic metabolism would be calculated based per gram of body mass. This approach was taken for the turkey research trials that are described in the following sections.

It is also recommended that future research examine different incubation temperature profiles for eggs from different genetic strains and flock ages. For example, testing low, normal, and high incubation temperatures at early and late stages of incubation where most embryonic mortality occurs may provide further insight into tailoring incubation conditions to optimize embryonic survival. This type of research would be of assistance to hatchery managers in deciding which eggs to place together in the same incubator exposed to similar incubation environment profiles.

VII. HOW GENETIC STRAIN AND FLOCK AGE INFLUENCE TURKEY EMBRYONIC METABOLISM

Eggshell conductance and embryonic metabolism were investigated in two modern turkey genetic strains (Hybrid and Nicholas) at four parent flock ages: Young (Y – 30 wk), Peak (P – 34 wk) Mature (M – 55 wk) and Old (O – 60 wk). In this study (Hamidu et al., unpublished) an additional 270 eggs per genetic strain at each flock age were incubated in order to obtain daily embryonic weights on days four to 28 of incubation. This additional step was added so that daily embryonic heat production could be calculated per gram of embryo body mass.

Eggshell conductance was highest in Hybrid eggs from an old parent flock, and Nicholas eggs from both mature and old flocks, indicating that eggs from each strain at these flock ages have the highest capacity for gasses to be exchanged between the embryo and the environment external to the egg. However, since egg weight for each strain also increased with flock age, differences in G may be influenced by egg size rather than solely flock age.

As was reported in broiler embryos (Hamidu et al., 2007), turkey embryonic heat production increased as the parent flock age increased (Hamidu et al., unpublished data). Embryos from the young flock were had higher embryonic heat production per gram of embryo mass during early and mid incubation compared to embryos from the other parent flock ages. This data provides evidence that embryos from the young flock were actually metabolizing substrates at a higher rate in order to gain the same gram of body mass as embryos from other parent flock ages. Based on this, it is suggested that future research examine if exposing eggs produced by young parents to slightly higher incubation temperatures could improve hatchability. Conversely, eggs produced by older parents may require lower incubator temperatures during the final days of incubation in order to maximize embryonic survival. This type of research will be necessary in order to establish optimum incubation temperature profiles for eggs from different genetic strains and parent flock ages.

Unlike the broiler strains previously examined, there were very few daily differences in daily CO_2 production, O_2 consumption and heat production between the two strains examined. This indicates that the genetic selection of the two turkey strains examined may not have resulted in differences in embryonic metabolism. However, further research is needed to establish whether eggs from different turkey parent flock ages could benefit from being incubated separately, and whether different incubation temperature profiles are required in order to optimize hatchability and poult performance. Previous research has shown that early and late embryonic mortality is highest in very young and very old turkey flocks (Fairchild et al., 2002). Thus, future research is needed to investigate whether there is a relationship between embryonic metabolism and mortality in turkey embryos from eggs produced by different flock ages.

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INCUBATION PRINCIPLES: WHAT DOES THE EMBRYO EXPECT FROM US?

R. MEIJERHOF[1]

Summary

To obtain optimum incubation results and maximum chick quality, the conditions during incubation must be adjusted to meet the requirements of the embryo. One of the most important conditions during incubation is temperature, and then especially the temperature inside the egg, the so-called embryo temperature. The temperature inside the egg is not identical to the temperature of the air, but is a balance between heat production and heat loss, and is also determined by stage of incubation, air velocity, relative humidity and evaporation. Control and fine-tuning of this balance will result in an optimum incubation process. To achieve this in a field situation, a basic understanding of the physics of heat exchange and its influence on embryo development is needed.

I. INTRODUCTION

Artificial incubation is a very delicate process, which requires an excellent control to maximize results. Over time, the technology and control equipment used in hatcheries have improved significantly. At this moment, hatcheries setting up to 2 million eggs weekly are no exception any more, as well as incubators setting over 100,000 eggs in one machine. The technology installed to make this process run constantly and reliable is enormous and complicated. We are able to read and follow all the different machines remotely, control and adjust the settings from central computers, receive alarm warnings by telephone, store and process all relevant data automatically. Modern hatchery management aims at creating optimal environmental conditions for eggs and chicks from egg storage at the breeder farm until the moment of chick delivery at the farm. Climatic conditions in any room can be controlled very accurate at any point and at any time.

However, in spite of all these technical improvements, it must be questioned if we are actually controlling the key factors for the embryo itself to the level that we think we are. To understand these key factors, we have to look into what happens during incubation itself.

II. WHAT HAPPENS DURING INCUBATION

From a biological point of view, incubation, changing an egg into a live chicken, is of a complexity far beyond our imagination and nothing less then a miracle. However, from a technical point of view we can simplify the process to a very straight forward model. In essence, the embryo builds up the body from the content of the egg. For this process energy is needed. The energy is mainly derived from burning the fat sources in the yolk. To burn the fat, the embryo needs oxygen, which has to come from outside of the shell. During burning, three waste products are produced: carbon dioxide, metabolic water and metabolic heat. These waste products have to be removed from the egg, by ventilation, by evaporation and by cooling. Last but not least, this whole process is controlled by temperature. Concluding it means that for adequate incubation we need temperature, ventilation for oxygen and carbon dioxide exchange and ventilation for moisture loss. When we arrange this adequately, the embryo will utilize the yolk and change it into body tissue in the most optimal way. Besides

[1] HatchTech BV, Veenendaal, The Netherlands

these conditions we also need turning, but that interesting subject is not in the scope of this presentation.

For many years we know that from all involved factors, temperature is by far the most important one (Deeming and Ferguson, 1991). For this reason, incubators are designed to control air temperature in every spot of the machine in a very uniform way (Owen, 1991).

However, the real importance for the embryo is not the air temperature, but the temperature inside the shell, as this is the only temperature that the embryo experiences. This means that air temperature control is only adequate as long as it reflects embryo temperature.

A complicating factor in this respect is that an embryo of modern high yielding strains seems to produce more heat during incubation than the more classical type of birds (Hulet and Meijerhof, 2001). The consequence of this is that the internal egg temperature (embryo temperature) of modern high yielding strains will be higher than of classical strains, if incubator conditions are not adjusted.

III. EMBRYO TEMPERATURE VERSUS AIR TEMPERATURE

The embryo temperature, being the temperature inside the egg, is a balance between the heat production of the embryo and the heat loss of the egg (Meijerhof and van Beek, 1994). If the egg loses more heat than it produces it cools down, until an new equilibrium is reached, and vice versa.

This means that heat transfer to and from the egg is important for the temperature of the embryo (French, 1997). We must realize that this heat transfer is not only a result of the difference in temperature between eggs and surrounding air, but that air velocity and evaporation will have a high influence on the rate of heat transfer as well (Meijerhof and van Beek, 1994; Meijerhof, 2002). A high air velocity will give a high heat transfer, a low air velocity will give a low heat transfer. This means that when there is a difference in temperature between egg and air, the rate of air velocity will determine the actual embryo temperature at a given moment. This is important both at the start of incubation, when the eggs are warmed, as in the second part of incubation, when the embryo starts to produce significant levels of metabolic heat. This surplus of heat has to be removed from the egg, in order to keep the embryo temperature at the desired level. If water needs to be evaporated in the incubators, either water coming from the egg shells or water sprayed by the machines, this evaporation will have a cooling effect on the eggs as well, as evaporation requires high amounts of energy.

As embryo temperature is a balance between heat production and heat loss and heat loss depends on temperature, air velocity and evaporation, it is clear that just controlling air temperature is not sufficient to control embryo temperature.

IV. START OF INCUBATION

At the start of the incubation process, the eggs have to be warmed to the set point incubation temperature. This means that relative cool eggs are placed in a warm environment, and energy has to be transferred from the air to the egg. Eggs experiencing a high air flow will be warmed rapidly, eggs at a low air flow will take several hours. Depending on the type of machine, the difference in time between first and last eggs to reach the final incubation temperature can be substantial. After several hours, depending on the uniformity of the airflow over the eggs, the last are warmed to the air temperature and the incubation process has started at full speed for all eggs.

During incubation, eggs experience a more or less constant moisture loss. This moisture loss will cool the eggs because this process uses energy for evaporation (Meijerhof and van Beek, 1994). During the start of incubation this will result in an egg temperature slightly below the set point (air) temperature. When humidifiers are used in machines to control relative humidity, it should be realized that evaporation of water uses a tremendous amount of energy. This energy (heat) has to be provided through the air and will specifically cool the eggs close to the humidifiers. When humidifiers are used at the beginning of incubation, the eggs will be cooled more and will take more time to reach set temperature.

V. METABOLIC HEAT PRODUCTION

After some days, the embryo will start to produce metabolic heat, and from this moment onwards, the temperature inside the egg will start to rise above air temperature. A recognizable amount of heat production already starts at approximately day 4 of the incubation process. From approximately day 7-8 onwards, the heat production results in significant increase of embryo temperature above air temperature. From this moment onwards, the machines will have to start cooling when single stage setting is used.

Also in this stage, air velocity plays an important role in heat transfer from eggs to the environment (Lourens, 2001). At high air velocity, more heat will be removed from the egg shell, thus forcing the embryo temperature to decrease. At low air velocity, the egg temperature will rise. This problem increases with increasing egg size. When eggs are bigger, they produce more metabolic heat, have a relatively limited surface for heat transfer and air flow over the eggs will be limited (Meijerhof, 2002).

A complicating factor is the increased production of metabolic heat by the embryos of modern yield birds. Although adequate scientific data is still not largely available, field observations and first experiments indicate that the heat production of yielding breeds is substantially increased compared to the heat production of embryos of more traditional breeds. This will make the differences in embryo temperatures at different spots more pronounced, both in multi stage and single stage machines.

Measurements on embryo temperatures in commercial setters have confirmed this. At Praktijkonderzoek Pluimveehouderij, measurements were done in a commercial single stage machine. At 17 days of incubation, differences of more than 4oF in embryo temperature were observed, although air temperatures were more or less even at all spots (Lourens, 1999). Elibol and Brake (2008) observed as well that the position of the eggs relative to the ventilator influenced the temperature of the eggs.

An additional effect on embyro temperature is also the cooling effect of the humidifiers in the machine, due to the evaporation of water. This will provide a more or less constant cooling force to the eggs in the area close to the humidifiers. In some type of machines, this influence can be enormous.

VI. PRACTICAL IMPLICATIONS

In the past years, there has been done a lot of research which shows that embryo temperature in most commercial setters and hatchers is very variable, mainly due to differences in air velocity and spraying. This raises two questions:

(i) Is it of practical importance, in other words, does it affect the embryo or the chick?
(ii) If it is important, can we do anything about it?

To answer the first question, yes, it is a very relevant factor affecting both hatch and chick performance. Practical experience shows that trying to control embryo temperatures between acceptable ranges will result in a better hatchability and especially a better chick quality (Wineland et al, 2000a). The influence on yolk uptake and closure of the navels is high, resulting in differences in first week mortality due to navel/yolk sac infections and e-coli infections. However, research also shows that a sub-optimal embryonic temperature has a negative influence on the development of the embryo, and with it on the performance of the resulting broilers (Lourens et al, 2005). It was shown that a difference in embryo temperature of 2oF resulted in a significant difference in embryo growth and feed conversion of broilers at 6 weeks of age (Gladys et al, 2000). It is also demonstrated that differences in embryo temperature resulted in a difference in development of both the whole chicken as also specific organs like the heart muscle. Wineland et al, 2000a; 2000b)

There is still a lot of research going on to better understand the influence of temperature on the development of the embryo. One of the involved factors seem to be the availability of oxygen for the embryo. If the embryo temperature is increased the metabolism of the embryo is increased as well, demanding for higher oxygen supply at the end of incubation. If this demand is not met, more negative consequences of the high temperature are observed than when there is enough oxygen available (Lourens et al, 2007).

The question on how to achieve an accurate and uniform embryo temperature is more difficult to answer, especially in existing machines. Because the major important factor for embryo temperature is air velocity and to a lesser extent the place and function of the humidifier, a substantial part of the problem is in the design of incubators and hatchers, and will challenge incubator manufacturers. With given incubators and setters, it is not always easy to find a good solution. When a good uniformity of temperature throughout the machine cannot be achieved, obtaining an adequate average is not of much use as part of the eggs will be too cold where others are to warm. When embryo temperature difference in a machine are as high as 4^{o}F or more as sometimes observed, it is difficult to find a good average

Once the embryo temperature is as uniform as possible, we have to adjust the air temperature as a factor of embryo temperature. At this moment we try to aim at a starting embryo temperature of 99.5oF-100.0oF and an end temperature of 100.0 – 100.5oF. It is questionable if this is optimal in every situation, but practical experiences show that at least it is a reasonable aim.

The influence of having a correct embryo temperature is high. Not only on hatchability, but especially on chick quality and broiler performance. Research has shown differences in feed conversion as high as 100 gram of feed for a 2 kg broiler (Gladys et al, 2000). If we calculate the number of birds produced by a single incubator per year, the costs of not being able to control embryo temperature to the desired level are enormous.

Especially with modern high-yielding breeds, which tend to have a high metabolic heat production during incubation, control of embryo temperatures is the key factor to get to a better hatchability and especially a better chick quality.

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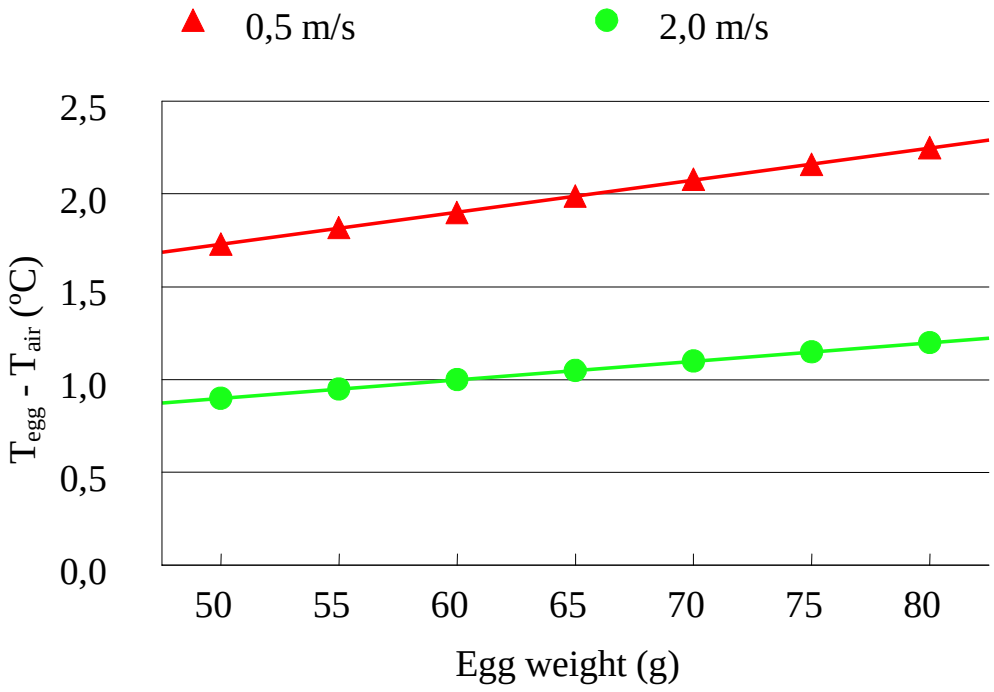

Figure 1 Theoretical relation between egg weight, air velocity and embryo temperature (metabolic heat production 4000 w/m3)
[Meijerhof and van Beek (1993)]

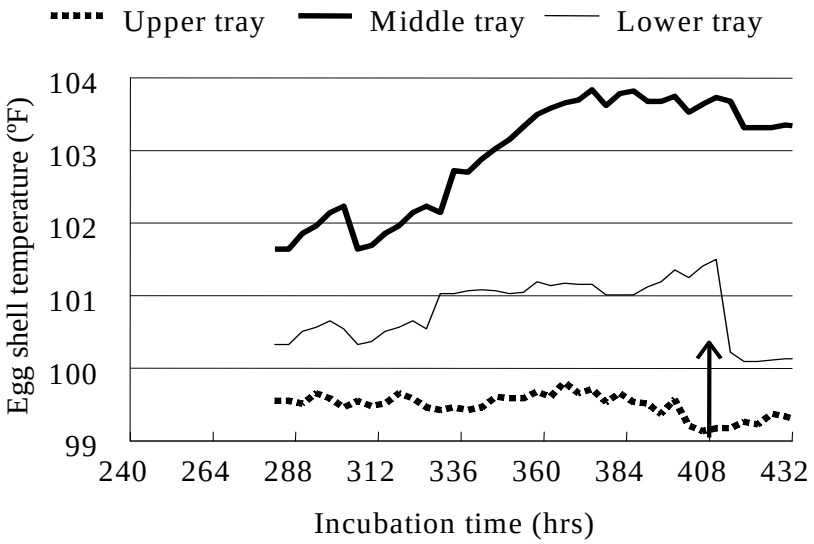

Figure 2 Egg shell temperatures in a commercial single stage setter
(Praktijkonderzoek Pluimveehouderij 99/1)

THE EFFECTS OF HATCHING EGG STORAGE

G.M. FASENKO[1]

Summary

In commercial poultry species, eggs are often cold stored until as they can be placed in an incubator. The cool temperature induces embryonic diapause, allowing the embryo to survive until it is incubated and the optimum temperature and humidity conditions for embryonic growth are provided. It is usually necessary to store eggs at both the breeder farm and at the hatchery. However, it is known that hatchability declines when eggs are stored for longer than seven days. On the cellular level, long term hatching egg storage causes cell death via both necrosis and apoptosis. This likely induces embryonic mortality, therefore resulting in decreased hatchability. Long term storage of hatching eggs also affects both embryonic development and embryonic metabolism. Previous research has shown that embryos from long term stored eggs do not initiate growth, even when optimum temperature and humidity conditions are provided.

I. INTRODUCTION

Due to the fact that most hatching egg farms are located at considerable distances from each other as well as from the hatchery, transportation of the eggs from the farm to the hatchery (via environmentally controlled trucks) does not occur on a daily basis. Because of this eggs are cold stored at the breeder farm for approximately three days. Once the eggs are transported to a hatchery, they are stored again. The length of time which eggs will be stored at the hatchery depends on the current supply and demand conditions in the industry. If the demand for hatchlings is high and there is space available in the incubators, the eggs will be set reasonably fast. If demand for hatchlings is low and there is a surplus of hatching eggs, the eggs will be stored in the cooler for a longer period. It is widely known within the poultry industry that storing eggs at cool temperatures for longer than seven days has a negative impact on hatchability. What follows in this paper is a review of various research that has examined how embryonic development and hatchability are affected by egg storage.

II. TEMPERATURE DURING EGG STORAGE

Whether at the breeder farm or the hatchery, hatching eggs are stored at temperatures below 21°C to inhibit further development of the embryo. The temperature below which embryonic development does not occur is called physiological zero. Different temperatures have been reported for physiological zero. Early research determined that the minimum temperature required for embryonic development was 21°C (Edwards, 1902). Forty years later, a higher temperature of 28°C was reported (Funk and Biellier, 1944). Fasenko et al. (1992) used microscopic techniques which classify embryonic development into stages (Eyal-Giladi and Kochav, 1976) to establish that storing fertile eggs at 14°C for different lengths of time stopped all embryonic development. However, Gupta and Bakst (1997) used a similar microscopic technique for turkey embryos (Gupta and Bakst, 1993) to determine that some embryonic development does occur in turkey embryos stored, for a range of storage lengths, at 15°C.

[1] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

The discrepancy between reported minimum temperatures for embryonic development may be due to the fact that different tissues within the embryo may have different minimum temperatures for development to be visible (Kaufman, 1948). The entire concept of what is perceived as physiological zero is also an exceptionally important factor which requires further research. It may be that there are cellular metabolic processes occurring when embryos are cooled, but that embryonic development is not readily observable using microscopic techniques. For this reason, the author of this paper recommends using the term embryonic diapauses as an alternative to the term physiological zero.

III. HOW EGG STORAGE AFFECTS HATCHABILITY

It is well known that hatchability is significantly reduced in domestic fowl when eggs are stored for longer than seven days (Scott, 1933; Asmundson, 1947; Kosin, 1950; Becker, 1963; Merritt, 1964; Sittman et al., 1971; Whitehead et al., 1985; Fasenko et al., 2001a,b). In research conducted by the author of this paper, eggs were stored for two, four, six, eight, 10, 12, 14, and 16 days, and it was found that storage for less than eight days had no significant effect on hatchability. However, hatchability was significantly lower in eggs stored for eight, 12, and 16 days (Fasenko and Robinson, 1999).

IV. HOW EGG STORAGE AFFECTS EMBRYONIC MORTALITY

It has been known for several decades that embryonic mortality is negatively impacted by egg storage (Merritt, 1964; Arora and Kosin, 1966; Sittman et al., 1971; Mather and Laughlin, 1976). It has also been found that embryonic mortality can be measured even before incubation begins, and that mortality increases with storage length (Fasenko et al., 1992). In an examination of broiler hatching eggs, total embryonic mortality increased in eggs stored for 14 days (27.7% mortality) compared to eggs stored for four days (10.7% mortality) (Fasenko et al., 2001b). In turkey eggs stored for four days (22.9%) compared to 14 days (29.3%), a similar pattern was observed (Fasenko et al., 2001a).

V. CHANGES IN EMBRYONIC DEVELOPMENT DUE TO STORAGE

Storing eggs long-term increases the incubation period that is needed for a chick to hatch (Kosin and Konishi, 1973; Mather and Laughlin, 1976, 1977). There are two possible reasons that have been suggested for this increase in incubation time. Some research (Arora and Kosin, 1966) has shown that embryonic development in long-term stored eggs does not start immediately even after proper incubation conditions are provided. Other research (Mather and Laughlin, 1977) has shown that the embryonic development of embryos from eggs stored for 14 days lags behind the development of embryos from unstored eggs by approximately 12.2 hours. In addition, the embryonic development progressed at a slower pace in embryos of stored eggs compared to embryos from unstored eggs.

Research undertaken by Fasenko and Robinson (1998) showed both of these hypotheses to be correct. In this research, the biological age of embryos from eggs stored for 14 days was found to lag behind that of embryos from eggs stored for four days (Fasenko and Robinson, 1998). This occurred despite the fact that the chronological ages of the embryos examined were the same. When embryonic development was examined at three hour intervals for the first 12 hours of incubation, the development of embryos from eggs stored for 14 days was observed to lag behind the development of eggs stored for four days as soon as six hours into incubation. The researchers also observed that embryos did not all respond

to long-term storage in the same way. After being exposed to incubation temperatures for 12 hours, some embryos had still not initiated development. Other embryos began to develop, but at a reduced rate compared to embryos from short-term stored eggs. However, some embryos from long-term stored eggs did develop at the same rate as embryos from short-term stored eggs. Identifying the factors that are able to provide these embryos with resistance to long-term storage is critical to alleviating the negative impacts of long-term egg storage.

VI. THE RELATIONSHIP BETWEEN EMBRYONIC DEVELOPMENT AND SURVIVAL

Hays and Nicolaides (1934) were the first to link embryonic development at the time of oviposition and hatchability with the ability to survive cold egg storage. They found that hens with a consistent record of high hatchability laid eggs with blastoderms that were at more advanced stages of development at the time the eggs were laid. Building on this observation, Kosin, (1956) looked at the effects of incubating turkey and chicken eggs prior to storage. In both chickens and turkeys it was found that if eggs were incubated prior to storage (of at least seven days) hatchability was improved.

Based on this finding, further research was undertaken by Fasenko et al (2001a). Hatchability was found to decline when turkey breeder eggs were stored for 14 days (64.4%) compared to four days (70.9%) (Fasenko et al., 2001a). However, when the eggs were first incubated for 12 hours prior to 14 days of storage, hatchability was improved (70.9%) and the embryos had developed to the stage where hypoblast formation was complete. In broiler breeder eggs, hatchability was lower in eggs stored for 14 days (72.2%) compared to eggs stored for four days (89.7%). Hatchability in eggs stored for 14 days improved significantly (78.1%) when eggs were incubated for six hours prior to storage (Fasenko et al., 2001b). After six hours of pre-storage incubation the broiler embryos were at the same stage of development as the turkey embryos after 12 hours of pre-storage incubation (hypoblast formation was completed). This study also found that the improved hatchability was not simply due to providing extra incubation time for the embryos to hatch, since when eggs were incubated for 18 hours prior to being stored for 14 days, hatchability was dramatically reduced to 11.5% (Fasenko et al., 2001b).

This research provides evidence that there are certain stages of embryonic development at which embryos have an improved ability to survive cool storage. The author of this proceedings has put forth the hypothesis that when embryos have completed hypoblast formation, they may be at a relatively inactive developmental period. Thus, when cold storage is imposed at this stage of development, the embryos are not undergoing active periods of cellular division, metabolism or differentiation, which are more vulnerable to reduced temperatures.

One practical consideration for pre-storage incubation is that breeder farms are not equipped with incubators. Since all the above studies incubated eggs prior to any storage, logistically, the eggs would have to be incubated at the breeder farm. Thus, an additional study was conducted by the author of this paper to determine if the same effects could be achieved by incubating eggs after three days of storage, but before they are stored at the hatchery (unpublished data). It was found that incubating eggs after three days of storage and prior to an additional 11 days of storage did not improve hatchability. Thus the beneficial effects of pre-storage incubation only have an effect when the eggs are incubated prior to any storage occurring.

VII. WHERE WE GO FROM HERE? THE EFFECT OF EGG STORAGE ON EMBRYONIC ABNORMALITIES, CELL NECROSIS, AND APOPTOSIS

I has been determined that the number of necrotic cells increases as the length of the egg storage increased (Arora and Kosin, 1968). More recently, it has been demonstrated that the number of apoptotic cells (cells programmed to die) increases in eggs stored for 14 days (Bloom et al., 1998). Preliminary research conducted by the author of this paper has found that the proportion of apoptotic cells is increased in long-term stored eggs compared to short-term stored eggs (unpublished data). It is hypothesized that when eggs are exposed to long term cool storage the number of necrotic (dead) cells may be increased, and embryonic cells may be undergoing inappropriately regulated apoptosis. Either way, the result would be an increase in the ratio of nonviable to viable embryonic cells. This is important as there is likely an optimum number of viable cells that is required for normal growth and development.

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DISPOSAL AND TREATMENT OF HATCHERY WASTE

P.C.GLATZ[1] and Z.H. MIAO[1]

A project funded by the Rural Industries Research and Development Corporation Chicken Meat Program was undertaken to identify how hatchery waste is currently managed by major chicken meat hatcheries in Australia. In addition a literature review was conducted to identify alternate methods of handling and processing of hatchery waste.

A weekly average of 10.4 tonnes of waste is produced by chicken meat hatcheries in Australia. The average cost of disposal is A$127 per tonne. An emerging issue is the decline in the availability of disposal sites. The majority of hatchery waste is sent to landfill or for composting with some rendered for use as pet food. Hatchery wastewater is mostly used for irrigation or disposed directly into the sewer. Most of hatcheries have no environmental issues with hatchery waste on site but some report odour problems. Some hatcheries would like to treat the waste on site so that it could be sold as a commodity or to use methods to separate liquid from solid waste and recycle water.

The hatchery waste literature review (Glatz and Miao, unpublished) identified that waste can be separated into solid and liquid waste by centrifuging or by using screens. Potential methods for treating hatchery waste on site include use of a furnace to heat the waste to produce steam to run a turbine generator or to use an in line composter to stabilise the waste. There is also potential to use an anaerobic digester system at hatcheries to produce methane and designer fertilisers. Hatcheries disposing wastewater into lagoons could establish a series of ponds where algae and zooplankton utilise the nutrients in the wastewater. After the pond treatment the water is cleaner making it more suitable for irrigation.

The ideal system to establish in a hatchery would be to incorporate separation and handling equipment to separate waste into its various components for further treatment. This would save disposal costs, produce biogas to reduce power costs at plants and produce a range of value added products. However, the scale of hatchery operations in Australia is too small to contemplate development of a hatchery waste treatment system on site.

[1] Pig and Poultry Production Institute, South Australian Research and Development Institute, Roseworthy Campus, SA, Australia, 5371

THE EFFECT OF COMMERCIAL PRODUCTION METHODS ON THE CARBOHYDRATE AND AMINO ACID COMPOSITION OF CORN DISTILLERS DRIED GRAINS WITH SOLUBLES

M. CHOCT[1] and S.T. PETERSEN[2]

Summary

Six samples from ethanol plants that used different processing conditions were obtained from the Midwest, USA and were analysed for nutrient composition, focussing on the contents of carbohydrates, including starch, non-starch polysaccharides (NSP) and free sugars. The main differences in the processing conditions were temperature, dryer type and duration of drying. The samples varied widely in protein, fat, ash and free sugar contents, with the minima and maxima being 24.2% and 42.2% for crude protein, 2.1% and 16.6% for fat, 3.7% and 10.7% for ash, and 1.4% and 8.3% for free sugars. The total carbohydrate contents averaged around 40% with total NSP and starch both at around 18%, remaining relatively stable across the six samples. Most of the NSP was insoluble with less than 10% being soluble. The main sugars making up the NSP were glucose, xylose, arabinose, galactose and mannose in decreasing order. The essential amino acid levels, when expressed relative to the crude protein level, were similar among all samples. It may be concluded that the major differences in the quality of DDGS samples arise mainly from differences in the protein, ash and fat levels.

I. INTRODUCTION

During the past several years, distillers dried grains plus solubles (DDGS) has become a major feed ingredient in North America as a result of ethanol production from corn. This is because ethanol fermentation utilises the starch component, leaving other components in the grain, such as oil, protein, fibre and minerals, relatively enriched. This by-product's suitability as animal feed and its effects on animal performance, feed efficiency and gut health in livestock have been the subject of many recent studies (Shurson and Whitney, 2004; Ward et al., 2008). However, what has been lacking is a more in-depth analysis of DDGS from various ethanol production methods. Perhaps the difficulty in predicting quality relates to the analysis of the carbohydrates, particularly the NSP present in the fibre fraction. Therefore, we obtained six DDGS samples from the Midwest, USA and analysed them for major nutrient constituents, including the composition of fibre, ie, the soluble and insoluble NSP, as well as starch and free sugars.

II. MATERIALS AND METHODS

a) Sample collection

Samples of corn DDGS were collected from six dry-milling ethanol plants across the Midwest, USA that produced different quality levels of DDGS according to marketing standards as follows: Sample 1, classified as Good, was dried using a Bar-Rose ring type dryer at an exhaust temperature of 230ºF for approximately 45 min; Sample 2, classified as Excellent, was dried using two rotary drum dryers where the 1st dryer reduced the moisture from 65% to 40% at 800ºF over 10 min, and then the 2nd dryer added syrup and reduced the moisture further to 11% at 825-850ºF initially and down to 170-210 ºF over a 10-minute

[1] Australian Poultry CRC, P.O. Box U242, University of New England, Armidale, NSW 2351, Australia
[2] Land O'Lakes Purina Feed LLC, P.O. 64281, St. Paul, MN 55126, USA

period; Sample 3, classified as Good, was dried using an ICM rotary dryer at an exhaust temperature of 700 ºF initially, then down to 500ºF over 5-15 min; Sample 4, classified as Average, was dried using a Bar-Rose ring type dryer at 850-900ºF over 40 sec; Sample 5, classified as Good, was dried using two rotary drum dryers where the corn entered the 1st dryer at 850–900ºF and exited it at 180-190ºF over 15 min, and then it entered the 2nd dryer at 650-700 ºF and exited it at 190-205ºF over 15 min; Sample 6, classified as Excellent and Hi-Protein, was dried using a rotary drum dryer at 250ºF for approximately 40 min.

b) Nutrient analyses

Total ash, fat and moisture were analysed using the AOAC procedures (Method 945.18). Total starch was measured using the AOAC Method 996.11, and soluble and insoluble NSP levels were determined using the AOAC Method 994.13.

c) Amino Acid Analysis

Amino acids were analysed by Evonik Degussa (Hanau, Germany) using near-infrared spectroscopy for Sample 1-5 and via HPLC methods for Sample 6, as described by Llames and Fontaine (1994) and Fontaine et al. (1998).

III. RESULTS AND DISCUSSION

The proximate composition of the samples is shown in Table 1. The total value ranged from 97.4% in Sample 6 to 105% in Sample 2. It is understood that corn has other components, such as phenolics and pigments, which are not accounted for in the proximate composition presented here. Lignin, for example, the major part of the phenolics in DDGS, has been reported to range from 2-4% (Dong and Rasco, 2006). Thus, there appears to be a 5-10% overestimation for most of the samples.

The samples varied widely in protein, fat, ash and free sugar contents, with Sample 6 having the minima and maxima in all four nutrients (highest for protein and lowest for the others). Unfortunately, most studies fail to characterise the carbohydrates in DDGS. The current paper focussed on the carbohydrate contents of DDGS produced in different plants. Indeed, in the current study the total NSP and starch contents, both at around 18%, remained relatively stable across the six samples. Together with the free sugars (carbohydrates of less than 10 sugar units), the NSP and the starch make up the carbohydrates and amount to 40% of DDGS in this study. Most of the NSP is insoluble with less than 10% being soluble. The predominant constituent sugars for the NSP were glucose, xylose, arabinose, galactose and mannose in decreasing order (data not shown).

It is surprising to find that all the DDGS samples examined in the current study contained nearly 20% starch. This means that approximately 30% of the total starch in corn is left unfermented during ethanol production, suggesting that the current method of producing ethanol from corn starch is very inefficient. It is possible that most of the starch remaining in the DDGS is resistant starch, which behaves like NSP and escapes fermentation. Unfortunately, the current study did not measure the level of resistant starch in the samples. It is also worth noting that Sample 6 had the highest level of crude protein and the enrichment of protein came at the expense of fat, ash and free sugars rather than starch or NSP. This is because this particular sample is from a new ethanol process that aims to remove the oil for bio-fuel. This leaves a hi-protein DDGS product which will become increasingly available in the market. Unfortunately, further details of the process are confidential and not available for publishing.

Table 1 Proximate Composition of six DDGS samples (%)

Sample Number	Moisture	Ash	CP	Starch	Total NSP	Free Sugars	Fat	Total
1	14.4	10.7	28.2	17.8	14.6	4.7	9.2	99.5
2	14.5	7.3	24.2	22.4	17.4	8.3	10.9	105.0
3	15.0	9.7	28.1	16.9	18.8	4.3	8.6	101.4
4	11.1	9.1	25.1	16.7	17.4	3.9	16.4	99.5
5	9.8	7.9	28.8	15.5	18.9	3.8	15.4	100.1
6	9.0	3.7	42.2	19.7	19.3	1.4	2.1	97.4
Mean	12.3	8.1	29.4	18.2	17.7	4.4	10.4	100.5
Min	9.0	3.7	24.2	15.5	14.6	1.4	2.1	97.4
Max	15.0	10.7	42.2	22.4	19.3	8.3	16.4	105.0

The essential amino acid contents of the samples are shown in Table 2. The total protein contents determined using the NIRS at Degussa and the wet chemistry method at UNE agreed closely. A statistical evaluation of the data was not possible due to lack of replicates. However, in general, the amino acid levels, when expressed as a percentage of CP on dry matter basis, did not differ among the samples, although Sample 6, which was analysed using the wet chemistry method, had higher numerical values for MET, M+C, LEU, and PHE and lower numerical values for ARG and LYS compared with the NIR analysed samples, ie, Samples 1-5. These discrepancies probably reflect the differences in the analytical methods used rather than intrinsic differences between Sample 6 and the other samples.

It may be concluded that the current production method(s) for ethanol from corn is inefficient, leaving a large amount of starch as well as a considerable amount of low molecular weight sugars in the DDGS unfermented. There are also major differences in the chemical composition of DDGS samples, which arise mainly from differences in the protein, ash and fat levels.

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Table 2 Individual amino acid contents (%) of five DDGS samples obtained using Near Infrared Reflectance Spectroscopy (NIRS) and of one sample using chemical analysis

	No.	DM	CP	MET	CYS	M+C	LYS	THR	TRP	ARG	ILE	LEU	VAL	HIS	PHE
							As is basis								
NIRS															
	1	88.95	29.78	0.56	0.53	1.09	0.88	1.13	0.24	1.32	1.10	3.44	1.44	0.78	1.44
	2	88.96	24.21	0.45	0.45	0.91	0.67	0.91	0.20	1.08	0.85	2.77	1.13	0.65	1.16
	3	88.62	29.36	0.55	0.52	1.07	0.84	1.09	0.21	1.20	1.09	3.38	1.42	0.74	1.46
	4	90.76	26.69	0.51	0.50	1.02	0.81	1.01	0.22	1.21	0.98	2.99	1.30	0.72	1.28
	5	92.20	29.20	0.53	0.50	1.04	0.86	1.10	0.23	1.28	1.10	3.39	1.43	0.76	1.45
NIRS							g/100g CP on DM Basis								
	No.	DM	CP	MET	CYS	M+C	LYS	THR	TRP	ARG	ILE	LEU	VAL	HIS	PHE
	1	100.00	33.48	1.88	1.78	3.66	2.96	3.79	0.81	4.43	3.69	11.55	4.84	2.62	4.84
	2	100.00	27.21	1.86	1.86	3.76	2.77	3.76	0.83	4.46	3.51	11.44	4.67	2.68	4.79
	3	100.00	33.13	1.87	1.77	3.64	2.86	3.71	0.72	4.09	3.71	11.51	4.84	2.52	4.97
	4	100.00	29.41	1.91	1.87	3.82	3.03	3.78	0.82	4.53	3.67	11.20	4.87	2.70	4.80
	5	100.00	31.67	1.82	1.71	3.56	2.95	3.77	0.79	4.38	3.77	11.61	4.90	2.60	4.97
Mean		100.00		1.87	1.80	3.69	2.91	3.76	0.79	4.38	3.67	11.46	4.82	2.62	4.87
STD				0.04	0.07	0.10	0.10	0.03	0.05	0.17	0.10	0.16	0.09	0.07	0.09
Wet Chemistry															
As is	6	91.50	42.23	0.92	0.77	1.70	1.09	1.59	n/a	1.49	1.67	5.90	2.04	1.13	2.27
DM g/100g CP		100.00	46.15	2.18	1.82	4.03	2.58	3.77	n/a	3.53	3.95	13.97	4.83	2.68	5.38

INFLUENCE OF PELLETING TEMPERATURE ON THE PERFORMANCE AND NUTRIENT UTILISATION OF BROILER STARTERS

M.R. ABDOLLAHI[1], V. RAVINDRAN[1], T.J. WEBSTER[1], G. RAVINDRAN[1] and D.V. THOMAS[1]

Published data on the effect of pelleting temperature on poultry performance are limited (Creswell and Bedford, 2006). The objective of present study was to examine the effect of pelleting temperature on the performance of broiler starters fed maize- and wheat-based diets. The experimental design was a 2 × 3 factorial arrangement of treatments, involving two grain types (maize and wheat) and three pelleting temperatures (60, 75 and 90 ºC). Two diets, based on maize or wheat, were formulated to meet the Ross 308 strain recommendations for major nutrients for broiler starters. Both diets were formulated to be isocaloric and isonitrogenous. Each formulated diet was divided to three equal batches and pelleted in a pellet mill at three temperatures (60, 75 and 90 ºC). The pelleting temperature was measured at the outlet of the conditioner. All diets contained titanium oxide as an indigestible marker. Each of the six dietary treatments was offered ad libitum to six replicate cages (8 birds per cage). Body weights and feed intake were recorded at weekly intervals throughout the 21-day trial. From day 17 to 20, feed intake and excreta output were measured quantitatively per pen for the determination of apparent metabolisable energy (AME). On day 21, ileal digesta were collected for determination of apparent ileal digestibility of nitrogen and starch. Pellet durability index (PDI) was determined in a Portable Pellet Tester (New Holmen Pellet Tester, TekPro Ltd., Norfolk, UK).

Table 1 Effects of pelleting temperature on broiler performance (1-21 days post-hatching) and PDI

Grain type	Pelleting temperature (ºC)	Weight gain (g)	Feed intake (g)	Feed per gain (g/g)	PDI (%)
Maize	60	1040^{a}	1272^{ab}	1.228	81.4^{ab}
	75	960^{b}	1203^{c}	1.265	81.6^{ab}
	90	1015^{a}	1279^{a}	1.261	79.3^{ad}
Wheat	60	1021^{a}	1338^{d}	1.315	76.7^{d}
	75	925^{c}	1235^{bc}	1.344	82.7^{bc}
	90	908^{c}	1255^{ab}	1.383	85.1^{c}
SEM		11.6	14.9	0.009	0.94

$^{a-d}$Means in a column without a common superscript are significantly different ($P < 0.05$).

The performance of broiler starters was influenced ($P < 0.05$) by pelleting temperature. In general, the birds fed diets pelleted at 60 ºC consumed more feed, grew faster and were more efficient than those fed diets pelleted at 75 and 90 ºC.

[1] Institute of Food, Nutrition and Human Health, Massey University Palmerston North, New Zealand.

However, a significant ($P < 0.05$) grain type x pelleting temperature interaction was observed for weight gain and feed intake due to birds fed the maize-based diet pelleted at 90 ºC having greater gain and intake than those fed the maize-based diet pelleted at 75 ºC. Cowieson et al. (2005) also found that increasing conditioning temperature from 80 to 90 ºC resulted in lower weight gains and poorer feed efficiency.

Pellet quality was not affected ($P > 0.05$) by grain type. But an interaction ($P < 0.05$) between grain type and pelleting temperature was observed for PDI. Increasing the pelleting temperature improved PDI in wheat-based diets, but no effect on PDI in maize-based diets.

Table 2 Effects of pelleting temperature on the ileal digestibility coefficients of starch and nitrogen, and AME for broiler starters

Grain type	Pelleting temperature (ºC)	Ileal nitrogen digestibility	Ileal starch digestibility	AME (MJ/kg DM)
Maize	60	0.843[a]	0.970[a]	14.16
	75	0.819[b]	0.975[a]	14.19
	90	0.848[a]	0.976[a]	14.29
Wheat	60	0.823[b]	0.933[b]	13.08
	75	0.811[bc]	0.922[b]	13.18
	90	0.802[c]	0.898[c]	13.10
SEM		0.006	0.006	0.071

[a-c]Means in a column without a common superscript are significantly different ($P < 0.05$).

In general, ileal nitrogen digestibility was lowered ($P < 0.05$) at higher pelleting temperatures. The only exception was the higher nitrogen digestibility of maize-based diet pelleted at 90ºC than that pelleted at 75 ºC, resulting in a significant grain type x pelleting temperature interaction. A significant grain type x pelleting temperature interaction ($P < 0.05$) was also observed for ileal starch digestibility. In maize-based diets, pelleting temperature had no effect on the starch digestibility. Starch digestibility, however, was lowered in the wheat-based diet pelleted at 90 ºC compared to those pelleted at 60 and 75 ºC. Treatments had no effect ($P > 0.05$) of the AME values. Overall, the present data demonstrate that increasing the pelleting temperature from 65 to 90 ºC has adverse effects of the performance of broiler starters fed wheat- and maize-based diets.

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EFFECTS OF GRAIN SOURCE, COMMINUTION TECHNIQUE AND PARTICLE SIZE ON NUTRITIVE VALUE, FEED UTILIZATION AND GROWTH OF BROILER CHICKENS

M.M. BHUIYAN[1], P.A. IJI[1] and L.L. MIKKELSEN[1]

It is generally thought that smaller particle size will increase the surface area and improve access to digestive enzymes for digestion of nutrients (Waldroup, 1997). The influence of particle size appears to be confounded by the complexity of the diet and nature of feed processing, such as milling, pelleting and crumbling (Goodband et al., 2002). In the present study, a 2 x 2 x 3 factorial experiment was designed to study the effect of milling technique (hammer versus roller) with differing particle size (fine or coarse) of three cultivars of maize (Down, Emerald or Moree) on nutrient composition, growth performance, ileal digestibility and intestinal microbial population of broiler chickens.

A total of 420 day-old male Cobb broiler chicks were randomly allocated to 12 treatments of 5 replicates (7 birds per replicate) in brooder cages set up in an environmentally controlled room. An indigestible marker, Celite, was incorporated to enable assessment of nutrient digestibility. Temperature was regulated according to standards while feed intake and body weight were monitored on a weekly basis. On days 7 and 21, samples were taken for assessment of visceral organ weight, digesta pH, microbial profiles and nutrient digestibility. All data were analyzed using the general linear model.

Table Digestibility of nutrients at 21 days of age

	Protein	Energy	Starch
Effect of maize varieties			
Downs	0.80	0.72^{b}	0.94^{b}
Emerald	0.81	0.76^{a}	0.96^{a}
Moree	0.81	0.76^{a}	0.97^{a}
Effect of Particle size			
Coarse	0.79^{b}	0.73^{b}	0.96
Fine	0.82^{a}	0.76^{a}	0.96
SEM	0.004	0.006	0.004

There was no significant difference in body weight gain, feed intake or FCR at 21d of age due to maize variety, milling, particle size or their interactions. Liver weight was higher ($P < 0.01$) on coarse particle size diet than on the fine particle diet while bursa weight was increased ($P < 0.05$) in chicks on the fine particle diet. Energy and starch digestibility was highest ($P < 0.01$) in the diets based on Emerald and Moree varieties. Protein and energy digestibility was increased ($P < 0.01$) due to particle size reduction. The microbial profiles of chickens were not affected by the main effects or their interactions.

In conclusion, the present study indicates that grinding some varieties of maize would be beneficial in terms of improvement in nutrient digestibility.

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[1] School of Envirnoment & Rural Science, University of New New England, Armidale NSW 2351

IMPACT OF MANNO-OLIGOSACCHARIDES AND DIETARY THREONINE ON FEED PASSAGE RATE IN BROILER CHICKENS

S.H.CHEE[1], P.A. IJI[1], M.CHOCT[1], L.L. MIKKELSEN[1] and A. KOCHER[2]

It was postulated by Ferket *et al*. (2002) that the decrease in thickness of intestinal external muscularis in relation to the feeding of manno-oligosaccharides (MOS) is likely to be associated with an overall improvement in gut viability where there is a reduced need for gut motility to control gut microbial activities. Therefore, the objective of this study was to evaluate the interactive effects of MOS and dietary threonine on feed passage rate.

A 3 x 2 factorial experimental design was used to investigate the interaction between threonine levels at 70, 100 and 130% of NRC (1994) recommendations and MOS (Bio-MOS®, Alltech Inc.) at 0 and 2 g/kg on feed passage rate. Ninety six day-old male Cobb broiler chicks were group-brooded according to treatment in a multi-tier brooder for 14 days. At d 15, 72 and 24 birds were reallocated and randomly assigned to the six treatment groups kept in metabolic and individual cages, respectively. Birds kept in the metabolic cages, comprising 4 replicates per treatment with 3 birds per replicate, were used for the assessment of feed passage rate using cumulative and non-cumulative titanium dioxide (TiO_2) excretion methods as described by Ferrando *et al.* (1987). Times of 1% (T_1) and 50% (T_{50}) excretion, which were the respective times required to excrete 1 and 50% of the marker administered were determined for each replicate. T_1 is regarded as the time (h) taken for the first appearance of TiO_2 and T_{50} is the total tract mean retention time (h). The individually caged birds with 4 replicates per treatment were used for the evaluation of mean retention time (h) as described by van der Klis *et al.* (1990) assessed by intestinal region of duodenum, jejunum and ileum.

Estimations of the first appearance and total tract mean retention time as indicated by the excretion time for 1 and 50% TiO_2 (T_1 and T_{50}) averaged at 2.33 and 6.21 h, respectively. There was no interaction between MOS and dietary threonine on total tract transit (first appearance) time. However, MOS tended ($P = 0.08$) to interact with dietary threonine to reduce the time taken to excrete 50% of the TiO_2 (T_{50}) with the shortest retention time noted in birds given the adequate-threonine plus MOS diet. In addition, MOS also interacted with dietary threonine to reduce ($P<0.01$) the ileal mean retention time with shortest transit time of 1.61 h noted in MOS-treated birds fed with adequate level of dietary threonine. Although the increase in ileal transit time induced by MOS and threonine interaction is somewhat unexpected and contradicts with the postulations made by Ferket *et al*. (2002) and Biggs and Parsons (2007), an increase in ileal feed passage rate is arguable. It is likely that an increased passage rate through the distal part of the digestive tract may favour more the control of intestinal microbial activities than the reduction of time available for digestion.

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[1] School of Environmental and Rural Science, University of New England, Armidale NSW 2351
[2] Alltech Biotechnology P/L 64-70 Nissan Drive, Dandenong South, Vic 3175

WATER HOLDING CAPACITY OF WHEAT MAY BE INDICATIVE OF VOLUNTARY FEED INTAKE IN BROILER CHICKS

P.H. SELLE[1], K. GERMAINE[2] and T. MIDDLEBROOK[3]

Summary

The *in vitro* water holding capacities (WHC) of 32 wheat samples, which had been previously evaluated in a series of broiler bioassays, were determined. There were positive correlations between wheat WHC and voluntary feed intakes in non-supplemented diets ($r = 0.245$; $P < 0.02$) and in diets ($r = 0.205$; $P < 0.05$) to which phytase plus xylanase were included. In non-supplemented diets, wheat WHC capacities were also positively correlated to energy intake ($P < 0.001$), dietary AME ($P < 0.005$) and weight gain ($P < 0.01$), but were not significantly related to feed efficiency ($P > 0.35$). Phytase plus xylanase significantly increased weight gain (12.0%), feed intake (7.8%), feed efficiency (5.1%), dietary AME (11.55 versus 11.02 MJ/kg) and energy intake (0.489 versus 0.427 MJ/day). Responses to enzymes appeared more pronounced in diets based on 'low-WHC' wheats. These preliminary findings suggest that wheat WHC may be indicative of feed intakes in broilers and may be predictive of weight gain and energy utilisation in broilers offered non-supplemented diets. Further research is justified to investigate the validity of wheat's WHC an indicator of broiler performance.

I. INTRODUCTION

The rate at which wheat is hydrated in the gastrointestinal tract may be partially responsible for variation in voluntary feed intakes of broilers and the *in vivo* hydration rate may be related to the *in vitro* WHC of what (Scott, 2004; 2005). Increases in WHC of celluloses have been shown to accelerate digesta passage rates in rats (Kikuchi and Yajima, 1992) so wheat with a high WHC and a rapid hydration rate may promote increases in gut transit rates and feed intakes, and, in turn, enhanced growth performance of broiler chickens. An initial assessment indicated that wheat WHC was correlated to feed intakes in broilers. Therefore, for verification, the WHC of archival samples of 32 wheats, which had been previously assessed in broiler bioassays as part of a RIRDC-funded project (Scott, 2006), was determined in order to compare wheat WHC with the data already generated in broilers.

II. MATERIALS AND METHODS

Archival samples of 32 wheats were successfully retrieved and their WHC determined. Samples were ground through a 1 mm sieve and weighed into centrifuge tubes with 50 ml deionised water. After stirring the samples were left to hydrate for 15 minutes and then centrifuged at 3000 rpm for 10 minutes. Then the water was decanted and the tubes re-weighed to calculate WHC by the following equation:

$$\text{WHC} = \frac{[\text{tube weight with wheat} - \text{empty tube weight}] - \text{sample weight (g)}}{\text{sample weight (g)}}$$

[1] Faculty of Veterinary Science, The University of Sydney. Camden NSW 2570.
[2] Weston Technologies. Braidwood Street, Enfield NSW 2136
[3] Weston Animal Nutrition. Braidwood Street, Enfield NSW 2136

In the broiler bioassays, each wheat had been hammer-milled and incorporated into mash diets at 750 g/kg, which were sequentially offered to three replicates of six male birds per pen from 4-17 days post-hatch. Other dietary components (g/kg) included soy protein isolate (69), corn gluten meal (50) and fish meal (60). The effects of these wheat-based diets, without and with an enzyme combination at standard inclusion rates (Phyzyme phytase plus Avizyme 1302 xylanase: Danisco Animal Nutrition), on growth performance and energy utilisation had been determined, as described by Scott (2006). An SPSS® Inc. statistical program was used to analyse the data from the three replicated broiler bioassays to complete analyses of variance and establish Pearson correlations between wheat WHC and performance parameters.

III. RESULTS

The mean values, and their ranges, of the relevant parameters from broilers offered non-supplemented diets are shown in Table 1. The 32 wheats had an average WHC of 0.816 g H_2O/g sample (coefficient of variation: 19.8%), which ranged from 0.701 to 1.654 units. As shown in Table 2, wheat WHC was significantly correlated ($r = 0.245$; $P < 0.02$) to feed intake of broilers offered non-supplemented diets and it is of interest that feed intake was not related to AME ($P > 0.30$). Wheat WHC was also positively correlated with weight gain ($r = 0.264$; $P < 0.01$), dietary AME ($r = 0.295$; $P < 0.005$), metabolisable energy intake expressed as MJ/day ($r = 0.425$; $P < 0.001$), but not feed efficiency ($P > 0.35$). Dietary inclusions of phytase plus xylanase significantly improved weight gain (12.0%), feed intake (9.3%), feed efficiency (5.1%), increased AME by 0.53 MJ/kg and energy intake by 14.5% from 0.427 to 0.489 MJ/day (Table 3). Also, it appears that enzyme responses were more pronounced in diets with low wheat WHC. However, following enzyme addition, while wheat WHC was still correlated to feed intake ($r = 0.205$; $P < 0.05$), it was no longer significantly related weight gain, AME and energy intake (Table 4).

Table 1 Mean and range of values for wheat WHC, growth performance and energy utilisation in broilers offered diets without enzymes

Item	Mean	Standard deviation	Minimum	Maximum
Water holding capacity (g H_2O/g sample)	0.816	0.1616	0.701	1.654
Weight gain (g/bird)	459	48.1	346	570
Feed intake (g/bird)	667	61.3	489	809
Feed efficiency (g/g)	1.48	0.120	1.16	1.81
AME (MJ/kg DM)	11.02	1.571	6.2	14.9
AME intake (MJ/day)	0.427	0.0751	0.223	0.681

Table 2 Pearson correlations (r) between wheat WHC and growth performance and energy utilisation in broilers offered diets without enzymes

	WHC	Weight gain	Feed intake	Feed conversion	AME (MJ/kg)	ME intake (MJ/day)
Gain	0.264					
	P = 0.009					
Intake	0.245	0.750				
	P = 0.016	P < 0.001				
Conversion	-0.092	-0.609	0.015			
	P = 0.371	P < 0.001	P = 0.883			
AME	0.295	0.162	-0.099	-0.308		
	P = 0.004	P = 0.114	P = 0.338	P = 0.002		
ME intake	0.425	0.601	0.508	-0.321	0.792	
	P < 0.001	P < 0.001	P < 0.001	P = 0.001	P < 0.001	1.000

Table 3 Main effects of enzyme supplementation (phytase plus xylanase) on parameters of growth performance, AME and energy intake (MJ/day)

Main effect	Gain (g/bird)	Intake (g/bird)	FCR (g/g)	AME (MJ/kg DM)	AME intake
Nil	459	667	1.48	11.02	0.427
Enzymes	514	719	1.40	11.55	0.489
SEM	22.48	31.43	0.0522	0.5571	0.0299
Significance (P =)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 4 Pearson correlations (r) between wheat WHC and growth performance and energy utilisation in broilers offered diets with phytase plus xylanase

	WHC	Weight gain	Feed intake	Feed conversion	AME (MJ/kg)	ME intake (MJ/day)
Gain	0.117					
	P = 0.256					
Intake	0.205	0.758				
	P = 0.045	P < 0.001				
Conversion	0.034	-0.576	0.029			
	P = 0.739	P < 0.001	P = 0.780			
AME	-0.025	-0.005	-0.151	-0.157		
	P = 0.809	P = 0.959	P = 0.143	P = 0.126		
ME intake	0.133	0.581	0.604	-0.232	0.664	
	P = 0.198	P < 0.001	P < 0.001	P = 0.023	P < 0.001	1.000

IV. DISCUSSION

This study lends some support to the proposition that WHC of wheat is predictive of voluntary feed intake in broilers offered diets based on 'raw' wheat, presumably by influencing gut passage rates. It is also possible that storage of the wheat samples may have influenced subsequent WHC determinations. Pirgozliev et al. (2003) also found that wheat WHC was positively correlated to feed intake (r = 0.443); however, unlike the present study, WHC was negatively correlated to AME (r = -0.488). In this study the diets were not steam-pelleted and excessive pelleting temperatures of wheat-based diets may compromise broiler performance by solubilising NSP and increasing gut viscosity (Cowieson et al., 2005). However, steam-pelleting partially gelatinises starch, which should increase WHC (Pinnavaia and Pizzirani, 1998). The related issue of particle size is of relevance as reductions in wheat bran particle size have been shown to diminish WHC (Cadden, 1987). Phytase plus xylanase inclusions reduced variation between wheats as coefficients of variation for AME were 14.3% without enzymes but 9.5% with enzymes. This would have contributed to the diminished WHC correlations in supplemented-diets. Also, the physical structure of wheat fibre is a determinant of the grain's WHC (Robertson and Eastwood, 1981) and NSP-degrading enzymes have been shown to reduce WHC of wheat bran (Aulrich and Flachowsky, 2001). Thus the impact of enzymes on fibre structure in wheat may have influenced its effective WHC in the gut. The WHC of sorghum also could be of interest as Buffo et al. (1998) reported several significant correlations between three criteria for water absorption properties and attributes of sorghum quality in 46 samples. There is the need to evaluate WHC of wheat (and sorghum) in conventional, enzyme-supplemented diets that have been steam-pelleted and the extent of starch gelatinisation determined over a range of grain particle sizes and pelleting temperatures. The outcome would be of considerable relevance if wheat WHC is shown to be significantly correlated to feed intake and broiler performance in this practical context.

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THE USE OF ALTERNATIVE RAW MATERIALS IN POULTRY FEEDS

A. LEARY[1] and K. FILER[2]

Summary

Over the past eighteen months the price of materials making up a large proportion of animal feeds has fluctuated dramatically, firstly increasing by as much as two to three times and lately decreasing although not to prices common two years ago. A consequence of these price fluctuations is a related variability in availability and quality of ingredients. To overcome these major challenges the feed industry must both optimise the digestibility of the ingredients already used in feed, but also consider the use of materials that in the past may have been thought alternate or unusual. The current paper will discuss the nutritive value, concerns and practical applications for two alternative feed materials, cassava (tapioca) and palm kernel meal.

I. INTRODUCTION

Corn and wheat prices continue to fluctuate as does total output. Grain prices have recently fallen from record highs and the future projections may not reach record highs, but prices are not expected to move much lower (www.world-grain.com). In addition to price, supplies continue to fluctuate as well. The total wheat output could reach record highs after several years of decline and corn output is expected to be below recent record levels. These fluctuations also occur at a time when uncertainty has caused global financial markets to post sharp declines. All of these factors have continued the interest in the use of alternative raw materials in poultry feeds. This interest is especially prevalent in Asia, particularly Southeast Asia, where a number of tropical plants produce potential sources of energy for poultry. Being able to utilise these materials would lessen reliance on wheat or corn imports and provide a more constant supply of grains.

II. CASSAVA

Cassava, also known as tapioca, is grown in Southeast Asia, tropical Africa and Central America. The plant is grown for starch production in human food as well as animal feed. Starch is deposited in the tuber or root portion of the plant. Chips and pellets are products that are utilised in the animal feed industry. Prime quality cassava products contain 70% starch, of which 80% is amylopectin. Cassava is low in protein (2%) and has low levels of amino acids and fat. The crude fibre content of cassava increases from about 2% to 5% as the quality decreases. With this increased fibre is a decrease in ME content as well. In broilers the reduction is from about 3,800 to 3,200 Kcal/kg and in swine the reduction is from 3,700 to 3,200 Kcal/kg.

A variety of studies have been performed to demonstrate the utilisation of cassava in animal feed over the past 30 years, although very few recent peer reviewed articles are available. Results indicate that cassava can be substituted for standard energy feeds including corn and wheat from 10% level to as high as 60% in broilers (Garcia and Dale, 1995). Cassava starch is completely digested in the intestine and the difference between faecal digestibility and ileal digestibility of dry matter is 3.4 and 4.4% for cassava chips and pellets

[1] Alltech Biotechnology Corporation, 209/1 K Tower B, Sukhumvit 21 Rd (Asoke), Bangkok, 10110
[2] Alltech Asia-Pacific Bioscience Centre, 113, Thailand Science Park, Pathumthai, Bangkok, 12120

compared to 13.6% for corn (Promtong et al., 2005b). Starch in cassava diet is primarily digested in the upper part of the small intestine and less is digested in the ileum. This compares to corn starch that is digested in the lower part of the small intestine and may not be completely digested. Changes in the position of starch digestibility have also been shown to lead to differences in gut morphology. Broilers on a cassava diet have been shown to have a larger percentage of butyrate in the volatile fatty acid profile as compared to birds on corn diets (Promtong et al., 2004). Increases in lactic acid bacteria have also been demonstrated in broilers as well as a lower pH in the digestive tract (Promtong, 2005a).

Cassava is low in protein content and requires protein supplementation in diets. Increased soybean meal (SBM) offers a method to supplement protein. SBM has high content of non-starch polysaccharides (NSPs) and phytates which impair the digestibility of nutrient in digestive tract of the animals. The use of enzymes to improve SBM is common practice. A few studies have demonstrated that enzymes can improve diets that are high in cassava (Kanto, 2008).

The use of cassava in layers has been evaluated as well. Egg weight for layers feed cassava (50.05 g) was similar to layers feed corn (50.55 g) and broken rice (50.30 g) (Khajaren and Khajarern, 1986). In layers fed a diet containing 36% cassava, feed intake was similar to a control diet with no cassava (113 versus 115 g/hen/day) and the mortality was reduced (0.14 versus 0.42%) (Saentaweesuk et al., 2000). The eggs produced by layers feed cassava diets tend to have a thicker egg white (Separattananan et al., 2005).

Although cassava has been shown to be a good energy source in broiler and layers, concerns exist that producers must be aware of. Cassava is low in protein and when feeding at high levels supplementation of protein is required. Full-fat soy can supplement the nutritional deficiencies of cassava in a feed formulation. Diets containing a large percentage of cassava are also low in fat and may cause hard fat accumulation in the animal. In order to avoid this, the diet can be supplemented with soft fat by adding full fat rice bran or full fat soybean meal. Some varieties of cassava also have high levels of hydrocyanic acid which is reduced during processing. Chronic cassava toxicity has been reported to lower egg production (Jalaudin and Leong, 1973). When included in layer diets, cassava does not contain pigment and the egg yolks and skin may be pale compared to standard diets. In order to prevent this, the diets can be supplemented with corn gluten meal, marigold meal or synthetic pigments.

III. PALM KERNEL CAKE

Palm kernel cake (PKC) is the major by-product of palm kernel oil extraction. PKC is obtained from the kernel after the oil has been extracted. It can supply both energy and protein. The protein content is low at 15–19% and the average amino acid availability is 64.4% (Yeong, 1981). It does have poor palatability and a high fibre content, crude fibre range is 13 to 16% (Jaafar, 1993). PKC contains a large amount of mannan and galactomannan. Of the carbohydrates in PKC, 81% are in the form of NSPs. The make up of the NSPs is 78% linear mannan with low galactose substitution, 12% cellulose, 3% arabinoxylan and 3% glucoronoxylans (Dusterhofi et al., 1992).

With such a large amount of NSP present the use of PKC in commercial diets has been limited although research studies have suggested levels as large as 20% can be utilized in broiler diets. Although the feed to gain was larger, the difference was not statistically significant when a diet with 10% PKC (2.85) or 20% PKC (2.92) was feed when compared to a control diet (2.77). In the same study, feeding at 30% and 40% PKC resulted in a significant increase in feed to gain (3.22 and 3.46) compared to control (Yeong, 1981).

In a study comparing 10%, 20% and 30% to a control diet a consistent decrease in average daily gain was seen as the level of PKC in the diets increased. Feed conversion was lowest on the control diet (2.35 ± 0.02^{ae}) and numerically increased at 10% (2.04 ± 0.01^{ce}), 20% (2.54 ± 0.02^{d}) and 30% (2.72 ± 0.02^{ce}) (Yusoff, 1982). A similar study at the same feeding levels showed a decrease in weight gain at a 20% and 30% PKC level compared to a corn soy control and a 10% PKC diet (Ahmad, 1982). Yeong and Mukhejee (1983) showed that supplementation of a broiler diet containing 20% PKC with 9% palm oil resulted in growth and feed efficiency similar to that obtained with the control diet.

For layers, Onwudike (1986) demonstrated that diets containing up to 34% PKC could be fed to starter pullets without any adverse effects on performance. He also showed that diets containing up to 38% PKC could be fed to grower pullets without affecting the rate of egg production, egg weight, weight of first egg dropped and feed intake. Longe (1984) found that layers fed a 20% PKC diet ate significantly more feed and produced fewer eggs than control birds. Layers were also less efficient in utilizing feed containing PKC. In layers, one study suggested that up to 25% could be feed without significant effect on egg production or daily egg mass (Chong et al., 2008). It was noted that high levels of PKC reduces the egg yolk colour and supplementation with pigments is required.

Enzymes have been used successfully to improve performance in the poultry industry for a number of years and offer a method to improve the performance characteristics of PKC. A limited number of studies have evaluated the use of enzymes with PKC. The components of PKC suggest that mannanase and cellulase could improve performance in poultry. A limited number of studies have evaluated the use of enzymes to improve the nutritional characteristics of PKC. A number of in vitro studies have suggested that mananase can improve the digestibility of palm kernel. Treating a poultry diet with 0.1% mannanase has demonstrated an increase in AME of 8.5% (Daud et al., 1997). In the same study cellulase supplementation increased the AME value numerically but not significantly. In a study investigating the use of multi-enzyme complexes that included mannanase and galactosidase, the enzyme complex numerically improved feed intake (818.4 versus 805.3 g/bird) and weight gain (596.8 versus 567.7 g/bird) in a diet that included 30% PKM compared to the same diet with no enzyme (Sundue and Dingle, 2003).

Additional concerns with PKC are related to the overall quality of product that can be purchased. Processing involves a large amount of heat and pressure and could lead to the formation of Maillard compounds between reducing sugars and amino acids. PKC can also become rancid quickly if it contains a large amount of oil residue from the processing. Poor processing can also lead to impurities such as shell and dirt in the product. As these concerns are related to processing it is important that PKC only be purchased from a producer that is able to supply a consistent quality product.

IV. CONCLUSION

The interest in feeding alternative raw materials has increased due to increased grain prices and supply issues. These raw materials commonly have feeding limitations. The limitations can usually be reduced if they are well understood. Cassava and palm kernel meal have demonstrated application in poultry feeds as replacements for corn. The nutritive value of these raw materials are well understood as are the limitations, which need not be barriers because it is possible to reduce or even eliminate the limitations. Work will continue on the use of alternative raw materials in poultry feeds but the information that has been generated has demonstrated they are viable replacements in standard diets.

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EFFECT OF GRAIN PARTICLE SIZE AND MILLING METHOD ON BROILER PERFORMANCE AND APPARENT METABOLISABLE ENERGY

N. RODGERS[1], P.A. IJI[2], L.L. MIKKELSEN[2], B. SVIHUS[3], H. HETLAND[3] and M. CHOCT[4]

Summary

An experiment was conducted to determine the effect of sorghum particle size and milling type on broiler performance and feed apparent metabolisable energy (AME). Results show that AME was improved by feeding a pelleted diet containing whole sorghum, but the best performance (lowest FCR) was elicited by feeding a rolled sorghum diet at a common commercial grind size. Feed particle size may influence the rate of excretion of different fractions of the digesta and AME of a feed. AME may not be an accurate indicator of the nutritive value of grain as the same feed can have a different AME values based on physical structure.

I. INTRODUCTION

A substantial quantity of work has been carried out to determine the effect of feed particle size on the gut development and performance of poultry with varied results (Amerah et al., 2007, Deaton et al., 1995, McIntosh et al., 1962, Nir et al., 1994, Proudfoot and Hulan, 1989). Most of the work on feed particle size for poultry has focussed on gut development, with some work on enzyme activity and digestibility (Svihus et al., 2004), GIT microbiology (Bjerrum et al., 2005, Engberg et al., 2002) and feed passage. Recent feed passage work, however, has focussed on insoluble fibre rather than feed *per se* (Hetland and Svihus, 2001, Hetland et al., 2003). The link between particle size, feed passage and AME and their influence on performance has not been studied with reference to milling type.

Comparison of mill type within particle size is not often considered in experimental design, and when mentioned previously (Deaton et al., 1989), the resultant mean grain particle size and distribution from each mill type was not equivalent. Work on the effect of milling type at an equivalent particle size is needed to adequately determine the effect of milling procedures on broiler performance and gut development.

The aims of this study were to gain a more thorough understanding of how feed particle size influences the performance of the broiler as influenced by AME and feed passage. The hypotheses were that a feed based on a coarse grain particle would coincide with higher AME and improved performance than when feeding a fine grain particle. Rolling grain was hypothesised to improve performance at the same geometric mean diameter as a hammer-milled grain.

II. MATERIALS AND METHODS

An experiment was conducted at the Agricultural University of Norway's Department of Animal and Aquacultural Sciences on campus. Feed was processed at the Agricultural University Center for Feed Technology Ltd. (FôrTek). Diets (Table 1) for four treatments were made, differing only in mean particle size and processing method used to modify the

[1] Alltech Biotechnology Pty. Ltd., Dandenong South, Vic.
[2] Department of Animal Science, University of New England, Armidale, NSW.
[3] Department of Animal and Aquacultural Sciences, University of Life Sciences, Norway.
[4] Australian Poultry Cooperative Research Centre, Armidale, NSW

sorghum grain component. The treatments were (i) whole sorghum (WS), (ii) hammer-mill - 3.0 mm screen (HM3), (iii) roller-mill - 0.15 mm roller spacing (RM0.15), (iv) hammer-mill - 1.0 mm screen (HM1). The RM0.15 sorghum was ground to have an equivalent mean particle size of the sorghum used for HM3 (the control). Diets WS and HM1 were used to represent the extreme variations from the intermediate particle sizes used in diets HM3 and RM0.15. Commercial feed milling equipment was used for the feed manufacture. All diets were steam conditioned in a double conditioner for 60 seconds at 75 ˚C and then steam-pelleted using a 3 mm die.

Table 1 Composition of the semi-purified diet ($g.kg^{-1}$ diet on an 'air dry' basis)

Ingredient	Inclusion level
Sorghum[A]	750.0
Fishmeal 70 %	40.0
Isolated Soy Protein 90 %	120.0
Soybean oil	39.8
Monocalcium phosphate	2.00
Ground limestone	8.00
Sodium chloride	2.50
Vitamin premix[1]	3.00
Mineral premix[2]	1.50
Manganese oxide	0.10
Titanium dioxide	5.00
DL-Methionine	3.00
L-Lysine	3.00
L-Threonine	1.00
Choline chloride	1.10

[A]Sorghum differing in particle size and milling type according to treatment.[1]Vitamin premix supplied per kilogram of diet: Retinyl acetate, 5.15 mg; cholecalciferol, 0.10 mg; DL-tocopheryl acetate, 75 mg; menadione, 9 mg; pyridoxine, 6 mg; riboflavin, 24 mg; Ca-pantothenate, 26.3 mg; biotin, 0.39 mg; thiamine, 3.75 mg; niacin, 75 mg; cobalamin, 0.03 mg; folic acid, 3.75 mg.[2]Mineral premix supplied per kilogram of diet: Fe, 75 mg; Mn, 60 mg; Zn, 105 mg; Cu, 15 mg; I, 0.75 mg; Se, 0.3 mg.

Three hundred (300) day-old male broilers (Ross 308) were brooded until 10 d in brooders under the same conditions. The birds were maintained on a commercially available wheat-based starter diet during the brooder phase, fed ad libitum. At 11 days of age, birds were weighed and selected for the experiment if within 15% of the mean group bodyweight. Pairs of birds were placed into 96 wire mesh cages (24 replicates of two birds, 192 birds total). Temperature and light conditions were managed according to the Ross 308 Management Guide (21˚C by 21 days and 16 hours of light per day).

Bodyweights were recorded at 11 d, 14 d and weekly thereafter (21 and 35 d results shown). Feed refusals were weighed at the time of bird weighing, and weighed additions to each feeder were allocated on a needs basis. Excreta for nitrogen corrected apparent metabolisable energy (AME_n) determination (total collection method) were collected for three consecutive days from 25 d after 6 hrs fasting. Daily collections of excreta were pooled and frozen at -20 ˚C. The birds and feeds were weighed at the commencement and completion of excreta collection.

III. RESULTS

Table 2 shows broiler performance parameters from 11-21 d and 11-35 d. The body weight gain to 21 d was highest ($P < 0.05$) for the two intermediate particle sized sorghum treatments. The FCR at 21 d was significantly dependant on the type of sorghum processing

used (P < 0.001). Birds fed roller-milled sorghum performed better than those fed hammer-milled grain, which were better than those fed whole sorghum as

Table 2 Growth performance and nitrogen-corrected AME.

Treatment	WS	HM3	RM0.15	HM1	SEM	P value
11-21 days						
BW gain[1] (g)	614.3[b]	643.9[a]	638.2[ab]	615.2[b]	8.8	0.032
Feed intake[2] (g)	868.7[a]	875.7[a]	837.5[b]	823.8[b]	10.1	<0.001
FCR[3] ($g.g^{-1}$)	1.41[a]	1.36[b]	1.31[c]	1.34[b]	0.014	<0.001
11-35 days						
BW gain[1] (g)	1823.3[b]	1905.2[a]	1916.4[a]	1865.7[ab]	26.3	0.047
Feed intake[2] (g)	2853.8	2902.2	2795.9	2804.5	37.4	NS
FCR[3] ($g.g^{-1}$)	1.57[a]	1.52[b]	1.46[c]	1.50[b]	0.007	<0.001
AME_n[4]	13.85[a]	13.47[b]	13.51[b]	13.60[b]	0.08	0.005

[a,b]Vaules in a row with unlike superscripts differ significantly (P < 0.05). [1]Cumulative bodyweight gain from 11d of age. [2]Cumulative feed intake from 11d of age. [3]21d FCR calculated and averaged for the two birds from 11-21d of age, 35d FCR calculated from 11-35d of age and averaged for the two birds for the period 11-21d of age. [4]AME_n calculated using excreta taken over three consecutive days ($MJ.kg^{-1}$ DM).

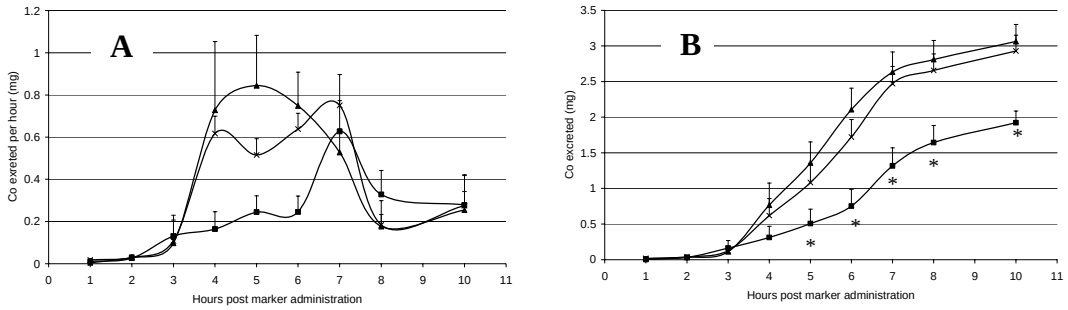

Figure 1 Liquid (Co; graphs A, B) and solid phase marker passage rate of birds fed HM1 (■), HM3 (✕) and WS (▲) diets. A: excretion pattern. B: cumulative excretion. Error bars represent the pooled SEM, *represents a significant difference (P < 0.05).

part of the diet. By 35 d, the birds fed sorghum with some degree of pre-pelleting processing (hammer or roller milled), achieved a higher (P < 0.05) bodyweight gain than those fed the WS treatment diet. The RM0.15 treatment allowed a four point improvement (P < 0.001) in FCR over the HM1, six points over the HM3, and 11 points over the WS treatment. Overall, birds fed the roller-milled sorghum to an equivalent particle size of a commercial grind, achieved a higher bodyweight gain and a lower FCR than all other treatments.

Tabulated AME data shows that processing sorghum grain, as opposed to leaving the grain whole prior to pelleting, decreases (P = 0.005) dietary AME. The marker excretion patterns in figure 1 show a significantly slower (cumulative) liquid marker excretion when the whole sorghum diet was fed.

IV. DISCUSSION

Feeding an intermediate grain particle size to broilers elicited the best performance response in this experiment which is in agreement with the work of Nir et al. (1990, 1994) and Deaton et al. (1995). The whole sorghum diet, however, had the highest AME, which was in line with the findings of Preston *et al.* (2000) and Svihus et al. (2004) (in part), but the poorest performance by 35 d. A explanation for the poor performance of the WS birds despite higher AME values may be due to a perceived higher investment of energy to extract nutrients from less mechanically degraded (prior to ingestion) grain. This result does however indicate that the WS birds were more effective at liberating more energy from the feed.

The liquid phase components of the feed were shown to move at a slower rate when birds were fed coarse particles than when fed fine particles. This result confounds the observations of and Hetland and Svihus (2001), where coarse particles (oat hulls) tended to increase titanium oxide excretion rate ($P < 0.08$). Although AME was shown not to be related to performance, the cumulative excretion of liquid phase marker over time may be indicative of the particle size and AME.

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FREE AMINO ACIDS CAN IMPROVE EFFICIENCY OF UTILISATION OF RESOURCES IN LIVESTOCK PRODUCTION

M. PEISKER[1], J. McLEISH[2] and Y. DERSJANT-LI[1]

Summary

The livestock sector faces challenges related to the ongoing environmental discussion. FAO estimates that the livestock industry produces 18% more CO_2 equivalents than transport. The advent of first generation biofuels, driven by the need to tackle Global Warming Potential (GWP) of the transport sector, has further increased pressure for livestock producers by moving feedstock away from feed to fuel. The major source of greenhouse gases (GHG) from the livestock sector is the provision of resources (feedstuffs, energy) followed by animal related emissions (methane, nitrous oxide). Therefore the major potential to reduce GHG from livestock is seen in improving resource efficiency and reducing direct animal related emissions. Free amino acids can be used for both purposes. They improve the efficiency of transforming feedstuffs into high quality animal protein for human consumption not only by making better use of existing feedstuffs but also by enabling efficient utilization of by-products like distillers' grains and solubles (DDGS) and rapeseed meal. Amino acids contribute to lower eutrophication of water bodies and rivers and impact on nitrous oxide emission from livestock directly by reducing the output of N-containing compounds (nitrates) that are denitrified to nitrous oxide (N_2O).

I. INTRODUCTION

Global increasing demand for edible protein of animal origin coincides with increasing awareness of ecological issues and mandates scrutinising current livestock production systems. According to FAO (2006), the livestock sector generates 18% more greenhouse gas emissions as measured in CO_2 equivalent than the transport industry. It is also a major cause of land and water degradation. The livestock industry will need to address their resource utilisation including water use, GWP, eutrophication and other environmentally related issues. Generating more accurate information may change the GWP-ranking of different species currently used to produce animal protein. It would take the best available techniques, including potential shift to net energy systems, phase feeding, further reduction in dietary crude protein levels, application of current and new feed additives, to meet this challenge.

The three major greenhouse gases are CO_2, CH_4 and N_2O. Their relative-to-CO_2 GWP depends on their atmospheric lifetime and is pegged for a 100-year period as 1 for CO_2, 21 for CH_4 and 310 for N_2O (US Greenhouse Gas Inventory Program 2002). Due to its GWP of 310, N_2O represents an important factor in monogastric animals. Nitrous oxide is formed by bacterial de-nitrification and nitrification processes in soil mainly from nitrate. The amount of nitrous oxide released largely depends on the amount of N added to soils as fertilizer or manure. Up to 4% of the N in fertilizer or manure can be emitted as N_2O (Woitowitz, 2007). Thus reducing N-content in manure could contribute to reduce N_2O emission, provided good fertilisation practices are followed.

[1] ADM Specialty Ingredients (Europe) B.V. Stationsstraat 76, 1541 LJ Koog aan de Zaan, The Netherlands.
[2] ADM Australia, Suite 1003 1 Newland Street Bondi Junction NSW 2022, Australia

II. GWP OF LIVESTOCK PRODUCTS

GWP, excretion of relevant pollutants and water use for different animal products or protein are shown in Table 1. Important differences with respect to GWP, environmental load and water use exist when comparing animal protein products from ruminants and monogastric animals. Monogastric animals are more efficient in turning organic feed material into edible protein with less specific pollutants excreted. Poultry meat and eggs appear to be the most environmentally friendly animal protein, showing the lowest specific excretion.

Table 1 GWP (g/kg product), excretion (kg/kg edible protein) of N, P, CH_4, CO_2 and water requirement (m^3/kg edible protein) of different meat types (Heissenhuber, 2008; Flachowsky, 2008; Faostat, 2006; Hoeckstra and Chapagain, 2007)

	GWP (g/kg)		Excretion (kg/kg edible protein)				Water use
	conventional	ecological	N	P	CH_4	CO_2	(m^3/kg edible protein)
Milk[1]	826	843	0.24	0.04	0.4	12	29
Beef[2]	10066	10223	1.0	0.14	1.2	35	81
Pork[3]	4109	4965	0.55	0.08	0.05	10	32
Poultry[4]	1978	2846	0.25	0.03	0.01	3	20
Eggs[5]	1724	1592	0.3	0.05	0.02	4	28

[1]40 kg milk/d, [2]1500g/d LWG, [3]900 g/d LWG, [4]60 g/d LWG, [5]90% laying performance

Ruminants are penalised by their methane production, which contributes significantly to GWP (21 CO_2 equivalents). But it has to be considered that ruminants are capable of utilising fibre-rich feedstuffs that cannot be used by non-ruminants, thus they have an important function for resource utilisation. It is also evident from Table 1 that ecological production schemes, with the exception of egg production, do not necessarily demonstrate better specific greenhouse gas balances compared to conventional schemes. With increasing performance the specific environmental load decreases; therefore, improving resource efficiency is coupled with increasing animal performance (Flachowsky, 2008).

Table 2 Breakdown of GHG (g/kg product) for different species/product under conventional or ecologic scenarios (Woitowitz, 2007)

	CO_2		N_2O		CH_4	
	conventional	ecologic	conventional	ecologic	conventional	ecologic
Milk	182	184	0.5	0.5	21.3	22.5
Beef	1960	1710	9.6	9.7	244.2	262.1
Pork	1000	1550	7.4	9.0	43.5	32
Poultry	1125	2190	1.8	1.3	9.4	10.4
Eggs	1080	1315	1.8	0.8	5.6	5.4

Table 2 shows that for poultry CO_2 plays a major role whereas nitrous oxide and methane are less important in relation to beef and pork. The relative contribution of the different GHG to GWP varies for the different species. For milk and beef, methane comprises more than 55%, whereas for pork nitrous oxide makes 53% and for poultry carbon dioxide represents 58% (meat) or 60% (eggs) of total GWP. N_2O makes about 33% of GWP for poultry, meat and eggs alike. Nitrous oxide emission from ecological poultry schemes are less compared with

conventional schemes. This is due to the fact that provision of resources (feed) represents the major source of specific nitrous oxide emissions in conventional production schemes. Therefore the major starting point to reduce this part of GWP in poultry production is seen in improving resource efficiency. Commercially available free amino acids can significantly contribute to meet this objective.

III. FREE AMINO ACIDS

a) Resource utilization

Comparing the global market for free amino acids in 1990 with its forecast 2010, it increased by 600% for lysine, 570% for methionine, 1200% for threonine and tryptophan went from zero to 2000 tonnes annually. In the European Union, amino acids represent the largest market share of all feed additives, accounting for more than 40% of the market. Currently 7 out of the 10 essential amino acids are commercially available (methionine, lysine, threonine, tryptophan, arginine, histidine and valine).

These amino acids are needed to make the best use of by-products from biofuels like DDGS and rapeseed meal in poultry diets. For Europe, van der Aar (2007) estimates a 30% reduction in the use of soybean meal (33 to 25 mio. tonnes), an increase of 77% rapeseed meal (7.5 to 13.3 mio. tonnes) and 1400% DDGS (0.5 to 7 mio. tonnes) from 2005 to 2010. The downside of the use of these by-products is their inherent variation in amino acid level and true ileal digestibility. Parsons et al. (2006) estimate that the nutrient composition varies substantially amongst DDGS. The greatest concern is the bioavailability of lysine, as during the process of drying DDGS the material is typically exposed to high temperatures. The adverse effect of excess heat on amino acid availability, and especially on lysine, is established. Table 3 provides information about the variability of amino acids in wheat DDGS.

Table 3 Total amino acids content and amino acids digestibility of wheat DDGS (36.6% CP in DM) in poultry (Gady, 2007)

	Mean	SD	CV%
Amino Acids as % of Protein			
Lysine	1.93	0.36	18.4
Methionine	1.45	0.07	5.1
Threonine	3.32	0.57	17.1
Tryptophan	1.07	0.12	11.5
True Amino Acid digestibility coefficients for poultry (%)			
Lysine	38.2	10.4	27.1
Methionine	77.7	5.1	6.6
Threonine	67.9	7.0	10.4
Tryptophan	71.6	7.5	10.5

Given the high CV of true amino acid digestibility, clearly it is necessary to (i) determine the content and availability of amino acids in the feedstuffs (NIRS systems are available for on-line testing) and (ii) compensate for this variation by adding free amino acids. The dietary inclusion level of wheat DDGS for poultry is capped at 10% (van der Aar 2007) and up to 11% of corn DDGS (Parsons et al. 2006).

b) Impact on GWP

The real impact of free amino acids on nitrogen excretion has already been demonstrated. It is accepted that a reduction of one percentage point in dietary crude protein reduces the nitrogen content in manure by 10 %, the ammonia emission into the ambient air by 10%, the animals` water consumption by 3% and the manure volume by 5%. The question arises to what extent can reduced dietary crude protein levels contribute to lower GWP in production of foods from animal origin, particularly from monogastrics. Waguespack et al. (2007) identified arginine and valine as equally limiting after methionine, lysine and threonine in corn-soy diets for broilers. With the availability of these amino acids available, some constraints concerning further reduction of dietary crude protein levels (below 20% in starter feed) are removed. Corzo et al. (2007) demonstrated in milo-soybean meal diets that dietary crude protein level could be reduced to 17.5% when methionine, lysine, threonine, tryptophan and valine are added to broiler diets (21-42 days), without compromising performance. A 2%-step reduction in dietary crude protein in broiler feed would lead to 50 g reduction in N-excretion per kg edible protein. This translates to 162 g less CO_2 equivalents atmospheric nitrous oxide emission per kg product. In relation to the GWP for conventional broiler production (1978 g, Table 1) this represents a potential reduction of 8%. For pork a 4% reduction in dietary protein would lead to 13% reduction of GWP per kg product.

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NEW BIOETHICAL CHALLENGES IN POULTRY PRODUCTION: THE WAY FORWARD

P.R. CHEEKE[1]

Summary

Bioethics is concerned with doing the right thing in animal production. How does one decide what is the right thing? It is not merely a matter of opinion. There are several tests one might apply: (i) The theory of reciprocity. Would you like to be the recipient of a particular action? In terms of layers, would you want to spend your life in a small cage? If not, can you justify doing this to another subject of a life? (ii) The test of publicity. Can you accept having your views publicized? (iii) Are your views consistent with moral philosophies dealing with suffering, pain, etc.? (iv) Would your peers support your position? Application of these bioethical tests to intensive production of broilers and layers leads to the conclusion that industrial poultry production is not ethical. This is the conclusion of an increasingly large segment of the general public, and has contributed to rising interest in vegetarianism and veganism. The vegetarian movement has reached the status of a functional religion with many adherents. Concerns about animal welfare in industrial production systems have swelled the ranks of those opposed to modern poultry production. As a consequence, the food industry is responding to these concerns with the establishment of animal welfare standards (e.g. McDonald's and other fast food chains). There is room for change in the poultry industry. The way forward may be to move backward, to search for a middle ground. The industry may need to reject certain technological advances (e.g. featherless broilers, blind layers) that inflame public opinion in order to reach common ground with consumers. The education of poultry science students must now include courses in bioethics, if tomorrow's leaders are to deal effectively with the inexorable rise in consumer concerns regarding the ethical treatment of livestock and poultry. Poultry scientists may have to admit "we've gone too far."

Bioethics in the context of animal agriculture is the philosophical study of ethical controversies that arise in the production of livestock and poultry for food, fiber and other applications (e.g. entertainment). Put another way, bioethics is concerned with doing the right thing in animal production. What is the right thing? It is not merely a matter of opinion, with poultry producers having one opinion and animal rights advocates having a diametrically opposed viewpoint. There are some bioethical tests that can be applied: (i) The theory of reciprocity. Would you like to be the recipient of a particular action? In terms of laying hens, would you want to spend your life in a small cage? If not, can you justify doing this to another subject of a life? What exactly is a "subject of a life?" Many bioethicists define "subject of a life" in terms of sentience or self-awareness. This would probably differentiate the degree of ethical concern about poultry farming versus oyster farming. A related concept is the avoidance of suffering. Humans have an obligation that animals whose lives they impact do not suffer. This is the concept of animal welfare. (ii) The test of publicity. Can you accept having your views widely publicized, or are you not entirely proud of them? (3) Are your views consistent with moral philosophies dealing with suffering, pain, etc.? (iv) Would your peers support your position? Application of these bioethical tests to intensive production of broilers and layers leads to the conclusion that industrial poultry production may not be ethical.

[1] Department of Animal Sciences, Oregon State University, Corvallis, Oregon, 97731, USA.

There is evidence that the general public considers industrial poultry production to be unethical (Davis and Cramer, 2006). For example, Hall and Sandilands (2007) investigated public attitudes towards the welfare of broiler chickens. They reported "at the outset the majority of participants admitted that they knew little about how broiler chickens are reared and were shocked at some of the facts presented to them." Webster (1994) put it well: "an excellent test of animal welfare is to discover whether their owner can display his animals with pride to any fair-minded observer. The special pleading required to suggest that the welfare of broiler fowls or laying hens is satisfactory, despite their appearance, is deeply unconvincing to almost any unbiased observer." For example, one of my colleagues remarked when viewing a layer house "this is a concentration camp for chickens!" In my judgment, there is little that the poultry industry can do to overcome these perceptions.

There is abundant evidence that the general public is becoming disenchanted with industrial animal and poultry production. All of the global fast-food restaurants (e.g. McDonald's, Burger King, Wendy's, etc.) have initiated animal welfare programs, specifying that eggs and meat must be produced in a welfare-satisfactory manner. Supermarkets (e.g. Safeway in the U.S.) have begun similar programs. As I have remarked previously (Cheeke, 2004), "one of the best things modern animal agriculture has going for it is that most people haven't a clue how animals are raised and 'processed.' For modern animal agriculture, the less the consumer knows about what's happening before the meat hits the plate, the better."

The adoption of animal welfare standards by fast food restaurants, restaurants in general, and supermarkets is not due to sudden attacks of conscience. They are a response to real or perceived public attitudes, and represent an attempt to "get a leg up" on competitors.

Concerns about industrial production of livestock and poultry are probably major factors in the rise of vegetarianism, particularly among young people. Concern about the interests of non-human animals is a logical extension of the expanding concept of human rights. Clearly, the public is extending the concept of rights to an ever-expanding circle of life, beginning with companion animals. Other domesticated animals are close behind. The animal rights movement represents a natural extension of society's concern for fair play. Protection of the rights of animals has led to the development of a new field of study, animal law. An organization called the Great Ape Project is campaigning for the United Nations to adopt a Declaration on Great Apes, which would extend to apes the protection of three basic interests: the right to life, the protection of individual liberty, and the prohibition of torture. 0This may be a first step towards granting legal rights to other animals, including poultry. Rollin (1995) suggests that a new social ethic concerning raising of animals has developed, the basis of which is the satisfaction of an animal's telos. Telos is the suite of natural behaviors characteristic of an animal. For example, natural behaviors of chickens include exercise, nest building, dust-bathing, perching, wing flapping, vocalization, etc. Intensive production of layers and broilers denies birds the opportunity to engage in many of these behaviors.

Of particular concern to the poultry industry should be the rise in vegetarianism and its more extreme form, veganism, in young people, who represent the consumers of tomorrow. Animal rights activism exhibits many attributes of a functional religion (Jamison *et al.*, 2000). There is an initial process of conversion or enlightenment, and the adoption of a new belief system. There is an element of community. New converts are welcomed into the fold. "Veganism provides an elaborate superstructure with which activists support their lives" (Jamison *et al.*, 2000). They have disciplined behavior codes, with strict avoidance of animal products. "Their publications are filled with advice for vegetarian and vegan cooking, cruelty-free shopping, cruelty-free entertainment, and cruelty-free giving." They have an evangelical bent, continuously on the look-out for new converts. Jamison *et al.* (2000) conclude: "in politics, intensity matters. Organizations and movements that are able to muster

and sustain intense support generally are able to effect change over time; hence, the passionate participation of true believers has always marked the politics of successful mass movements. The animal rights movement exemplifies this political fervor and has met with varying degrees of success." Persistent efforts in European and U.S. politics by animal rights activists to introduce legislation to modify or ban various animal production techniques (sow stalls, layer cages, etc.) illustrate the tenacity and intensity of "true believers." In the United States, a ballot measure in California to ban the keeping of layers in cages was passed by voters with a wide margin in 2008. In general, as California goes, so does the rest of the country.

How can the poultry industry respond to its critics, or should it? Defenders of industrial animal and poultry production often assert that the problem is that most people today have an urban background, and are several generations removed from agriculture, so they don't understand why we raise animals the way we do. Get over it! This is the way it is going to be in Europe and North America. Never again will most people have a rural upbringing. If that is the case, then "we just have to educate them." The problem with that approach is that most people do not respond well to "I'm right and you're wrong," which is what we really mean by "educating them." If we really want to educate them (the public), then we'd have to tell them exactly how animals are raised in industrial systems. Hall and Sandilands (2007) did this, and found that consumers "were shocked at some of the facts presented to them." Showing consumers caged layers and industrial broilers is not likely to win them over. Recently in the U.S. state of Arizona, there was a ballot initiative to ban the use of sow stalls (it passed). It was suggested in the run-up to the election that reporters be taken to visit swine farms to actually see sow stalls. The swine industry was against this idea. As Webster (1994) noted, "an excellent test is to discover whether their owner can display his animals with pride to any fair-minded observer." Obviously, swine producers in Arizona were acutely aware of the fact that the last thing they needed was for reporters (representing the general public) to actually see what sow stalls are. The reality wouldn't win over any fair-minded observer.

I suggest that the way forward is to take an entirely different approach. Bioethical concerns about industrial poultry production are real, legitimate, and increasing. Instead of belittling and attacking the activists, a better approach might be to accept that perhaps they are right. Maybe we have gone too far down the industrialization road. Take, for example, the development of featherless chickens. Poultry scientists can identify many advantages for featherless chickens in certain environments. However, it is intuitively obvious that the featherless chicken "won't fly." The public will simply not accept it, and efforts to force these kinds of developments will simply result in more resistance and more activists. The featherless broiler or the blind layer are excellent recruiting tools for the animal rights and vegetarian movements. Getz and Baker (1990) summed up the situation well: "animal rights and welfare groups must be recognized as organizations involved in the animal industry of the future. They are likely to influence future policies. In fact, they ultimately may improve animal agriculture programs because challenging current methods, procedures and assumptions usually leads to improvements."

I suggest that the poultry industry should embrace change. The way forward is to begin by looking backward. The development of industrial poultry production has led to a dead end. The consuming public doesn't like it or want it. An optimist would recognize this as a tremendous opportunity. The discipline of Poultry Science has been given the opportunity of a new life. Engage young minds in the search for new production methods that retain production efficiency while improving the welfare of the birds. A new field of research opens up. The food industries (restaurants, supermarkets) are setting welfare standards for animal products. This provides opportunities in a new field: animal welfare auditing. Welfare

auditors employed by McDonald's (for example) to monitor welfare conditions and producer compliance with welfare standards ought to be Poultry Science graduates, not History or Art majors!

The way forward, in summary, is to recognize that the poultry industry has painted itself into a corner. Poultry scientists should embrace their critics, and develop production methods that allay consumer concerns while maintaining production efficiency. Great opportunities exist for those willing to accept this challenge. As Davis and Croney (2004) point out, there is a middle ground. Bear in mind that it is the responsibility of the producer to produce what the consumer wants, not the other way around. A time-tested maxim in the retail business is "the customer is always right."

Perhaps these final words from me (Cheeke, 2004) are appropriate:

"Do we, as humans, having an ability to reason and to communicate abstract ideas orally and in writing and to form ethical and moral judgments using the accumulated knowledge of the ages, have the right to take the lives of other sentient organisms, particularly when we are not forced to do so by hunger or dietary need, but rather do so for the somewhat frivolous reason that we like the taste of meat? In essence, should we know better?

When human population growth has stabilized, petroleum reserves have been depleted, and new processes developed for producing palatable meat substitutes from plant products, our descendants might look back a couple of hundred years from now and ask, 'how could they do that to animals?'"

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CONCEPTUAL UNCERTAINTY IN ANIMAL WELFARE ASSESSMENT AND THE LAYWEL REPORT

J.L. BARNETT[1] and P.H. HEMSWORTH[1,2]

Summary

There is considerable uncertainty on the concept of animal welfare with differing opinions on how animal welfare should be measured. There are three prominent concepts of animal welfare and these different concepts can lead to scientists using different criteria or methodologies to assess animal welfare. This conceptual uncertainty is the basis of the controversy on housing for laying hens and is reflected in both scientific recommendations and policy decisions. A recent EU scientific review using the 'five freedoms' concept as a baseline for animal welfare assessment concluded that while all alternative systems have the potential to provide satisfactory welfare for laying hens, conventional cages cannot meet the welfare requirements of hens. A different interpretation of the same data is reached using the functional approach to welfare assessment.

I. INTRODUCTION

There is considerable uncertainty within science on the concept of animal welfare (Fraser, 2003; Sandøe et al., 2004). Scientists differ in their views on how animal welfare should be measured or judged, with three prominent concepts of animal welfare: the welfare of animals is judged on the basis of (1) how well the animal is performing from a biological functioning perspective; (2) affective states, such as suffering, pain and other feelings or emotions; and (3) the expression of normal or 'natural' behaviours. These concepts have been reviewed (Barnett and Hemsworth, 2003) but are briefly described.

a) Animal welfare concepts

The biological functioning concept is underpinned by the definition "The welfare of an individual is its state as regards its attempts to cope with its environment" (Broom, 1986). The 'state as regards attempts to cope' refers to both (1) how much has to be done to cope with the environment and includes responses such as the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses and (2) the extent to which coping attempts are succeeding and this includes the lack of biological costs to the animal such as deterioration in growth efficiency, reproduction, health and freedom from injury. Thus, using this approach, the risks to the welfare of an animal imposed by an environmental challenge can be assessed at two levels (1) the magnitude of the behavioural and physiological responses and (2) the biological cost of these responses.

The second concept, affective states, defines animal welfare in terms of emotions and emphasizes reductions in negative emotions, such as pain and fear, and increases in positive emotions such as comfort and pleasure (Duncan and Fraser, 1997). Duncan (2004) has argued as follows that animal welfare ultimately concerns animal feelings or emotions: all living organisms have certain needs that have to be satisfied for the organism to survive, grow and reproduce and if these needs are not met, the organism will show symptoms of atrophy, ill-health and stress and may even die. Measuring preferences of animals, using preference tests

[1] Animal Welfare Science Centre, The University of Melbourne, Parkville, Vic 3010
[2] Animal Welfare Science Centre, Department of Primary Industries, Werribee, Vic 3030

and behavioural demand testing (Dawkins 1980; Matthews and Ladewig 1994), has been used to assess animal welfare on the basis that these preferences are influenced by the animal's emotions, which have evolved to motivate behaviour to avoid harm and facilitate survival, growth and reproduction.

While not well enunciated, the third concept promotes allowing animals to express their normal behaviour. In the early literature, the view that animals should perform their full 'repertoire' of behaviour was very common, however there is broad agreement within science that it is often difficult to attribute actual suffering when the expression of certain behaviours is prevented or is absent when it would be expected to be present (Dawkins, 2003). Furthermore, as discussed by Dawkins (1980), 'wild' behaviour may represent an animal's efforts to survive in a life and death struggle or contest and therefore some 'natural' responses are adaptations to cope with extreme adverse situations. More recently the emphasis has been on behavioural indicators of poor coping such as fearfulness, aggression and stereotypies (EFSA, 2005).

In addition to these three concepts, the 'five freedoms' proposed by the UK Farm Animal Welfare Council to protect the welfare of animals (FAWC, 1993), were derived from a report to the UK Parliament (Brambell *et al.*, 1965). These freedoms include aspects of the aforementioned concepts. However, in terms of a consensus on animal welfare assessment, there has been little attempt to define the levels of freedom that are desirable together with the adverse consequences of not providing such freedoms.

These different concepts on animal welfare can lead scientists to use different criteria or methodologies in assessing an animal's welfare. For short term animal welfare issues involving acute stress, such as painful husbandry procedures, there is considerable agreement on the need to assess animal welfare from a perspective of biological functioning (Mellor et al., 2000). However, for longer term issues disagreement over these welfare concepts lead to contentious debates concerning animal welfare and the varying interpretations. The effect of confinement on laying hens is an obvious example of the consequences of disagreement on the concept of animal welfare.

b) Conceptual uncertainty

This uncertainty surrounding the concept of animal welfare has implications for identifying and resolving genuine risks to an animal's welfare. Firstly, while there are several concepts of animal welfare in the literature, scientists have basically used two methodologies to study animal welfare, either biological functioning or animal preference methods. The first approach is an integrated one measuring behavioural, physiological and health and fitness responses to assess biological functioning on the basis that difficult or inadequate adaptation will generate welfare problems for animals. The second uses animal preference (and behavioural demand) testing on the basis that animal preferences are influenced by the animal's emotions, which have evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction. Differences in concepts and definitions of animal welfare lead to differences in the methodology used to assess animal welfare under different husbandry or housing practices.

Secondly, differences between policy makers on the concept and definition of animal welfare can lead to disagreement on animal welfare-related policy and legislation. While decisions on specific animal use are affected by a number of considerations including scientific information of the harms and benefits to the animal, differences in concepts, definitions and, in turn, assessment lead to differences between policy makers in industry, community groups and Government in their interpretation of the validity of scientific information arising from a specific methodology. Consequently, these differences between policy makers in interpreting similar information can lead to disagreement on setting or

accepting specific animal welfare standards.

Thirdly, it is important in any welfare monitoring scheme in the field that the emphasis should be on the animal itself and thus on those measures that best reflect lack of animal suffering. The welfare measures or 'tools' that science develops to evaluate the welfare implications of husbandry and housing practices will obviously be incorporated into welfare assessment and screening tools in the field. Thus any uncertainty about the validity of the scientific measures on which the field measures are based will affect community, consumer, industry, community group and Government confidence in compliance with specific welfare standards.

These conceptual differences at both scientific and policy levels are illustrated in developments in housing systems for poultry. A comprehensive review by scientists of the literature (LayWel, 2006) provided recommendations on the welfare implications of housing systems for laying hens Using the five freedoms as a baseline for animal welfare assessment, the review considered 39 welfare risks under four main categories: 1) Injury, disease and pain (overall mortality, mortality due to feather-pecking/cannibalism, mortality due to disease, infectious disease and use of therapeutic drugs, predation, internal parasites, external parasites osteoporosis/low bone strength, keel bone deformation, bone breaks during lay, bone breaks at depopulation, bumble foot and beak trimming); 2) Hunger, thirst and productivity (feed intake, water intake, FCR and egg production); 3) Behaviour (nest box eggs at peak lay, hens on a perch at night, use of a dust bath, foraging, social, behavioural restriction and injurious pecking); and 4) Fear, stress and discomfort (fearfulness, corticosterone concentration, heterophil to lymphocyte ratio, crowding/suffocation, feather pecking, feather loss, plumage soiling, bumble foot, thermal discomfort, dust, ammonia and dirty eggs). Risks were tabulated for conventional cages, furnished cages, single and multi-level non-cage systems and systems with an outdoor run. The authors concluded that while all alternative systems have the potential to provide satisfactory welfare for laying hens, conventional cages cannot meet the welfare requirements of hens. However, from the documentation presented in the LayWel Report, it can be argued that conventional cages perform better in 18 of the 39 risk areas, including those involving mortality, while non-cage and outdoor systems perform better in 9 and 10 categories, respectively, than conventional cages. The conventional cages perform worse for 6 of the 7 categories of behaviour, but no evidence is presented that behaviour is more important to welfare than for example, mortality. Using similar interpretations, the European Union Council Directive 99/74/EC proposed that the use of conventional (unenriched) cages will be banned in the European Union by January 2012. In reviewing the development of hen welfare standards in the EU, Savory (2004) concluded that the freedom to 'perform normal behaviour' is often given more weight in interpreting welfare risks than the other four freedoms. Thus, using the functional approach to welfare assessment the data in the LayWel and related reports can be interpreted to suggest that the welfare of hens in conventional cages is generally equal to or better than that for hens in other systems. The major limitation is on the hens' behavioural repertoire, but this is within the context that the behaviours that are important for hen welfare have neither been adequately defined nor the consequences of denying them adequately determined.

II. CONCLUSIONS

While there is scientific uncertainty in relation to animal welfare concepts or views, it does not necessarily diminish the robustness of the research utilising criteria or methodologies promulgated by these different concepts. Furthermore there is some overlap between the differing views. However, it does raise the question of the relatedness of these concepts and further work is required to determine how these differing views align when determining

important resources for hens. Nevertheless, until there is better agreement on these views, it is important that the basis of the methodology used by scientists to assess animal welfare should routinely be provided so that individuals using science in their decision-making appreciate both the rationale for the methodology and its limitations (Fraser, 2003; Sandøe *et al.,* 2004).

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THE EFFECTS OF GROUP SIZE ON THE PROPORTION OF NEST BOX EGGS LAID BY HENS IN CAGES

G.M. CRONIN[1,2], S.S. BORG[2] and J.L. BARNETT[2,3]

Summary

We previously found that only 66% of hens in cages with a nest box became 'consistent' nest-box layers, that is laying at least 80% of their eggs in the nest box. To begin to understand how social factors might limit nest box use in 'modern' cages, we housed birds either singly or in groups of eight and recorded the incidence of nest box and floor eggs. The proportion of nest box eggs was greater (P = 0.022) for singly- compared to group-housed birds between 19 and 33 weeks of age (84.2 vs. 66.6% of eggs). The experiment confirmed that social factors contribute to the reduction in the proportion of eggs laid in nest boxes.

I. INTRODUCTION

The welfare of laying hens in cages is a current international topic of ethical, political and scientific debate. From 2013 in the European Union, egg production from caged hens will only be acceptable if 'furniture' (viz. a nest box, dust bath and perch) is provided in the cage. Australian research on furnished cages by Barnett and Cronin (2005) however, reported only 62% of eggs were laid in nest boxes. Cronin et al. (2007) reported that 45% of 'first eggs' were laid in the nest box by hens in cages. The proportion of nest box eggs increased linearly to about 70% at the eighth egg and thereafter remained relative static. The literature suggests hens are highly motivated to lay in a nest box. With the possible exception of the study by Cooper and Appleby (1997), most experiments measuring motivation of hens to reach a nest box selected hens on the basis that they were already 'consistent' nest-box layers. With this in mind, it is perhaps not surprising that the reported high motivation of hens to lay in a nest box does not necessarily reflect the proportion of nest box eggs reported in observational studies.

The incidence of nest box eggs in cages has been reported to range from 43-100% (Wall et al., 2002; Guesdon and Faure, 2004; Tauson and Holm, 2005: Cronin et al., 2007). A number of factors have been shown to affect both the attractiveness and access to nest boxes by hens, including specific features of the nest, strain, age and rearing experience of the bird and social factors. Social factors are reported to affect both access to a nest site (Sherwin and Nicol, 1993; Friere et al., 1997, 1998) and the time hens remain at the site after oviposition (Lundberg and Keeling, 1999). During observations of hen behaviour in 25 commercial aviaries, Odén et al. (2002) reported considerable aggression occurred outside nest boxes, suggesting competition for nest boxes. Similarly, Shinmura et al. (2007) found that dominance status of hens influenced use of resources in furnished cages. Since the timing and synchrony of egg laying in hens tends to be regulated by light (Appleby et al., 2004), increased competition for preferred egg-laying sites may occur in cages with a single nest box. These authors suggested that nest boxes should accommodate multiple hens simultaneously engaged in pre-laying behaviour and they presented a theoretical model of nest area requirement of 300 cm^2 per hen. Thus, a group of eight hens would require 2,400 cm^2 of nest space, double that provided in 'modern' furnished cages (see below). As a first step to investigate the attractiveness of nest boxes for laying hens in cages, the present experiment examined the effects of group size on utilisation of nest boxes.

[1] Faculty of Veterinary Science, The University of Sydney, Camden NSW 2570 (present address)
[2] Animal Welfare Science Centre, Department of Primary Industries, Werribee VIC 3030
[3] Animal Welfare Science Centre, The University of Melbourne, Parkville VIC 3010

II. MATERIALS AND METHODS

A total of 96 Hy-Line Brown hens were housed either singly or in groups of eight in cages measuring 1.2 m wide, 0.5 m deep and 0.45 m high at the rear. All cages contained a nest box located on the right side (viewed from the front), which measured 0.24 m wide, 0.5 m deep and 0.27 m high at the front. The floor of the nest box was overlain with 'Astro turf' (0.22 m x 0.37 m x 15 mm thick). The cages were located within an insulated shed divided into two experimental rooms. Thermometers positioned at four locations in each room recorded the daily minimum and maximum temperatures to assist in the manual control of room temperature, which was independently controlled in each room. Each room contained a bank of 20 cages, with ten cages per tier arranged as two rows of five cages in a back-to-back formation. The two tiers (i.e. upper and lower) were separated by the height of a cage. Both tiers of each bank were used in the experiment, providing a total of 40 cages.

Birds were transported to the Werribee facility at 13 weeks of age, beak trimmed and placed at random in the cages. Nest boxes were present but access was closed off for the first two weeks. At entry to the cages, birds were exposed to a 12 h L:12 h D (light:dark) schedule, which was increased to 16 h L:8 h D by 24 weeks of age. Treatments were allocated at random to tiers within rooms, so that within each tier there were eight cages containing single birds and two cages of eight birds. Egg locations were recorded daily to determine the occurrence of eggs laid in the nest box compared to on the wire floor. Data were collated and differences due to group size were analysed using Analysis of Variance in Genstat 11.1 (VSN International Ltd.), after blocking for room, tier and row. The experimental unit was the cage. Proportional data, i.e. hen day production (HDP) and eggs laid in the nest box between 19-33 weeks of age, were analysed following angular transformation of percentage values. Due to a strong positional effect on the proportion of nest box eggs, the effect of group size on the proportion of nest box eggs was also analysed after omitting the data for one row (Row 4).

III. RESULTS

HDP between 19-33 weeks of age did not differ due to group size (angular transformed means {ATM} with back-transformed means {BTM} in parentheses were 77.23 (95.1%) and 74.16 (92.4%) degrees, respectively; sed 2.491, P = 0.23). The change in HDP per week for the two group size treatments is shown in Figure 1. Group size however, affected the proportion of nest box eggs between 19-33 weeks of age (ATM with BTM in parentheses were 71.5 (89.9%) and 54.1 (65.6%) degrees, respectively; sed 6.57, P = 0.012). Figure 2 shows the change in the mean proportion of nest box eggs per week in the two treatments. From 19-33 weeks of age, the proportion of nest box eggs ranged from 4.1-100% (median 95.3%) and 48.6-93.8% (median 69.3%) respectively, for 1- and 8-bird cages.

Further investigation of the data showed a strong positional effect on nest box eggs, with most cages in Row 4 (in Room 2) having a low proportion of nest box eggs. Of the ten cages in this row, nine housed single birds with a mean proportion of nest box eggs of 53.3% (std dev 40.64). Minimum and maximum values were 4.1% and 100%, respectively. Although the three cages with the lowest proportion of nest box eggs (4.1%, 4.8% and 5.5%) were end-cages, the fourth end-cage in this row had 100% nest box eggs. Of the remaining 1-bird cages, three were considered 'inconsistent' nest box layers (46.8%, 54.6% and 73.2% nest box eggs). In comparison, the mean proportion of nest box eggs for the seven 1-bird cages in the adjacent row (Row 3) was 97.1% (std dev 5.77) and the minimum and maximum values were 84.2% and 100%, respectively. There was only one 8-bird cage in Row 4 and

this cage had the lowest proportion of nest box eggs (48.6%) recorded for group treatment cages. In comparison, there were three 8-bird cages in Row 3 and these averaged 69.3% (std dev 2.16) nest box eggs. After omitting the data for Row 4 cages, the difference due to the group size treatment was stronger (ATM with BTM in parentheses were 79.3 (96.6%) and 59.7 (74.5%) degrees, respectively; sed 3.58, $P < 0.001$). The mean (std dev) maximum and minimum temperatures recorded in Room 1 were 23.7°C (1.39) and 21.2°C (1.71), and in Room 2 were 25.6°C (1.78) and 21.6°C (1.66), respectively.

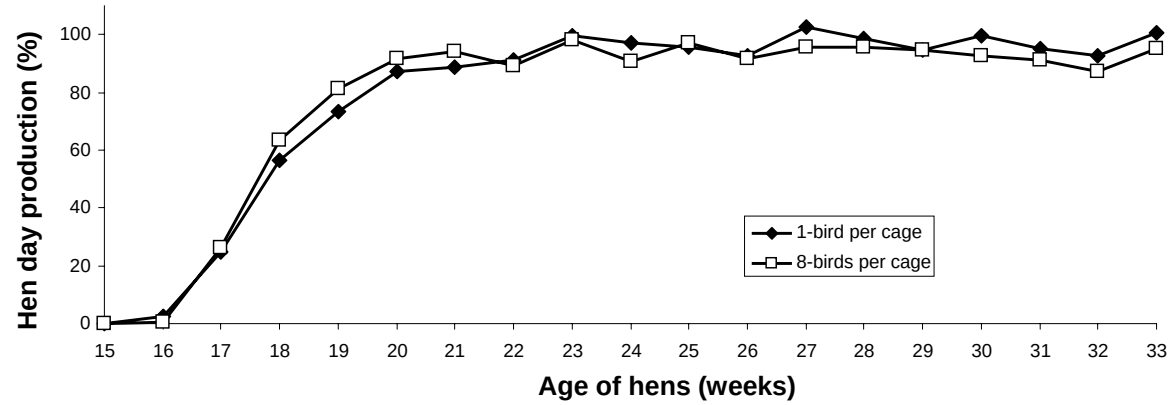

Figure 1 Hen day production per week for the two group size treatments.

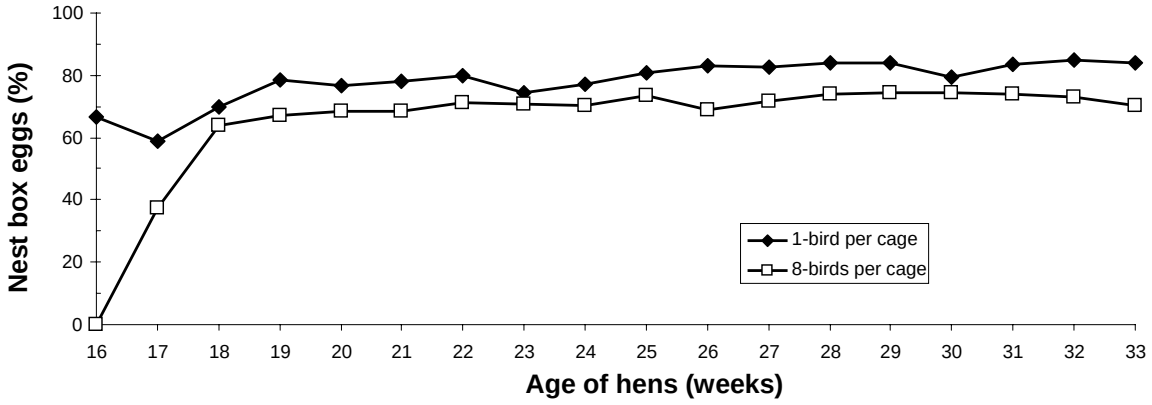

Figure 2 Proportion of eggs per week laid in nest boxes in the two group size treatments.

IV. DISCUSSION

The proportion of nest box eggs recorded for group-housed hens in the present experiment was similar to findings in our previous experiments using the same cages and similar Hy-Line Brown birds (62%, Barnett and Cronin, 2005; 70%, Cronin et al., 2007). Nevertheless, the proportion was lower than overseas studies by Tauson and Holm (2005), in which almost 100% nest box eggs were reported using cages of the same design as the present experiment. Sherwin and Nicol (1993) and Friere et al. (1997, 1998) have suggested that social factors contribute to hens laying outside the nest box. The results of the present experiment support this suggestion. Group-housed hens laid proportionally fewer eggs in the nest box than singly-housed hens. We did not video record bird behaviour in this experiment and thus are not able to conclude whether those group-housed birds that did not use the nest box were subordinate, as suggested by Shinmura et al. (2007). If subordinate birds avoid using the nest box, Appleby et al. (2004) recommended increasing the nest box floor area. Whether this

would be effective requires testing. For example, subordinate hens may not enter the nest box if a dominant is near the entrance or inside the nest box. Potentially a second entrance or two separate nest boxes per cage might be required. While the cages in the present experiment were designed for eight birds, Appleby (1984) stated that ratios of up to eight birds per nest box were used in industry for breeder flocks. In the latter situation however, larger groups of hens and multiple nest boxes were involved, providing hens with a choice of nest boxes.

An interesting result in this experiment was the lower incidence of nest box eggs from cages in Row 4. Rows 1 and 4 were mirror images and were equidistant (~1.2 m) from the shed's outer east and west walls, respectively. While the recorded mean maximum temperatures between the rooms differed by less than 2°C averaged over the experiment, Row 4 faced west and may have received radiant heat from the afternoon sun (although the shed wall was insulated). Nevertheless, how this would affect choice to lay in the nest box, which typically occurs in the morning, is unclear. Other possibilities are that hens were affected by other (human?) activities in Row 4 within the shed, or by noise from outside the shed (human, livestock, nocturnal animals). Whilst there was a laneway for moving cattle or sheep outside the west of the shed, it was rarely used during this experiment. In conclusion, while social factors strongly contributed to hens not laying in the nest box, it was apparent other (unknown) factors also influenced birds' choice of egg-laying site.

ACKNOWLEDGEMENTS

Financial support of AECL and DPI Victoria is gratefully acknowledged. The experiment was conducted with the assistance of Bruce Schirmer, Tracie Storey, Judy Nash, Lauren Edwards and Bronwyn Stevens, who cared for the birds and recorded and collected the eggs, and Kym Butler, who provided advice on experimental design and statistical analysis.

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DOES THE QUANTITY OF REWARD IN A Y-MAZE PREFERENCE TEST AFFECT HEN CHOICE AND MOTIVATION?

S.M. LAINE[1], G.M. CRONIN[2,4], J.C. PETHERICK[3] and P.H. HEMSWORTH[1]

Summary

The quantity of reward in a Y-maze preference test refers to the period of time the animal is allowed contact with the chosen resource. Fifteen hens (Hy-Line Brown strain) were given eight preference test trials (conducted on alternate days) for their choice between dust (a tray of peat moss) or social contact (the presence of a familiar subordinate hen). The quantity of reward for social contact was 5 minutes for all birds. However, the hens were allocated to three treatments (n = 5), which differed in the quantity of reward when dust was chosen: Long (45 minutes), Intermediate (20 minutes) and Short (2 minutes). During testing, hens were housed individually and were deprived of both social contact and dust. Hens on all treatments chose dust significantly more often than expected at chance level ($P < 0.001$ for all). The choice behaviour was not significantly different between treatments, however the Intermediate hens had a tendency to choose social contact more often ($P = 0.07$). Intermediate birds also took significantly more time to move to their choice compared to both the Short and Long birds ($P = 0.0015$). In addition, there was a significant treatment effect on the proportion of dust-chosen trials in which a dustbathing bout was interrupted ($P = 0.0079$), with the Long treatment having the lowest proportion interrupted and the Short treatment having the highest proportion of dustbathing bouts interrupted. Results indicate that dust was an attractive resource in the Y-maze for all birds. The tendency to choose dust less often and take longer to move through the Y-maze suggests that Intermediate treatment birds were less motivated to gain access to dust. This could perhaps have been due to the period that these birds had access to dust, which was less than the average time for a dustbathing bout, causing a greater proportion of dustbathing bouts to be interrupted in comparison to the Long treatment. Therefore, while the quantity of reward did not significantly influence choice behaviour, the Intermediate treatment appeared to reduce hen motivation for accessing dust.

I. INTRODUCTION

Animal preferences may tell us what is important to an animal and thus provide an indication of its welfare. Preference tests carried out in a Y-maze apparatus, where an animal makes a choice between two resources, may appear to be straightforward. However, aspects of the design of Y-maze preference tests may have the potential to influence motivation and thus choice behaviour, leading to spurious results that are not reflective of the animal's true preferences.

One such factor is the quantity of reward. The quantity of reward in a Y-maze preference test refers to the period of time the animal has with its chosen resource. Although it has been previously proposed that the quantity of reward may affect how 'attractive' a resource is to an animal in a preference test (Nicol, 1997; Kirkden and Pajor, 2006) this concept has yet to be examined. This experiment set out to determine the effects of the

[1]Animal Welfare Science Centre, Melbourne School of Land and Environment, University of Melbourne. Parkville Vic 3010.
[2]Animal Welfare Science Centre, Department of Primary Industries. 600 Sneydes Road, Werribee Vic 3030.
[3]Department of Primary Industries and Fisheries. PO Box 6014, Rockhampton Qld 4702.
[4]Faculty of Veterinary Science, University of Sydney, Camden NSW 2570.

quantity of reward on the choice behaviour and motivation of laying hens in a Y-maze preference test where hens were offered the choice between dust and social contact.

Dustbathing is a series of behavioural components that aids the maintenance of plumage (van Liere and Bokma, 1987; Olsson and Keeling, 2005). On average, the duration of a dustbathing bout is 27 minutes and occurs every second day with peak activity being around midday (Vestergaard, 1982). Laying hens have been found to have preferred substrates in which to dustbathe, with peat moss being the most preferred material (Petherick and Duncan, 1989; de Jong et al., 2007).

Laying hens are highly social animals that flock together and form a stable dominance hierarchy, which may remain stable for a number of years (Schjelderupp-Ebbe, 1922 cited in Mench and Keeling, 2001). Social contact is presumably important for laying hens as many behaviours such as feeding, dustbathing and preening are often performed in synchrony by many individuals (Hughes, 1971; Webster and Hurnik, 1994; Duncan et al., 1998). Additionally, social isolation is known to cause stress, both behaviourally and physiologically, in domestic chicks (Jones and Merry, 1988).

Thus a dustbathing substrate and social contact are important resources for laying hens. We hypothesised that the quantity of reward would impact on how attractive the resources were perceived by laying hens and this would ultimately impact on hen choice behaviour. To test this hypothesis, we gave a choice in a Y-maze for access to a familiar, subordinate hen for a fixed period of time and a dustbathing substrate for three different periods of time.

II. MATERIALS AND METHODS

Fifteen hens (Hy-Line Brown laying strain) aged 31 weeks were housed in individual cages (0.57 m x 0.50 m x 0.48 m). Each cage contained a dustbath that was refilled daily with peat moss. Throughout the experiment, hens had *ad libitum* access to food and water. The birds were firstly familiarised to the Y-maze apparatus (for a description of the apparatus, refer to Laine et al., 2007). Hens were then trained on alternate days for a total of five training sessions. During training and testing, hens had their home cage dustbath removed and were deprived of social (visual) contact by placing opaque rubber partitions between cages. All training and testing sessions were conducted from 1100 h each day. Immediately following training, hens were tested for eight trials (one per day) conducted on alternate days for their choice between social contact (the presence of a familiar subordinate hen) or dust (a tray of peat moss). The quantity of reward for social contact was 5 minutes for all birds. However, birds were allocated to three treatments which differed in the quantity of reward for dust; Short (2 minutes), Intermediate (20 minutes) and Long (45 minutes). 'Time to choice' was defined as the time taken from when the start box gate was opened to when the hen moved into one of the Y-maze arms. A choice was defined as the resource that the Y-maze arm contained that the hen moved into on each test trial. A dustbathing bout was defined as interrupted if the hen was picked up and taken back to her home cage by the experimenter prior to the completion of the dustbathing bout.

III. RESULTS

Hen choice behaviour was compared to chance level (i.e. 50:50) using a Chi-Square test. After log transformation to reduce skewness of data, an analysis of variance (ANOVA) was used to compare the choice behaviour between treatments. The hens' choice for dust was higher than would be expected by chance for all treatments ($P < 0.001$). However, the Intermediate treatment hens had a tendency ($P = 0.07$) to choose social contact more than the

other treatments. The back transformed proportion of trials in which dust was chosen by the Short, Intermediate and Long treatments were 0.98, 0.83, and 0.98 respectively.

After negative reciprocal transformation, an ANOVA was used to compare the time to choice between treatments. The Intermediate treatment was significantly slower to make a choice compared to both the Short and Long treatments (P = 0.0015), with no difference between the Short and Long treatments (Fig 1).

After log transformation, an ANOVA was used to compare the proportion of dust-chosen trials in which a dustbathing bout was interrupted between treatments. There was a significant treatment effect, with the Long treatment having the lowest proportion of dustbathing bouts interrupted and the Short treatment having the highest proportion of bouts interrupted (P = 0.0079). The back transformed proportion of dustbathing bouts that were interrupted for the Short, Intermediate and Long treatments were 1, 0.995 and 0.17 respectively.

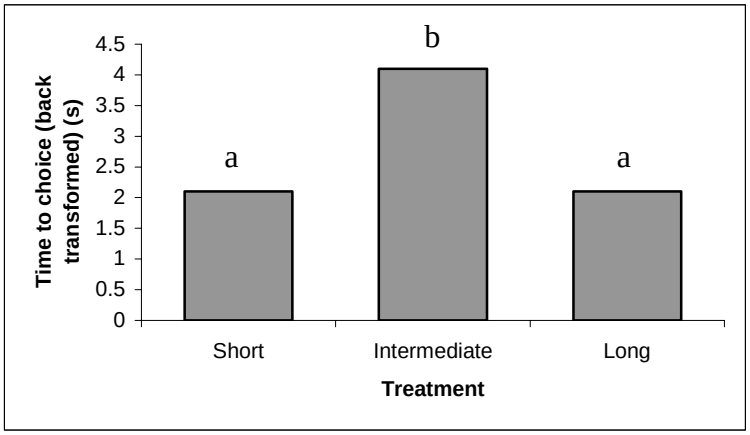

Figure 1 Time taken (back transformed) for hens to move through the Y-maze to make a choice. (Different letters indicate significant differences, P = 0.0015).

IV. DISCUSSION

The results indicate that dust was an attractive resource for all birds, as hens in all treatments chose dust significantly more often than would be expected by chance. Peat moss was chosen as the dust substrate as it has previously been found to be the preferred dustbathing substrate by laying hens (Petherick and Duncan, 1989; de Jong *et al.*, 2007). In addition, preference test trials were conducted on alternate days, during the late morning/early afternoon to coincide with peak dustbathing activity (Vestergaard, 1982) and therefore presumed peak dustbathing motivation. These factors may have made very attractive, especially when hens were deprived of dust in their home cage. Moreover, even though the hens were deprived of social contact in their home cages, they had auditory and olfactory contact with other birds. Perhaps this contact, coupled with the brief visual contact hens had with the 'social contact' bird while they were in the start box of the Y-maze (prior to making a choice) was sufficient social contact for hens.

The speed of movement through the Y-maze and other tests where an animal must move to a resource can be equated to motivation (e.g. Petherick et al., 1992). It would be expected that an animal with a high motivation for a resource would move faster than an animal with low motivation. This, as well as having a tendency to choose dust less compared to the other treatments, suggests that birds in the Intermediate treatment were less motivated to gain access to dust in the Y-maze. This could be due to the quantity of reward that these hens had with dust (20 minutes), which is less than the reported average dustbathing bout

duration of 27 minutes (Vestergaard, 1982). In the present experiment, Intermediate treatment hens had a greater proportion of dustbathing bouts interrupted compared to the Long treatment. Mason et al. (1998) suggested that repeated interruption of an activity might devalue the resource. Perhaps repeated interruption of the Intermediate birds' dustbathing bouts devalued the dust for these hens. If so, this devaluation could explain the apparent low motivation for dust access in the Y-maze.

Interestingly, the Short treatment did not differ from the Long treatment in terms of choice behaviour or time to choice. However, Short treatment hens had the highest proportion of dustbathing bouts interrupted compared to the other treatments. This repeated interruption, however, did not seemingly affect Short treatment hen choice behaviour or motivation (in terms of speed of movement) when compared to that of the Long treatment hens. Perhaps interruption at the very beginning of the dustbathing bout was less aversive to the hen compared to interruption further into the bout. This could explain why the small quantity of reward for dust seemingly had no impact on hens in the Short treatment.

While the quantity of reward did not significantly alter hen choice behaviour, it did apparently impact on motivation to access dust. Further research examining the effects of dustbathing bout interruption on motivation may aid in interpreting results obtained in this experiment. If interruption of an activity does prove to have an influence on animal choice behaviour or motivation, this may have important implications for the selection of reward quantities in animal preference tests in the future.

ACKNOWLEDGEMENTS

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THE EFFECTS OF HOUSING LAYING HENS AS GROUPS IN CONVENTIONAL CAGES ON PLASMA AND EGG ALBUMEN CORTICOSTERONE CONCENTRATIONS

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

Summary

The limited space available to hens in conventional cages remains an issue for the egg industry. Stress and the plasma concentrations of corticosterone continue to be frequently used as part of welfare assessment. In the present study individually housed hens were moved to new cages and housed 5, 4, 3 and 2 birds/cage or singularly. Plasma and egg albumen were collected at various times up to 110 days after the hens were move. Following the relocation and housing in groups, the plasma concentrations varied throughout the study. The corticosterone concentrations in egg albumen were higher over the first 16 days than at later sampling times. There were no significant differences in plasma or egg albumen corticosterone concentrations when hens were housed in different group sizes and provide with differing space allowance.

I. INTRODUCTION

In any production system that houses hens as groups, the space available to an individual is limited by flock mates and the specifications of the enclosure. Therefore, important considerations in assessing hen welfare are the size of the enclosure, number of hens and the availability of resources (Mench and Keeling, 2001). There are reports indicating that egg production decreases as the cage density increases (Anderson and Adams, 1991; Brake and Peebles, 1992; Bell et al., 1998). While production is often used as an indicator of welfare, a more comprehensive evaluation requires assessment using a number of criteria. There appears to be a relationship between cage density and plasma corticosterone concentrations (Craig et al., 1986). Decreasing the area per bird increased corticosterone concentrations (Mashaly et al., 1984), as does a decrease in personal space (Compton et al., 1981). While plasma corticosterone is one physiological measure of stress, and chronic stress is associated with poor welfare, it is difficult to measure because continuous blood sampling is not an option. Measuring corticosterone in egg albumen can eliminate most of the problems associated with having to take blood samples (Downing and Bryden, 2008). The objective of the present study was to evaluate what the combined effects of group size and density of hens housed in conventional cages have on plasma and egg albumen corticosterone concentrations.

II. MATERIALS AND METHODS

Isa Brown hens, 58 weeks of age were used in the study. They were fed *ad libitum,* a commercial layer diet containing 12.3 MJ/kg metabolisable energy and 160g/kg protein. During the study hens were maintained on 16h light with lights on at 0600h. Hens previously housed individually in conventional layer cages (1175 cm^2/bird) were transferred to group cages and housed 5, 4, 3 and 2 hens per cage or as a single hen per cage. The floor space allocated to each of the treatments were 460, 575, 767, 1150 and 2300 cm^2/bird,

[1] Faculty Veterinary Science, University of Sydney, Camden, NSW 2570
[2] School of Animal Studies, University of Queensland, Gatton Campus QLD 4343

respectively. There were ten replicate cages for each of the housing treatments. The hens were moved between 0800h and 0930h on day 1 of the study.

Two weeks and one week before the hens were transferred to group cages a 1 ml blood sample was taken by jugular venipuncture from 36 of the hens to be used in the study. On days 2, 4, 8, 11, 16, 26, 43, 54, 83, and 110 of the study a 1mL blood sample was taken from one hen in each cage. Samples were collected between 1530h and 1630h. The blood was centrifuged and the plasma harvested and stored at -20^0C until assayed. Daily egg production was recorded for the entire period of the study. On the days following the blood sampling periods, all eggs were collected and oviposition times recorded. The first two eggs laid by hens in cages housing 5, 4 or 3 birds and all eggs laid by hens in cages housing 2 birds or a single bird were identified and weighed. These were then broken open and the albumen collected, weighed and then stored at -20^0C until assayed. The corticosterone concentrations in plasma and egg albumen were determined by RIA as described previously by Downing and Bryden (2008).

Values are given as means ± SEM. Differences between treatments were assessed by ANOVA and if significant ($P < 0.05$) then multiple comparisons were made using the Tukey test. All analysis was conducted using the 'Statview' computer program (SAS Institute Inc, NC, USA).

III. RESULTS AND DISCUSSION

The mean egg production for the study period were 5.78 ± 0.09, 5.88 ± 0.09, 6.04 ± 0.08, 5.94 ± 0.11 and 6.30 ± 0.11 for hens housed 5, 4, 3, 2 or 1 bird(s) per cage, respectively. The overall mean production was higher in birds housed singly compared to those housed 5, 4 or 2 birds per cage. Koelkebeck and Cain (1984) using different group sizes and space allowances found that in general, egg production was favoured by having one hen per cage. In the present study, no significant effects of hen number/cage on egg weight or oviposition time were observed.

At two weeks and one week before being transferred to the group cages the mean (± SEM) plasma corticosterone concentrations were 0.55 ± 0.04 ng/mL and 0.82 ± 0.09 ng/mL, respectively. The mean (± SEM) plasma corticosterone concentrations during the experimental period are shown in Figure 1. The effect of hen number per cage failed to reach significance ($P = 0.075$). However, the effect of day was significant ($P<0.0001$) but there was no significant treatment x day interaction. Plasma concentration of corticosterone was higher on days 4 and 43 than all other days except days 2 and 83 ($P < 0.05$). The concentrations on days 2 and 4 were higher than on days 8 and 11 ($P < 0.05$). On any individual collection day, the plasma corticosterone concentrations were not different for hens housed 5, 4, 3 or 2 per cage or singularly.

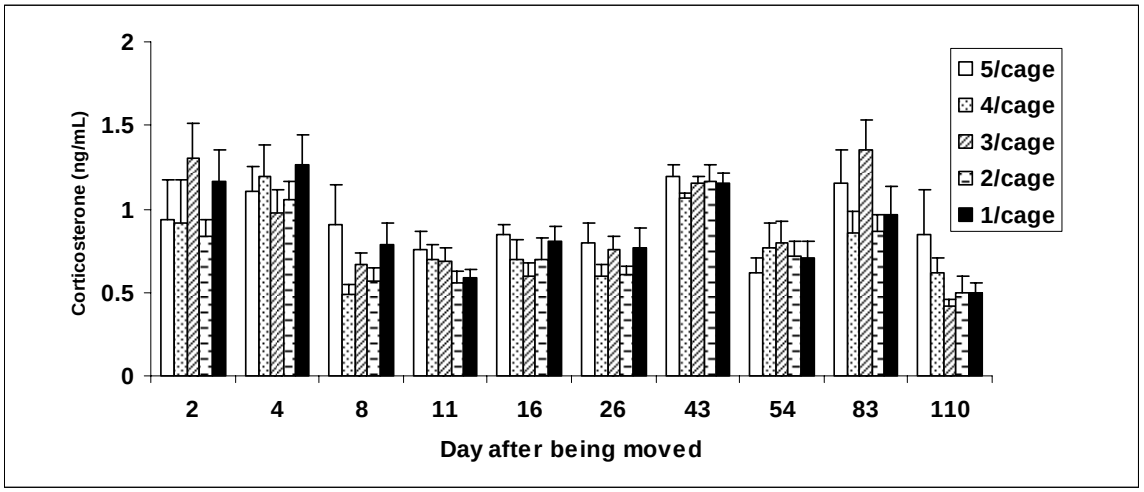

Figure 1 The mean (±SEM) plasma corticosterone concentrations for hens housed individually and then moved and housed in groups of 5, 4, 3 and 2 birds/cage, or singularly for 110 days.

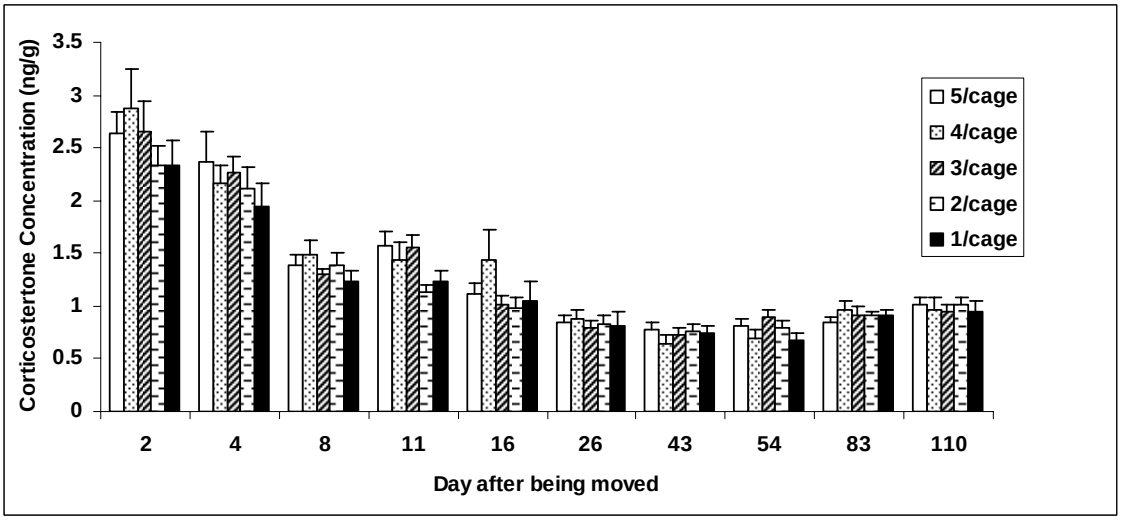

Figure 2 The mean (±SEM) egg albumen corticosterone concentrations for hens housed individually and then moved and housed in groups of 5, 4, 3 and 2 birds/cage, or singularly for 110 days.

The mean (± SEM) albumen corticosterone concentrations are shown in Figure 2. The effect of hen number per cage just failed to reach significance ($P = 0.06$). However, the effect of day was significant ($P < 0.0001$) and there was no significant treatment x day interaction. The albumen corticosterone concentrations were higher on days 2 and 4 than all other days ($P < 0.05$). On days 8 and 11 the concentration was higher than on later collection days while the concentration on day 16 was higher than on days 43 and 54 ($P < 0.05$).

There is direct relationship between the plasma and egg albumen corticosterone concentrations (Downing and Bryden, 2008). In the present study, the blood samples were taken on the day the albumen would have been deposited during egg formation but after it had been deposited, as the samples were collected after 1530h. Because of the difference in timing it can not be assumed that the plasma concentrations are representative of those existing during albumen accumulation in egg formation. The plasma concentrations are those existing at a single time point. The hens used in this study were housed in a large facility with other hens and it is possible that some unforeseen event or activity caused the elevation in plasma corticosterone concentration observed on days 43 and 83 and were not related to movement of hens to group cages. Craig et al. (1986) reported that moving hens and housing them individually or in groups of 4 or 6 had varying effects on plasma

corticosterone concentrations. Following the relocation the corticosterone concentrations were elevated during the first 5 days but had declined by 2 to 3 weeks. In the present study, as the group size increased, and the space allowance decreased, no significant effects on plasma corticosterone concentrations were observed. The large variation in plasma corticosterone concentrations is a major issue associated with using single point samples in measuring stress hormones concentrations.

On individual sampling days the number of hens in the cage had no effect on the egg albumen corticosterone concentrations. The egg albumen concentrations were higher on days 2 and 4 after the hens were moved to the group cages. These gradually decreased by day 16 and then remained at a similar level for the remainder of the study. The increase in albumen concentrations observed during the first 16 days are most probably a result of stress associated with the moving of hens to a new novel environment and housing them in groups. After this period the combination of group size and space allocation had no effect on egg albumen corticosterone concentration. Subtle changes in plasma corticosterone concentrations could be amplified in albumen because of the period over which the albumen accumulates and could provide a better indication of corticosterone concentrations than single point blood samples.

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EVALUATION OF CYTOKINE AND CHEMOKINE PROFILES IN CHICKENS TREATED WITH CORTICOSTERONE: A POTENTIAL INDICATOR OF STRESS IN POULTRY

S. SHINI[1] and A. SHINI[1]

In chickens, the major neural pathways activated by stressors are the hypothalamo-pituitary adrenal (HPA) and the sympathetic-adrenal-medullary (SAM) axes. The pituitary gland produces adrenocorticotropic hormone (ACTH) which in turn stimulates the production of glucocorticoid (GC) hormones, including corticosterone. Leukocytes, such as T, B, natural killer (NK) and antigen-presenting cells carry receptors on their nucleus and cell surface for stress hormones produced by the adrenal and pituitary glands. These hormones can therefore modulate the activities of these cells, in particular the production of proinflammatory cytokines and chemokines. The cytokines and chemokines themselves can in turn modulate the activity of the hypothalamus, and thus alter hormone production. We hypothesize that stress evokes a specific combination of molecular mediators (i.e. cytokines and chemokines) as precursors of physiological processes to allow the animal to 'cope' with elevated levels of corticosterone. In this study we evaluate cytokine and chemokine profiles in corticosterone-stressed chickens and delineate their roles in controlling leukocyte traffic.

At 7 weeks of age, 120 chickens were exposed for 1 week to the following treatment in drinking water: corticosterone dissolved in ethanol and then in water (20 mg/1 ml ethanol/1 l water), ethanol (1 ml/1 l water), or untreated water. Whole blood was collected from eight randomly selected chickens per group at 0 h, 3 h, 24 h, and 7 days post-initial treatment. Cytokine and chemokine mRNA expression levels were evaluated in peripheral blood lymphocytes and heterophils. The expression of mRNA for interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-10, IL-12α, IL-12β, IL-13, IL-18, interferon (IFN)-γ, transforming growth factor (TGF)-β4, chemokines (CC) CCLi1, CCLi2, CCL5, CCL16, CXCLi1, CXCLi2 (formerly IL-8) and chemokine receptor (CXCR1 and CXCR4) in lymphocytes and heterophils were measured using real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR) assays.

Administration of corticosterone significantly increased circulating corticosterone concentrations and total heterophil counts, and decreased total lymphocyte counts at 3, 24 h and 1 week post-initiation of treatment. Expression levels of IL-1β, IL-6, IL-18 and TGF-β4 mRNAs were significantly up-regulated in lymphocytes 3 h post-treatments with corticosterone. TGF-β4 and IL-18 remained elevated 1 week post-repeated treatment. Compared with controls, corticosterone-treated birds showed greater expression levels of chemokine mRNA, particularly for CCLi2, CCL5 (RANTES), CCL16 and CXCLi1, in peripheral lymphocytes 3 h post-initial exposure. The mRNA expression levels for IL-1β, IL-6, IL-18 and IL-12α in peripheral heterophils were also initially upregulated by corticosterone, and thereafter (starting at 24 h) downregulated. Repeated treatment with corticosterone (for one week) stimulated the expression of cytokine TGF-β4 and chemokine CCL16 and suppressed IL-12 α and IFN-γ in heterophils. It was demonstrated that conditions associated with significant changes in plasma corticosterone concentrations might affect the immune response by increasing pro-inflammatory responses, and leading to potential modulation of the Th1/Th2 balance. We conclude that evaluating cytokine and chemokine mRNA profiles could help to assess stress in chickens.

[1] School of Animal Studies, University of Queensland, Gatton QLD 4343, Australia

CHICK QUALITY

G.M. FASENKO[1], E.E. O'DEA CHRISTOPHER[1], A. ULMER FRANCO[1] and L. KAWALILAK[1]

Summary

The research projects described in the following proceedings cover only a few of the factors which can influence hatchling survival and growth, but there are many more. What happens during incubation and in the first week of the hatchling's life has the potential to impact the bird's health and growth, and ultimately carcass composition. While neonate health is essential to subsequent growth, defining and assessing the characteristics that will result in a healthy bird during grow-out is challenging. One of the physical attributes that has been linked to hatchling health is navel condition. Incomplete withdrawal of the residual yolk sac into the abdominal cavity of the neonate can result in unhealed navels. This proceedings will outline the relationship between unhealed navels, omphalitis, intestinal maturation, and chick quality. Ongoing chick quality research will be discussed as well as the need for scientists to develop objective measures of chick quality. The use of infrared thermography (IRT) as a tool for use in potential disease diagnosis will also be examined.

I. INTRODUCTION

There are many factors which can have an influence on hatchling survival and post-hatch performance. Some of these factors can be influenced by hatching egg producers, hatcheries, and/or broiler producers. Thus, it is in the best interest of the various players in the industry to collectively try to manage these factors.

The health of avian neonates has subsequent effects on bird growth and ultimately meat characteristics and economic returns. Currently, the health of hatchlings is evaluated using subjective, visual measures, however, automation in modern hatcheries has made close observation of individual chicks and poults extremely difficult. The visual subjective measures of neonate health can include factors such as activity, eating and drinking, alertness, lack of injuries, and a closed navel.

Hatcheries in Canada regularly include two extra chicks in every box of 100 sent to broiler farms as there is an acknowledgement that 2% of the broiler mortality in the first week of production may be attributed back to the hatchery. It is recognized in the poultry industry that the health and viability of poults at hatching is even poorer than that of broiler chicks. Two of the most prevalent causes of mortality (especially during the first week of grow-out) are omphalitis (yolk sac infections), and red hocks or leg problems. In many cases, hatchlings that visually appear healthy at the hatchery may have the beginnings of bacterial infections, or inflammation in the legs. One province in Canada, recorded that yolk sac infections accounted for 30% of cases of disease reported by the broiler farmers in 2005. Although these cases may initially be sub-clinical at hatching, the infections may worsen once the chicks or poults are placed and exposed to stressors in the grow-out barn. Even if the poultry neonate survives the infection, the consequence is that some nutrient intake that would be directed towards growth now has to be directed towards launching an immune response. This infection early in the life of the hatchling negatively influences feed efficiency and body weights at processing.

[1] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

II. HOW DOES GENETIC STRAIN, PARENT FLOCK AGE, AND EGG SIZE AFFECT CHICK QUALITY

As a broiler breeder flock ages, egg size increases and egg characteristics and chick quality change. A research project was carried out in the Fasenko lab to look at how production parameters related to incubation and broiler production influence chick health (Ulmer Franco et al., unpublished). In this study, eggs from 2 broiler breeder strains at 3 flock ages were incubated. At each flock age, eggs were categorized into three distinct egg weight groups. Irrespective of genetic strain, eggs from young breeders had smaller yolks and the egg yolks and chick yolk sacs (removed post-hatching) had different lipid profiles compared to egg yolks and yolk sacs from eggs produced by older flocks. This is important because the lipids in the yolk provide the main energy source for the embryo and early neonate. These lipid composition differences may be related to hatchling health and survival, especially in offspring from young breeder flocks that seem to be more susceptible to health issues.

Egg size influenced the number of culled chicks; chicks from larger eggs had a higher propensity to be culled than other egg weight categories. This agrees with previous research (Lawrence et al., 2004). This demonstrates that, although broiler producers in general prefer receiving larger chicks, bigger is not always better. One of the focuses of future research should be to further define the relationship between lipid availability in neonates from young flocks, early growth, and rearing conditions specifically provided for these potentially susceptible hatchlings.

III. THE EFFECT OF UNHEALED NAVELS AT HATCHING ON BROILER PERFORMANCE

Once eggs hatch, assessing chick quality is key to predicting which hatchlings will survive and thrive once placed on farms. Automation in hatcheries has meant that hatchery personnel have less time to assess the quality and health of each individual chick. The result is that only weak or obviously abnormal chicks are removed, and are not shipped to broiler farms. Automation makes it more difficult for hatchery personnel to check for problems such as unhealed navels, and inevitably, some chicks with navel conditions will pass through the screening process.

The yolk sac is a highly vascularized membrane that starts to develop and surround the yolk at around 2d of incubation. This extraembryonic membrane enables the transfer of nutrients from the yolk to the developing embryo (Romanoff, 1960). At around 19 d of incubation, the yolk that has not been absorbed by the embryo (residual yolk sac) starts to be internalized through the navel into the chicken embryo's body cavity. Upon hatching it should be fully withdrawn with the skin of the navel completely healed and closed over (Romanoff, 1960). However, this is not always the case. When the residual yolk sac is not fully internalized, the protruding tissues prevent the navel from healing properly. If withdrawal of the yolk is complete, at hatching the navel is fully closed with no yolk protruding or scab ("navel button") and no leakage of fluid ("leaky" navel).

Recently published research has provided evidence that broiler chicks with small navel buttons (less than 3 mm in diameter) or fluid leaking from the navel have significantly lower body weights (BW) at processing (6 wk later) (Fasenko and O'Dea, 2008). Initially, chicks from both the leaky navel (39.0 g) and navel button (38.9 g) groups were heavier than chicks with healthy navels (37.8 g) at hatching. However, in Trial 1, after one week, chicks with navel buttons were lighter (83.8 g) than either healthy chicks (89.3 g) or chicks with leaky navels (87.8 g) which were not different from one another. The same pattern was

observed in a second trial. By 41 days of age, both the navel button (Trial 1: 1921 g, Trial 2: 1799 g) and the leaky navel (Trial 1: 1932 g, Trial 2: 1812 g) broilers had lower body weights than the broilers with healthy navels (Trial 1: 2029 g, Trial 2: 1898).

The authors hypothesized that the growth reduction may have been the result of undetected yolk sac infections (omphalitis) present at the time of chick placement. If the infection is sub-clinical, there is no reason to cull these chicks. However, nutrients that would have been used by the broilers for growth, were subsequently directed towards fighting the infections. This hypothesis was supported by the fact that broiler mortality over the entire grow-out period was higher in broilers that had navel buttons at hatching (12.7%) compared to broilers with healthy navels at hatching (5.7%). Mortality in broilers with leaky navels (10.1%) was not significantly different from either of the other treatment groups.

This research showed that even minor navel conditions result in reduced broiler performance during grow-out. This is especially important due to the fact that an increased degree of automation in modern hatcheries has reduced the opportunity for these chicks to be detected and removed from the broiler production chain. An additional issue is that a definitive diagnosis of omphalitis cannot be made until after the bird has died. Beyond subjective visual evaluations, or removing the yolk sac to test for bacteria, there are currently no reliable, non-invasive methods to diagnose yolk sac infections.

IV. IMPAIRED INTESTINAL VILLI GROWTH IN BROILER CHICKS WITH NAVEL BUTTONS

The residual yolk sac is very important to the newly hatched avian neonate. Withdrawal of the yolk sac into the abdomen of the embryo provides the hatchling with nutrients during the first few days of life. This residual yolk sac comprises approximately 14% of the chick's body weight at the time of hatching (Mikec et al., 2006). The contents are absorbed and utilized for growth of the small intestine and to supply energy (Noy and Sklan, 1999) and nutrient reserves for several days (Uni et al., 2003).

Omphalitis (yolk sac infection) is one of the main causes of first week mortality (Rai et al., 2005). It has been reported that bacteria associated with omphalitis cause deterioration and decomposition of essential RYS nutrients that should have been used as a source of energy in the post-hatch period (Rai et al., 2005). In post-hatch chicks, the residual yolk sac (RYS) contents are mobilized simultaneously by 2 different routes: via the YS stalk into the intestine (Esteban et al., 1991) and by absorption through the YS membrane into the chick's circulation (Noy and Sklan, 1998). The absorption of nutrients from the YS is essential to initiate body growth (Chamblee et al., 1992; Murakami et al., 1992) and also for development of the small intestine (Noy and Sklan, 1999).

Previously conducted research has described the details of the normal gastrointestinal development of the chick (Sklan, 2001; Uni et al., 2003). During the last 3 d of incubation (in preparation for the intake of feed) an increase in the number of enterocytes in the small intestine causes a large increase in villi height (Uni et al., 2003). As a consequence, the surface area available for absorption of nutrients as well as the weight of the small intestine are greatly increased (Uni et al., 2003). The immediate post-hatch period is also critical for intestinal growth (Geyra et al., 2001). The small intestine grows rapidly in the first 24 hours after the intake of carbohydrate-rich grain-based feeds (Sklan, 2001).

A project was carried out to study the relationship between the presence of small navel buttons and residual yolk sac weight and intestinal villi growth during the first 5 d post-hatching in broiler chicks (Kawalilak et al., 2008). It was established that chicks with navel buttons had larger yolk sacs than chicks with healed navels. This provides an indication that

there may have been an impaired ability of neonates with navel buttons to absorb the yolk sac contents. It was also determined that, using intestinal villi height as a measure of intestinal growth, the intestine was more developed in chicks with healed navels. Since development of the intestine is crucial to growth of the neonate, hatchlings with unhealed navels may be at a disadvantage with respect to their ability to absorb nutrients ingested in the feed.

The next step in this research is to determine if the cause for the low absorption of the yolk sac contents and slower intestinal growth observed in chicks with navel buttons is due to an infection of the yolk sac.

V. CURRENT AND FUTURE CHICK QUALITY RESEARCH

a) Omphalitis and broiler chick quality: morphology and microbiology of yolk sacs

The two previous studies discussed provide evidence that even minor broiler navel conditions are linked to changes in intestinal physiology, and ultimately a reduction in broiler growth and survival. It was hypothesised that this may be linked to the presence of sub-clinical omphalitis. A recently initiated study is designed to determine if contamination through unhealed navels is resulting in systemic infections.

Omphalitis is known to increase first week mortality in broilers, decrease broiler growth rate, decrease flock uniformity, and increase the need for antibiotic treatments to be administered to broiler flocks. However, the route by which bacteria (including *E. coli*, *Salmonella*, and *Campylobacter*) enter the chick to cause omphalitis has not been scientifically proven. It is important to reduce the possibility of bacterial contamination throughout all levels of the broiler production chain because the bacteria mentioned above are the main causes of food borne illnesses. Over the past few years, the incidence of omphalitis has been increasing in broiler production in western Canada.

In addition to examining the mode of entry of bacteria into the yolk sac, it is also important to understand if the hen can protect the chick against bacterial invasion by incorporating antibodies against the bacteria into the egg. Although egg yolk antibody concentrations (IgY) against specific bacteria have been measured, research has not been conducted to link yolk antibody concentrations and chick health.

The research that is currently ongoing is aimed at answering four main questions: (i) Is the bacterial mode of entry into the yolk sac, via the navel? (ii) Do younger or older breeder hens provide better protection to their chicks against disease? (iii) Does the degree of broiler barn cleanliness and sanitization influence the incidence of omphalitis? (iv) Are the bacteria found in the barn environment the same bacteria found inside the chick?

b) Using infrared thermography as a method of detecting omphalitis

Infrared thermography (IRT) may provide an objective method by which bacterial infections can be accurately detected in poultry neonates. IRT measures the heat emitted from an object and displays the temperature information as a color picture (Eddy et al., 2001). The surface temperature of the animal can then be used to evaluate the animal's internal state (Berry et al., 2003.) IRT has proven effective in early diagnosis of lameness in horses (Eddy et al., 2001) mastitis in dairy cows (Berry et al., 2003) and viral diarrhoea in beef calves (Schaefer et al., 2004). IRT can identify physical changes in the animal before the clinical (visible) appearance of disease is apparent (Eddy et al., 2001). Early disease detection is imperative in order to implement treatment quickly to reduce costs associated with loss in production and the need for repeated antibiotic treatments. Especially in poultry grow-out barns where stocking density is high, early disease prevention has great importance. The impact of successfully identifying disease using IRT would have much greater benefit in the turkey

industry where poult health and mortality during the first week of production is even more of a problem.

While IRT has proven effective in early disease diagnosis of other livestock species, to date, this technology has not been used extensively in poultry disease detection. Tessier et al. (2003) successfully used IRT to measure abdominal skin temperature in broiler chickens. An unpublished pilot study conducted by Fasenko and colleagues successfully used IRT to measure the temperature of the navel areas of broiler chicks. This pilot study examined if IRT could be used to capture images of chicks with normal and unhealed navels and correlate those IRT images to final body weights at shipping. The data obtained did show navel temperature differences between chicks with healthy and chicks with unhealed navels. We anticipate that with further research, IRT could ultimately be used as a diagnostic tool by poultry veterinarians, flock supervisors, industry field personnel, and hatchery managers.

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THE INFLUENCE OF INCUBATION ON CHICK QUALITY AND BROILER PERFORMANCE

R. MEIJERHOF[1]

Summary

Chick quality is highly influenced by the conditions during incubation, and have a significant impact on subsequent performance. Especially embryo temperature has shown to have a significant influence on embryo development and broiler performance. To evaluate the quality of day-old chicks and to predict performance potential, an objective and repeatable method for measuring chick quality is required. Several methods are developed, but not all methods have the same repeatability or predictive value. One of the methods that has shown to useful in field situations is chick length, as it reflects embryo development during incubation. Research has shown that a positive correlation between chick length at day of hatch and broiler performance exists.

I. INTRODUCTION

The efficiency of hatcheries is often measured in terms of hatchability. The more chickens are produced from a batch of fertile eggs, the more efficient and cost-effective the hatchery is. This is true, but at the same time it under-estimates the importance of the hatchery in the total production chain. If the hatch of fertiles is reduced, not only the cost of the un-hatched chicken is a negative factor for bottom line profits. Non-optimal incubation results in a loss of hatch but also in a loss of chick quality, because the development of the surviving chicks will also be impaired. As a result of that, it can be expected that the performance of these birds later on will be non-optimal as well. In the field and in experiments we see that the negative influence of the poor incubation conditions on broiler performance is of much higher economic importance then hatchability by itself.

II. IMPORTANCE OF TEMPERATURE

The process of converting the content of an egg into a day old chick is driven by temperature. An increase in temperature will result in an increase in development, but with it also in an increase in demand for nutrients. If the demand for some nutrients can not be met, or the process of converting egg content into embryo can not accommodated by the system, a reduced hatchability and chick quality will result.

Important is to realise that it's the temperature inside the shell, the so called embryo temperature, which drives the embryo development. This internal temperature is not equal to the air temperature and can not be fully controlled by controlling the air temperature. The temperature of the embryo is the result of the balance between the heat production of the embryo and the heat transfer between shell and environment.

The heat production of the embryo is not a constant factor. High-yielding breeds seem to produce more heat as an embryo then classical strains, but also bigger eggs produce more heat. However, the biggest influence has the stage of incubation. During the start of incubation, almost no heat is produced. After about 4 days we can observe some heat production, which increases to a maximum around 18 days of incubation. When the eggs should maintain the same embryo temperature throughout the incubation process, the heat

[1] HatchTech BV, Veenendaal, The Netherlands

transfer has to increase over time as well. This heat transfer is not only a result of the difference in temperature between eggs and surrounding air, but especially air velocity has a high influence on it as well (Meijerhof and van Beek, 1993). If there is a temperature difference between egg and air, for instance because of embryonic heat production, the air velocity will determine the actual embryo temperature. Besides air temperature and air velocity, also the evaporation of water and to a lesser extent the heat capacity of the air play a role in heat transfer more. As a result the embryo temperature can vary substantially (Lourens, 2001) and with it the development and the quality of the hatched chick. Although almost all machines control air temperature very well, the other factors affecting heat loss are much less controlled and vary between and within machines much.

III. DEVELOPMENT AND CHICK QUALITY

Practical experience and scientific research shows that controlling embryo temperatures between acceptable ranges results in a better hatchability and a better chick quality. Especially the influence on yolk uptake and closure of the navels is high, resulting in differences in first week mortality due to navel/yolk sac infections and e-coli infections. Gladys et al. (2000) showed that a difference in embryo temperature of $2^{o}F$ resulted in a significant difference in embryo growth and feed conversion of broilers at 6 weeks of age.

Wineland et al. (2000a, 2000b) demonstrated that differences in embryo temperature resulted in a difference in development of both the whole chicken as well as specific organs. As bigger eggs have more problems loosing heat, they will often have a higher temperature at embryo level. It is often observed in the field that the quality of the day old chick deteriorates with older age of the breeder flock, and that yolk sac residues increase. Lourens et al. (2006) showed that when the temperature of the egg shell is kept constant, embryos of small and big eggs are relatively to their egg mass identical.

Incubation is a process of converting the content of an egg into a chicken. The content of that egg supplies both the building stones for the chicken body and the energy that is needed to build up that body. Especially the temperature during incubation influences the process of development and how well the content of the egg is converted into a chicken. Several experiments (Hulet, 2001; Wolanski et al., 2005; Luiten, 2003; Molenaar et al., 2007) showed that maximizing the development of the embryo during incubation results in better chick quality and especially in a better broiler performance.

IV. MEASUREMENT OF CHICK CHICK QUALITY

The quality of the day-old chick is important for a good start of the chick and with that for the final performance of the bird. In the field we realize more and more that incubation is not only a matter of producing as much chicks as possible, but that especially the quality of the day-old chick is a money maker. However, if we want to quantify the quality of the day old chick, we often must do that in a rather subjective way. Every hatchery manager has an internal picture of what he or she sees as a good chick quality, but it is difficult to describe and especially to measure.

a) Visual score

Most people use a visual score in terms of good, average or poor. Although this score is subjective, it is often rather accurate, as most people will look at chicks more or less the same way. Factors that people consider when chicks are scored this way are:
Colour: more yellow chicks are appreciated over more white chicks
Development: a large, well developed, long feathered chick will be considered to be better

Navel quality: well closed navels reduce the risk of navel infection and mortality
Vitality: alert and vital chicks will start more easily in finding feed and water
Although a visual score of an experienced hatchery manager gives a good estimate of the quality of the day old chick, and although there are good reasons why a hatchery managers scores the chicks that way, the system remains subjective and poorly repeatable.

b) Tona or Pasgar score

Recently the university of Leuven developed the Tona score, which was adjusted by Pas Reform into a simplified and more practical Pasgar score. Both methods apply a standardised scoring method to a number of chicks, looking at measurements as: chick viability, yolk sac uptake, navel closure, ability of the chick to get up after being placed on its back etc. Both methods put the visual score of a hatchery manager into a measurable and more repeatable figure. Until sofar, a strong positive correlation between Tona- or Pasgar score and broiler performance has not been demonstrated, but it can be assumed that there is a positive correlation between these scores and chick survival in the first week.

c) Day old chick weight

Although easy to record and highly repeatable, day-old chick weight has limited value as an indicator for chick quality. Day-old chick weight is highly correlated with egg weight, but not with chick development. This is because chick weight is a combination of the real chick weight and the remaining yolk residue. Embryos use the fat in the yolk as fuel for their development, so if a lot of yolk is left over, less development has occurred and the chick quality should not be considered as optimal.

d) Yolk free body mass

The yolk free body mass (body weight without residual yolk) is a better indicator for chick development and therefore for quality, especially when corrected for initial egg weight. It indicates how much of the egg content is actually converted into embryo, and therefore how much development has taken place. However, taking yolk free body mass is rather labour intensive and a lot of chicks have to be sacrificed to determine it.

e) Chick length

Another, more practical way to measure chick development is determining the length of the chicken, measured for instance from tip of the beak to the middle toe. Wolanski et al. (2005) showed that the length of the chicken is indicative for its development, and can be checked quickly. It has a substantially higher positive correlation with broiler performance then day-old chick weight, especially when corrected for egg size. Molenaar et al. (2007), showed that in male broilers originating from equal egg size an increase in chick length at hatch resulted in an increased body weight. Embryo length at 18 days can also be used as an indicator for the efficiency of in-ovo injection, as it is correlated with the place where the vaccine is administrated (allantios, amnion, breast muscle or neck).

V. CONCLUSION

From a comparison of the different methods to measure chick quality, it seems that the Tona- or Pasgar score and the chick length are having advantages in terms of repeatability, practical applicability and relation with chick quality. However, we have to realize that the two method are measuring different things. The Pasgar score is mainly influenced by the conditions in the hatcher, as factors as navel closure, yolk uptake and vitality have a large influence on the score. This will influence mainly the condition of the day-old chick and its ability to start and survive the first week. As hatcheries are often blamed for first week mortality, this score is a useful method especially in this area, for independent hatcheries. Chick length deals more with development, which is related with the conditions in the setter, and has less influence on the survival change in the first week but more on the performance of the broiler during the grow-out. This method will be of more value for totally integrated companies which make their money on broiler performance.

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EFFECT OF A HIGH PROTEIN HIGH FAT PRE-STARTER FEED (NUTRIFUL) ON BROILER CHICKEN PERFORMANCE

H. ENTING[1], J. DE LOS MOZOS[2], Á. GUTIÉRREZ DEL ÁLAMO[2] and P. PÉREZ DE AYALA[2]

Summary

The effect of a high protein high fat pre-starter feed on early growth and final body weight was studied. Early feed intake was significantly enhanced by a red colour of the feed and by providing the feed as a 2 mm pellet instead of a crumble. Inclusion of yeast derived β-glucans and n-3 and n-6 polyunsaturated fatty acids enhanced early growth significantly, especially in malabsorption infected chickens. A high protein high fat pre-starter including these components (Nutriful) improved early performance when 2.5-5 g/bird was provided as a hatching supplement during transport as compared with 24 hour fasted chickens. This pre-starter diet also significantly improved final body weight when provided on the farm (15 g/bird), which is due to an increased feed intake during the entire grow-out period.

I. INTRODUCTION

During the last decade, the importance of providing feed and water as soon as possible after hatching has been emphasized (Dibner et al., 1998; Noy and Sklan, 1998; Sklan and Noy, 2000). Several studies have shown that a delay in the access to feed after hatching has a negative effect on the uptake of residual yolk (Noy and Sklan, 1998), the development of the intestinal tract (Noy and Sklan, 1998; Noy et al. 2001; Sklan and Noy, 2003), enzyme activities in the intestinal tract (Sklan and Noy, 2000), the immune system (Dibner et al., 1998) body weight gain and breast meat percentage (Noy and Sklan, 1999). More recently, it has been hypothesized that nutrient levels in eggs might limit early growth of broiler chickens, especially in chickens from young broiler breeders (Enting et al., 2007a; b). This might increase the need for early feeding. *In ovo* administration of nutrients can improve broiler performance, as has been explained by Uni and Ferket (2004). Uni et al. (2005) and Foye et al. (2006) showed that *in ovo* feeding can result in elevated glycogen levels in the embryo, which was assumed to improve hatch body weight and pectoral muscle weight. Peebles et al. (1997) and Lilburn (1998) indicated that high fat corn based starter diets improved body weight and pectoral muscle weight. Since earlier performed experiments at Nutreco PRRC with high protein low fat starter diets only showed improved performance during the starter period but not thereafter, the effect of the early intake of a high protein high fat pre-starter feed on broiler performance was investigated.

II. STIMULATION OF EARLY FEED INTAKE

Since early feed intake can affect broiler performance later on, the effect of feed colour and feed form on feed intake was studied first. A red feed colour increased early feed intake (Table 1), which not only had a positive effect on early body weight, but also on final body weight (Table 2). The improved body weight gain was due to a higher feed intake during the entire grow-out period. Besides feed colour, also feed structure might affect early chicken performance. A 2 mm pellet can significantly improve performance in comparison with a

[1] Trouw Nutrition International, P.O. Box 200, 5830 AE Boxmeer, The Netherlands
[2] Nutreco Poultry and Rabbit Research Centre, Ctra. CM-4004, Km. 10.5, 45950 Casarrubios del Monte, Spain

crumbled pre-starter diet, also at age of slaughter (Table 3). As with feed colour, the higher final body weight was the result of a higher feed intake during the entire grow-out period.

Table 1 Feed colour preference test in young broiler chickens (relative figures compared to control group; 8 replicates of 25 chickens each per treatment)

Feed colour	Control (corn-wheat based diet)	Red	Yellow
Day 1	100[b]	151[a]	113[b]
Day 1-3	100	104	99
Day 1-7	100	97	105

Table 2 Effect of starter feed colour on broiler chicken performance (8 replicates of 7 chickens each per treatment)

Starter feed colour	Control	Red
Body weight, day 3, g	74.3[b]	76.4[a]
Feed conversion ratio, day 0-3	0.96	0.90
Final body weight, day 42, g	2019	2059
Feed conversion ratio, day 0-42	1.74	1.74

Table 3 Effect of feed form of a pre-starter feed (day 0-7) on broiler performance (8 replicates of 75 chickens each)

Feed form pre-starter	Crumble	2 mm pellet
Body weight, day 7, g	145[b]	156[a]
Feed intake, day 0-7, g/day	17.9	18.1
Feed conversion ratio, day 0-7	1.19[a]	1.09[b]
Body weight, day 42, g	2028[b]	2066[a]
Feed intake, day 0-42, g/day	80.9	82.0
Feed conversion ratio, day 0-42	1.71	1.70

III. EFFECT OF PRE-STARTER FEED UNDER LESS OPTIMAL CONDITIONS

Dibner et al. (1998) emphasized the importance of early nutrition on the development of the immune system. Several nutrients can modulate immune responses (Klasing, 1998; Goddeeris and Mast, 2003), among which β-glucans from yeast (Den Hartog et al., 2005; Morales et al., 2005) and n-3 and n-6 polyunsaturated fatty acids (Korver and Klasing, 1997; Korver et al., 1998; Sijben et al., 2001) seem to have consistent effects on immune responses and early performance. Therefore, β-glucans (Fibosel, Trouw Nutrition International) and fish and soybean oil were included in a pre-starter feed and this feed was tested in healthy and malabsorption (MAS)-infected broiler chickens (Table 4). The inclusion of Fibosel and fish and soybean oil had a more pronounced positive effect on performance in MAS-infected chickens than in non-infected control birds. Recent findings of Nutreco PRRC demonstrated that n-3 and n-6 polyunsaturated fatty acids modulate IgA, IgM and IgG responses in broiler chickens. These responses were strongly dependent on the amounts of n-3 and n-6 polyunsaturated fatty acids in the feed.

IV. EFFECT PRE-STARTER FFED (NUTRIFUL) AS HATCHING SUPPLEMENT

On the basis of the earlier performed experiments, an extruded, red coloured feed containing yeast extracted β-glucans, n-3 and n-6 fatty acids, high vitamin levels and highly digestible

Table 4 The effect of the inclusion of Fibosel and fish oil in a pre-starter feed on the performance of malabsorption (MAS)-infected and non-infected broiler chickens (24 replicates of 11 chickens each per treatment; chickens were infected on day 1)

Pre-starter feed			Day 0-7
Starter feed		Day 0-21	Day 8-21
MAS-infected chickens	body weight, day 7, g	92[b]	114[a]
	feed conversion ratio, day 0-7	1.56[a]	1.17[b]
	body weight day 21, g	526[b]	566[a]
	feed conversion ratio, day 0-21	1.54[a]	1.50[b]
Non-infected chickens	body weight, day 7, g	151[b]	173[a]
	feed conversion ratio, day 0-7	1.26[a]	1.03[b]
	body weight day 21, g	736	746
	feed conversion ratio, day 0-21	1.47	1.44

protein, fat and carbohydrates was developed (Nutriful, Trouw Nutrition International). This feed contained 230 g/kg of protein and 100 g/kg of fat, which provided 13.4 MJ AME/kg. The feed was mixed with 0.5 g/g (experiment 1, Table 5) or 0.3 g/g (experiment 2, Table 6) of its weight with water and provided in transportation boxes after hatching. The early provision of this feed during transport improved body weight significantly compared to broilers that has been fasted for 24 hours. This is in line with findings of Dibner et al. (1998), Noy and Sklan (1998) and Sklan and Noy (2001). The improved body weight was due to a higher feed intake, and this effect was already obtained when 2.5 g/bird of the pre-starter feed was provided.

Table 5 Effect of provision of a high protein high fat pre-starter feed (Nutriful) in transportation boxes; the control birds did not receive feed during transport and were fasted for 24 hours (150 replicates of 1 chicken each per treatment)

Nutriful, g/chicken	0	10
Body weight, day 0, g	44.1	44.1
Body weight, day 7, g	148.2[b]	153.6[a]
Feed intake, day 0-7, g/day	18.1	18.8
Feed conversion ratio, day 0-7	1.217	1.202

Table 6 Effect of provision of a high protein high fat pre-starter feed (Nutriful) in transportation boxes; the control birds did not receive feed during transport and were fasted for 24 hours (20 replicates of 75 chickens each per treatment)

Nutriful, g/chicken	0	2.5	5
Body weight, day 0, g	41.1	41.5	41.5
Body weight, day 21, g	698.6[b]	726.7[a]	726.9[a]
Feed intake, day 0-21, g/day	43.5[b]	44.8[a]	44.5[a]
Feed conversion ratio, day 0-21	1.389	1.373	1.364

V. EFFECT PRE-STARTER FFED (NUTRIFUL) ON FINAL BODY WEIGHT

The extruded feed that was used as a hatching supplement was also tested as pre-starter diet. The effect of this pre-starter diet on final body weight is demonstrated in Table 7. The data in table 7 indicate that providing 15 g/bird of Nutriful improved final body weight. Moreover, Nutriful improved body weight uniformity (data not shown). The higher final body weight

was due to a higher feed intake after the pre-starter diet was provided. Large scale field experiments with Nutriful demonstrated more pronounced positive effects on performance, which might be due to the fact that the immune modulating components in Nutriful exert an additional effect under less ideal conditions (table 4).

Table 7 Effect of Nutriful on final body weight of male broiler chickens (36 replicates of 13 chickens each per treatment)

	Control	Nutriful
Body weight, day 42, g	2711[b]	2761[a]
Feed intake, day 0-42, g/day	111.6[b]	114.9[a]
Feed conversion ratio, day 0-42	1.755	1.773

VI. CONCLUSIONS AND APPLICATION

Nutriful improves overall performance of broiler chickens, both when it is used as a hatching supplement (2.5-5 g/bird) and as a pre-starter diet (15-25 g/bird). The positive effect on final body weight is mediated by an increased feed intake. Effects seem to be more pronounced under less optimal (field) conditions due to its immune modulating components. Therefore, application in layer and breeder rearing can also be very useful. Since nutrient levels in eggs might limit early growth of broiler chickens, application of Nutiful can also show more pronounced effects in chickens from young breeders.

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MANAGEMENT STRATEGIES TO REDUCE OSTRICH CHICK MORTALITY

P.C.GLATZ[1] and Z.H. MIAO[1]

A literature review funded by the Rural Industries Research and Development Corporation New Animal Products Program was undertaken to identify management strategies to reduce ostrich chick mortality. High chick mortality in commercial ostrich farming is a problem around the world (Glatz and Miao 2008). Chicks are especially vulnerable during the first few weeks after hatching and frequently succumb to disease, various disorders and stress. Other factors affecting survivability include starvation, leg problems, navel and yolk sac infections.

The review examined the effect of management on chick mortality. Issues examined included: egg collection time, egg cleaning, egg pre-storage, incubation and hatching conditions, litter type, housing, heating, stocking rate, feeding fibre, probiotics, enzymes and preventative health measures. The review identified that the following issues need to be addressed by industry to reduce ostrich chick mortality: (i) collect eggs soon after lay and keep them under proper storage conditions, (ii) maintain incubator and hatch settings as specified and keep both areas clean, (iii) eliminate cold stress to reduce susceptibility of birds to infection and disease, (iv) keep the brooding area clean with appropriate bedding, stocking density and temperature, (v) allow chicks to exercise in outdoor runs, (vi) use recommended nutrition and feeding systems, (vii) condition chicks to human presence and handling, (viii) use gentle restraint, handling and transport of chicks, (ix) maintain biosecurity and hygiene and (x) use recommended vaccinations to minimise bird infection.

Total chick costs in Australia have been reported to be $239 per chick. Using appropriate management strategies could reduce mortality and make savings of $50 per chick which equates to an annual saving of $1.125 million for the ostrich industry.

Glatz PC, Miao ZH (2008) *Australian Journal of Experimental Agriculture* **48**, 1257-1265.

[1] Pig and Poultry Production Institute, South Australian Research and Development Institute, Roseworthy Campus, SA, Australia, 5371

DETERMINATION OF INFECTIVITY OF VIRAL PATHOGENS IN POULTRY LITTER USING A BIO-ASSAY: EFFECT OF CHICKEN TYPE AND AGE OF EXPOSURE

A.F.M.F. ISLAM[1], S.W. WALKDEN-BROWN[1], P.J. GROVES[2] and B. WELLS[3]

Summary

An experiment was conducted to develop and optimize a chicken bioassay to detect the presence of infective viral pathogens in poultry litter from a variety of sources. The experiment also aimed to determine the effect of type of chickens and age of exposure to litter on the level of viral infectivity. The bioassay detected chicken anaemia virus, infectious bursal disease virus and fowl adenovirus from chicken litters. SPF chickens showed higher sensitivity than commercial broiler chickens without any effect of age of exposure, however, the assay was more sensitive in broiler chickens when exposed at day 8. We conclude that the bioassay based on exposure of day-old SPF chickens is a viable assay of viral infectivity of poultry litter.

I. INTRODUCTION

A large recent survey suggests that 98% of Australian broiler chickens are reared on new litter materials (East et al., 2006). In single batch cleanout system 645g of litter is produced for the production of each kilogram of marketed broiler meat whereas reuse of the litter for subsequent batches of chickens can reduce litter production to 324 g/Kg at 5th batch cleanout, 257 g/Kg at the 9th batch (Coufal et al., 2006). The reluctance to reuse litter in Australian broiler industry is primarily based upon concerns on the animal health and productivity issues (Groves, 2003) particularly due to carry-over infection with viral pathogens.

It is more difficult to detect and quantify viruses than bacteria in the poultry shed environment as viruses does not grow in non-living media and are difficult to isolate from heavily contaminated material. Molecular tests for viral presence suffer from the limitation that they may not be measuring viable virus or infectivity as they cannot differentiate infective from non-infective virus. A chicken bioassay based on exposing young chickens to the litter material in isolation and detecting pathogens or sero-conversion in them could be an effective approach of measuring litter infectivity (Witter et al., 1970).

To develop and optimize such a bioassay, an experiment was conducted to detect the presence of infective viral pathogens in poultry litter from a variety of sources. The study also aimed to determine the effect of type of chickens (broiler versus specific pathogen free or SPF) and age of exposure to litter, on the level of viral infectivity detected. The effect of litter transportation from the poultry farms to the experimental laboratory was also tested.

II. MATERIALS AND METHODS

The experiment utilized a 5×2×2 factorial design plus an uninfected control treatment with two replicates (isolators) of each treatment combination. There were five litter sources UNE-Dir, UNE-Tran, field litter (FL) 1, FL2 and FL3, two chicken types (SPF leghorns and female Cobb broiler) and two ages of chickens (days 1 and 8) at initial exposure. Chickens were kept in 22, positive pressure isolators in the UNE isolator laboratory, 20 for the main factorial

[1] School of Environmental and Rural Sciences, University of New England, Armidale NSW
[2] Zootechny Pty Limited ,Bringelly NSW
[3] Cordina Farms, Girraween, NSW.

experiment and 2 for the controls. Chickens of the two ages of the same type were kept together in the same isolator with toe web-marking to identify exposure ages.

On day 7 ten day-old chickens of either SPF or Cobb broiler chickens were permanently marked by toe-web cutting and placed in each isolator (Day 8 exposure group). On day 0 a further 10 day-old chickens of each type were added to the chickens placed a week before (Day 1 exposure group), providing a total of 20 chickens per isolator, 440 chickens in total. On the day after the second placement, chickens were exposed to the litter material by placing 8 litres of litter in two plastic cat litter trays in each isolator. Thus each litter sample was used in 4 isolators (2 SPF and 2 Cobb). The litter in these trays remained in the isolators until it was completely depleted, about three weeks later. The two control isolators had no litter material or trays placed in them.

Field litter material was collected from three commercial farms in NSW and Queensland, placed in eskies in cloth bags with ice bricks (4L) to maintain coolness and transported to UNE by overnight courier. UNE litter materials were generated at UNE by rearing 40 broiler chickens on wood shavings to 28 days of age. These were vaccinated with live vaccines against Newcastle disease (ND), infectious bursal disease (IBD), infectious bronchitis (IB), and chicken infectious anaemia (CAV) viruses on days 14, 14, 21 and 21 respectively. The UNE-Dir litter was taken directly to isolators while the UNE-Tran was used following a 24 hour transportation simulation, in which temperature was varied from 15 to 34^{o}C.

At day 42 of the experiment, blood samples were collected, serum separated and stored. Chickens were then humanely killed, body weight taken, spleen samples collected and birds examined post-mortem. Serum samples were then analysed for serology for IB (ELISA), CAV (ELISA), IBD (ELISA), FAV8 (ELISA) and ND (HI). MDV was detected using real-time quantitative PCR of DNA extracted from spleen.

The effects of litter source, chicken type and age at exposure and their interactions on quantitative variables were tested using general linear models in JMP 5.1. For body weight data in SPF chickens, the effect of sex was also fitted. Differences in the proportion of chickens with positive serology for the different diseases was analysed by either Chi-square analysis or Fischer's exact test.

III. RESULTS AND DISCUSSION

There was a significant effect of chicken type ($P>0.002$) but not the age at exposure ($P = 0.16$) on chicken survival with a significant interaction between the effects of chicken type and age such that the effect of age at exposure was only evident in broilers. There was higher mortality in the broiler (12%) than SPF chickens (4%) with highest mortality (21%) observed in day 8 challenged broilers. This was observed mostly between days 14 and 35 of age when maternal antibody levels would have declined to low levels. The higher survival of SPF chickens may due to genetic resistance or it may reflect the pathogen burden borne by commercial chicks right from hatch.

Body weights (BW) of broiler and SPF chickens were analysed separately. For SPF chickens, there were significant effects of age of exposure (and thus age of chicken), sex and litter source ($P>0.001$) with no significant interaction. BW was higher in male than female chickens (male 392±6.0g versus female 340±6.0g). Chickens exposed to FL2 (333±5.0g) and FL3 (295±5.0g) had significantly lower BW than controls (366±5.0g). Moreover, the BW of chickens on FL3 litter was significantly lower than that of chickens on all other litter groups except FL2. In broilers, there was a significant effect of age at exposure, litter source ($P>0.001$) and their interaction ($P=0.003$). The BW of Control chickens (1841±25g) and the chickens exposed to UNE-Tran (1750±25g) and FL2 (1740±25g) were higher than the BW of the other three treatments. The BW of broiler chickens reared on UNE-Dir (1498±46g) litter

treatment was significantly lower than the chickens reared on UNE-Tran (1750±52g) litter. While this may suggest that some pathogens (other than those under investigation) were inactivated during the transportation simulation of the UNE-Tran litter, the lack of a similar finding in SPF chickens does not support this. The interaction between age at exposure and litter source was significant because the effect of litter treatments on BW was more pronounced in chickens exposed at day 1 than those exposed to litter at 8 days of age.

A significant proportion of chickens were positive for CAV, FAV and IBD (Table 1), whereas a very low proportion of chickens was positive for IB, and none were positive for ND or MDV. The control groups were negative for all the pathogens under investigation.

The bioassay detected CAV successfully in all participating field litters (14-93%) including both UNE test litters (7-97%). Both field and vaccinal (Steggles strain 3711) strains of CAV were transmitted through litter to both SPF and broiler chickens, although the transmission rate was higher in SPF (76-97%) than broiler (7-57%) chickens.

Table 1 Serological results for CAV, IBD and FAV by chicken type and litter source. FAV ELISA was assayed only for SPF chickens.

Litter	CAV		IBD		FAV	
	SPF	Broiler	SPF (%)	Broiler (%)	SPF (%)	Broiler (%)
None	0/14 (0%)	0/13 (0%)	0/14 (0)	0/13 (0)	0/14 (0)	0/10 (0)
FL 1	19/25 (76%)	7/36 (19%)	0/25 (0)	0/36 (0)	13/24 (54)	18/20 (90)
FL 2	27/29 (93%)	5/37 (14%)	2/29 (7)	1/37 (3)	16/29 (55)	10/20 (50)
FL 3	26/31 (84%)	21/37 (57%)	0/31 (0)	0/37 (0)	30/30 (100)	19/19 (100)
UNE Dir	28/29 (97%)	6/36 (17%)	27/29 (93)	0/36 (0)	0/27 (0)	3/25 (13)
UNE Tran	28/30 (93%)	2/30 (7%)	30/30 (100)	0/30 (0)	0/28 (0)	2/21 (10)

Sera positive for FAV were found in chickens exposed to all three field litters (54-100%) but not in the UNE litters or control. Field IBDV strains were picked up by both SPF and broiler chickens from the FL2 litter only although the proportion of positive chickens was very low. Vaccinal IBDV (strain V877A) in the UNE test litters was transferable to SPF chickens only, but not broilers, indicating the protective effect of maternal antibody.

Vaccinal IBV (Ingham's strain) was unable to transmit through UNE litters to either SPF or broiler chickens, although a significant number (25%) of donor chickens were positive for IBV antibody 7 days after vaccination. Only two broiler chickens were positive for IB from the field litter groups and it was not repeated in SPF chickens, suggesting that the IBV transmits poorly in litter. No ND virus was detected in any of the litter samples. While this may reflect a lack of NDV in field litters, the UNE litters also failed to transmit vaccinal ND (strain V4) to chickens although 100% of the shedder chickens were sero-positive for ND. This strain of ND appears not to transmit readily through litter.

Spleen samples of SPF chickens from each litter type were negative for MDV. This clearly shows that the three field litters had no or low MDV infectivity. The UNE test samples were not contaminated with MDV, which is known to be highly stable in environment (Carrozza et al., 1973). The negative field results are not surprising as the work of our research group is increasingly demonstrating that MDV is not as ubiquitous as commonly believed (Walkden-Brown et al., 2006; Groves et al., 2007).

Overall SPF chickens proved a more sensitive detector of viral pathogens. Vaccinal IBDV was only detectable in SPF but not in broiler chickens. A significantly higher proportion of SPF (81%) than broiler (22%) chickens was positive for field CAV strains ($P<0.001$) and this was also true of the vaccinal CAV in UNE litters (>90% v. 7-17%).

The age of exposure had no effect on the proportion of chickens positive for IBD or FAV (Table 2). However, in broilers a higher proportion was positive for CAV when exposed to litter at day 8 compared to day 1 ($P>0.01$). This almost certainly is due to the decline in

maternal antibody against CAV by day 8. Thus if SPF chickens are chosen for use in the bioassay the age at exposure does not appear to be important. If commercial chickens are used, exposure at a later age would maximise sensitivity, but at the risk of elevated mortality.

There was no significant difference in the proportion of IBD and CAV positive chickens observed in the UNE-Dir (CAV 97% and IBD 93%) and UNE-Tran (CAV 93% and IBD 100%) treatments. These results suggest that transportation simulation did not affect the transmissibility of these viruses. This is consistent with the known extreme resistance of these viruses to physical and chemical inactivation. In the case of more fragile viruses such as IBV and NDV, both failed to be transmitted by fresh (UNE-Dir) or transported (UNE-Tran) litter. The significant reduction in LW of broiler chickens reared in UNE Dir compared to UNE Trans could be due to the transmission of labile pathogens (possibly bacteria) in fresh rather than the transported litter, but this line of reasoning is not supported by the fact that SPF chickens on the same litters failed to show any such effect. On balance we can only conclude that there was no evidence of loss of viral pathogen infectivity of litter transported by the protocol used for this experiment.

There appeared to be a general relationship between chicken performance and litter infectivity. Chickens on FL3 had the lowest LW and the highest proportion of chickens positive for CAV and FAV and consequently the highest overall mean titres (data not shown) against these viruses. In contrast, chickens on FL1 had the best growth performance in SPF chickens and the lowest proportions of chickens positive for CAV and FAV and the lowest overall mean titres for FAV. Chickens on FL2 were intermediate between the others on most measures and were the only field litter in which IBDV was detected.

Table 2 Serological results of CAV, IBD and FAV according to exposure day.

Chicken	Exposure day	CAV	IBD	FAV
SPF	1	62/77 (81%)	28/79 (35%)	31/76 (41%)
	8	66/81 (82%)	31/79 (39%)	30/78 (39%)
Broiler	1	13/104 (13%)	1/104 (1%)	26/54 (48%)
	8	28/85 (33%)	0/85 (0%)	28/53 (53%)

IV. CONCLUSIONS

Both the test and field samples exhibited significant infectivity for a range of poultry viral pathogens. Control groups remained free of infection and the use of isolators allowed significant differences between litter samples to be detected effectively. Taken together the results indicate that a bioassay of litter infectivity based exposure of day-old SPF chickens is likely to a viable assay of viral pathogen infectivity of poultry litter.

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COMPARISON OF CHALLENGE METHODS FOR EXPERIMENTAL INFECTION WITH MDV: INTRA-ABDOMINAL INJECTION OF CELL CULTURED MDV VERSUS EXPOSURE OF INFECTIOUS DUST

K.G. RENZ[1], S. W. WALKDEN-BROWN[1] and A.F.M.F. ISLAM[1]

Summary

The aim of this study was to investigate whether infection of chickens with MDV1 by exposure to small amounts of infective dust (500 mg) under defined conditions is suitable as an alternative challenge method to intra-abdominal injection with infective cellular material. The infection via intra-abdominal injection induced 100% infection in chickens at 14 dpi and higher mortality with gross MD lesions (32-49%) whereas exposure to infective dust resulted in 67-83% infectivity and (13-14%) mortality. The body weight and immune organ data at 14 dpi revealed that infection by injection induced an earlier onset of immune responses and related changes to MDV infection in these organs compared to infection by exposure.

I. INTRODUCTION

Pathogenic Marek's disease virus (MDV) naturally transmits via the respiratory route by inhaling infectious dust and dander containing cell-free MDV released from the feather follicle epithelium (FFE) (Calnek et al., 1970; Carrozza et al., 1973). Most experimental studies of MDV in chickens use intra-abdominal (IA) injection of various infectious materials such as cell-culture grown isolates, whole blood, lymphocytes or cell-suspensions of organs (spleen, bursa, skin etc) (Purchase and Biggs, 1967). There are reports of infecting chickens via the airborne route, either large amounts of dust of up to 15 g have been used to infect groups of birds (Islam et al., 2001) or a much smaller dose of 2 mg given intra-tracheally (Hussain et al., 2005). Dust infection model can be very attractive if the infection work effectively.

There is no direct comparison between the dust infection method with the conventional IA injection model. In this study we compare the effectiveness of the dust MDV infection method with the IA injection of cell culture adapted MDV in terms of pathology, mortality and immune responses.

II. MATERIAL AND METHODS

In a 2x2 factorial experiment 8 groups of 33-34 commercial unvaccinated ISA-BROWN chickens (N=265) were either infected via intra-abdominal injection at 5 days of age with 500 pfu cell culture adapted MDV (strains MPF57 and 02LAR) or exposure to infectious dust (Exp) of the same two strains of MDV. For the infection by dust exposure, three dust aliquots, each sampled at different days post challenge, were used. The aliquots were pooled before chickens were infected. For dust exposure, 54 chickens per treatment were placed in a custom made box. A different infection box was used for each isolate. Each individual chicken had 3 mg of dust dispersed near the external nares using a 5 ml syringe and a blunt 18 g needle. 100 mg of dust was then circulated into the box after the completion of individual infections. A further 4 aliquots of 100 mg each were introduced into the box at 20 minute intervals. Chickens were carefully disturbed within boxes at 5 minute intervals

[1] Animal Science, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia

throughout to circulate dust. Two hours after the initial circulation of 100 mg dust, the chickens were transferred to isolators with great care to prevent cross-contamination. Intra-abdominal infection occurred by injection.

A total of 53-54 chickens per treatment combination were available to be reared beyond 14 days post infection (dpi) (26-27 per isolator) and kept until 56 dpi or 61 days of age. The uninfected control isolators had a total of 50 chickens.

III. RESULTS

The infection with MDV both by injection intra-abdominally and by dust exposure was successful as confirmed by qPCR of spleens at 14 dpi (100% for the injection groups, 67-83% for dust exposure groups). The control isolators were free of MDV throughout the experiment confirmed by qPCR of weekly dust samples.

Ten of 265 eligible chickens (3.7%) died of miscellaneous causes (mostly bacterial infections) up to 5 dpi (10 days of age) and these chickens were excluded from the analysis as were 60 chickens sacrificed at 14 dpi. The next mortality occurred on 24 dpi and this and all subsequent mortality is included in the analysis of mortality. Thus of the 195 eligible chickens at risk of death from 6 dpi onwards, 50 died up to 56 dpi (25.6%) while the remainder were sacrificed on this date. The first MD lesions were detected at 24 dpi and 42/50 chickens (21.5% of all eligible chickens) showed gross MD lesions (Table 1). Logistic regression analysis revealed that the mortality was significantly higher (P=0.0004) in the groups which were infected by intra-abdominal injection. The effect of challenge virus was not significant (P=0.1) although there was a trend towards higher mortality in the groups challenged with 02LAR. The interaction between the two main effects was not significant.

Table 1 Mortality and incidence of MD from 6 to 56 dpi of the experiment.

Viral strain	Infection method	n	Total mortality n (%)	Mortality with MD lesions n (%)	Total MD n (%)
Unchallenged	Injection	37	0 (0%)	0 (0%)	0 (0%)
MPF57	Injection	38	14 (36.8%)	12 (31.5%)	26 (68.4%)
02LAR	Injection	39	20 (51.3%)	19 (48.7%)	33 (84.6%)
MPF57	Exposure	43	7 (16.3%)	6 (13.9%)	23 (53.5%)
02LAR	Exposure	38	9 (23.7%)	5 (13.1%)	16 (42.1%)
	Total	195	50 (25.6%)	42 (21.5%)	98 (50.3%)

Of the 195 chickens alive at the time of the 1st MD tumour at 24 dpc, 98 (50.3%) had MD tumours on post mortem examination following death or sacrifice on 56 dpi. The 1st death with gross MD lesion occurred in a chicken from the injection treatment at 24 dpi whereas the 1st death with MD tumours in the dust exposure treatments occurred at 27 dpi. There was no significant effect of viral strain (P=0.44), However, a significant effect of challenge method (P=0.001) and the interaction between the effect of viral strain and challenge method was observed (P=0.04). Chickens challenged by injection had a significantly higher MD incidence than those challenged by dust exposure (P=0.0001). MD incidence by treatment is summarised in Table 2.

Table 2 MD incidence (% with gross MD lesions) grouped by treatments.

Viral strain	Infection method	MD-Yes	MD-No	MD incidence %
Unchallenged	Injection	0	37	0
MPF57	Injection	26	12	68.4
02 LAR	Injection	33	6	84.6
MPF57	Exposure	23	20	53.5
02 LAR	Exposure	16	22	42.1
	Total	98	97	

At 14 dpi, the live weight of chickens challenged by injection was lower then the dust challenged chickens (P<0.05, Figure 1, upper panel). There was a significant effects of infection method and viral strain with higher relative bursal weight in dust challenge than IA challenge and higher bursal weight in 02LAR than MPF57 challenge group (P=0.05). There was a significant operator effect on relative thymus weight (P=0.01). Taking into account the effect of operator, there was a significant effect of infection method, viral strain with an interaction between the two main effects (P<0.05). The thymic weight was lower in IA challenge with the strain MPF57.

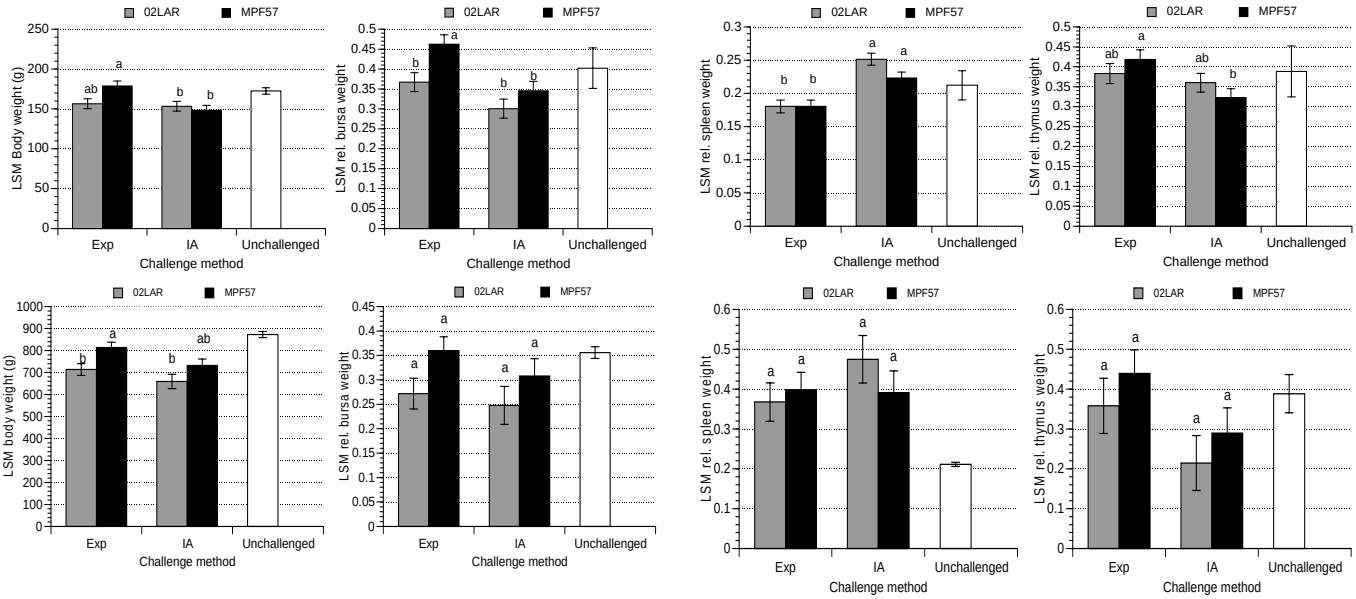

Figure 1 Interaction plots from bodyweight and immune organ data at 14 dpi (upper panel) and 56 dpi (lower panel), LSM±SEM. Means not sharing a common letter differ significantly using Tukey's HSD (P<0.05). The unchallenged group was excluded from the analysis, shown is the mean ±SE of the mean.

At 56 dpi, chickens challenged with 02LAR had a significantly lower body weight, regardless of challenge method (P<0.05, Figure 1, lower panel). Similarly, chickens challenged with 02LAR showed a strong trend towards lower bursa weights compared to chickens challenged with MPF57, although this was not significant (P>0.05). There was no significant operator effect on relative thymus weight. Chickens challenged with 02LAR showed a strong trend towards lower thymus weights compared to chickens challenged with MPF57 although it was not significant (P>0.05). Chickens challenged by injection showed a strong trend towards lower thymus weights compared to chickens challenged by dust exposure, but again, this was not significant.

IV. DISCUSSION

The infection by exposure was successful thus confirming earlier studies which have successfully used dust or dust-like infectious material to infect chickens by exposure (Calnek et al., 1970; Islam et al., 2001; Hussain et al., 2005).

There were marked differences in the early stages of MDV infection, which was evident in the organ data of sacrificed chickens at 14 dpi. Despite the differences, the infection by exposure to small amounts of infective dust (approximately 9 mg/chicken) showed a high primary infection rate and therefore, could be considered as a suitable tool for primary isolation and monitoring of MDV from the field where dust is often the only available source of infectious material and small amounts only may be available. This infection method does not require propagation and titration in cell culture which is laborious and time consuming.

However, major disadvantages of this infection method are that it is impossible to accurately determine the individual dose of MDV each chicken received by dust exposure, that the immune response is not uniform and pathology and mortality is much lower compared to infection by injection. As well, the primary infection (% MDV positive chickens at 10-14 dpi) on the day of infection was incomplete as only 67-83% of these chickens were positive by qPCR of spleens at 14 dpi. The infection of those chickens which were negative at 14 dpi would therefore have occurred from their flock mates and would therefore have been delayed by another 2 weeks. In contrast, the infection on the day of challenge in the injection groups was complete as all spleens assayed at 14 dpi were positive for MDV by qPCR and this might also explain why the onset of infection was quicker in the injection groups. This may therefore limit the application of the infection by dust exposure for experimental use where the infectious dose needs to be accurate, and there is a requirement for uniform infection of chickens on the day of challenge.

ACKNOWLEDGEMENTS

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RATE OF NATURAL TRANSMISSION OF MAREK'S DISEASE VIRUS (MDV) TO SUSCEPTIBLE CHICKENS IN THE POST-CHALLENGE PERIOD: EFFECT OF VACCINATION WITH HVT AND ASSOCIATION WITH MDV GENOME COUNT IN AIR

J.R. CRABB[1,2], S.W. WALKDEN-BROWN[2,3] , S. BAIGENT[2], L. SMITH[2] and V. NAIR[2]

Summary

Marek's disease virus (MDV) is transmitted by inhalation of contaminated feather dander released by infected chickens. There is ongoing evolution of MDV to greater virulence. Investigation of the underlying causes requires determination of viral fitness under different conditions, in turn requiring determination of viral transmission rate between chickens. This study investigated the transmission rate of MDV to in contact chickens. MDV was quantified in blood, feather, spleen and dust of infected and HVT-vaccinated and unvaccinated in contact chickens in several treatments over a period of 28 days using real-time PCR. MDV was transmitted to 1 of 8 chickens (12.5%) prior to 12 days post infection (d.p.i.) and to 9/34 (26.5) and 2/34 (5.9%) unvaccinated and vaccinated chickens respectively between 12 and 28 d.p.i. Daily infection rate of uninfected chickens did not increase significantly after 21 d.p.i. MDV load in air increased significantly over time but was not clearly associated with infection rate.

I. INTRODUCTION

Infection with pathogenic Marek's disease virus (MDV-1) is via inhalation of cell-free virus shed in feather dander. Infectious virus is shed for the life of the bird (Carrozza et al., 1973) with shedding commencing in both resistant and susceptible birds from around 7 days post infection (d.p.i) (Islam and Walkden-Brown, 2007), although effective transmission does not generally occur until around 14 d.p.i. (Islam and Walkden-Brown, 2008). There has been a marked increase in the virulence of MDV over time (Witter, 1997), and a number of management factors have been postulated as contributory causes of this. One approach to testing hypotheses relating to the effects of such factors on the evolution of MDV virulence is to compare the fitness of different MDV pathotypes under different vaccination or management scenarios. The fitness of a pathogen represents a trade off between the costs and benefits of harming the host. Increased multiplication and transmission must be balanced against potential death of the host and truncation of transmission, so lifetime reproductive success (R0) is a key determinant of pathogen fitness. In turn, a key determinant of R0 is the rate of transmission of MDV from an individual infected chicken to uninfected cohorts. In order to investigate this parameter and to understand in more detail the dynamics of MDV transmission, the current experiment was designed to measure the transmission rate of MDV to in-contact susceptible or vaccinated chickens and determine the association with MDV shedding rate and content in air.

[1] The Royal Veterinary College, University of London, Royal college street, London, NW11UT, UK
[2] The Institute for Animal Health Compton, Newbury, Berkshire, RG20 7NN, UK.
[3] School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia

II. MATERIAL AND METHODS

The experiment utilised 208 individually identified, specific pathogen free, maternal antibody negative day-old Rhode Island Red chicks at the Institute of Animal Health in Compton in the experimental design summarised in Table 1.

Table 1 Experimental design: Summary of the main features of individual treatment groups.

Description	Treatment group			
	ID (Infected donors)	ES (Early sentinels)	LS (Later sentinels)	FS (Fixed period sentinels)
Number of chickens	30	8	72	96
Treatment	Challenged with 1000 p.f.u vvMDV strain RB1B by intra-peritoneal (i.p) injection at 7 days of age	Exposure to early post-challenge ID chickens	Half vaccinated with 2000 p.f.u HVT strain FC126 by intramuscular (i.m) injection at 7 days of age. All were then exposed to ID chickens.	4 groups of 24 chickens were exposed to ID and LS chickens for 72 hours, then removed and reared for another 8 days.
Room	10 chickens from room 1 moved into rooms 2 and 3	Room 1	Rooms 2 and 3	Exposed in rooms 2 and 3, then moved to rooms 4 and 5.
Contact with ID		Direct	Direct contact (cage 6 = indirect contact)	Direct (Cage 6 = indirect contact)
Housing	1 ID chicken in 5 of 6 cages in both rooms	Housed in 2 cages 4 to a cage	3 vacc. plus 3 unvacc. birds per cage in each room	Housed in pairs in a sectioned off area of each cage in both rooms.
Period of exposure to ID		12 days	Continuous (16 days)	72 hours
Introduction to ID (d.p.i)		At challenge	12 d.p.i (0d.p.e)	15, 18, 21, 25 d.p.i.
Removal (d.p.i) from ID		12 d.p.e	At end point (16 d.p.e)	18, 21, 25, 28 d.p.i.
End point	12 d.p.i (20 chickens) 28 d.p.i (10 ID chickens)	22 d.p.i	16 d.p.e (28 d.p.i)	8 days post exposure period
Sacrificed at end point?	Yes	Yes	Yes	Yes
Post mortem	Yes	Yes	No	Yes (spleens removed)

Sample timing is in units of d.p.i. for ID chickens. Samples collected included peripheral blood, whole spleen, feathers from the axillary tract and dust sampled the room exhaust air prefilters, or from the glass fibre filter of an air sampling apparatus set up in rooms 1 and room 3 to measure dust content (>1µm diameter) in air. Peripheral blood leukocytes (PBL) were separated by centrifugation over histopaque. DNA was extracted from all tissues using DNeasy tissue kits according to the manufacturer's instructions (Qiagen, UK) and subjected to qPCR for MDV1 detection as previously described (Baigent et al., 2005). MDV load in PBL was used as the sole determinant of infection in all treatment groups, with values greater than 1 genome copy per 10^4 cells taken as positive. Data investigation and statistical analysis was performed using appropriate models in JMP v6.0 (SAS Institute, NC, USA). Discrete data, such as infection status were analysed using generalized linear models with a logistic link function. For LS chickens both a cumulative measure of infective status and a daily infection rate of chickens available for infection were calculated. The latter was expressed as a percentage, arcsine transformed and analysed as described for continuous variables below. Continuous variables such as MDV load in PBL, feather tips (FT) and spleen were log transformed [Log10 (y+1)] and subjected to analysis of

variance after fitting appropriate models to test the effects of room and vaccination status. For LS chickens, the PBL and FT data were repeated measures so a REML model was fitted with room and vaccination as fixed effects, individual chickens as a random effect, and day post infection as the repeated measure.

III. RESULTS

MDV challenge of ID chickens was highly successful and by 7 d.p.i. 28/30 chickens were positive for MDV. A high level of early mortality (43.3%) was observed prior to 12 d.p.i (MD early mortality syndrome) and MD lesions and lymphomas were observed on post mortem in all 10 chickens surviving beyond this period. Four of 10 donor chickens used in rooms 2 and 3 died of MD before 28 d.p.i. MDV-1 load in donor chickens increased significantly with d.p.i in both PBL (P<0.0001) and feather samples (P<0.0001) (Figure 1 A).

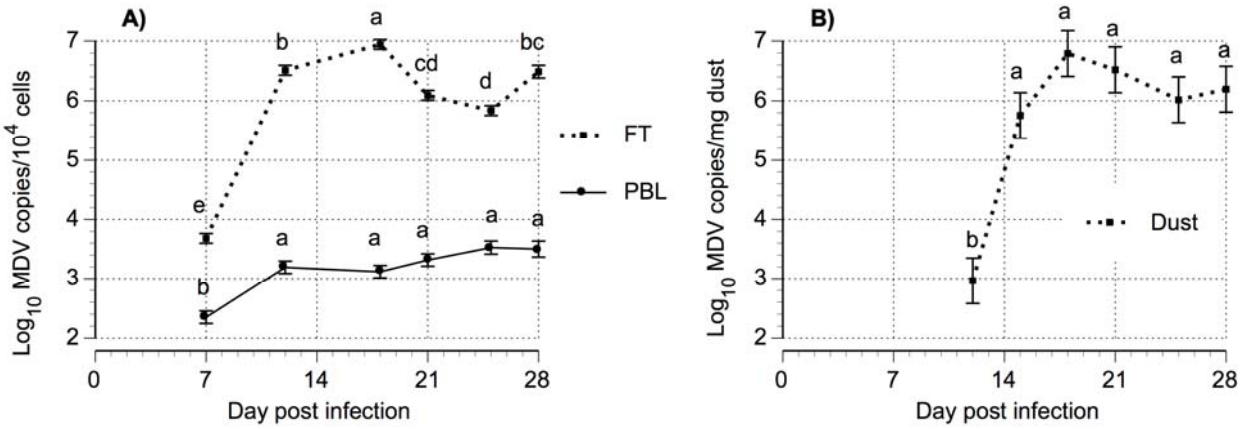

Figure 1 A) Mean MDV-1 load (LSM±SEM, log scale) in PBL and feather tips (FT) over time in ID chickens infected with 1000 pfu of MDV-1 strain RB1B i.p at 7 days of age and that survived to 28 d.p.i. (n=6). B) Mean MDV-1 load (LSM±SEM, log scale) in dust collected from rooms 2 and 3 containing ID and LS chickens. Each point is the mean of two rooms.

In the ES group exposed to donor birds up to 12 d.p.i., one bird of eight was positive for MDV1 infection (Figure 2). In LS chickens exposed to ID chickens from 12 d.p.i (0 days post exposure, d.p.e) the number of positive samples increased significantly over time in non-vaccinated (P = 0.004; 0, 2, 6 and 9 of 34 chickens positive on days 6, 9, 13 and 16 d.p.e. respectively) but not vaccinated (P = 0.684; 0, 0, 1 and 2 of 36 chickens positive on days 6, 9, 13 and 16 d.p.e. respectively) sentinel chickens. Vaccination with HVT significantly reduced (P = 0. 022) the probability of a chicken becoming infected and the final proportion of chickens with infection at 16 d.p.e was significantly (P = 0.018) higher in the non-vaccinated group (9/34, 26.5%) than in the vaccinated group (2/34, 5.9%). In the FS group exposed to ID birds for fixed 3 or 4-day periods, 1/24 chickens (4.2%) was positive for MDV-1 at each of the four contact periods (15-18, 18-21, 21-25 and 25-28 d.p.i.). Infection rate amongst non-infected LS chickens was assessed and analysed two ways. 1) The probability of a PBL sample becoming positive for the 1st time in MDV negative LS chickens at each sampling period was analysed using a generalised linear model. This revealed a significant effect of vaccination (P = 0.06) but not d.p.e (P = 0.40). The probability of becoming infected after 9, 13 and 16 days of exposure in unvaccinated chickens was 0.051, 0.132 and 0.121 respectively and for vaccinated chickens 0.009, 0.025 and 0.022 respectively. 2) The true daily infection rate of uninfected chickens in each cage was calculated as a percentage and analysed after arcsin transformation. Again the analysis revealed a significant effect of vaccination (p=

0.01), with no significant effect of d.p.e (P = 0.51). The overall mean daily infection rate between 9 and 16 d.p.e (21-28 d.p.i for the donors) was low, being 1.66 ± 0.51 % of eligible chickens. Vaccination had a profound effect on daily infection rate with mean values of 2.8±0.91 % for unvaccinated and 0.54 ± 0.38 % for HVT-vaccinated chickens respectively.

MDV-1 load in dust from rooms 2 and 3 increased significantly with d.p.i (P = 0.007, Figure 1B). Neither room (P = 0.161) nor dust source (room filter or pump filter) (P = 0.602) had a significant effect on MDV1 load. Dust production measured in room 3 increased steadily with time, as did MDV content per m^3 of air (Log_{10} values of 4.72, 4.90, 5.25, 5.45, 6.31 and 6.41 copies of MDV/m^3 on 14, 16, 19, 24, 26 and 28 d.p.i. respectively). There was no clear association between dust content in air and daily infection rate as the latter did not increase over time.

IV. DISCUSSION

MDV is generally considered to be ubiquitous and highly contagious. These results suggest that it is less contagious than generally thought with relatively low infection rates of in contact chickens during the first 28 days post challenge. They also show that vaccination with HVT has a marked inhibitory effect on infection rate. These findings are broadly consistent with other recent findings of comparatively low daily infection rates in unvaccinated broiler chickens (8.4%/day) with a markedly reduced rate (0.8% day) in HVT-vaccinated chickens (Islam et al., 2008). The higher infection rates in that report probably relate to the much greater challenge level of MDV (Log_{10} MDV load/m^3 air range 6.5-7.5) relative to that of the present study (range 4.7-6.4) due to the much higher ratio of infected to uninfected in contact chickens. The possibility of underestimating true infection rate cannot be discounted, although taqman PCR detection of MDV in lymphoid tissue is the most sensitive measure of infection available.

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LABORATORY AND FIELD STUDIES ON THE SAFETY AND EFFICACY OF AN EXPERIMENTAL BIVALENT MAREK'S DISEASE VACCINE, VAXSAFE® SBH.

C.A.W. JACKSON[1] and R.J. JENNER[2]

Summary

Whilst there is limited evidence that Marek's disease (MD) viruses in Australia are evolving towards increased virulence in the face of vaccination, the availability of a bivalent MD vaccine for broilers that could delay that trend prompted Bioproperties Pty Ltd to import a masterseed of the non-oncogenic serotype 2, MD virus from Merial, France. An experimental cell-associated bivalent vaccine, Vaxsafe® SBH, was produced in which the imported serotype 2 SB-1 strain was combined with the already Australian registered serotype 3 HVT FC-126 strain, using the same manufacturing methods. Studies have been completed on the safety of single, repeat dose and ten times dosing of susceptible chickens. Evidence of the stability of the attenuation characteristics of the SB-1 vaccine virus component has been provided through reversion to virulence and retention of immunogenic property studies. Safety studies have also been completed on spread to in-contact chickens and dissemination in the host. In addition, laboratory safety and efficacy testing has been undertaken using commercial meat and layer chickens (Walkden-Brown et al., 2007). A large-scale field trial involving two commercial broiler hatcheries located in Queensland was completed in 2008. Data from the laboratory and field studies are presented demonstrating the safety and efficacy of the experimental bivalent vaccine.

I. INTRODUCTION

Although there has not been a clear evolution in the virulence of field MD viruses (MDV) in Australia as has been described in the USA, the continued recovery of isolates of very virulent MD viruses (vvMDV) from sporadic outbreaks (Walkden-Brown et al., 2007) has raised concerns about the increasing risk of vaccine failures. In addition, the total reliance on serotype 1 Rispens vaccine in layers and breeders and the use of low doses of HVT in broilers was of similar to the concern of Witter (1999). Whilst a role for similar vaccines in Australia was evident some 30 years ago (Jackson et al., 1976), a continued role for serotype 2 vaccines was shown more recently (Karpathy et al., 2002) where Maravac® (Fort Dodge Australia) was found to enhance protection by serotype 1 vaccine viruses, BH16 and CVI 988 in the presence of HVT. In this light, Bioproperties Pty Ltd imported the masterseed of serotype 2 strain SB-1 to permit the manufacture of a combination vaccine with FC126 strain of HVT. The experimental vaccine has been formulated to take advantage of the claimed protective synergism when serotype 2 and 3 vaccine viruses are combined in the one vaccine (Witter and Lee, 1984).

A combined serotype 2 (SB-1) and serotype 3 (FC 126) has been registered in the USA since 1983 by Merial (Select Laboratories) and is used to vaccinate over 10 billion broilers annually worldwide, mostly via the *in ovo* route. The high level of efficacy of this vaccine has recently been described (Wakenell et al., 2002). Bioproperties has produced a similar vaccine and subjected it to the range of tests required for registration including local field trials.

[1] Biological Technology Transfer Pty. Ltd, 2 Victory Avenue, Camden, NSW 2570, Australia
[2] Golden Cockerel Pty. Ltd, Queensland

II. PEN TRIALS ON THE SAFETY AND EFFICACY OF VAXSAFE® SBH

Bioproperties has completed single, repeat dose and ten times dosing of susceptible chickens to demonstrate the safety of the experimental vaccine. Evidence of the stability of the attenuation characteristics of the SB-1 vaccine virus component has been provided through reversion to virulence and retention of immunogenic properties studies. Safety studies have also been completed on spread to in-contact chickens and dissemination in the host.

The efficacy of the bivalent vaccine in terms of its minimum protective dose had previously been established overseas by Merial in challenge trial both *in ovo* and in day-old chickens. A further pen efficacy study was undertaken in Australian by Walkden-Brown et al. (2007) who compared the efficacy of Vaxsafe® SBH (bivalent SB-1 and HVT) and of Vaxsafe® HVT in commercial broiler and layer chickens by challenge with isolates of very virulent MDV (vvMDV) and virulent MDV (vMDV) from Australian poultry flocks. A total of 648 broiler and 540 layer chickens were used in these experiments. Vaccines were administered on the day of hatch (0-day of age). Challenge was undertaken at five days post vaccination Real time quantitative polymerase chain reaction (qPCR) analysis of spleen samples collected at seven days post challenge confirmed the presence of vaccine and challenge viruses in all vaccinated and challenged chickens, respectively. All chickens that died or were euthanized during the experiment were subjected to post-mortem examination for the presence of gross MD lesions. It was evident from the two experiments that the bivalent vaccine, Vaxsafe® SBH was efficacious in commercial broiler and layer chickens after challenge with four virulent Australian MDVs. In broiler chickens, Vaxsafe® SBH provided significantly lower total mortality and lower MD mortality compared to the unvaccinated control chickens. The vaccine also significantly increased mean broiler body weights. In layer chickens, Vaxsafe® SBH also provided significantly lower total mortality and MD mortality compared to the unvaccinated control chickens. However, the vaccine did not significantly increase mean body weights of layer chickens. When compared to Vaxsafe® HVT, Vaxsafe® SBH was not inferior to HVT for any of the parameters that were measured. These findings indicted that Vaxsafe® SBH was able to provide enhanced protection for those parameters that are important to the broiler industry (higher body weights) and to the layer industry (greater efficacy).

III. FIELD TRIALS ON THE SAFETY AND EFFICACY OF VAXSAFE® SBH

A field trial was conducted on broiler farms owned by a Queensland broiler company. Broiler chickens were vaccinated *in-ovo* as 18 day-old embryos with two different vaccine treatments at hatcheries A and B. The vaccines were administered at close to the minimum release titres of the SB-1 vaccine component to assess efficacy and safety in the face of natural field challenge from wild-type MD viruses. However, it was not possible to confirm natural field challenge by wild-type MD viruses when broiler shed fluff samples were collected on completion of the trial and tested by conventional and real-time PCR possibly due to the low incidence of MDV at the time of the trial.

Analysis of the broiler flock productivity data of broilers derived form hatchery A (Table 1) found that there was a significant effect of vaccination treatment on both first week mortality (FWM) ($P = 0.004$) and total mortality ($P = 0.03$). In both cases the reduction was in favour of Vaxsafe® SBH. There was no significant difference in the effect of vaccination treatment on seven-day body weights or on weight gain.

Table 1 Productivity data of broilers from hatchery A.

Vaccine treatment	No. sheds	No. broilers	% FWM*	7-day wt. (g)	Mortality (%)	Wt. gain (g/day)
Vaxsafe® SBH	26	694,000	1.09	166	5.76	55.9
Vaxsafe® HVT	32	879,789	1.46	166	6.98	54.8

*FWM = first week mortality

Analysis of the broiler flock productivity data of broilers derived from hatchery B (Table 2) found that there was a significant effect of vaccination treatment on 7-day body weights (p=<0.0001). The reduction was in favour of Vaxsafe® HVT. There was no significant difference in the effect of vaccination on any of the other parameters that were compared.

Table 2 Productivity data of broilers from hatchery

Vaccine treatment	No. sheds	No. broilers	% FWM	7-day wt. (g)	Mortality (%)	Body Wt (g)	Gain (g/day)
Vaxsafe® SBH	12	264,100	1.08	145.0	5.93	2113.8	52.92
Vaxsafe® HVT	17	351,800	1.27	159.7	5.11	2224.1	53.96

*FWM = first week mortality

Overall, the data analysis showed that there was no significant difference between vaccine treatments on weight gain except for 7-day body weights in favour of Vaxsafe® HVT at one hatchery most likely due to the use of smaller eggs from younger breeding flocks. However, there was a significant difference in first week mortality and in final batch mortality in favour of the Vaxsafe® SBH treatment at one of the two hatcheries. It was concluded that the data showed that Vaxsafe® SBH was at least as safe and efficacious as Vaxsafe® HVT under the conditions of this field trial.

IV. DISCUSSION

A combination vaccine with HVT has generally been adopted in many countries and particularly in the USA where the bivalent vaccine of SB-1 and HVT has continued to be widely used for *in ovo* broiler vaccination. The safety and efficacy data obtained in Australia from the pen and field trials described above further supported the value of this type of vaccine. Overall the pen trial data indicated that Vaxsafe® SBH provided protection from challenge with virulent and very virulent Australian MDV, and provided equivalent or better protection than that obtained with Vaxsafe® HVT in Australian commercial broiler and layer chickens. However, productivity gains in field trials were not clearly demonstrated possibly due to the limited exposure to MDV at the time of the trial.

The development of a combined serotype 2 and 3 vaccine using imported master seeds that grow to a high titre should provide the broiler industry with a useful alternative to HVT alone especially where more frequent challenge with vvMDV arises. Whether an evolution in increased virulence of field viruses occurs in Australia, as has occurred in the USA, and requires a change to a combined serotype 2 and 3 vaccine, such as Vaxsafe® SBH, may depend upon the ways in which the broiler industry applies the existing vaccines.

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Bioproperties Pty Limited is acknowledged for providing access to data from pen and field trials studies of Vaxsafe® HVT and Vaxsafe® SBH. Reference to the pen trial studies undertaken by Dr Steve Walkden-Brown, University of New England and his co-workers is gratefully acknowledged.

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A NOVEL APPROACH TO SALMONELLA CONTROL BASED ON THE ACTION AND METABOLISM OF ORGANIC ACIDS IN POULTRY

D. JOARDAR[1] and T.J. WERTELECKI[2]

Summary

Only some selected organic acids have a strong bactericidal effect on salmonella at low pH - formic < HMTBa < propionic. At neutral pH the most effective organic acids are sorbic and fumaric acid with formic acid being the most effective in an un-dissociated form. To achieve optimal control of Salmonella and Campylobacter in poultry the organic acid blends should be based on a combination of appropriate organic acids and a correctly targeted site of application via feed and water.

I. INTRODUCTION

Salmonella belong to the enterobacteriaceae family, a leading cause of food borne illness in humans. Emphasis on the control of Salmonella in the pre-harvest live production system will result in reduced levels at the processing plant. With many potential sources to introduce Salmonella into poultry flocks, any strategy to control these organisms in live production must ensure water hygiene, feed hygiene and gut hygiene. This paper examines the effect of organic acids against salmonella in drinking water, reduction of salmonella contamination in feed, and the effect of protected organic acids on Salmonella at neutral pH, which is similar to lower gut environment. Use of organic acids is capable of assisting in the control of the shed and spread of Salmonella.

II. MODE OF ACTION OF ORGANIC ACIDS AGAINST SALMONELLA

The change of pH from neutral towards more acidic conditions is adverse for bacterial growth and proliferation. Reduction in pH is achieved by dissociation R-COOH = R-COO- + H+. The protonation results in decreased pH which destabilizes the membrane transport system of the bacteria. The direct anti-microbial property of un-dissociated organic acid molecule (R-COOH) is achieved with lipophilic property resulting in penetration of bacterial cell wall, and following entry starts dissociating inside the bacterial cell. This results in the disturbance of the ionic balance inside the cell and bacteria expend considerable energy to remove the H+ to maintain pH balance, and the rest of the acid molecule RCH_2COO^- upsets DNA synthesis. Loss of energy and lack of ability to perform protein synthesis in organelles results in bacterial cell death.

Based on the above, the anti-microbial mode of action of un-dissociated organic acids depends on two parameters - the pKa value which is the pH (pH 3 to 5) at which 50% of the acid is in un-dissociated form and the molecular weight (MW). Lower MW results in lower MIC to penetrate bacterial cell wall.

[1] Novus International (Thailand) Co. Ltd, 193/62, Lake Rajada, 16th Fl, Bangkok 10110.
[2] Novus Polska, PL54-109 Wroclaw, Hodowlana 27, Poland

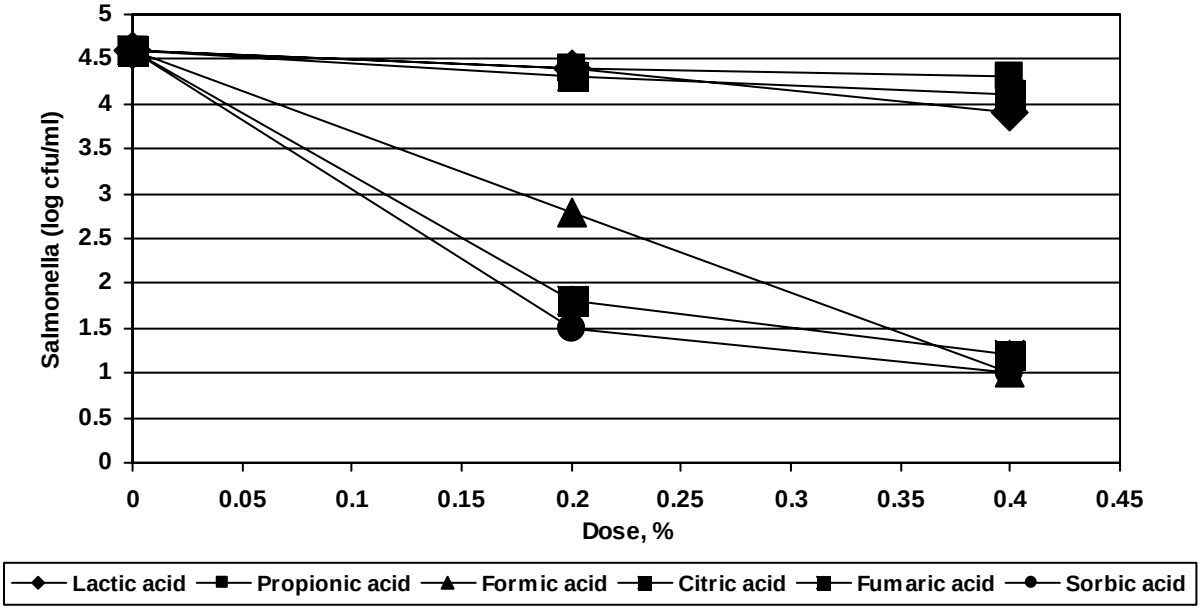

Figure 1 Anti-salmonella effect of different organic acids at 3.5 pH.

Organic acid blends based on Formic acid and propionic acid are most effective in salmonella inhibition. Formic acid, with its pKa 3.75 and lower MW 46 gives effective penetration inside the bacterial cell. MIC of un-dissociated propionic acids against pathogenic bacterial cell is 70micro mol and MIC of dissociated propionic acids against pathogen cell is 100-350 micro mols when compared to MIC of dissociated lactic acids (Na) against pathogen cell is 1250 micro mol**.**

The proper ratio of formic and propionic acids enhances the anti-salmonella effect with a lower dose rate. The synergy of both acids is very important in controlling the proliferation of some types of Salmonella f.e PT4 which have developed a specific acid resistance mechanism in cells.

III. FEED HYGIENE BY APPLICATION OF ORGANIC ACIDS BLENDS

Principles of effective feed hygiene should be based on organic acid blends having the highest synergy between low pKa acids, which reduces the pH of the environment of the bacteria, with high pKa acid with low molecular weight to ensure a pronounced anti-microbial effect inside the cell. Salmonella prevention in feed is important because feed is one of the major vectors for pathogen transmission of bacteria i.e. salmonella to the farm. During compound feed preparation, the combination of thermal feed treatment (Pelleting, expanding, etc.) with the addition of organic acids, has proven successful. Organic acids ensure feed sterilization and offer protection from re-infection with salmonella. Since most bacteria cannot grow at pH values of less than 6.0, by acidifying feed with formic acid, the pH of the feed is reduced and bacterial growth is eliminated.

An in vitro assay for inhibition of Salmonella and E.Coli by organic acids in feed is relevant. In this assay, A feed sample and product sample are combined, pH is adjusted with HCl (pH 3.5, pH 4.0), Salmonella (40,000 cfu/g) or E. coli (400,000 cfu/g) are added.

Incubation at 37°C for 90 min for killing effect and the assay is diluted, plated on agar and incubated overnight for enumeration.

Anti-bacterial activity of organic acids measured in feed at low pH with the following treatments (in 50 ml sterile tubes):

	Treatments	Dose	Reps.	Feed	Inoculants (cfu/g of feed)
1	control		2-3	5 gram	40,000
2	Activate DA	0.3%	2-3	5 gram	40,000
3	Acidomix	0.3%	2-3	5 gram	40,000

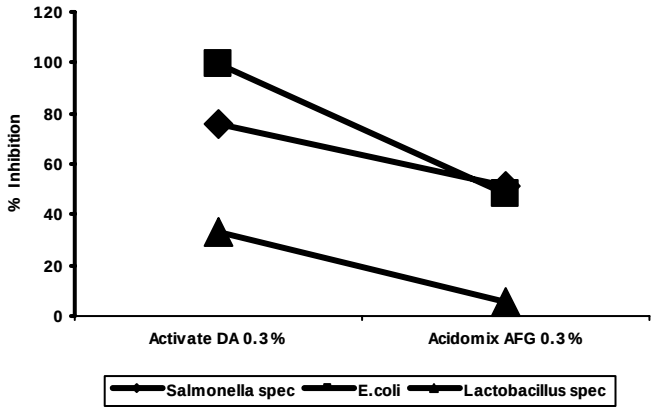

Figure 2 Based on the above in-vitro tests, with a dose of 0.3% of blended organic acid e.g. Activate DA & Acidomix AFG, can be used for feed sanitation against Salmonella.

IV. WATER HYGIENE BYAPPLICATION OF ORGANIC ACID BLENDS

Organic acid blends can reduce Salmonella spread, ensuring water hygiene and acidifying the upper gut to reduce contamination in the crop. When used from day 1, broilers can establish beneficial gut micro flora in the small intestine and caecum.

Effective salmonella reduction can be achieved using an organic acid blend as tested by the cloacal swab method which showed a reduction in salmonella positives of 64.2% (25 versus 70%).

Organic acids can be an effective tool in reducing the shed and spread of salmonella in live production when applied in drinking water. This can be a pre-harvest intervention tool to reduce salmonella horizontal spread.

V. LOWER GIT HYGIENE BY PROTECTED ORGANIC ACIDS

The concept of using a specific blend of acids embedded in a fat matrix, so that they are released slowly during transit through the lower gut, has been shown to have optimum efficiency in inhibiting pathogenic bacteria under the physiological conditions in the intestine at neutral pH.

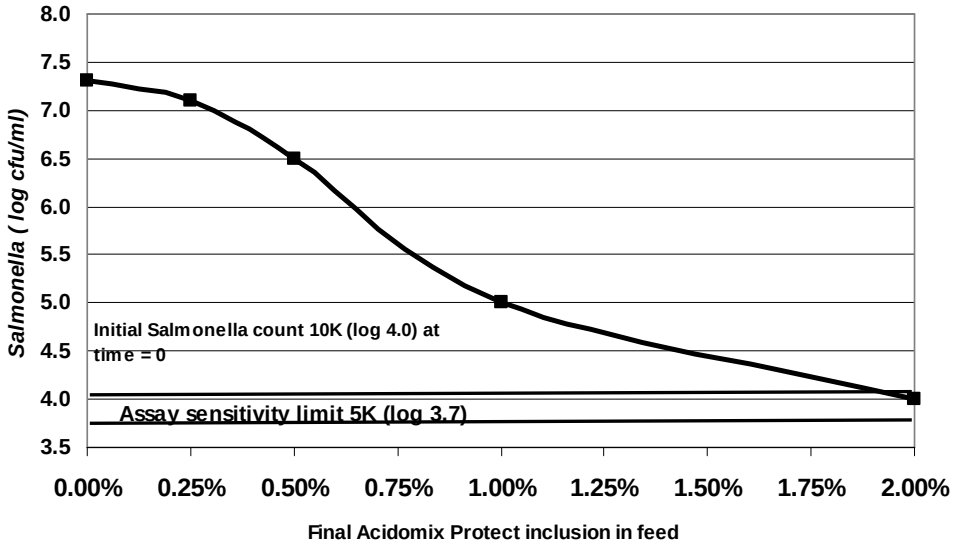

Figure 3 Anti-Salmonella effect of protected organic acid blends tested by intestinal model at neutral pH.

VI. CONCLUSIONS

Thus free organic acids work in the feed and the upper part of the intestinal tract (acidification & antimicrobial action), the sites of action can be extended to the lower intestinal tract by combining acidifiers with protected organic acid blends.

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SALMONELLA SEROTYPING COMPARSION BETWEEN THE STANDARD KUAFFMAN-WHITE SCHEME AND COMMERCIAL (DIVERSILAB™) Rep-PCR

A. PAVIC[1] and G. BAILEY

Summary

Salmonella serotyping of poultry isolates is routinely performed at the Australian *Salmonella* reference Laboratory (IMVS). Increased government pressure on the poultry industry to control and reduce *Salmonella* prevalence, has resulted in an increase in through chain sampling. This has drastically increases the number of serovars submitted to the reference laboratory resulting in an increased lead time from isolate to serovar of approximately 10 weeks. To reduce lead time, to two weeks, the differentiation of *Salmonella* serovars was performed using a molecular technique, Rep-PCR, on the DiversiLab™ platform. Comparison of these techniques (rep-PCR vs. Serotyping) gave consistent results. Additionally incorporating the easyMag NA automated extraction platform into the process greatly reduced extraction time and resulted in higher DNA yields and purity when compared to manual extraction.

I. INTRODUCTION

The serotyping of poultry *Salmonella* is performed under an agreement between the Australian Chicken Meat Federation (ACMF) and the Australian *Salmonella* Reference Centre at the Institute of Medical and Veterinary Science (IMVS). This serotyping at the moment is at no cost to the laboratories servicing the poultry industry. However, with the incidence of human salmonellosis increasing and pressure from the Health Departments is being applied to the poultry industry to control and reduce *Salmonella* prevalence. Hence the amount of *Salmonella* testing has increased drastically. This has increased lead time from submission to final serovar identification to approximately 10 weeks. The long lead time has made it difficult for poultry companies to be proactive in the control of *Salmonella*.

Initially, Birling Avian Laboratories obtained a copy of the Kauffman-White scheme (Grimont and Weill, 2007) and purchased the specific antibodies to differentiate the most common serogroups (B, C, D and E) and flagella antibodies to differentiate *S. typhimurium* and *S. montevideo.* The number of *Salmonella* serovars currently known, according to Grimont and Weill (2007), is 2579, with approximately 2036 belonging to the two poultry significant sub-species *S. enterica* and *S. salmonae*. There are 35 somatic lipopolysaccharide O antigens with numerous phase 1 and 2 flagella antigens. This makes serotyping a costly and time consuming exercise. The manufacture and assembly of these antigens is controlled by DNA (Whitfield, 1995;Valvano, 2003). Therefore, the identification of the variable region within the bacterial chromosome that regulates this expression would be highly desirable as a differentiation tool.

Rep-PCR, according to Healy et al. (2005), uses primers that target non-coding repetitive sequences interspersed throughout the bacterial genome and is an established approach for subspecies classification and strain determination of bacteria. The DiversiLab™ system was initially designed for clinical application, especially identification and control of antibiotic resistant strains of bacteria in hospitals. Over the years the system has diversified to target many different bacterial genera found in industrial and pharmaceutical environments. The kits supplied now include numerous bacterial species, fungi, yeasts and Mycobacterium.

[1] Birling Avian Laboratories, 975 The Northern Road, Bringelly NSW 2116

The bioMerieux nucliSENS easyMag NA platform is an automated nucleic acid extraction system based on silica extraction technology. This system was evaluated against Roche's MagNA Pure LC (MPLC) by McNally *et al*. (2007). These authors' concluded that easyMag had higher genetic yields, was easier to use and contained intuitive software.

The initial objective of the current research was to compare molecular methods to serotyping for the differentiation of *Salmonella* serovars. A subsequent objective was to compare manual DNA extraction to an automated technique.

II. METHOD

a) Salmonella Isolation

All testing was performed at an accredited laboratory in accordance with ISO: 6579:2002. The samples were emulsified 1:10 in buffered peptone water (Oxoid, CM509), and incubated at 37ºC/24hrs (standard incubation temperature unless otherwise stated). Aliquots of 1000µl and 100µl were transferred into selective Muller Kauffman (MK) (BioMerieux, 42114) and Rappaport-Vassiliadis (RV) (BioMerieux, 42110) (42°C/24hrs) broths respectively.

The following validated modifications were used: the selective and differential agar plates used were XLD/Hektoen (Oxoid, PP2027) and confirmation was performed on chromogenic SMID2 (BioMerieux, Ref 43621). The target organism was confirmed serologically with poly-O and poly-H antisera (Pro-Labs Diagnostic Refs TL6002, TKL6001) obtained from a nutrient agar (Oxoid CM3) slope and employing the slide agglutination technique. The confirmed *Salmonella* isolates were forwarded to the Australian *Salmonella* Reference Laboratory for complete serological and phage typing.

b) Molecular Typing

Prior to DNA extraction the isolate was sero-grouped B to E (Pro-Labs Diagnostic – PL6011, PL6012, PL6013, PL 6015 & PL6017) and prepared for manual extraction as indicated in the manufacturers kit (MO BIO Ultra Clean, 12224-250, DiversiLab™ Protocol 41-0007-00 rev 9). Post extraction the DNA was hybridised with manufactures primer (DiversiLab™ *Salmonella* Kit, SA060701). The replicated DNA was loaded into a chip and read on the DiverisLab™ platform. The results were compared to the sero-group specific library and a serovar determination was based upon similarity at the 98% level comparing the analyte to the library utilising Kullback-Leibler and Pearson correlation.

c) Verification

The first verification was performed by using the method as stated by the platform manufacturer of DiverisLab™. The same clone was then shipped to an Accredited *Salmonella* reference laboratory (IMVS) for traditional serotyping according to the Kauffman-White scheme. The results were matched and analysis using a 2x2 contingency table and statistical difference was determined by performing the McNemar's tests.

The subsequent verification was a modification of the extraction method from a manual process to an automated process using the Easy Mag instrument and protocols (bioMerieux - Nuclisens®). There were no other changes to the procedure mentioned above.

III. RESULTS AND DISCUSSION

The results summarised in Table 1 were analysed on the assumption that serological identification was 100% correct (i.e. the Gold Standard), thus indicating no agreement between the two methods (McNemar's P = 0.001). However, upon closer examination, 24 of the 28 pairs that disagreed were due to the following reasons: initial serovar was not in the

library (Adelaide and Tennessee) or did not serogroup. This second error is a result of the Library analysis being based upon a comparison of serovars within the same serogroup. As mentioned earlier, the vast number of serovars present for *Salmonella* means that comparing the unknown to the library would require a lengthy timeframe.

Table 1 Summary of Salmonella isolates typed by Molecular (DNA) and serological (LPS) methods over a 6 month period using DiversiLabs manual DNA extraction method.

	DNA	LPS	%	Incorrect	No.	Comments
Agona	14	14	100%			
Infantis	38	40	95%	rough r:1:5:-	2	Did not Group
Kiambu	13	13	100%			
Mbandaka	3	3	100%			
Muenster	4	5	80%	Zanzibar	1	Same Group E
Montevideo	4	4	100%			
Sofia	3	3	100%			
subsp 1 ser 4,12:d:-	11	13	85%	Agona	2	Same Group B
subsp 16:I:v:-	0	9	0%	rough r:1:5:-	9	did not Group (N)
subsp 1 ser rough r:1,5:-	24	30	80%	Zanzibar	1	Grouped as E+
Tennessee	7	10	70%	Infantis	3	Not In library
Typhimurium	31	31	100%			
Zanzibar	48	52	92%	rough r:1,5:-	4	Grouped as E+
Adelaide	0	1	0%	Zanzibar	1	Not In library
total	200#	228	88%			
Non Library errors	200#	217	92%			
Non Library & Grouping Errors	200	205	98%			

Denomination with # are statistical significant at <0.01 probability level

The serovar Subsp 16:1:v:-, according to the Kauffman-White scheme, belongs to sero-group N which is not routinely tested at this laboratory (only sero-groups B, C, D and E) and this was tested in a cluster of isolates. This serogroup has been added to the routine testing and the serovar is now in the library. The other sero-grouping error is for the rough r:1,5:- serovar which does not posses an O antigen. This latter serovar is identical in molecular structure and flagella antibody structure to *S.* Infantis. IMVS identifies this serovar as "rough" whereas the NSW reference laboratory (ICPMR) has techniques that will reactivate the LPS O antigen and thus indentifies it as *S.* Infantis. When these two errors have been removed from the calculations the two methods are not statistically different (McNemar's P = 0.13).

Table 2 Summary of Salmonella isolates typed by Molecular (DNA) and serological (LPS) methods from the 1/8/08 to the 3/9/08 period using Easy Mag automated DNA extraction method.

Salmonella subspecies	DNA	LPS	% Correct
Agona	4	4	100%
Infantis	4	4	100%
Kiambu	4	4	100%
Mbandaka	2	2	100%
Sofia	2	2	100%
subsp 1 ser rough r:1,5:-	4	4	100%
Zanzibar	22	22	100%
total	42	42	100%

The data summarised in Table 2 is for a one month period as we are still waiting for serovar results. The Easy-Mag, an automated DNA purification and extraction method, has demonstrated very favourable agreement (100%) to traditional serotyping. The DNA profile is clearer after automated extraction compared to manual extraction thus enables more accurate comparison to the library.

According to Grimont and Weill (2007) only 30 *Salmonella* serovars account for approximately 90% of all serovars isolated in any specific country. Australian serovar data, comparing all production animals and humans, identifies 33 serovars that account for 82% of all isolates (Davos, 2007). The current Birling operational library has 28 individual serovars which were isolated from poultry, feed and miscellaneous samples.

In conclusion both methods can successfully type *Salmonella*. The automation of the time and labour consuming extraction method resulted in improved clarity in the Rep-PCR bands thus increasing accuracy. This will enable shorter lead-times and lead to improved control measures that will assist in decreasing human Salmonellosis.

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FEED ADDITIVE STRATEGIES TO CONTROL GASTRO-INTESTINAL COLONISATION OF SALMONELLA IN POULTRY

L. LI[1]

Summary

Salmonella infections in poultry cause a number of serious problems – impairing bird's welfare and performance, damaging consumer confidence in the safety of poultry products, distorting public perception of intensive farming and ultimately poultry farmers suffering economic losses. Due to the awareness of the negative impact that pathogenic Salmonella have on the poultry industry, there have been intensive efforts, with assistance from the feed additive industry, to search for natural, cheap and effective products to control Salmonella. Without doubt, every nutritionist and poultry farmer is aware of the products and technologies being developed to target Salmonella contamination. To date, the possibilities include vaccination, feed additives such as antibiotics, possible dietary alternatives to antibiotics; for example, organic acids and salts, formaldehyde, essential oils and specific carbohydrates (lactose, mannose, glucose and fructooligosaccharide). Unfortunately, time and space does not allow this paper to cover all feed additives, their application concepts and mode of actions on the future of the poultry industry. However, those feed additives, which are most used or most likely to make a significant, positive impact on the poultry industry, will be addressed here. As the public become increasingly aware of food safety and health issues there is no room for complacency in the ongoing battle against Salmonella.

I. SALMONELLA INFECTIONS IN POULTRY

Salmonella was named after the United States Department of Agriculture veterinarian, Daniel E Salmon. More than 2,500 Salmonella serotypes have been indentified in nature. Salmonella serotypes can grow at temperatures ranging from 7 to 47 °C, and at pH values of between 4.0 and 9.5. Optimal growth condition is between 35 – 37 °C and pH 7.0 - 7.5. They are easily destroyed by heating. The minimum water activity value for growth is 0.96, but the organism can survive for few months in foods containing fats and at low water activities.

Salmonella infections in poultry can be divided into host specific infections and non-host specific infections. For instance, *Salmonella pullorum* and *Salmonella gallinarum* are highly adapted to the host species and are of little public health concern. Infections with paratyphoid Salmonella are non-host specific, the paratyphoid serotype includes *Salmonella enteritidis* and *Salmonella typhimurium* (Rabsch *et al.*, 2002). These are capable of causing Salmonella food poisoning in humans. Paratyphoid Salmonella infections in poultry can impair bird's welfare and performance, damage consumer confidence in the safety of poultry products and distort public perception of intensive farming; and ultimately, poultry farmers suffer economic losses.

II. FEED ADDITIVES USED TO CONTROL SALMONELLA CONTAMINATION

Contamination of poultry by Salmonella is multi-factorial, as contamination might be due to contaminated feed and water, environmental sources and transmission from contaminated eggs. It is difficult to find information on the relative importance of one factor compared with

[1] Technical Manager, Kemira Asia Pacific, Singapore

another. The bottom line is every factor should not be over-looked as any negligence will increase the prevalence of contamination and will aid in amplifying the potential risk of food-borne illness.

Feed is considered as a potential source for Salmonella contamination of poultry flocks. Although the industry is monitoring Salmonella contamination levels in raw materials, the lack of consistent measures (national or international standard) to prevent Salmonella-contaminated feed being fed to poultry might contribute to the lingering risk in the poultry primary industry (FSANZ, 2006).

To some extent, the heat treatment applied during the pelletting is effective at lowering Salmonella to safe levels, but this is not the case when contamination levels are high. Even if freshly prepared compound feed had low levels of Salmonella contamination, severe recontamination might occur during the storage or transport process (Wierupa *et al.*, 1995); Salmonella might enter the poultry farm via drinking water, dust mites, rodents and wild birds etc, and the situation can be more complicated in free-range farming system. Practically speaking, even if the smallest amount of Salmonella passes to the bird's GI system and the Salmonella count in any infected faeces will escalate rapidly. Therefore, the preferred product should be used in conjunction with heat treatment in order to protect the feed from contamination and recontamination. In other words, the product should work continuously from the feed mill to the gastrointestinal tract of the bird.

Antibiotics – Use of growth promoting antibiotics has been associated with an increase in resistance of bacteria to therapeutic agents; and a fear that this could reduce the ability to treat diseased humans (McEwen, 2006). This has caused an increased awareness of the use of antibiotics and a general wish to reduce the use of antibiotic growth promoters. For instance, the European Union has totally banned antibiotics use in animal feed since 2006. In Asia, There are currently no antibiotics registered for growth promotion in Japan. South Korea is to ban the use of certain antibiotics in poultry feed in 2009. It is understood that the Australian poultry industry still uses specific antibiotics for growth promotion and improvement of feed utilisation (JETACAR, 1999).

Formaldehyde (CH_2O) – Historically, formaldehyde has been used as a feed additive to protect dietary protein in ruminant (Madsen, 1982). In poultry, formaldehyde fumigation is widely used to minimise bacterial contamination of eggs and hatching chicks of to destroy potential pathogenic contaminations. In one case, formaldehyde fumigation increased the severity of mould infection rather than reducing it (Wright *et al.*, 1961). In 1996, US Food and Drug Administration (FDA) stated that formaldehyde, at the dose rate of 0.9 kg per ton, maintained complete poultry feed Salmonella free for about 14 days and FDA also concluded 0.9 kg per ton is safe to use in poultry feed. Based on one tolerance study on fattening broilers, the EU Panel on Additives and Products of Substances used in Animal Feed (FEEDAP) concluded that formaldehyde, at a dose rate up to 0.99 kg/ton, had no effect on feed intake and body weight during the entire experimental period; however, the study also found 0.99 kg/ton inclusion rate significantly reduced feed conversion ratio compared to that of controlled chickens (EFSA, 2004). Some researchers also proposed that formaldehyde can be used to control Salmonella contamination in poultry feed (Brown, 1996; Khan *et al.*, 2006; Summers, 1985). Babar *et al.* (2001) reported that formalin (37% formaldehyde), at dose rate of 10 g/kg, fed to broiler birds decreased feed intake and body weight. Zulkifli *et al.* (1999) studied the effect of formaldehyde fumigation during hatching on the performance and behaviour of hatched chicks. This study found that formaldehyde fumigation resulted in overall lower feed conversion ratio, but the birds' behaviour, bodyweight and mortality rate were not affected by formaldehyde fumigation.

From the metabolic standpoint, formaldehyde is involved in several metabolic reactions in the body. The oxidation into formic acid and carbon dioxide, the reaction with

glutathione, and also the reaction with proteins and nucleic acids, which are partly reversible, are of importance (Kitchens *et al.*, 1976). Since formaldehyde reacts with lysine and other amino acids (Broderick and Lane, 1978), the nutritive value of protein treated with formaldehyde might be reduced. Formaldehyde is highly corrosive and volatile, has a pungent smell, strongly irritates the mucous membrane and has a toxic potential. Therefore, there are concerns about the palatability of treated feed, efficacy on performance of birds and possible adverse health considerations resulting from inhalation of formaldehyde fumes. Formaldehyde is either banned or not used at all in the EU, Japan and NZ.

Organic acids/salts/esters – Short chain fatty acids and salts (Hinton and Linton 1988) (AL-Tarazi and Alshawabkeh, 2003; Heres *et al.*, 2004; Van Deun *et al.*, 2008); medium chain fatty acids (Skrivanova *et al.*, 2006; Van Immerseel *et al.*, 2004) and esters (Thormar *et al.*, 2006) have been widely evaluated for their usefulness in manipulating intestinal biochemistry to either directly kill or inhibit Salmonella and campylobacter colonisation and to support the growth of protective bifidobacteria and lactobacilli bacteria (Dibner and Buttin, 2002; Owens et al., 2008).

Apart from being particularly effective against acid-intolerant Salmonella, Campylobacter or E. coli, feeding organic acids can also improve nutrients (protein and energy) digestibility, reduce the digesta pH, increase pancreatic secretion and trophic effect on the gut mucosa. Hence, the application of organic acids in poultry feed or drinking water enhance the bird performance (Abdel-Fattah *et al.*, 2008; Byrd *et al.*, 2001; Dibner and Buttin, 2002; Viola *et al.*, 2008).

From a review of the literature for poultry, there is a lack of consistency in demonstrating organic acid benefits in animal performance (Dibner and Buttin, 2002). Several factors influencing responses to organic acids have been identified. Briefly, the efficacy of the antimicrobial effects varies from one acid to another and is dependent on concentration of the acid applied, the level of contamination and buffering capacity of the diet, pH of the gastrointestinal tract (Chaveerach *et al.*, 2002; Salsali *et al.*, 2006) and animal factors such as stress and a low threshold level of tolerance against pathogenic bacteria from feed (Burkholder *et al.*, 2008). Therefore, further research is needed to specify the interaction between these factors and different organic acids.

Given different countries have different legislations towards the use of antibiotics or feed additives, application of different additives needs more detailed scenarios of pathogens response and host-pathogen interaction under different environmental conditions. Gathering this detailed information should yield clues for developing new products to better exploit vulnerabilities of food borne Salmonella during feed processing and application of feed additives.

III. CONCLUSION

From the practical point of view, the most efficient way for Salmonella control should be the combination of monitoring, biosecurity, vaccination and using the correct feed additive. All feed additives used for Salmonella control have pros and cons. To produce Salmonella-free products, one needs to consider not only the efficacy of the product, but also the long-term rewards.

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Hughes, R.J.	31, 73, 81	hughes.Bob@saugov.sa.gov.au
Iji, P.A.	44, 56, 77, 85,123, 124, 133	piji@une.edu.au
Islam, A.F.M.F.	176, 180	fislam2@une.edu.au
Jackson, C.A.W.	188	cawjacko@ozemail.com.au
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Kocher, A.	44, 70, 124	akocher@Alltech.com
Kawalilak, L.	162	
Kumar, A.	47	ak65@uq.edu.au
Laine, S.M.	153	s.laine@pgrad.unimelb.edu.au
Leary, A. M.	129	alison.leary@Alltech.com
Li, L.	200	li.li@kemira.com
Liu, K.	36	Kevin.liu@adisseo.com
Lunam, C.A.	69	chris.lunam@flinders.edu.au
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McLeish, J.	137	John_Mcleish@admworld.com
Meijerhof, R.	106, 167	Ron.Meijerhof@hatchbrood.nl
Mikkelsen, L.L.	44, 77, 85, 123, 124, 133	lmikkels@une.edu.au
Miao, Z.H.	116, 175	
Middlebrook, T.	125	todd.middlebrook@gwf.com.au
Molan. A.L.	66	a.l.molan@massey.ac.nz
Muir, W.I.	90	wmuir@camden.usyd.edu.au
Nair, V.	184	
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Offer, J.	67	
Olnood, C.G.	85	colnood@une.edu.au
Ophel-Keller, K.	73	ophelkeller.kathy@saugov.sa.gov.au
Partridge, G.G.	40	
Pavic, A.	196	tony_pavic@baiada.com.au
Peisker, M.	137	m_peisker@admworld.com
Perez De Ayala, P.	171	