21ˢᵗ ANNUAL AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

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Organised by

THE POULTRY RESEARCH FOUNDATION
(University of Sydney)

and

THE WORLD'S POULTRY SCIENCE ASSOCIATION
(Australian Branch)

AUSTRALIAN POULTRY SCIENCE SYMPOSIUM
2010

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AUSTRALIAN POULTRY AWARD

The Australian Poultry Award is presented annually to an Australian resident who has made a long-term outstanding contribution to poultry science and/or the Australian poultry industry. The Award is made by the Australian Branch of the World's Poultry Science Association (WPSA) and takes the form of a suitably inscribed plaque which includes the winner's name, together with a framed citation. Nominations are called for early each year from the membership of WPSA, and completed nominations require to be forwarded to the Secretary of the Australian Branch no later than 31st July. The selection committee consists of the Australian Branch Management Committee of WPSA (10 members) as well as Award recipients from the previous 10 years who are still active in the Australian poultry Industry. Voting is by secret postal ballot, and if more than two candidates are nominated, a preferential voting system is used. The Award is made to the winner at suitable forums where poultry industry people are gathered, such as the annual Australian Poultry Science Symposium, the biennial Poultry Information Exchange (PIX), and the triennial Australian Poultry Convention.

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1983	Dr R.K. Ryan	2006	Dr Juliet R.Roberts
1984	Mr C. Donnelley	2007	Dr Vivien Kite
1985	Dr P. Gilchrist	2008	Mr Rowly Horn
1986	Dr C.A.W. Jackson		

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RECOGNITION OF 100TH ANNIVERSARY OF THE FACULTY OF VETERINARY SCIENCE – UNIVERSITY OF SYDNEY

It is my pleasure, as the Faculty of Veterinary Science's new Dean, to welcome you to the 21st Annual Australian Poultry Science Symposium. This symposium promises to continue in the Poultry Research Foundation's proud tradition of forging new research directions and facilitating collaborative links between poultry scientists, producers, researchers and veterinarians. The symposium will draw together leaders from Australia and our local region with international experts and provide participants with access to the most current insights into improving poultry health and production. The symposium topics span the breadth of nutrition, metabolism, physiology, behaviour, welfare, microbiology, husbandry, toxicology and immune function in intensive production systems. Presented papers will also address the issues facing poultry production in developing countries, helping our near neighbours to achieve greater food security for their people. It is particularly appropriate that the PRF symposium is one of our first celebrations of our Centenary Year as the PRF is one of the longest established Foundations of the Faculty and the University. It is also a Foundation that grows in strength every year, thanks to the dedicated efforts of the Foundation members and supporting staff. We appreciate your contributions and look forward to stimulating discussions on poultry production in a time of global uncertainty and to welcoming you to other events in the Faculty Centenary Program, 2010.

HISTORY OF THE FACULTY

In 1909 the University of Sydney, with the support of the New South Wales Government, established a Veterinary School and appointed James Douglas Stewart, MRCVS, the Director and Professor. The school officially opened in 1910 with 16 students enrolled in the 5 year Bachelor of Veterinary Science. The teaching moved from the basement of the southwest corner of the Quadrangle (original Fisher library), to the new JD Stewart Building late in 1913.

The school's development was slow in the first world war, but with the leadership of Professor Stewart, who remained Dean until 1939, it grew steadily to 25 undergraduates (1928), and over 100 in 1935. The Sydney University Veterinary Faculty was the only one in Australia to operate continuously from its establishment.

In 1936 the University, in association with the McGarvie Smith Institute, purchased a 160 hectare property at Badgery's Creek, for Animal Husbandry teaching, and at this time the degree reverted to a 5 year long course. Building extensions on main campus (1939), were followed by the temporary Department of Veterinary Pathology and Bacteriology building (1946) the Veterinary Teaching Hospital (1949). In 1954 the Camden farms were acquired to provide final year students with animal units for the teaching of husbandry and disease control, and with a Veterinary Clinic and Hospital, Lecture Theatres and Teaching Laboratories, and a hall of residence (Nepean Hall).The extensive Hospital and Clinic buildings (Evelyn Williams Building), an Animal Science building (RMC Gunn Building) were added, the McMaster building acquired and the Veterinary Science Conference Centre (opened 1998) were erected at the Camperdown Campus (Sydney).

The Faculty expanded and internationalised its veterinary student intake in 2000 with the introduction of the new curriculum, which coincided with major changes to the Faculty structure, removing existing Departments. To meet increased demand, the faculty offered a new undergraduate degree, the Bachelor of Animal and Veterinary Bioscience in 2005. This 4-year

degree provides a quality education in structure and function of animals, their management and welfare in an agricultural, para-veterinary, laboratory or wildlife context. We have also introduced a number of postgraduate diplomas and coursework programs, leading to Master of Animal Science, Master of Science in Veterinary Science, Master of Veterinary Science, Master of Veterinary Studies, Master of Veterinary Clinical Studies and Doctor of Philosophy. The current undergraduate student profile is approximately 80% female, with special entry programs for indigenous, rural-origin, mature age and disadvantaged students. In the past decade the Faculty's staff numbers, research funding, productivity and postgraduate student training have more than doubled, along with the substantial growth in student numbers to more than 900 in 2010.

The Wildlife Health and Conservation Centre (2004), General Teaching Block (May 2007) and the Liz Kernahan Conference Centre (2009) were added to the Camden campus. The Camden 2020 master plan has commenced with current developments in Sample Biobanking facilities, Surgical Training facilities and student accommodation (duplexes for 70) due for completion in 2010.

Professor JD Stewart,
Founding Dean, 1909-1939
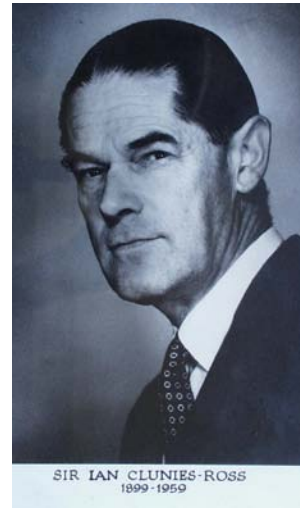
Sir Ian Clunies Ross,
Dean 1940-1946

Anatomy dissection in basement of Quadrangle, 1910

Old veterinary surgery (demolished 1977)

HISTORY OF THE POULTRY RESEARCH FOUNDATION

The PRF was one of the original University of Sydney foundations, established in October 1958. It has been a resounding success in its mission to support liaison and research collaboration between the University and the poultry industry. Its success has come through the strong partnerships forged among the Foundation members, poultry and stock food industry leaders and Faculty research staff and students. Together, particularly through the annual symposium, they have contributed to improvements, particularly in the feeding and husbandry of chickens, turkeys, ducks and other commercial poultry. The symposium has achieved greater international recognition and regularly is linked with other leading events in the world poultry research calendar. The research supported by the Foundation has assisted in refining the nutrient requirements and optimal diet formulations for poultry production, issues of great concern as feed is the major cost in production. The early Foundation Chairs, particularly Mr John Darling, Mr Val Parkinson, Dr Balkar Bains and of course our current President, Ms Linda Browning, have forged the productive relationships that have been central to the Foundation's success.

The Foundation has also had a marked impact on research directions, postgraduate student training and undergraduate education in the University, through the appointment of outstanding poultry research scientists and educators. The Directors have provided imagination, energy and intellectual drive for new research directions and developments, particularly Professor Terry Robinson, Professor Frank Annison, Professor David Fraser, Professor Tom Scott and Dr Peter Groves. Not to mention the insight of inaugural directors from the Department of Animal Husbandry, later to become the Department of Animal Science, Dr. Harold McNary, Associate Professor Charles Payne and Associate Professor Derick Balnave. These directors have been supported by a capable and committed team of technical, administrative and academic staff, who ensure the diverse range of the Foundations' goals are achieved, and we are most fortunate to have Dr Wendy Muir, Dr Jeff Downing, Dr Greg Cronin and Dr Peter Selle, Mrs Jo-Ann Geist, Mrs. Joy Gill and Mrs. Melinda Hayter currently on staff.

The PRF has a bright future, with many opportunities to further improve the health, welfare and productivity of poultry ahead. Thank you for your diverse contributions and we welcome your close and productive association with the PRF, Faculty and University.
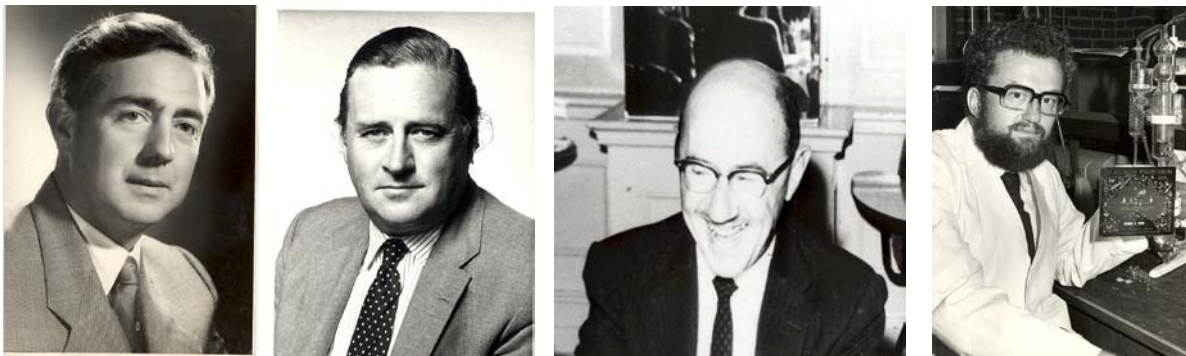
L to R: Professor Terry Robinson, Mr John Darling, Dr Harold McNary, Associate Professor Charles Payne 1975 Poultry Science Award,

Construction of the first layer shed 1959
(Dr. Harold McNary & Mrs.Janet McNary)

Layer shed in operation, early 1960s,

All my best wishes for a most successful meeting,
Rosanne Taylor
Dean, Faculty of Veterinary Science, 2010

JOHN L. BARNETT IN MEMORIAM

The tragic loss of Associate Professor John Barnett and his wife Jenny Barnett in the Victorian bush fires on February 7th 2009 deeply affected all those who knew and loved them. John was a true gentleman, a brilliant scientist, a world leader in the field of animal welfare science and above all a true humanitarian.

John's main area of expertise was stress physiology and its application to the study of domestic animal welfare. This research over 30 years provided a timely balance on discussions within science and the livestock industries on welfare methodology and interpretations and this impact will continue to improve animal welfare methodology in the future. John's research on poultry and pigs have also made a critical contribution to our understanding of the welfare risks associated with confinement housing, highlighting the major risks of confinement that arise from spatial and social restriction. He worked extensively with the livestock industries in developing welfare components of livestock industry QA programs and in assisting to achieve improvements in awareness and practices to safeguard animal welfare standards. His outstanding scientific efforts have been highly acclaimed nationally and internationally by both science and the livestock industries and animal welfare science will greatly miss his important contributions.

John was major contributor to the annual Australian Poultry Science Symposia and triennial Australian Poultry Conventions, and was an active contributor to symposia and meetings of the WPSA European Federation Working Group 9 on *Poultry Welfare and Management.* He led the Australian Poultry Cooperative Research Centre's Welfare program since 2003. John was also a major contributor to the Animal Welfare Science Centre's research and teaching programs at the University of Melbourne, Monash University and the Victorian Department of Primary Industries. His wise counsel on matters of science as well as life will be sadly and irreplaceably missed.

DEVELOPMENTS AND INNNOVATIONS IN BROILER NUTRITION IN THE NETHERLANDS

L.L. DE LANGE[1]

Summary

During the last decades important triggers for innovation have been the reduction of the output of minerals and nitrogen to the environment and the ban by the EU on the preventive addition of antibiotic growth promoters (AGP's) to feeds. A good protein evaluation system with an accurate estimation of requirements will minimise the output of nitrogen to the environment, improve intestinal health and performance. AGP's have a direct effect on the numbers of bacteria in the gut but also on the composition of the microbial community in the different segments of the gastro-intestinal tract of broilers. Relative high quantities of DNA from *Lactobacillus Acidophilus* in the upper gut of young broilers are related to a bad feed utilisation. The effect of immune modulators on performance is not always consistent and depends on source, age and challenge. Future challenges in animal nutrition research are about animal welfare and how to reduce the output of green house gasses in animal husbandry to prevent global warming.

I.   INTRODUCTION

The Netherlands (NL) is a small country, but big in agriculture. With an agricultural export volume of about 58.5 billion Euro NL is the world's second largest exporter of agricultural and food products after the USA. The net trade surplus of the agricultural sector in NL of 23 billion Euro in 2007 is after the US the second largest in the world, and represents 57% of the total Dutch trade surplus (LEI, 2009). This strong position is built on the leading role of The Netherlands in primary agricultural production, logistics and agricultural research.

In the seventies and eighties of the last century the livestock industry in The Netherlands has developed rapidly. The main reasons for this development were the low transport costs due to the favourable infrastructure and waterways, the high prices of grains within the European Union (EU) combined with the use of cheap imported alternative feed ingredients as tapioca from Thailand and maize gluten feed from the US. All this has led to low feed prices and low feed costs per kg meat, milk or eggs in The Netherlands compared to other European countries. So the turnover of the feed industry in NL could increase for many years with 5 – 10 % annually.

In the nineties, the high stocking density close to a dense human population caused problems with pollution, and led to restrictions for the output of nitrogen, minerals and trace elements to the environment. In the last decade, the risks of a high stocking density have become clearer with the outbreak of contagious diseases. The advantage of the import of cheap raw materials disappeared when the prices of the cereals came down by a changing policy of the EU. By this reason and the environmental restrictions the feed production went down from 16.5 million tons at the start of the nineties to a stable volume of about 14 million tons during the last five years. The production of poultry feeds has been for many years quite constant on a level of 3.5 million tons a year, disregarding the severe outbreak of avian influenza in 2003. Of the 3.5 million ton poultry feed about 1.5 million ton is for broilers and about 2 million ton concerns feed for layers (FEFAC, 2009).

[1] De Heus Feeds BV, P.O. Box 396, 6710BJ Ede, The Netherlands

All this made the animal feed industry in NL very competitive, innovative and expanding abroad. The knowledge about the evaluation of raw materials coming from all over the world (CVB-tables), about the nutritional requirements of farm animals, about how to minimise the output of nitrogen and minerals to the environment (phytase is a Dutch invention) has developed well during the last decades within The Netherlands and more specific within De Heus Feeds.

The gross domestic production of poultry meat in NL is 700,000 tons per year. The consumption of broiler meat in The Netherlands comes mainly from breast and processed meat, while the local consumption of grillers and leg meat is relatively low. The average consumption of broiler meat in NL is 18.4 kg per capita or 303,000 ton total per year. The main export markets are Germany and the UK (PVE, 2009). The 44 million broilers in NL are housed on 700 farms. So the average size of the family owned farms is around 70,000 broilers, taking into account the time that the houses are empty and cleaned. The life weight at slaughter varies from 1.7 till 3.5 kg with an average of about 2.3 kg. The broiler houses are well insulated with a good climate control, and cleaned and disinfected after each flock. Feed costs are about half of all production costs. The costs for housing are almost 10%.

The production of broiler meat in NL is not integrated. Slaughter houses, broiler farmers, hatcheries and feed mills operate independently, but they make short term contracts and exchange information. De Heus Feeds has in this highly competitive broiler market a market share of 25 – 30% of the compound feed.

II. DEVELOPMENTS

In an open competitive market economy is always a good trigger for rapid developments and innovations. With the decline of prices of cereals in the EU at the nineties adding whole wheat on the broiler farm has become popular saving processing and transport costs. At the beginning about 10% whole wheat was used combined with standard feeds without affecting the performance negatively. Nowadays frequently 20 – 35% whole wheat is used together with concentrates rich in protein.

Environmental problems induced the development of phytase by Gist Brocades and Dutch research institutes. The use of this enzyme has become widespread all over the world when the use became economic by replacing expensive mineral phosphorous and by the beneficial side-effects of phytase by reducing the anti nutritional effects of phytate.

The announcement of the ban of Antibiotic Growth Promoters (AGP's) in feeds starting in 2006 by the EU has given an enormous boost to research on intestinal health and how to replace these feed additives. Many alternatives for AGP's have been tested without questioning the mode of action of AGP's. More recently, efforts have been put to reduce the therapeutic use of antibiotics at farm animals on prescription by veterinarians. Especially the increasing concern about life stock farmers carrying resistant bacteria in their body is an important motive to reduce also the therapeutic use of antibiotics.

Another concern is about animal welfare starting in the UK more than 10 years ago. In The Netherlands a one issue political party on animal welfare is even represented in the parliament. EU regulations for animal welfare especially about housing are nowadays common practise. A new EU regulation starting July 2010 about stocking density related to mortality and foot pad dermatitis will affect the broiler industry. If the norms for mortality or foot pad dermatitis are exceeded the maximum stocking density has to be reduced (EU, 2007).

Also retailers in The Netherlands try to profile themselves recently with themes as sustainability and animal welfare. Special niche markets are being developed with extra added value and more attention for animal welfare.

III. INNOVATIONS

(a) Protein evaluation

In The Netherlands the protein requirements for poultry are usually based on the faecal digestibility of amino acids (AA). Minimum requirements are set for the essential amino acids as lysine, methionine + cysteine and □hreonine. The other essential amino acids are normally not limiting. If some nonessential AA are not supplied sufficiently these can be synthesized from non limiting essential and other nonessential AA. To ensure that the requirements for all AA are provided, one can formulate requirements for both essential amino acids and crude protein (NRC, 1994). An indirect way to ensure the supply of nonessential AA is by maximising the use of synthetic AA. At De Heus we prefer to formulate diets with protein requirements for essential AA and the sum of all digestible AA (De Lange *et al*, 2003). Doing so, also the surplus of non limiting essential AA is utilised to meet the requirements for nonessential AA. By this approach and by using well digestible protein sources, the amount of nitrogen at the end of the intestinal tract is minimised to prevent proteolytic fermentation. The fermentation of protein can increase the growth of pathogenic bacteria such as sulphite reducing clostridia and the formation of toxic compounds as ammonia and phenol as is demonstrated after predigestion with in vitro research in the TNO Intestinal Model (TIM-2) to simulate the function of the hindgut (Table 1).

Table 1. End products proteolytic fermentation TIM-2

Feed	A	B	C	D
Feathermeal (% in feed)	0.0	0.0	1.5	3.0
Digestibility protein (%)	92.5	91.5	89.5	82.2
Fermentable protein (g/kg residue)	73	77	96	138
Cumulative production of:				
Ammonia (mmol)	9.5	13.8	13.1	15.4
Phenol (µmol)	0.0	0.0	8.9	14.0
Sulphite reducing Clostridia (^{10}log cfu/ml)	5.1	5.8	6.3	6.8

To avoid proteolytic fermentation one can minimise the quantity of fermentable protein (Cone, 2005), closely related to indigestible protein or the difference between ileal digestible protein as described by Bryden *et al* (2009) and faecal digestible protein.

The objective of a correct feed evaluation is to predict animal performance accurately with different compositions of the feed. The combination of the minimum for digestible AA and a maximum for indigestible crude protein gives a better prediction of animal performance than crude protein (De Lange *et al*, 2003).

The protein levels in feed depend on age/weight, breed, intake, gain and economy. With the modern broilers there is a good relation between protein level in the feed and animal performance as is shown in trial VK-062, performed by De Heus (Figure 1 and 2).
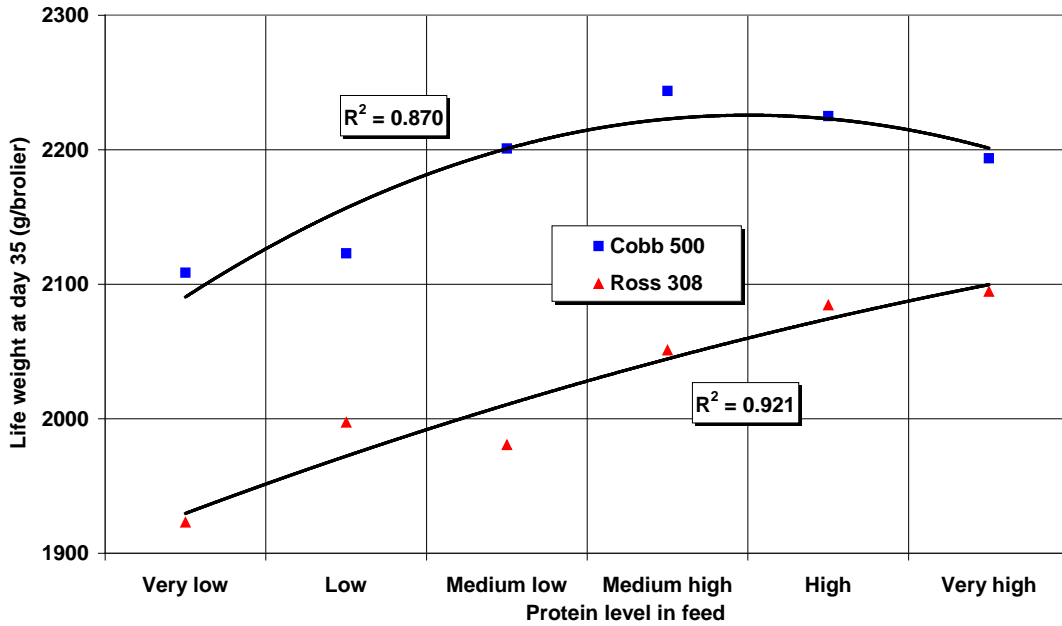
Figure 1. Relation between protein in feed and life weight at day 35 (VK-062)
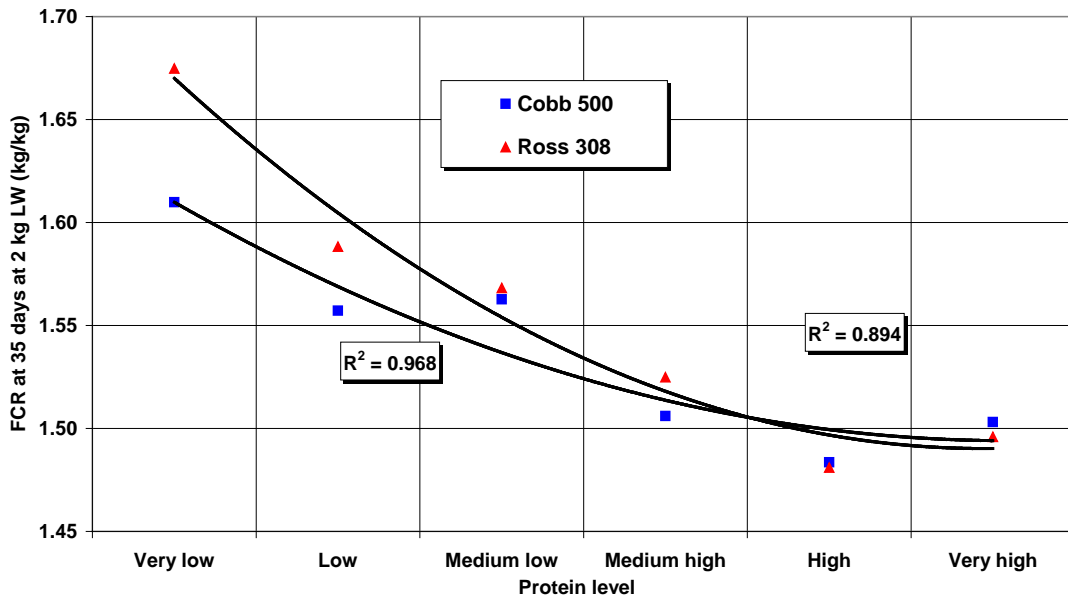
Figure 2. Feed Conversion Rate per cross breed and per protein level at day 35
at 2 kg Life Weight (correction 0.03 per 100 g LW; VK-062)

After correction for protein requirements for maintenance, the protein conversion expressed as protein requirements per 100 g growth is equal for the main cross breeds during the grower or finisher period (Figure 3). Taking into account the differences in intake and growth, the protein requirements expressed per kg feed are different between the main breeds. There seems to be a continuous improvement in protein efficiency in time when the protein requirements are expressed per 100 g daily gain.
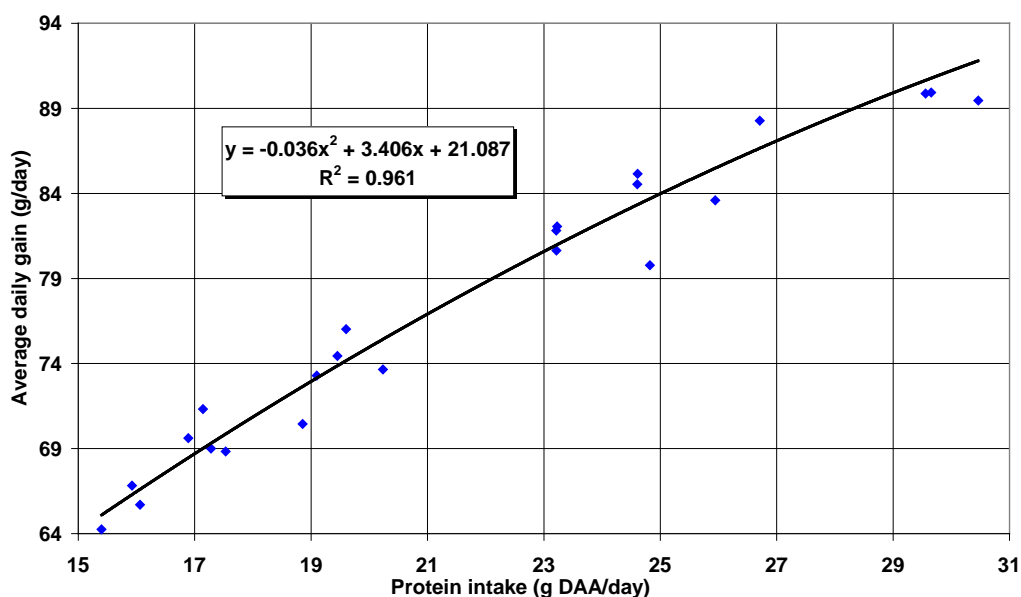
Figure 3. Relation daily protein intake and daily gain (VK-062)

The chart shows the equation:

$$y = -0.036x^2 + 3.406x + 21.087$$
$$R^2 = 0.961$$

with x-axis labeled "Protein intake (g DAA/day)" and y-axis labeled "Average daily gain (g/day)".

(b) <u>Intestinal health</u>

New molecular DNA based techniques like Terminal Restriction Fragment Length Polymorphism (T-RFLP), as described by Lu *et al* (1997) reveal the complex microbial societies in many biotopes as the broilers gut. In the ileum of broilers mainly *Lactobacilli* are dominant, while in the cecum *Clostridiaceae* related species are abundant (Lu *et al*, 2003). AGP's reduce the overall numbers and the numbers of species of gut bacteria in pigs and poultry (Jensen, 1998; Gaskins *et al*, 2002). Bacteria in the gut may be considered as parasites or commensals, dining at the same table as the host and consuming nutrients. Bacteria like *Enterococcus faecium* and *Clostridium perfringens* but also *Lactobacillus* species hydrolyse bile salts and cause impaired digestion of fat (Knarreborg *et al*, 2002). Bacteria also trigger the immune system as is shown in germ-free animals, which have a poorly developed immune system. Triggering the immune system leads to an acute phase response with loss of appetite and catabolism of muscle tissue (Gruys *et al*, 2006). Niewold (2007) hypothesizes that the growth promoting effect of AGP's relies on an anti-inflammatory effect by inhibiting the production and secretion of cytokines by intestinal inflammatory cells and so reducing the acute phase reaction.

In a cooperative research by Provimi and De Heus Feeds, funded by the Dutch Ministry of Economic affairs, is shown that AGP's and feed composition have an effect on the microbial composition in the gut, which is related to feed efficiency. Relative high quantities of DNA from *Lactobacillus Acidophilus* in the upper gut of young broilers are related to a bad feed utilisation, and are affected by feed and AGP's (De Lange and Wijtten, 2008). Davis *et al* (2009) showed a relation between the relative numbers of *L. Acidophilus* in the gut of pigs and a systemic immune response by measuring T-cells in the peripheral blood.

The alternative of De Heus Feeds for the use of AGP's is called Nutribiotics[®]. This concept is based on a high digestibility of protein to prevent the formation of toxic metabolites like ammonia and phenol and to prevent increased numbers of sulphite reducing *Clostridia*, on natural antimicrobial components to reduce bacterial growth, on a feed form with coarse particles to lower the pH and to increase the retention time of feed in the gizzard, and on a low viscosity of the intestinal content to facilitate digestion. After the ban De Heus has succeeded in continuing the improvement of broiler performance in practise with a FCR of 1.70 – 1.75 at a life weight of 2.3 kg.

The next challenge in research and application into practise is the modulation of the immune system of the broiler chicken. The best way of immune modulation is most probably by stimulating the development of the immune system at a young age and by reducing acute phase reactions at an older age when birds have to grow efficiently. Also modulating the immune reaction to an anti-inflammatory Th2 response instead of a pro-inflammatory Th1 response can help to improve feed and protein conversion. Modulating immunity of young chickens starts with the parent stock. A well developed innate immune system helps in initiating the adaptive immune response (Goddeeris, 2005). Feeding BioMos[®], a yeast cell wall (YCW) product to breeder animals changes the ratio yolk/egg white in breeding eggs and increases the quantity of specific immune globulins in blood against New Castle Disease after vaccination (De Lange, 2007).

Table 2. Effect of YCW products in breeder diet (VK-078)

Breeder diet	Control	BioMos[1])	Fibosel[2])	SEM	P-values	
					Br. diet	Br.*Chall.
Weight (g)	2117 [b]	2080 [ab]	2068 [a]	14	0.06	0.68
FCR	1.597	1.592	1.578	0.007	0.11	0.05
FCR 2 kg LW	1.562	1.568	1.557	0.009	0.65	0.28

[1]) BioMos®: YCW product of Alltech; added 1 kg per ton feed
[2]) Fibosel®: purified YCW product of Lallemand; added 200 g per ton feed
[a,b]Means within a row not sharing a common superscript differ significantly (P<0.05)

In a De Heus experiment (VK-078) adding Fibosel[®] to the breeder diet reduces the live weight of the off spring at day 35 compared to the control group ($P < 0.05$), and tends to improve the not corrected FCR ($P < 0.10$) compared to the control group (Table 2). Also the interaction between the addition of YCW based products to breeder diets and a challenge with a viscous diet during the grower phase on the FCR tends to be significant ($P < 0.10$) (Table 2 and 3).

Table 3. Interaction between YCW in breeder diet and challenge grower diet (VK-078)

	Non challenged			Challenged		
	Contr	BioMos	Fibosel	Contr	BioMos	Fibosel
FCR	1.569	1.582	1.573	1.625	1.602	1.582
FCR relative	100.0%	100.8%	100.3%	100.0%	98.6%	97.4%

The beneficial effect of an investment in the immune system on a young age seems to depend on a challenge later in life.

Table 4. Effect of YCW products in starter diet (VK-078)

Starter diet	Control	BioMos[1])	Fibosel[2])	SEM	P-values Br. diet	Br.*Chall.
Weight (g)	2093	2066	2107	14	0.14	0.29
FCR	1.595	1.595	1.577	0.007	0.15	0.68
FCR 2 kg LW	1.567 [ab]	1.575 [b]	1.545 [a]	0.009	0.08	0.82

[1]) BioMos®: YCW product of Alltech; added 1 kg per ton feed

[2]) Fibosel®: purified YCW product of Lallemand; added 100 g per ton feed

[a,b]Means within a row not sharing a common superscript differ significantly (P<0.05)

Adding Fibosel to a starter diet improves the corrected FCR at 2 kg live weight compared to the BioMos group significantly (P < 0.05) and the difference with the control group tends to be significant (P < 0.10) (Table 4).

IV. FUTURE CHALLENGES

The nearby challenge in broiler nutrition science is to improve animal welfare by reducing mortality and foot pad lesions. The most obvious way to lower mortality is improving health and robustness of the one day old chickens, starting at breeder farms and hatcheries, but also at the start on the broiler farms. Reducing feed intake and average daily gain might be also part of a concept to lower mortality and sudden death. Less foot pad lesions will be pursued by nutrition and improving litter quality by nutritional and other means.

The best way to respect the environment at the production of broiler meat is by reducing the output of nitrogen, minerals and trace elements, by improving feed utilisation and by maximising the use of by-products that are less attractive for direct human consumption.

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CHALLENGING CURRENT POULTRY FEEDING DOGMAS BY FEED INTAKE RESTRICTION AND THE USE OF COARSE FEED INGREDIENTS

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Summary

In Europe, there is a trend towards a coarser structure of the diet. In the broiler industry, there is also growing interest for restricted feeding. A coarser structure of the diet reduces the cost of feed production, and has also been linked to an improved feed utilization through a better functioning gizzard. A slightly restricted feeding has also been observed to result in improvement in feed efficiency, and this has been shown to be caused by an altered growth curve and composition of growth. Although feed overconsumption is observed in individual broiler chickens under certain conditions, the bird's ability to store large quantities in the crop and gizzard makes it uncertain whether feed restriction may be a suitable tool to reduce this problem. However, a coarser structure may reduce the problem of feed overconsumption through a better functioning gizzard.

I. INTRODUCTION

Currently, the dominating global principle of poultry feeding is to provide poultry ad libitum with one diet containing finely ground low-fibre ingredients. The advantage of this feeding practice is obvious; it allows for a simple management, and it assures a homogenous feed which is easily available for digestion by the bird. However, there is emerging evidence for that this feeding principle does not maximise performance and profits. In this paper, the paradigm that birds should be ad libitum fed a diet containing finely ground low-fibre ingredients will be challenged, using the broiler chicken as an example.

The practice of fine grinding has been challenged in Europe during the past 20 years, partly due to data which indicate that coarser grinding or the use of whole cereals saves cost and in some cases has positive effects on bird performance (Preston et al., 2000; Hetland et al., 2002: Plavnik et al., 2002; Svihus et al., 2004; Ravindran et al., 2006; Gabriel et al., 2008). In addition, similar beneficial effects have been observed when coarse fibre components such as oat hulls or wood shavings are added to the diet (Hetland et al., 2003a; Amerah et al., 2009). The beneficial effect of coarse ingredients has been linked to a more developed gizzard that ensures a complete grinding and a well regulated feed flow and digestive juice secretion.

Although ad libitum provision of feeds to broilers remains the dominating practice, different feed restriction programmes appear to be on the rise. In Norway for example, it has now become common practice with a slight restriction on feed availability. Although the restriction is so moderate that the growth curve is not significantly altered, a slightly improved feed efficiency and a reduced mortality is commonly observed, favouring increased use of this feeding method. A common management practice is to restrict feed intake slightly by letting the birds empty the feeders twice a day, and to provide feed according to a predetermined feeding plan. The beneficial effect of this type of feed restriction could simply be a management benefit due to the fact that feeders are emptied regularly and thus that fines are not accumulating. However, there are indications for that some birds under ad libitum

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feeding may develop a habit of feed over-consumption (Svihus & Hetland, 2001), which if correct calls for restrictions on feed availability. Also, there is overwhelming evidence for that intermittent feeding by the use of lighting control improves feed efficiency compared to ad libitum feeding (Buyse et al., 1996).

Since birds under restricted feeding depend on temporary storage of feed in the anterior digestive tract, the storage capacity of this digestive segment is of importance. It has been shown that broiler chickens use both the crop and the proventriculus/gizzard as storage organs for food when adapted to long periods of food deprivation (Buyse et al., 1993). Barash et al. (1993) observed a significant increase in weight and feed-holding capacity of both crop and gizzard when chicks were fed meals one or two times per day instead of ad libitum. It is well known that structural components in the feed result in a larger gizzard, which will also be able to hold larger quantities of feed. It is therefore possible that an interaction between feed restriction and the structure of the diet exists.

II. STRUCTURAL COMPONENTS IN THE DIET

It is a well established fact that the digestive tract adapts to changes in diet composition. Studies of wild birds with large variations in their diet throughout the year for example, show that both the small intestine and the caeca fluctuate in size (Klasing, 1998). Particularly the gizzard is known to respond rapidly to diet changes, something noted amongst others by Charles Darwin during his studies of pigeons (Farner, 1960). A rapid and conspicuous enlargement in size of the gizzard is observed when structural components such as hulls, wood shavings or large cereal particles are included in the diet, and the predominant hypothesis is that the beneficial effects sometimes observed is mechanistically linked to gizzard development.

The increase of size of the gizzard is a logical consequence of an increased need for particle size reduction, as the increased grinding activity of the gizzard increases the size of the two pairs of gizzard muscles. For example, Hetland et al. (2003a) observed a 50% heavier gizzard when whole cereals and oat hulls were included in the diet for broiler chickens. Coarse feed particles need to be ground to a certain critical size before they can leave the gizzard (Clemens et al., 1975; Moore, 1999), causing the volume of gizzard contents to increase when diets with whole cereals or insoluble fibre are fed (Hetland et al., 2003a,b). Thus, structural components do not only increase the size of the gizzard, but also causes an increased volume of gizzard contents in birds fed diets with whole grains or other structural components (Hetland et al., 2003a,b).

An increased amount of finely ground particles in the duodenum for diets with structural components indicates that one potential beneficial effect of structural components is smaller particles entering the small intestine and thus a more efficient and complete digestion (Hetland et al., 2002). This corresponds to the increased starch digestibility sometimes observed when structural components have been added (Svihus & Hetland, 2001; Svihus et al., 2004). However, the increased amylase activity and bile acid concentration observed by Svihus et al. (2004) may indicate that an increased secretory activity may also be part of the cause for improvements in nutritive value associated with structural components. This hypothesis is also supported by the fact that amylase activity in jejunum and starch digestibility in anterior, median and posterior ileum for individual birds had a correlation of 0.56, 0.54 and 0.47, respectively. The cause for this increased secretory activity remains unclear, but may be associated with a stimulation of pancreatic secretion caused by an increase in gizzard activity. Hetland et al. (2003a) found a significant increase in amylase activity and bile acid secretion when gizzard activity was stimulated by oat hulls.

The major stimuli causing an increased enzyme secretion by the pancreas are the vagus nerve and cholecystokinin (CCK). Cholecystokinin is mainly produced in the pyloric region of birds (Denbow, 2000), and acts together with the vagus nerve to stimulate pancreatic enzyme secretion (Li and Owyang, 1993). Thus, a stimulating effect of an increased gizzard activity on enzyme secretions may be mediated through increased CCK release in the pyloric region of the gizzard.

Since an increased volume of the gizzard and thus an increased amount of feeds is found in birds fed structural components (Amerah et al., 2009; Svihus et al., 2004), the resulting increased retention time may also affect nutrient digestibility. It has been shown that pH of gizzard content decreases when the diet contains structural components (Gabriel et al., 2003). The cause for this could be the increased retention time as a consequence of increased gizzard volume, which would allow for more time for hydrochloric acid secretion. Although pepsin production has not been measured directly, a common secretory pathway for hydrochloric acid and pepsinogen make an assumption of increased pepsin secretion probable. Thus, another possible explanation for improvements in digestibility with structural components could be a more complete digestion in the stomach. Increased retention time in the gizzard may also potentially improve efficacy of exogenous enzymes added to the diet. Although this surprisingly enough appears to not have been studied, it is often postulated that an exogenous enzyme such as phytase exerts its main function in the gizzard due to the favourable pH there (Garrett et al., 2004). Thus, it is a reasonable hypothesis that a more developed gizzard as a consequence of structural components may improve efficacy of this particular enzyme. Since pH optimum for most phytases are in the area 4 to 5 however, the reduced pH with structural components may counteract the effect of increased retention time. These important questions call for being addressed in future experiments.

A coarser grinding will save considerable energy and time in the grinding process. Reece et al. (1986) stated that energy cost for hammer grinding of maize could be reduced with 27 % by increasing the sieve size from 4.76 to 6.35 mm. Thus, the reduced production costs and the improvements in performance are major motivations for a coarser structure of the diet.

III. OVERCONSUMPTION AND FEED INTAKE RESTRICTION

Birds eat and swallow food without prior chewing. Although secretion of saliva do occur, the saliva do not contain amylase in the chicken and turkey, and the time exposed to saliva in the mouth is short (Duke, 1986). Thus, intact food particles enter the crop, or, if the gizzard is empty, pass directly to the proventriculus and gizzard. The crop may store large amounts of food for a considerable amount of time. Svihus et al. (2002) found up to 30 g feed in the crop 30 minutes after feeding starved broiler chickens weighing between 1100 and 1400 g, with half this feed still remaining in the crop after 3 hours. In a recent experiment carried out in cooperation with the Poultry CRC at University of New England, it was shown that crop contents are rapidly moistened (Figure 1). Although no digestive enzymes are secreted and no grinding occurs, feed may be softened and microbial and exogenously added enzymes may be activated. Denstadli et al. (2006) showed that at conditions similar to those in the crop (45 % moisture, 45 °C and a pH of 4.7), added phytase was able to degrade 86 % of the inositol 6-phosphates in the feed within 45 minutes of incubation. The effect of crop retention on the efficacy of exogenous enzymes is currently under investigation at our lab.
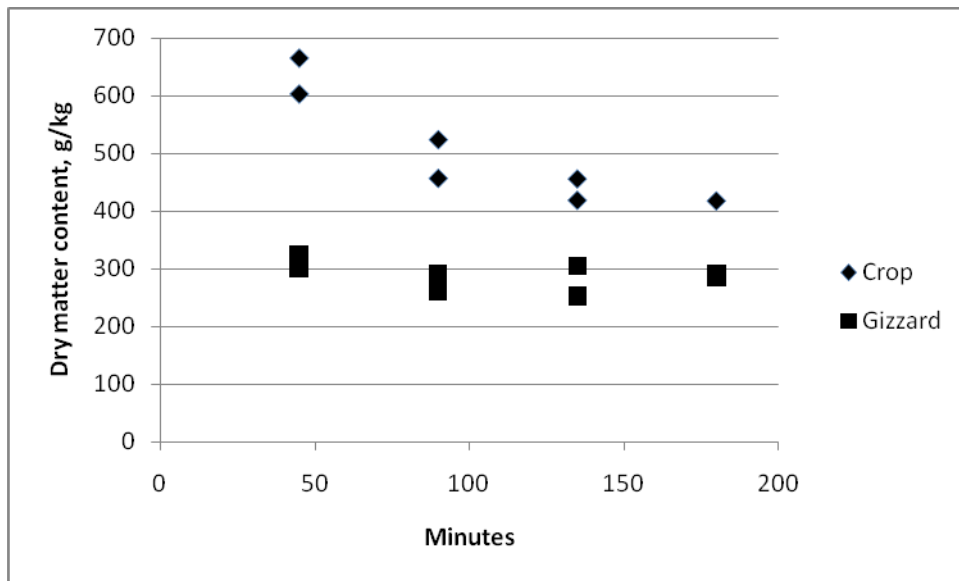
Figure 1. Dry matter concentration of contents of crop and gizzard at different times after feeding.

The main body of the gizzard comprises two thick, opposed lateral muscles and two thin anterior and posterior muscles. A thick layer of glyco-proteins that is hardened by the low pH covers the inside of the gizzard. The koilin layer is composed of a combination of rod-like and granular secretions from gizzard glands, and is continuously renewed by these glands as it is worn. Some birds, however, are known to shed the whole koilin layer at intervals (McLelland, 1979). None of the domesticated bird species have been reported to have such behaviour, but unpublished observations of gizzards lacking koilin layer at our lab indicate that this may happen in turkeys. The rod-like hardened glycoprotein will stand out from the remaining glyco-protein matrix and will give the surface the characteristic sand-paper like appearance (Hill, 1971). The thick muscles grind material by contractions that rub the material against the koilin layer on the inside of the gizzard. The small muscles move material between contractions of the large muscles.

Own research has led to the hypothesis that over-consumption of feeds may sometimes occur in broiler chickens, resulting in impaired nutrient utilization due to a too rapid feed passage through the small intestine. The first indication of this was in an experiment where it was observed that caged broiler chickens fed pelleted wheat-based diets showed improved nutrient utilization and smaller individual variation when feed intake was reduced by crushing the pellet (Svihus & Hetland, 2001). As reported in this forum earlier, plotting of feed intake for individual birds against AME frequently results in a slight inverse relationship; when feed intake increases, AME is reduced (Svihus, 2003). In the recent experiment mentioned above, this was very clearly observed (Figure 2). In this experiment, four out of ten ad libitum fed birds on the finely ground pelleted wheat diet showed signs of being feed overconsumers, characterised by a normal weight gain, a higher than average feed intake and an AME value below 10.3.
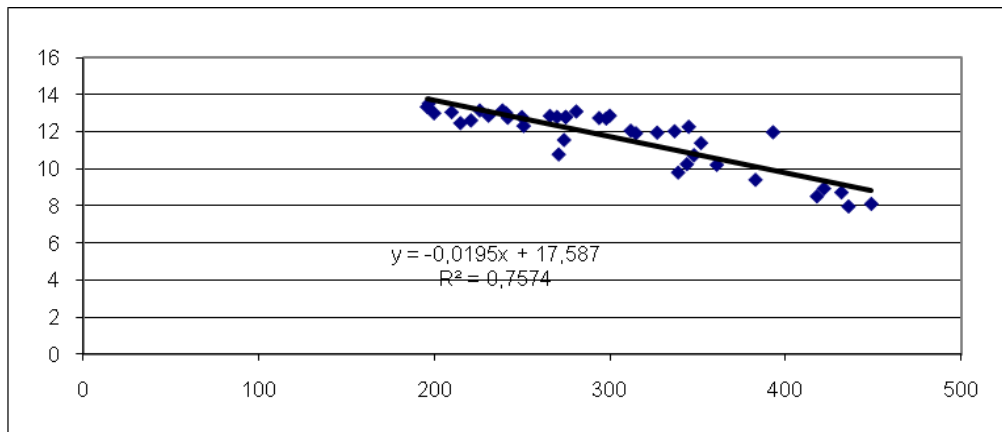
Figure 2.   Correlation between feed intake (horizontal axis, g) and AME (vertical axis, MJ/kg) for 40 individually caged broiler chickens receiving a wheat-based diet.

An equal number of intermittently fed birds with feed available only during 4 one hour feedings and one two hour feeding during the day exhibited the same signs of feed overconsumption. This indicates that intermittent feeding is not effective in prohibiting birds from feed overconsumption. Despite the very strong restrictions on feed availability through the five feeding times and only 6 hours with feed available compared to 18 for the ad libitum fed birds, the intermittently fed birds were able to adapt quite quickly to intermittent feeding, as indicated by the similar weight gain as ad libitum feeding after an adaptation period and no significant reduction in bird weight at the termination of the experiment. Obviously, this is due to an extensive use of the crop as an intermediary storage organ for feed. For example, on day 21 when the average daily feed consumption for all birds were 103 g, four out of 20 intermittently fed birds consumed between 28 and 30 g during one or several of the one-hour feeding bouts, and 12 out of 20 consumed more than 20 g. The fact that more than two-thirds of the feed were consumed during the first twenty minutes of the hour and that very little was consumed during the last twenty minutes of the hour also illustrates the ability of broiler chickens to consume large quantities of feed during a short time. This adaptability allows the intermittently fed birds to store large quantities of feed in the crop and gizzard, which can then be relied upon when feed is not available. Thus, it is possible that this mechanism hindered any prohibitory effect of the meal feeding on feed overconsumption.

The question may arise on the causes for over-consumption among individual birds. Peter Siegel was among the first to indicate that intensive breeding for weight gain had resulted in broilers that were capable of over-consuming feed due to disturbance of the appetite control centre in the brain (Siegel and Dunnington, 1987). Lacy et al. (1985) showed that broilers, as opposed to layers, did not respond to intra-hepatic glucose infusions with reduced feed intake. This indicates that even peripheral appetite control centres have been disturbed in broilers. It can thus be postulated that modern breeds of broilers may over-consume feeds, with a resulting impairment in nutrition utilization for some diets. Although Buyse et al. (1996) found improved feed utilization with intermittent feeding, it was hypothesized that a more concave growth curve for intermittently fed birds which would save maintenance cost of birds at an early age, was the main explanation, together with an altered growth composition. The lack of any effect of intermittent feeding in our experiment mentioned above indicates that compensation mechanisms through intermediate storage in the crop and gizzard allow even restrictedly fed birds to overconsume feeds. It is therefore possible that the main beneficial effects of restricted feed availability are an altered growth

curve, possibly together with beneficial effects associated with the altered management, such as less spillage and less accumulation of feed in the feeders. However, feed restriction through making the feed less easy to consume appears to be able to hinder feed overconsumption and improve nutrient availability. In the experiment of Svihus & Hetland (2001), grinding the pellet to mash resulted in a significant improvement in starch digestibility which was due to elimination of birds with extremely low ileal starch digestibility.

IV. DIET STRUCTURE AND FEED OVER CONSUMPTION

Results from our lab indicate that there may be an interaction between gizzard function and overconsumption of feeds. When feeds have more structure, either through the use of whole cereals or through addition of large fibre particles (oat hulls or wood shavings) into the diet, an improvement in starch digestibility and a reduced variation between birds is commonly observed (Svihus & Hetland, 2001; Hetland et al., 2002; Rogel et al., 1987). This was also observed in the previously mentioned experiment, where variation in feed/gain, AME and starch digestibility was strongly reduced for caged Cobb broilers when whole wheat was added to the diet (Table 1).

Table 1.   Broiler performance (16 to 25 days of age) and nutrient availability[1]

	Ad libitum		Restricted	
	Ground wheat	Whole wheat	Ground wheat	Whole wheat
Weight gain, g:				
Mean	543	527	505	523
Coefficient of variation	0.11	0.19	0.19	0.14
Range	443 – 627	369 – 667	344 – 629	407 – 644
Feed intake, g:				
Mean[2]	1022[a]	846[b]	890[ab]	795[b]
Coefficient of variation	0.17	0.19	0.27	0.15
Range	783 – 1347	636 – 1100	564 – 1202	622 – 1016
Gain/feed:				
Mean[3]	0.54[c]	0.62[ab]	0.58[bc]	0.66[a]
Coefficient of variation	0.16	0.05	0.10	0.07
Range	0.40 – 0.65	0.58 – 0.67	0.48 – 0.67	0.59 – 0.72
AMEn, MJ/kg:				
Mean[4]	10.8[b]	12.6[a]	10.9[b]	12.6[a]
Coefficient of variation	0.15	0.06	0.18	0.07
Range	8.1 – 12.3	10.7 – 13.5	8.0 – 13.1	10.2 – 13.2
Faecal starch digestibility:				
Mean[5]	0.81[b]	0.95[a]	0.82[b]	0.95[a]
Coefficient of variation	0.18	0.06	0.18	0.07
Range	0.59 – 0.94	0.81 – 0.99	0.56 – 0.97	0.75 – 0.98

[1]ANOVA main effects or interaction effects were non-significant unless mentioned. [2]P-value for wheat structure and feeding system were 0.0213 and 0.1111, respectively. [3]P-value for wheat structure and feeding system were <.0001 and 0.0379, respectively.[4]P-value for wheat structure was 0.0003. [5]P-value for wheat structure was 0.0004.[abc]Means within a row not sharing a common superscript differ at P<0.05.

Our hypothesis is that an active and well-developed gizzard will function as a food intake regulator that will assure that the bird does not over-consume feeds. This due to the active retention of large particles until they are broken down, which results in a larger filling of the gizzard and thus less room for more feed to be consumed. The hypothesis is that structural components in the diet will stimulate development of the gizzard, and that this will result in a better feed flow regulation such that feed overconsumption is avoided. Such a mechanism may also explain why the effect was so large in the current experiment, as birds were raised on wire floor with no access to litter material. Previous results indicate that birds eat litter to compensate for lack of structure in the diet (Hetland et al., 2004; Hetland et al., 2005).

V. CONCLUSION

The move towards a coarser structure of the feeds in Europe seems justified due to the reduced feed processing costs and the improvements in performance. Although restricted feeding has been documented to result in improved feed efficiency, the mechanisms for this are still unclear, and the value of this practice compared to other changes such as a coarser structure is thus uncertain. Investigations are currently underway at our lab to substantiate the feed overconsumption hypothesis further.

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CHALLENGES TO EUROPEAN POULTRY PRODUCTION

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Summary

European poultry production is regulated by many EU-Directives and national regulations that focus on food safety, animal welfare and environmental issues. In this paper animal welfare and control of environmental pollution are dealt with, as EU-27 average and differences between EU-27 countries. Implementation of the EU-Directives to improve animal welfare can have large consequences on the competitiveness of poultry production in Europe. Control of (early) mortality and wet litter of broilers are prerequisites for an economically feasible broiler production in countries where they are directly linked to stocking density in the near future. Moreover, direct costs for impaired intestinal health are substantial. Alternatives to antimicrobial growth promoters based on their potential non-antibiotic anti-inflammatory response have added value to broilers and floor-housed laying hens with impaired intestinal health. Dietary dilution of broiler breeder diets might help to reduce chronic hunger stress and improve broiler vitality especially in young broiler breeders.

I. INTRODUCTION

European poultry production is regulated by many EU-Directives and additional national regulations that focus on food safety, animal welfare and environmental issues. On the one hand legislation requires investments in e.g. best available techniques to reduce environmental pollution, or alternative housing systems, but on the other hand it limits farm size (e.g. via egg production rights in the Netherlands) and stocking density, specifying a minimum space allowance for layers and broilers. Especially the EU-Directives to improve animal welfare will have a large impact on the European poultry industry. Implementation of EU-Directive on the "Welfare of Laying Hens" will increase egg production costs in the EU by 12% in barn systems and even 20% in free range systems (GAIN Report, 2005), which was recently confirmed by Bagnara (2009). Bagnara (2009) estimates that abolition of the traditional battery cages by 1 January 2012 will reduce European productive potential by 40% as small-scale layer farmers will not be able to invest in aviary systems or enriched cage systems. If similar standards are not introduced in non-European countries as well by legislation or initiated by consumer demands, European legislations and regulations surely will reduce the competitiveness of the European poultry industry and impact world trade on processed poultry products. As welfare-based labelling might inform European consumers on the level of animal welfare for animal products, an EU funded research program "Welfare Quality" was started deliver the basics for such science-based welfare labelling.

II. EU-DIRECTIVES ON ANIMAL WELFARE

The EU-Directive on "Welfare of Laying Hens" (1999/74/EC) regulates the ban on traditional battery cages by 1 January 2012. This Directive specifies a minimum floor space per hen (being 750 cm^2 in enriched cages and 1100 cm^2 in aviary systems), availability of perches, laying nests and an environment in which hens are able to show their natural behaviour. In

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2007 68.6% of the eggs in the EU-27 were still produced by hens in traditional cages, however, showing a large variation among EU countries (IEC, 2007). Also regulations with respect to beak treatment of laying hens to prevent feather pecking and cannibalism is highly variable among EU-27 countries as indicated by Van Horne and Achterbosch (2008). Recent observations in the Netherlands indicated that floor-housed hens are more vulnerable to enteric disorders like chronic enteritis, which was observed in 10% of floor-housed layer flocks (De Bruijn et al, 2007). Chronic enteritis resembles focal duodenal necrosis, although involvement of *Clostridium colinum* was not (yet) confirmed in the Netherlands. It was estimated that such disorders might cost 2€/hen due to extremely high feed intakes and poor laying persistency.

The EU-Directive on "Welfare of Meat Chickens" (2007/43/EC) details farming conditions for broilers with respect to litter, feeders and drinkers, minimum length of a daily dark period and maximises stocking density in broiler barns at 33 kg/m^2. Higher stocking densities are allowed up to 39 kg/m^2 based on broiler management and housing and on climate control (ambient temperature and air humidity, CO_2 and NH_3 levels at bird level). The highest stocking density of 42 kg/m^2 can only be used when broiler mortality does not exceed a set maximum value for seven successive flocks being 1% plus 0,06% per day (which equals 3.4% during a 40 days growth period). Moreover, national guidelines have to be implemented on good management practise (e.g. limiting the occurrence of hock burns and foot pad dermatitis). This Directive will be effective by 1 July 2010. Although is introduced to improve broiler welfare, it but will have (initial) adverse effects profit at farm level and be a challenge for all parties of the broiler chain to improve broiler vitality and reduce mortality.

Apart from these EU-Directives, also additional national regulations are implemented e.g. to prevent welfare problems related to chronic hunger stress in broiler breeders farming.

III.   EU-DIRECTIVES ON POLLUTION PREVENTION

Several EU-Directives are implemented to control and reduce environmental pollution. EU-Directive 2008/1/EC on Integrated Pollution Prevention and Control (IPPC), requires industrial and agricultural activities to have a permit to produce, ensuring that environmental conditions are met and companies use Best Available Techniques (BAT) to reduce and prevent any pollution they may cause. IPPC applies to poultry farms with more than 40.000 bird places. Two other important environmental directives are the Nitrates Directive (91/676/EEC) that maximises the application of N from animal manure to max. 170 kg N/ha to control nitrate leakage from organic sources to ground and surface water and Directive 2001/81/EC on National Emission Ceilings (NEC) which sets upper limits for total emissions in 2010 on SO_2, NOx, volatile organic compounds and NH_3 for each EU member state. National regulations as part of the implementation of the NEC Directive limit e.g. the total production of ammonia per bird place per year for broilers and laying hens. Nutritional measures are part of the available techniques to reduce N and P excretions and ammonia emission.

In Table 1 the effects of different nutritional measures are quantified to reduce the excretion of N and P in animal manure.

Table 1. Reducing concentrations of N, P and DM in animal manure through feed supplements and feeding programs (adapted from Nahm, 2002, 2007 and Ferket, 2002).

Factor	% reduction in manure			Species
	N	P	DM	
Free amino acids and reduced protein	18-35			pullets and layers
intake	10-27			broilers
per % CP reduction	8.5			Broilers
Enzymes, general	5		12-15	Broilers
Phytases	5	25-35		Broilers
Growth promoters	5-30	5		Broilers
Phase feeding	10-33	10-25		Broilers
Choice of highly digestible feedstuffs	5	5		Broilers

IV. ALTERNATIVES FOR ANTI-MICROBIAL GROWTH PROMOTERS

Costs for intestinal disorders in broiler production can add up to 10€ per 100 broilers based on a reduced body weight gain by 5% and increased feed conversion ratio by 3% (Table 2), which estimates have been based on Schothorst Feed Research trials comparing production performances of broilers with and without feed antibiotics. Costs for housing, litter, manure and health care were obtained from statistics of the farmers' average in 2009.

Table 2. The costs of intestinal disorders in broilers (€ per 100 broilers), recalculated from Van der Klis and Veldkamp (2007).

	Healthy broilers			Intestinal disorders	
	Amount	Price/unit	Total	Amount	Total
Sales profit					
Body wt (kg)	220	0.83	182.60	209	173.47
Costs chicks and feed					
Chicks	100	0.28	-28.00	100	-28.00
Feed (kg)	352	0.32	-112.64	344	-110.08
Feed profit (€ 100 birds)			41.96		35.39
Variable costs					
Housing, litter, manure			-14.00		-15.65
Health care			-2.00		-4.00
Gross profit (€ 100 birds)			25.96		15.74

It has been demonstrated in Schothorst Feed Research experiments that inducing necrotic enteritis in broiler chickens via a successive *Eimeria maxima*/*Clostridium perfringens* challenge reduces daily feed intake due to an acute phase response (Figure 1). Comparing infected non-treated and non-infected non-treated indicated a decrease in albumin (10.5 vs 11.7 g/L, $P<0.05$) and numerically increase in AGP (382.6 vs 318.9 mg/L) and Ceruloplasmin (71.7 vs 54.4 mg/L) as shown by Ionescu et al. (2008). Niewold (2007) hypothesised that alternatives for feed antibiotics should be selected based on their non-antibiotic anti-inflammatory response, rather than on their antibiotic effects. In hens affected by chronic enteritis also clear positive effects were shown by anti inflammatory additives (data not shown).

Figure 1.   The effect of a successive *Eimeria maxima* and *Clostridium perfringens* (Cp) challenge on daily feed intake in broilers.
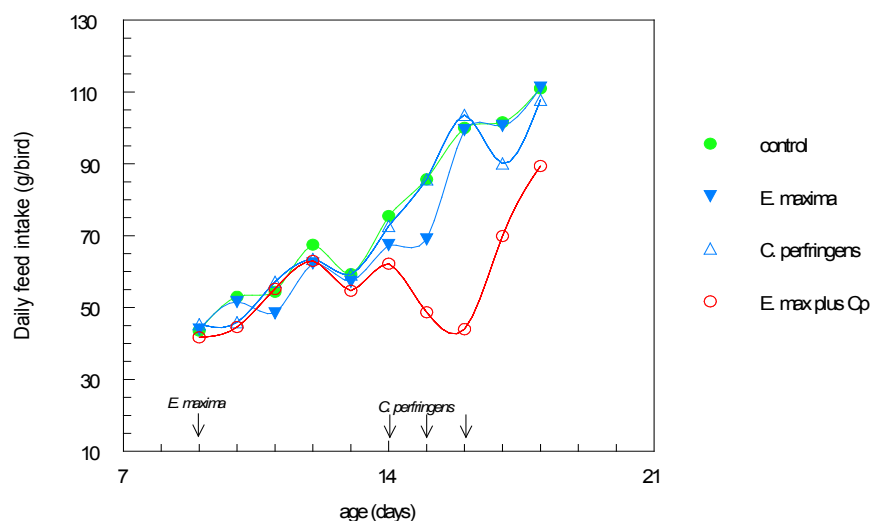
V.   NUTRITION OF BROILER BREEDERS AND BROILER VITALITY

National regulations that do not allow skip-a-day feeding programs in broiler breeder nutrition to prevent chronic hunger stress, being the case in the Netherlands, require different approaches to limit nutrient intake and body weight gain of broiler breeders. Dietary dilution of a normal density diets during rearing and laying (resp. 2600 and 2800 kcal/kg) by 10% and 20% increased eating time (De Jong et al., 2005), delayed maturation of the reproductive tract by two weeks (Enting et al., 2007a), improved white to yolk ratio in eggs and growth of the area vitellina externa and embryonic development, day-old chick weight of young breeder hens and offspring body weight gain, whereas mortality was low (3.1%) and not affected by dietary treatments (Enting et al. 2007c). Results are shown in Table 3.

Table 3.   Effect of dilution of broiler breeder diets on eating time, production performance, maturation of the reproductive tract and offspring performance.

	ND	ND-10%	ND-20%
Eating time of hens, min/day			
6 wk	45[c]	75[b]	115[a]
20 wk	28[c]	53[b]	68[a]
30 wk	306[c]	441[b]	585[a]
Weight oviduct,g			
24 wk	24,6	23,4	15,9
Δ wk 24-26	30,0[b]	37,6[ab]	53,3[a]
BW of 26-wk-old hen at, g	3106	3115	3106
Measurements on eggs of 29 wk old hens			
White: yolk ratio, 48 hoi	1,626[b]	-	1,801[a]
Area vitellina externa (mm^2), 48 hoi	700[b]	-	910[a]
Embryo wt (g), 264 hoi	1,67[b]	-	2,04[a]
Offspring performance from eggs of 29 wk old hens			
Wt one-day-old broiler, g	35,9	36,6	36,3
Wt 38-day-old broiler, g	2125[b]	2185[a]	2131[b]

ND: Normal Density; ND-10%: ND diluted by approx 10%; ND-20%: ND diluted by approx. 20%. Hoi: hours of incubation; [a,b,c] Mean values with different superscript letters within a row differ significantly.

Dietary dilution of breeder diets therefore seems not only to be an alternative for skip-a-day feeding programs, it also improves embryonic development and offspring performance of young broiler breeders.

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UPDATE ON CURRENT EUROPEAN BROILER BONE PROBLEMS

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Summary

Changes in genetic and management practices seem to be resulting in new patterns of bone problems in broilers on European farms over the last 2 years. Problems associated with endochondral bone growth include femoral head necrosis (FHN), rickets and tibial dyschondroplasia (TD). FHN seems to be the most prevalent and widespread problem involving bacterial infection that can be exacerbated by abnormalities of epiphyseal cartilage. Attention to hatchery hygiene seems to be important in trying to counter this problem. Rickets resulting from dietary errors with calcium, phosphorus or vitamin D or malabsorption of these nutrients is an occasional problem. TD still occurs, despite attempted selection against it. Dietary supplementation with 25-hydroxyvitamin D is the most effective dietary means of prevention. Black bone syndrome (BBS) is a recently identified problem that results from leakage of blood through porous areas of bone, particularly near the proximal tibia. The blood can darken during processing, particularly freezing of leg portions and can even spread into surrounding meat, causing consumer acceptance problems. Use of dietary 25-hydroxyvitamin D appears to be a promising approach to improving bone structure and minimising blood leakage in BBS.

I. INTRODUCTION

Modern intensively-reared broiler strains have been selected over many generations primarily for fast growth of muscle tissues combined with good feed efficiency. Concentration of selection effort on these two characteristics resulted in increasing incidences of defects in other physiological areas. Leg defects in particular became quite prevalent and led breeders to pay more attention to skeletal characteristics in their selection practices in recent years. The result has been, with the odd notable exception, a general improvement in skeletal characteristics of the more recent broiler strains. Changes in management practices, aimed at improving leg health, have also been proving effective. The net result of these improvements in genetic and management procedures has been a general enhancement in bone quality and leg health and welfare of broilers. Based on my recent observations in commercial broiler flocks around Europe, the greatest improvement seems to be in the non-specific rotational and angular deformities, of the valgus/varus type. However, bone problems have not yet been abolished. The increasingly greater growth rates and feed efficiencies of broilers put ever greater emphasis on optimising both nutritional and hygienic practices. Moreover, there have been genetic changes in bone structures, and these may need to be met by changes in nutrient specifications. There is also increasing evidence that some of these structural genetic changes may be resulting in new bone problems. The net result is that bone problems still occur, though their pattern is changing, and continued genetic, nutritional and management vigilance is needed to keep them under control.

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II. LEG PROBLEMS ASSOCIATED WITH ENDOCHONDRAL BONE GROWTH

a) Femoral head necrosis (FHN)

This seems to be currently one of the most widespread broiler leg problems. Strictly speaking, it is not a nutritional problem since it is a bacterial osteomyelitis associated mainly with *Stappylococcus aureus*, though sometimes with *Eschericia coli*. These bacteria can become trapped in the growth plate blood vessels, especially in the femoral head where blood flow may be weakest. But they may also become trapped in damaged or abnormal cartilage associated with nutritional problems such as rickets or tibial dyschondroplasia (TD). The infection spreads initially to the chondrocytes, causing necrosis of these cells, before extending into the bone itself. The condition is painful and affected birds can be identified by their extreme reluctance to stand or walk. FHN can be confirmed by attempted dislocation of the hip joint. In mild cases, epiphyseolyis takes place, in which the bone of the proximal femur separates from the cartilage cap, leaving the latter attached to the joint socket. In more severe cases, the bone of the proximal femur fractures.

An important solution to the problem seems to involve better hatchery hygiene, since a strong association has been shown between hatchery of origin and occurrence of FHN. The appropriate handling of dirty eggs is probably a key factor in minimising the likelihood of spreading infection among hatching chicks but avoidance of cartilage defects linked to nutrition in the growing broilers is also helpful. Antibiotic treatment is often tried, but seems to have only limited impact in preventing the condition.

A possible reason for the widespread occurrence of FHN could be a continuing reduction in the immune responsiveness of broilers as a consequence of further selection for faster growth. Perhaps the use of nutrients such as vitamins D and E to enhance immune status is an approach that could be considered to counter this tendency. Avoidance of immunosuppressive diseases such as infectious bursal disease would also be important.

b) Rickets

Deficiencies of calcium, phosphorus and/or vitamin D resulting in rickets in young birds still occur occasionally. The morphology of the growth plate can be used to distinguish between the rickets caused by calcium/vitamin D deficiency or phosphorus deficiency. In the former, there is a considerable increase in the thickness of the proliferative zone. In phosphorus deficiency rickets, the proliferative zone is relatively normal in size but there is a considerable increase in the size of the hypertrophic zone and a delay in mineralisation of this zone. Rickets of both types can be caused by dietary deficiency, an imbalance in the proportions of calcium and phosphorus or the use of feed additives that may inadvertently impair nutrient absorption. Rickets can also occur with diets that are not overtly inadequate in calcium/phosphorus/vitamin D and in these cases the problem is thought to be caused by infections that impair nutrient absorption (malabsorption).

c) Tibial dyschondroplasia (TD)

TD is primarily a genetic problem that can be influenced by nutrition. Breeders can select against TD, using a Lixiscope, an x-ray fluorescence device. This device can detect large TD lesions, though small lesions are less easy, and its use has resulted in a decrease in the general incidence of TD in some broiler strains. However, TD still occurs in commercial broiler production.

Several nutritional factors can influence the occurrence of TD. A low ratio of Ca:P can increase the incidence of TD, but there is no indication that a correct balance of these nutrients will necessarily prevent the condition (Edwards and Veltmann, 1983). Other nutrients, including monovalent cations and anions (Na^+, K^+, Cl^-) can also alter the

incidence, but the only nutrients that have so far been shown to completely prevent TD are vitamin D and its metabolites. 1,25-dihydroxyvitamin D (1,25-D) is particularly effective in the dose range 5 to 10 mg/kg but has potential practical hazards from toxicity (Rennie *et al*., 1995). 25-hydroxyvitamin D has also shown to be effective, though at higher dose rates (75-250 mg/kg) (Rennie and Whitehead, 1996). It has a much higher toxic threshold than 1,25-D and is available commercially as HyD.

More recent findings (Whitehead *et al*., 2004) indicate that vitamin D itself, when fed at high dose rates, can reduce or prevent the occurrence of TD at 14 d of age. Basal diets of different Ca and available P (avP) contents were supplemented with different concentrations of vitamin D, giving different incidences of TD. TD incidences responded to vitamin D supplementation, but concentrations above 5000 IU/kg were needed to minimise TD incidence. These results suggest that the vitamin D_3 requirements of young broilers can be quite high and that responses in performance and bone characteristics can occur to dietary concentrations above 5000 IU/kg at both conventional and abnormal dietary calcium and phosphorus concentrations or ratios up to 14 days. Practical vitamin D_3 supplements are normally in the range 3000-5000 IU/kg but, in view of the current findings, it would seem prudent to supplement at the higher end of this range or above if regulations permit, especially in broiler starter diets. Higher dietary vitamin D potencies can be obtained by replacement of a proportion of the feed vitamin D_3 supplement with 25-hydroxyvitamin D_3 (e.g. 3000 IU vitamin D_3 and 50 µg 25-hydroxyvitamin D_3/kg). Alternatively, additional vitamin D_3 or 25-hydroxyvitamin D_3 can be administered via the drinking water.

III. PROBLEMS OF INTRAMEMBRANOUS OSSIFICATION

<u>The black bone syndrome</u>

The blackening of broiler leg bones and the spreading of the discolouration into adjacent meat, especially after cooking, has been reported intermittently in recent years (e.g. Lyon and Lyon, 2002; Saunders-Blades and Korver, 2006). However, more recent observations on broiler processing lines and supermarket products are suggesting that the problem is much more widespread and fundamental than has been appreciated. It has implications for both broiler meat product quality and bird welfare and is now being described under the name black bone syndrome (BBS).

BBS seems to be associated with leaking of blood from the marrow through the bone of the proximal tibia and can be observed in freshly killed birds on processing lines, most frequently in the tibia. The problem of BBS seems to be made worse by freezing un-deboned leg portions. Presumably the freezing process forces more blood from the marrow through the bone and into adjoining meat. Cooking may contribute further to this process and also blackens the blood. The final result is a blackened and unappetising appearance of the meat surrounding the bone. This is already having market impacts because there are now reports that fast-food outlets are stopping using frozen broiler leg portions or using deboned meat instead.

BBS seems to be caused by a problem in intramembranous ossification. Cortical bone structure is becoming more porous in modern broilers (Williams et al., 2000). This structure in the midshaft is still strong and able to bear the weight of the bird. However, we have found a different situation in the shaft of the bone immediately adjacent to the proximal growth plate, the area through which the blood is seen to be leaking. Here, the bone is much thinner – it can often be depressed by moderate finger pressure - and we have confirmed this by histological examination. The overall mineralised area is very thin and comprised of poorly connected strands of bone, of more cancellous than cortical appearance. There are obvious routes in this structure through which blood could seep and collect under the periosteal layer.

The long-term solution to the problem would appear to require breeding companies to pay more attention to bone structure in their selection of future breeding stock. However, in the short-term it is possible that nutritionists might be able to help alleviate the problem by paying attention to factors that can maximise bone quality. We have followed this up by comparing various characteristics of bone from another study on the feeding of HyD carried out by IRTA in Spain. The treatments involved supplementation of the diets of broilers with either 0 or 69 μg 25-hydroxyvitamin D (as HyD), in addition to a normal dietary content of vitamin D_3. We measured the reflected optical density (as a measure of bone blackening) in the area of 10 to 15 mm from the proximal ends of frozen tibias. This measure of blackening was significantly less ($P < 0.05$) in birds given the HyD supplement. It is possible that further research may identify improved calcium and phosphorus nutritional practices which, when combined with the use of HyD, may further contribute to the alleviation of the BBS problem.

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UTILISATION OF METABOLISABLE ENERGY OF FEEDS IN PIGS AND POULTRY: INTEREST OF NET ENERGY SYSTEMS?

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Summary

The evaluation of the energy content of pig or poultry feeds has been most commonly based on their DE or ME contents. However, the closest estimate of the "true" energy value of a feed should be its NE content, which takes into account differences in metabolic utilization of ME of nutrients. This review first considers some methodological aspects of NE determination. Experimental data in pigs indicate that the NE/ME ratio varies greatly with the chemical composition of diets and nutrient, with ratios for fat (90%) and starch (80%) that are higher than for protein and dietary fibre (60%). This has marked consequences on the relative energy values of ingredients according to the energy system that is used. Consequently, the NE system is better in predicting the performance of pigs. With regard to poultry, the ranking between nutrients for NE/ME is similar to what is observed in pigs but with smaller differences between nutrients. This is consistent with results of energy balance trials that are unable to detect significant differences in NE/ME between diets that differ markedly for their chemical composition. In any case, the accuracy of the NE value is highly dependent on the accuracy of DE or ME values or digestible nutrient contents. Overall, there is an obvious advantage in using NE systems for pigs while further investigations are required for implementing a reliable NE system for poultry.

I. INTRODUCTION

The cost of feed is the most important cost of poultry or pig meat production (~60%) and the energy component represents the greatest proportion of this cost. Therefore, it is important to estimate precisely the energy value of feeds, either for least-cost formulation or for adapting feed supply to the energy requirements of animals. Evaluation of the energy content of pig or poultry feeds has been most commonly based on their DE or ME contents. However, the closest estimate of the "true" energy value of a feed should be its NE content, which takes into account differences in metabolic utilization of ME of nutrients for maintenance and production requirements. In addition, NE is the only system in which energy requirements and diet energy values are expressed on a same basis which should theoretically be independent of the feed characteristics. In many parts of the world, NE systems have been implemented in practical pig nutrition. On the other hand, NE systems are very little used for poultry. The objective of this review paper is to consider the recent contributions regarding the efficiency with which ME is used in both pigs and poultry and to suggest proposals for future evaluation of energy in poultry nutrition. More complete information can be obtained in recent reviews (Pirgozliev and Rose, 1999, Noblet and van Milgen, 2004; Noblet, 2006). The effects of feed characteristics (physico-chemical composition), animal factors such as body weight, physiological stage or species (in poultry productions) or technological factors such as particle size, pelleting, extrusion or the addition of enzymes, that affect primarily digestion will not be considered here (see Le Goff and Noblet, 2001; Noblet and Le Goff, 2001; Noblet and van Milgen, 2004; Noblet, 2006 for more information).

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II. METHODOLOGICAL ASPECTS

Not all gross energy (GE) of a feed is available for meeting the requirements of animals since variable proportions of GE are lost in faeces, in urine, as fermentation gases (i.e., methane, hydrogen) and as heat (or heat increment, HI). The DE content of a feed corresponds to its GE content minus faecal energy losses after digestion in the digestive tract. Even though they are related to digestion, energy of gas and heat originating from hindgut fermentation is not considered in the calculation of DE. The ME content of a feed corresponds to the difference between the DE content and energy losses in urine and gases. Most of the energy lost in gases is due to methane production, which typically is very small in growing pigs and poultry, but not so in adult sows (Le Goff et al., 2002). Consequently, most ME values in literature and tables for growing pigs and poultry ignore energy losses as methane.

Net energy is defined as the ME content minus HI associated with feed utilization (i.e., energy cost of ingestion and digestion and HI related to metabolic utilization of ME) and the energy cost corresponding to a "normal" level of physical activity (figure 1). However, the HI of a given feed (as % of ME) may not be constant over a large range of ME intakes for a given animal and depends on several physiological factors. For instance, the HI tends to be lower below than above maintenance energy supply (Noblet et al., 1993; 1994; 1994). The HI is also lower when ME is used for fat deposition compared with protein deposition (Noblet et al., 1999). Therefore, for comparing different feeds for their HI or the efficiencies of ME utilization, it is necessary to calculate these values under similar conditions (e.g., feeding levels), at protein and amino acid supplies meeting the requirement and/or constant composition of the gain and/or at a given physiological stage. This was implemented in the studies of Fraps (1946) in broilers or Noblet et al. (1994) in growing pigs.

Measurement of energy balance and heat production (HP) is rather complex and time-consuming so that animals are usually fed at only one "operational/practical" feeding level. For a growing animal, the NE intake is then calculated as the sum of retained energy (RE) at this feeding level and fasting heat production at zero activity (FHP) (Noblet et al., 1994). This NE value or the efficiency of ME for NE (k as NE/ME) then corresponds to a combined utilization of energy for meeting requirements for maintenance and for growth. The value of FHP is either measured directly in fasted animals or obtained from literature. It can also be calculated by extrapolating HP measured at different feeding levels to zero ME intake. However, this latter method, even though it has been widely used in the past, has important limitations, mainly because the growing animal adapts its FHP to its feeding/energy level prior to fasting (Koong et al., 1982; de Lange et al., 2006; Labussière et al., 2008) and questions the use of extrapolated HP at zero ME intake for getting an estimate of FHP. Consequently, direct measurements of FHP are preferable; the use of literature measurements of FHP is an alternative solution. In any case, the NE value is dependent on the FHP value.

From a practical point of view and to avoid bias in the calculation of NE for a series of feeds, it is necessary to carry out energy balance measurements in similar animals (i.e., same sex, same breed and the same body-weight range), to keep these animals in a temperature-controlled environment within their thermoneutral zone, to minimize variation in behaviour, and to feed the animals at the same energy level with balanced diets so that the animals can express their production/growth potential. Under these circumstances, an erroneous estimate of FHP will affect the absolute NE value but not the ranking between feeds. This also means that NE should not be measured in animals fed ingredients for which the chemical characteristics are very different from those of a complete balanced diet.

Heat production can be measured directly through direct calorimetry, estimated from gas exchanges through indirect calorimetry or calculated as the difference between ME intake and energy gain, this latter component being measured according to the comparative

slaughter technique (CST). The most commonly used method is indirect calorimetry, which consists in calculating HP mainly from oxygen consumption and carbon dioxide production, and to a lesser extent from metabolic nitrogen and gas energy losses (Brouwer, 1965). This method also allows measurements over a short period of time (i.e. a few days) with possibilities of combination of measurements at different energy levels (including fasting) on the same animal without adaptation. In addition, modelling methods can be implemented to partition the total HP between different components, which can be used in the further interpretation of energy balance data (van Milgen et al., 1997; figure 1).
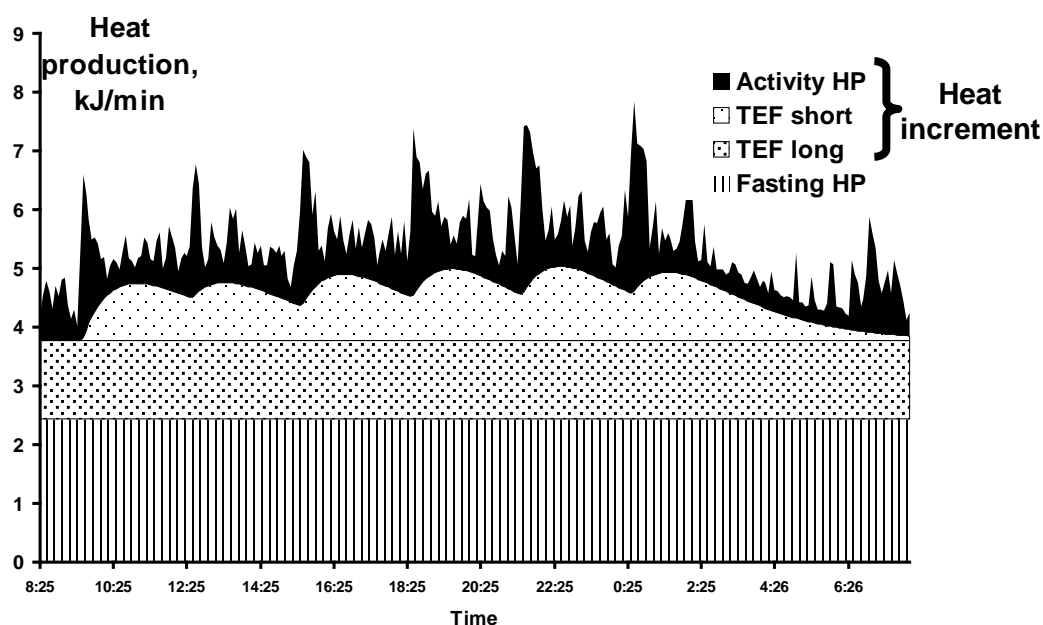
Figure 1.   Dynamics of components of heat production in a group of 9 broilers (1 kg BW) fed six meals per day (INRA data; HP: heat production; TEF: thermic effect of feed)

While measurements of DE and, to a smaller extent, of ME are easy and can be undertaken on a large number of feeds at a reasonable cost, the measurement of NE is far more complex and expensive. The best alternative is then to use information from prediction equations that were obtained from experiments carried out under similar and standardized conditions. In our laboratory, we measured NE on a wide range of different diets and proposed NE prediction equations, allowing to estimate the NE value of ingredients and complete diets based on available information of DE or ME content, combined with information of chemical characteristics (Noblet et al., 1994a). The latter information can be obtained from existing feeding tables or digestibility trials. The prediction equations obtained from these measurements can then be applied to any type of feed without further NE measurements (Noblet and van Milgen, 2004; Noblet et al., 2004).

III. UTILIZATION OF ME IN PIGS

Over the last 50 years, several experiments have been carried out by different laboratories to quantify the effect of diet and animal factors on HI or k in pigs (see the review of Noblet, 2006). The most recent and complete study was carried out by INRA with measurements on 61 diets (Noblet et al., 1994; Noblet, 2006). From their trials and other results, these authors

showed that the maintenance energy requirement (and FHP) in growing pigs is proportional to $BW^{0.60}$, and not to the commonly used metabolic BW ($BW^{0.75}$) (Noblet et al., 1999)). The FHP at thermoneutrality and zero activity averaged 750 kJ/kg $BW^{0.60}$/d. On this basis, the efficiency of ME for NE in growing pigs (k_g, %) averaged 74% but varied with chemical characteristics (g/kg DM) according to the following equation:

$k_g = 74.7 + 0.036$ x EE $+ 0.009$ x Starch $– 0.023$ x CP $– 0.026$ x ADF   (RSD = 1.2)[2].

A similar equation was proposed for adult sows fed at their maintenance energy level (Noblet et al., 1993). The variation in k_g is due to differences in efficiencies of ME utilization between nutrients with the highest values for fat (~90%) and starch (~82%) and the lowest (~60%) for dietary fibre (DF) and CP (Noblet et al., 1994). These values were confirmed experimentally in our laboratory (van Milgen et al., 2001). Measurements conducted in pigs having different BW and composition of BW gain, suggested that the efficiency of ME for NE was little affected by the composition of BW gain, at least under most practical conditions (Noblet et al., 1994). Similarly, the ranking between nutrients for their efficiencies was similar in adult sows fed at maintenance level and in growing pigs. Finally, the heat increment associated with protein utilization, either retained as protein or as lipid, appeared to be constant (van Milgen et al., 2001), which means that the NE value of dietary CP does not depend on its final utilization.

IV. UTILIZATION OF ME IN POULTRY

As for pigs, several trials or theoretical assumptions over the last 70 years have carried out to quantify the utilization of ME and its digestible nutrients for NE in poultry (see the review of Pirgozliev and Rose, 1999). Some studies were carried out on ingredients or unbalanced diets with some subsequent limitations in the interpretation of the results. The most comprehensive series of measurements were conducted in USA by Fraps (1946) and the Rostock group (Schiemann et al., 1972), mainly focussing on feed ingredients. Unfortunately, most attention in these studies was focused on starch, DF and CP with little variability in fat content of diets or feedstuffs. A recent study of Carré et al. (2002) was conducted on complete feeds (n=28) fed to 3 to 5 wk old broilers while varying the nutrient composition of the diets. The CST was used to quantify energy retention and NE was calculated according to a FHP value (500 kJ/kg $BW^{0.60}$/d) measured in respiration chambers (van Milgen et al., 2001). Later studies carried out at INRA suggested that FHP in 0.5 to 3.0 kg broilers is proportional to $BW^{0.70}$ with values ranging between 420 and 450 kJ/kg $BW^{0.70}$/d (Warpechowski et al., unpublished data).

In several literature studies, efficiencies of DE or ME for NE for maintenance + growth (or fattening) have been quantified, and some of these values are listed in table 1. The NE/ME ratio can also be calculated for a large series of feedstuffs in the studies of Fraps (1946). As for pigs, the lowest efficiency is observed for CP and the highest for fat. However, the difference between the most extreme values (i.e., CP and EE) appears to be somewhat lower in poultry (65 to 85%) than in pigs (60 to 90%). In addition, the values from Schiemann et al. (1972) were obtained in adult fattening birds, which are in a physiologically different stage while genotypes differed from modern birds. From that point of view, the study of Carré et al. (2002) is probably more representative of modern broiler production.

[2]EE: ether extract, CP: crude protein; ADF: Acid Detergent Fiber

Table 1.   Efficiencies of ME from digestible nutrients for NE in poultry (%)

Reference	Production[1]	CP	EE	Carbohydrates	Diet
Schiemann et al., 1972	M + fattening	61	84	75	73[2]
De Groote, 1974	M + growth	60	90	75	74[2]
Carré et al., 2002	M + growth	68	84	78	76

[1]M: maintenance [2]Assuming that 25, 20 and 55% of ME is provided as CP, EE and carbohydrates

An alternative approach to study the effect of diet on the efficiency of ME for NE was tested in recent trials conducted at INRA (Noblet et al., 2007; 2009). The approach consisted in preparing diets focussing each time on one specific nutrient (in exchange for starch). The effects of CP, EE and DF contents were evaluated and the trials were conducted in respiration chambers in group housed and ad libitum fed growing broilers over the 3rd to 7th week of age. The methods developed by INRA were implemented to partition total HP between its main components (figure 1). The most important results are presented in Table 2.

Table 2.   Effect of diet composition on heat production and efficiency of ME for NE in broilers: compilation of INRA data[1]

Trial	Diets	kJ/kg BW$^{0.70}$/d			NE/ME %
		ME	HP	AHP	
1	18% CP	1609	853	146	75.1
	22.7% CP	1609	846	153	74.8
2	22.5% CP	1457	892	173	67.7
	27.3% CP	1457	872	168	68.6
3	2.8% EE	1873	904	141	75.0
	9.7% EE	1877	901	152	75.7
4	9.5% NDF	1503	912	170	71.3
	17.7% NDF	1521	923	175	72.3

[1] Measurements carried out in groups of broilers weighing 1.3 to 1.5 kg BW on average for each trial; the indirect calorimetry method in respiration chambers was used; AHP: Activity heat production; complementary details by Noblet et al. (2007) for trials 1 and 2 and Noblet et al. (2009) for trial 3; trial 4: unpublished data. In trials 1, 2 and 3, the variation in CP or EE is associated with an inverse variation in starch content; in trial 4, the increased NDF level corresponds to a dilution by dietary fibre provided by wheat bran, maize bran and soybean hulls. In trials 1 and 2, data have been adjusted for a similar ME intake while observed values are given for trials 3 and 4. None of the differences between treatments within each trial were significant (P>0.05).

Table 3.   Comparative effect of dietary crude protein on energy utilisation in growing pigs and broilers (adapted from Noblet et al., 2003)[1.]

Species	Pigs		Broilers	
Dietary protein level	Normal	Low	Normal	Low
Body weight, kg	57.6	57.2	1.47	1.46
Energy balance, kJ/kg BW$^{0.60}$				
ME intake	2564	2566	1626	1642
Heat production	1402[a]	1346[b]	862	861
Fasting heat production	735	731	446	456
Heat increment[2]	667[a]	614[b]	417	404
NE/ME (x100)	73.9[a]	75.9[b]	74.8	75.0

[1]The reduction of dietary CP consists in a replacement of protein from soybean protein concentrate by maize starch with supplementation of amino acids in order to meet the requirements. [2]sum of TEF and AHP (see Figure 1)

The most surprising result of these trials is the absence of effect (within a trial) of dietary CP on HP, HI and NE/ME in broilers; a parallel study conducted in both pigs and broilers confirmed this major difference between pigs and poultry (Noblet et al., 2003; Table 3). Table 2 also indicates that the replacement of starch by fat was unable to increase

significantly the NE/ME ratio of the diet (Table 1). Either a higher difference in fat content between diets and/or a higher number of observations would be required for getting a significant difference. Finally, the presence of high levels of undigested DF in broilers diets did not significantly change the HP and the NE/ME ratio of the diet; this latter criterion was even numerically higher with the high DF diet. Anyway, this result does not confirm the hypotheses of Emmans (1994), who suggested attributing an energy cost to undigested organic matter for its excretion in order to calculate the so-called "effective" energy. Similarly, a recent study conducted in pigs did not indicate any additional energy cost of the presence of undigested DF provided by wheat straw (de Lange et al., 2006). Overall and unlike pigs, these studies indicate that major changes in diet composition do not affect the efficiency of ME for NE in broilers. The possible effects of dietary fat may deserve further studies, especially for an extrapolation of results to high fat single ingredients. This result also means that the hierarchy between diets for poultry would not be markedly affected by the energy system (ME vs. NE) and the design of trials such as those presented in table 2 did not indicate significant differences in the efficiency of ME utilization due to nutrients.

V. NET ENERGY SYSTEMS

An energy system corresponds to a method of prediction of the energy value of compound feeds and ingredients for a given type of animals. It then combines a step in energy utilization (DE vs. ME vs. NE) and a calculation method. With regard to NE systems, most of them combine the utilization of ME for maintenance and for growth or for fattening and they are based on prediction equations taking into account either digestible nutrients or DE (or ME) and some chemical characteristics (see reviews of Pirgozliev and Rose, 1999 for poultry and Noblet, 2006 for pigs). Some systems have been established from measurements on animals (Schiemann et al., 1972; Noblet et al., 1994; Carré et al., 2002) while others have been proposed from literature data and/or biochemical information and/or theoretical assumptions (Emmans, 1994). It should also be noticed that an adequate energy system is important to consider since most other nutritional criteria (protein, amino acids, minerals, etc.), either for feed value or for animal requirements are expressed relative to the energy content of the feed.

Table 4.   Relative DE, ME and NE values of ingredients for growing pigs[a]

	DE	ME	NE	NE/ME, %
Animal fat	243	252	300	90
Corn	103	105	112	80
Wheat	101	102	106	78
Reference diet	*100*	*100*	*100*	*75*
Pea	101	100	98	73
Soybean (full-fat)	116	113	108	72
Wheat bran	68	67	63	71
Soybean meal	107	102	82	60

[a]From Sauvant et al. (2004). Within each system, values are expressed as percentages of the energy value of a diet containing 68% wheat, 16% soybean meal, 2.5% fat, 5% wheat bran, 5% peas and 4% minerals and vitamins.

The system proposed by Noblet et al. (1994a) for pigs and applied in the INRA & AFZ feeding tables (Sauvant et al., 2004) is based on a large set of measurements (61 diets). The NE prediction equations that have been generated from these measurements include either DE values and chemical characteristics or digestible nutrients as predictors; they are applicable to ingredients and compound feeds and at any stage of pig production (Noblet, 2006). In this NE system and assuming that NE represents the best estimate of the "true" energy value of feeds, the energy value of protein-rich or fibrous feeds is overestimated when expressed on a DE (or ME) basis. On the other hand, fat or starch sources are underestimated in DE and ME systems (Table 4). Unfortunately, there is no comparable information for poultry, at least according to a general agreement on the prediction equations or systems to be implemented. According to the NE/ME ratios provided by Carré et al. (2002) for the main nutrients, the impact of moving from ME to NE for estimating the energy value of ingredients should be similar to what is observed in pigs (table 4) but to a smaller extent. However, our experimental results using indirect calorimetry failed to confirm this; additional measurements may be needed.

It is important to point out that specific and accurate DE (or ME) values or digestible nutrient contents should be used in the calculation of NE contents. For instance, energy digestibility differs between growing pigs and adult sows, with subsequent different NE values of feeds for both stages (Le Goff and Noblet, 2001; website: http://www.inapg.inra.fr/dsa/afz/tables/). In fact, reliable information on digestibility of energy or of nutrients is the most limiting factor for predicting energy values of feeds for pigs or poultry. The lack of comprehensive information on the effects of technology (e.g., pelleting, extrusion, enzymes addition) or about the differences in digestion between poultry species or physiological stages (growing vs. adult, etc.) is a major limiting factor for getting accurate estimates of energy values for pigs or poultry, irrespective of the energy system used.

VI. VALIDATION OF NET ENERGY SYSTEMS

An energy system, as any evaluation system for feeds, should be validated before being implemented. With regard to a NE system, a first level of validation is to measure the NE of feeds not included in the construction of the system and to compare these measured values to those calculated from the prediction equations proposed by the system. An illustration of this validation approach is given for the NE system proposed by Noblet et al. (1994a) for pig feeds (Figure 2). This figure indicates that this NE system appears quite robust. A satisfactory energy system should also be able to predict the energy value of any feed, especially ingredients for which the chemical composition is largely different from the composition of conventional ingredients or diets. The study by Noblet et al. (1993) on pigs confirmed that the NE system established on compound feeds is applicable to individual ingredients. In a review article concerning poultry, Pirgozliev and Rose (1999) compared the NE value of a series of feedstuffs as measured by Fraps (1946) to the values calculated according to different NE prediction equations. None of the literature NE systems for poultry was able to accurately predict the NE values measured on the feedstuffs; the biggest discrepancy was observed with the system proposed by Emmans (1994).

A last stage of validation of an energy system is its ability to predict the performance of animals. From that point of view, NE systems which take into account the final stage of energy utilization should be better able to predict animal performance than DE or ME systems. In addition, it is important to use the same energy system for expressing the diet energy values and the animal energy requirements; the energy system in which requirements

are the least dependent on diet characteristics is the NE system. In the case of pigs, the superiority of the NE system as proposed by Noblet et al. (1994) has been clearly demonstrated from results of growth trials. They indicate that the energy cost of growth or the daily energy requirement are independent of diet composition when expressed on a NE basis. On the other hand, on DE or ME bases, the energy cost is decreased when CP content is decreased or fat content is increased (Table 5). In other terms, unlike the NE system, the ability of DE and ME systems to predict the performance of pigs is lower than that of a NE system. For poultry, this exercise is difficult since no NE system is widely accepted and applied, so that there is no response trial in which BW or energy gain are related to either ME or NE intakes. Recent data by van der Klis (personal communication) did not show any superiority of a NE system partly based on the studies of Schiemann et al. (1972) on the ME system. This conclusion would be in agreement with our balance studies indicating that the NE/ME ratio was not significantly affected by diet composition (Table 2).
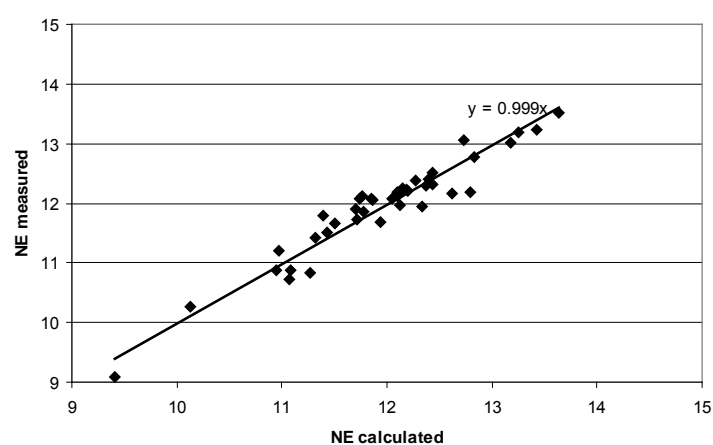
Figure 2. Relationship between measured NE values of compound feeds (n=41; indirect calorimetry method) and NE calculated according to NE prediction equations of Noblet et al., (1994) (adapted from INRA data)

Table 5. Performance of growing-finishing pigs according to energy system and diet characteristics[1]

Energy system	DE	ME	NE
Trial 1: Added fat (%)			
0	*100*	*100*	*100*
2	100	100	100
4	99	99	100
6	98	98	100
Trial 2: crude protein content (30-100 kg)			
Normal	*100*	*100*	*100*
Low	96	97	100
Trial 3: crude protein content (90-120 kg)			
Normal	*100*	*100*	*100*
Low	97	98	100

[1]Energy requirements (or energy cost of BW gain) for similar daily BW gain and composition of BW gain; values are expressed relative to the energy requirement (or energy cost of BW gain) in the control treatment (considered as 100; values in bold characters); from Noblet (2006) and unpublished data.

VII. CONCLUSION

This review indicates that NE is a better predictor than DE or ME of the "true" energy value of poultry or pig feeds. Available information for pigs indicates an obvious interest of formulating on a NE basis and NE systems should be implemented for getting a reliable prediction of performance of animals. In the case of poultry, the conclusions are less clear and convincing with no marked advantage of a NE system over a ME system for predicting the performance of broilers; further investigations are necessary to evaluate the potential interest of a NE system for poultry. Probably most attention should be focused on the impact of fat level with possible consequences on the relative energy value of fat-rich ingredients and subsequent results in least-cost formulation. Finally, even though NE is the final objective in energy evaluation of feeds, most attention should be paid to the accurate estimation of DE or ME values, which are the most important factors of variation of the energy value of poultry or pig feeds.

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AN EFFECTIVE ALTERNATIVE TO THE METABOLISABLE ENERGY SYSTEM

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Summary

There are several possible approaches to the development of a net energy system. All seek to provide a way of stating both how much energy is yielded by a given feed and how much of this energy a given animal needs to sustain some stated level of performance. Although the details of the systems which emerge from these approaches differ in detail they are all essentially ways of predicting the heat increment of feeding. In this paper the effective energy (EE) system of Emmans (1994), which is equivalent to a net energy system, is presented. The theory on which the system is based is outlined, the requirements for implementing the system are described, and the value of the system in terms of simulation modelling is discussed.

Because the EE contents of feed ingredients can be calculated within a feed formulation program, the system is relatively simple to implement and feeds can be formulated on an EE basis. Heat production by the bird as a result of consuming a given amount of a given feed is calculated as the difference between the ME and EE intakes. Many advantages result from being able to calculate this heat output, and some of these are given in the paper.

If a change to a net energy system is justified in poultry nutrition then adoption of the EE scale seems to provide the most readily available alternative.

I. INTRODUCTION

The purpose of an energy system is to describe the energy available in the feed for maintenance and growth, and for a given level of performance. The metabolisable energy (ME) system provides information on the amount of energy yielded to a given bird or animal by a given feed but does not account for the heat increment (HI) of feeding, i.e. it cannot distinguish between two feeds with the same ME content but differing in their chemical composition such that the increase in heat production from feeding differs between the two.

The original definition of net energy (NE = ME – HI) proposed by Armsby and Fries (1915) saw HI as a characteristic that was independent of the level of feeding, and thus as a characteristic of an ingredient or a feed that could be tabulated, in the same way as is ME. Similarly, NE could be tabulated also, if this view were accepted. However, this is a false assumption, as convincing evidence has accumulated demonstrating that HI differs with plane of nutrition (e.g. Forbes et al., 1928; 1930). Emmans (1984) addressed the problem of predicting the HI of feeding by first accounting for the fasting heat production, where no positive retentions of protein or lipid result from the feeding of a given feed, and then accounting for the extra heat that is produced when these retentions are positive. In his effective energy (EE) system, which applies to both single-stomached and ruminant animals, the heat increment of feeding is considered to be linearly related to five measurable quantities associated with the animal. The EE yielded by a feed or feed ingredient can be estimated from the ME, the faecal organic matter content and the amounts of digestible crude protein and lipid. According to Emmans (1994), as the EE values 'can be tabulated for ingredients, and are additive to the extent that ME values are additive, they can be used to formulate diets

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using linear programming'. For these purposes, and for describing animal performance, the effective energy scale is equivalent to a net energy scale.

In this paper the theory on which the EE system is based is briefly described, the requirements for implementing the system are outlined, and the value of the system in terms of simulation modelling are discussed. Some examples are given of the use of the EE system in describing the HI of feeds for broiler chickens.

II. DESCRIBING THE ENERGY CONTENT OF FEED INGREDIENTS

There are several possible approaches to the development of a net energy system. All seek to provide a way of stating both how much energy is yielded by a given feed and how much of this energy a given animal needs to sustain some stated level of performance. Although the details of the systems which emerge from these approaches differ in detail they are all essentially ways of predicting the heat increment of feeding.

A general equation for predicting heat increment has been proposed by Emmans (1994) in which he makes use of the following facts:

(i) Work is done, and hence heat produced, in the catabolism of protein.

(ii) Work is done, and hence heat produced, when protein and lipid are stored in the body. However, for fat deposition the work done is not directly proportional to the heat of combustion of the products stored. Less heat is produced when lipid energy in the body is derived from dietary fat as opposed to energy from the conversion of excess dietary protein (see Table 1).

(iii) More energy is needed to handle bulky feeds than highly digestible feeds due to the higher faecal organic matter content in bulky feeds.

(iv) Heat is produced when feed is fermented by ruminant animals to produce methane. This does not apply to simple stomached animals.

These work functions are summarised in Table 1, in which the units of each function are defined as is the work done per unit.

Table 1. Definitions of units of work functions (after Emmans, 1994)

Function	Unit of function	Symbol	Work, MJ/Unit
Excretion	1 kg urinary nitrogen (UN)	w_u	30.6 MJ/kg UN
Fermentation	1 MJ Methane (MTHE)	w_m	0.675 MJ/MJ methane
Defecation	1 kg faecal organic matter (FOM)	w_d	3.86 MJ/kg FOM
Growth	1 kg protein retention (PR)	w_p	34.2 MJ/kg positive PR
Fattening	1 kg lipid retention (LR)	w_l	16.3 MJ/kg LR (or)
		w_{ll}	4.3 MJ/kg LR[1]

[1]Where feed lipid is used directly for lipid retention by monogastrics

The equation to predict heat increment has five parameters, and these are based on two propositions:

(i) Apart from maintenance, which includes some level of activity (given), work is done in the immature animal for only five functions, namely, excretion, fermentation, defecation, growth and fattening.

(ii) The amount of work, measured as ME, needed for a unit of each function is constant across animals and diets.

$$HI = w_u (UN - FUN) + w_p.PR + w_l.LR + w_d.FOM + w_m.MTHE \text{ MJ/d}$$

where FUN is fasting urinary nitrogen, kg/d. This is the urinary nitrogen the animal would have produced had it not been fed. In monogastric animals MTHE = 0.

Allowance has to be made, in monogastrics, for feed lipid that is used directly for lipid retention. Of the digested lipid from the feed, DL kg/d, a proportion z is retained with the production of w_{ll} MJ/kg of heat (see Table 1). The lipid retained, other than from dietary lipid is (LR – z DL) kg/d and the heat produced is w_l MJ/kg. On the basis of pig and poultry experiments analysed by Emmans (1994) he suggests that 'suitable average values may be z = 0.3 for poultry and z = 1 for pigs'.

The total heat production of lipid retention H (LR) is thus:

$$H (LR) = w_{ll}. z \, DL + w_l (LR - z \, DL) \text{ MJ/d} = w_l. LR - z \, DL (w_l - w_{ll}) \text{ MJ/d}.$$

The EE content of a feed or feed ingredient for monogastric animals can thus be defined as

$$EE = ME - w_d. FOM - 0.16 w_u. DCP + z \, DL (w_l - w_{ll}) \text{ MJ/kg}$$
$$\text{or}$$
$$EE = ME_n - 3.8 \, FOM - 4.67 \, DCP + 12 \, z \, DL \text{ MJ/kg}$$

where ME is corrected to PR = 0 and z = 0.3.

The variable z, which may vary from zero (when LR = 0) to unity, introduces an 'animal' factor into the equation to calculate the EE content of a feed, with all other coefficients in the equation remaining constant across ages, physiological states and even species (Emmans, 1994). Noblet et al. (1994) made the point that 'One diet can theoretically be given several NE values according to the type of production for which it is used' and suggested that the digestibility coefficients of nutrients are influenced by such factors as body weight, physiological state, feeding level and 'interaction phenomena'. This leads to the unwieldy use of different NE values for the same ingredient depending on whether the feed is to be used for maintenance only, or for growth, fattening or reproduction. The value, z, in the EE system would appear to have the potential to achieve the same goal.

The effect on EE of a change in the value of z is seen in Table 2 for a range of feed ingredients. For those containing high levels of digestible crude lipid (DCL) the increase in EE content is predictably higher than where the ingredient is low in DCL. The resultant effect of an increase in z would in all cases be to reduce the heat increment of feeding, the effect being greater with some ingredients than with others.

Table 2. EE contents of some feed ingredients for three values of z, and the rate of change in z

	z = 0.3	z = 0.6	z = 1	Regression coefficient
Wheat bran	3.74	3.84	3.98	0.343
Soybean oilcake	5.52	5.54	5.58	0.086
Sunflower oilcake	5.24	5.27	5.32	0.115
Fishmeal	10.19	10.5	10.92	1.043
Gluten 60	11.63	11.72	11.84	0.300
Poultry byprod. meal	10.01	10.44	11.01	1.428
Soya full fat	12.14	12.7	13.44	1.857
Sorghum	12.92	13.01	13.13	0.300
Maize	13.06	13.18	13.35	0.415
Sunflower oil	40.75	43.99	48.27	10.740

III. COMPARING THE EE AND ME CONTENTS OF FEED INGREDIENTS

De Groote (1974) used a simplified NE system to differentiate feed ingredients into categories depending on their contents of crude protein, crude fat and starch + sugar, applying coefficients of 0.60, 0.90 and 0.75 to these components, which reflected differences in the efficiency with which the ME of these components is utilised for growth. Protein-containing ingredients had an NE:ME ratio averaging 0.63, cereals had values around 0.73 and for fats and oils the ratio was 0.90. Using a similar comparative approach, ten commonly used feed ingredients have been classified, in Table 3, according to their EE:ME ratio. Because the EE yielded to a monogastric animal by a feed ingredient will depend on the ME and the relative proportions of faecal organic matter and digestible crude protein and lipid, the EE:ME ratios differ to a greater extent between similar ingredients than did De Groote's NE:ME values. For example, fishmeal would be classified as a protein-containing ingredient and be given an NE:ME value around 0.63, but because of the high oil content the EE:ME ratio is considerably higher than this.

Table 3.   Comparison of EE and ME contents of some feed ingredients (using a value of z = 0.3)

Ingredient	Protein g/kg	Fat g/kg	Fibre g/kg	FOM g/kg	ME MJ/kg	EE MJ/kg	EE:ME
Wheat bran	155	40	100	514	6.11	3.74	0.61
Soybean oilcake	460	15	50	364	8.74	5.52	0.63
Sunflower oilcake	370	15	180	384	8.20	5.24	0.64
Fishmeal	655	100	0	149	13.08	10.19	0.78
Gluten 60	620	30	15	98	14.65	11.63	0.79
Poultry byprod. meal	580	140	25	202	12.56	10.01	0.80
Soya full fat	370	185	55	251	14.09	12.14	0.86
Sorghum	95	30	25	97	13.61	12.92	0.95
Maize	85	38	22	104	13.66	13.06	0.96
Sunflower oil	0	990	0	0	37.50	40.75	1.09

Because the difference between the ME and EE contents of a feed ingredient is the amount of heat produced (above maintenance) in the animal when the ingredient is consumed, those ingredients at the top of Table 3 will have a higher heat increment than the ingredients near the bottom of the table. This is a useful way of identifying ingredients that could be used to reduce the heat load on a broiler during hot weather, for example.

IV. CALCULATING THE EE REQUIREMENT OF GROWING BROILERS

One of the main purposes of an energy system is to be able to predict how much of a given food will be needed to meet the requirements of a bird for maintenance and growth. Having described how the energy available in the feed for these purposes can be calculated it now remains to describe how the requirements for maintenance and growth can be calculated.

a) EE for maintenance
The animal fed at maintenance will be producing heat due to the excretion of UN as, by definition, no protein is retained. This amount of heat will vary depending on the digestibility of the protein. Maintenance heat (MH) can be estimated from the current and mature protein

weights of the animal, as described by Emmans and Fisher (1986). They proposed the concept of a maintenance unit, based on the general scaling rule of Taylor (1970), which is calculated as $P_m^{-0.27}$. P, where P is the body protein weight (excluding feathers), and P_m the mature protein weight of the animal. The energy needed for maintenance (M_E) was predicted by Emmans and Fisher (1986) to be 1.63 MJ/unit day, which is used in the following equation to calculate MH.

$$MH = M_E. P_m^{-0.27}. P \ \ MJ/d$$

On reasonable estimates of P_m and body protein content the value is consistent with calculated values of MH for cattle, pigs, sheep and poultry at different values of P/P_m (Emmans, 1994).

The MH as calculated here would include some level of physical activity (Emmans, 1994), and could be modified to account, for example, for a cold environment and the additional activity associated with the consumption of mash as opposed to pelleted feed (Jensen et al., 1962).

b) EE for growth and fattening

A feed that leads to positive retentions of protein and lipid will be associated with the production of FOM and UN, and a portion of HI, relative to MH, caused by the feed will be associated with the rates at which these latter products are produced (Emmans, 1994). It can be assumed that all energy retention (ER) is accounted for as protein or lipid, so ER = h_p.PR + h_l.LR MJ/d, where PR and LR are the rates of retention of protein and lipid, kg/d, and h_p and h_l are their respective heats of combustion, MJ/kg. The values of h_p and h_l of 23.8 and 39.6 MJ/kg respectively are assumed to be chemical constants.

The quantity of effective energy required (EERQ) by an animal to support given values of MH, PR and LR can therefore be defined as:

$$EERQ = MH + PR ((h_p - a) + (w_p - 0.16 \ w_u)) + LR (h_l + w_l) \ \ MJ/d$$

where a = 5.63 kJ/g, which is applied to correct the classical ME (ME_c) to N-corrected ME (ME_n) using the equation ME_n (kJ/d) = ME_c – a (6.25 NR), where NR is N retention (g/d). After substituting the values of the constants into the above equation this reduces to:

$$EERQ = MH + 50 \ PR + 56 \ LR$$

In the case of poultry, protein retention is in body and feathers, so PR must include both of these components. The relevant coefficients apply equally to body and feather protein, making the calculation of EERQ less complicated than when determining the amino acid requirements for the growth of body and feather protein.

V. IMPLEMENTING THE EE SYSTEM

a) Calculating the EE of feed ingredients

The implementation of the EE scale in feed formulation is quite straightforward. The information required to calculate an EE value for each ingredient is AMEn, FOM, DCP and DCL. Starting with the familiar AMEn scale provides a natural evolution of present practice and also provides a practical basis for quality control. The quantities DCP and DCL (apparent faecal digestibilities) are not widely used but reasonable table values are available (e.g. WPSA, 1989) to provide a starting point. New experimental data can be accumulated at reasonable cost and it is to be expected that most variation in digestibility will be correlated

with variations in ME. Thus quality control of DCP and DCL may be achieved at the same time as control of ME data.

FOM may be calculated as OM – (DCP + DCHO + DCL), where OM is organic matter and DCHO is digestible carbohydrate. If we assume a fixed relationship between AMEn and digestible CP, CL and CHO (see WPSA, 1989 for an example) then DCHO can be calculated from the data required for the calculation of EE, and thus the digestibility of the total OM. Care must be taken to ensure that all unaccounted material in the calculation is CHO (for example anions in mineral sources will need to be called 'ash') but with this proviso the calculations are accurate.

b) Determining MH, PR and LR

The potential protein growth of an animal can be determined using a growth equation such as the Gompertz growth curve, and if this is done using the approach suggested by Emmans and Fisher (1986), it is a simple matter to determine the potential EE requirement for a growing bird on each day of the growing period. It is more difficult to determine the actual growth of protein and lipid, as this depends on the interaction between the genotype, the food being offered and the environment in which the bird is kept. A modelling approach is needed to take account of all these factors, and such a model is available (EFG Software, 2009). In this model the EE system is used to determine the heat increment of feeding and the heat associated with maintenance and growth, which enables the prediction of the actual intake of a given food by a given broiler in a given state under the given environmental conditions, and this then leads on to the prediction of the actual growth rates of body and feather protein and lipid.

As a result of using the EE system the model accurately predicts food intake and hence MH, PR and LR, as opposed to most simulation models in which food intake is an input to the model and not an output. It is not possible to optimise the way in which broilers are fed unless food intake is predicted, i.e. unless it is an output from a simulation model.

c) Using EE in place of ME in feed formulation

Within a given practical scenario the effect of implementing a change from ME to EE will not, in the short term, have a large effect since in a small sample of feed ingredients EE and ME will be closely correlated. Over time however small changes in ingredient economic values will lead to changes in ingredient evaluation and use. If a change to a net energy system is justified in poultry nutrition then adoption of the EE scale seems to provide the most readily available alternative.

VI. ADVANTAGES OF USING EE *vs*. ME IN SIMULATION MODELLING

Because the EE system provides a more accurate estimate of the energy available to the bird for maintenance and growth than does the ME system, there are many advantages to its use. Some of these are listed below.

a) Calculating the desired food intake (DFI)

To predict food intake the desired amount of food that an animal would need to consume to meet its potential requirement for each of the essential nutrients needs to be calculated. The DFI for lysine (DFI_{lys}) would thus be the daily requirement for lysine for maintenance and potential growth divided by the amount of digestible lysine in the feed. Similarly, the DFI for each essential nutrient is calculated, and that resulting in the highest DFI defines the first limiting nutrient in the feed, and would be the amount of food the animal would need to eat in order to grow or reproduce at its potential. Basing the energy requirement on ME, and

describing the feed energy content in terms of ME in order to calculate the DFI_{ME}, would introduce many inaccuracies and would thus not produce a valid estimate of requirement. Calculating the DFI_{EE} on the other hand is a more accurate means of predicting food intake for the reasons described above.

b) Estimating heat production and loss

The heat produced by the bird in consuming a given feed can be calculated as the difference between the ME and EE intakes. The larger the difference, the greater will be the heat output per g of feed eaten. One of the constraints that prevent broilers from consuming the amount of food necessary to achieve their potential output is the environmental heat demand: being able to maintain a constant body temperature through losing the heat generated to the environment. Under conditions in which the bird cannot lose all the heat that would be generated if the DFI were achieved, the only option open to the bird is to reduce food intake and hence heat production. Because it is possible, with the EE system, to predict the heat output of a bird in a given state being fed a given food it is also possible to determine whether the bird would be able to lose that amount of heat to the environment, and to determine the extent to which food intake would need to be reduced for thermal balance to be maintained. In this way, one of the most important constraints to the desired food intake can be addressed.

Similarly, where the environmental temperature is below the bird's lower critical temperature the additional amount of energy required by the bird to remain in thermal equilibrium can be determined, leading to a more accurate estimate of the energy required by the bird and hence of actual food intake.

c) Nutritional interventions in alleviating heat stress in broilers

It has been proposed that the adverse effects of high temperature on performance might be alleviated by dietary manipulation. Feeds containing highly digestible nutrients, minimal excesses of protein (amino acids), and a high proportion of the carbohydrate energy replaced with digestible fat energy would have the advantage at high temperatures. By increasing the EE:ME ratio of feeds, as described above, the heat increment of the feed is minimised, and, theoretically, a greater amount of this feed could be consumed by the bird at high temperatures, thereby improving performance.

In principle, to minimise heat increment, the EE content of the feed should be as close to the ME content as possible. With practical ingredients, the extent to which the heat increment of the feed may be reduced is quite constrained, and therefore, these strategies afford only marginal improvements in performance (Gous and Morris, 2005). When formulating feeds containing ingredients commonly used in the broiler industry (e.g. maize, wheat, soybean meal) the EE:ME ratios that are feasible range from about 0.86 to 0.91, with the higher ratio resulting when least-cost formulations are made. When the proportion of ME as lipid in a feed is increased, using commercially available feed ingredients, or when reducing the dietary amino acid excess as a means of reducing heat stress, the FOM is invariably increased unless an inert filler such as sand is offered in the formulation. By monitoring the effect of formulation changes on the EE:ME ratio it is possible both to explain the lack of response to such interventions when broilers are kept at high temperatures, and to predict whether any improvement would be possible, if at all.

Most nutritional strategies that have been proposed as a means of reducing the heat of digestion in the broiler result in a maximum theoretical saving in metabolic heat production equal to the effect of lowering the dry bulb temperature in the broiler house by about 1ºC (Gous and Morris, 2005). None of these strategies is as effective in terms of growth rate, feed conversion, liveability or carcass quality as reducing the radiant heat load on the birds

by making appropriate modifications to the structure of the broiler house and to the husbandry practices employed.

The ME system is clearly inferior to a NE system, which allows the heat increment of feeding to be calculated. This measurement is useful in poultry nutrition, especially in simulation modelling, as it leads to accurate predictions of food intake in different classes of poultry. For this reason it is almost obligatory to use a NE system when predicting food intake. If a change to a NE system is justified when formulating feeds for poultry then adoption of the EE scale seems to provide the most readily available alternative to ME.

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ENERGY IN POULTRY DIETS: ADJUSTED AME OR NET ENERGY

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Summary

Energy evaluation in poultry is generally based on metabolisable energy systems. For many decades it has been debated whether or not the development of a net energy system would enhance the prediction of energy partitioning in meat- and egg-producing poultry. Based on the extracaloric value of fat in laying hens and a potential lower energetic efficiency of protein utilisation, several modified ME systems were developed and used in commercial poultry nutrition. Recently, CVB initiated the development of an ATP-based NE system for broilers. Validation of this energy system did not show added value over the current modified AME system for broilers. Therefore, most probably frequent updates in nutrient digestibilities in feedstuffs would be of higher commercial relevance, as well as target animal dedicated digestibility data.

I. INTRODUCTION

Feed costs are the main costs for poultry production, of which 70 to 75% are related to dietary energy. To be able to reduce feed costs while maintaining bird performance, dietary energy values should be an accurate representation of the utilisable energy for both meat- and egg-producing birds in their successive production phases. This not only refers to the energy evaluation system used, but it should be realised that digestibilities of the energy delivering nutrients varies among bird species and age. Generally, energy evaluation systems in poultry are based on metabolisable energy (apparent metabolisable energy: AME, or true matabolisable energy: TME), being calculated from digestible protein, digestible fat and digestible carbohydrates. This carbohydrate fraction might be split into starch, sugars and non starch polysaccharides.

Discussions are ongoing whether adjustment of AME or TME systems would have added value for poultry, taking the efficiency of utilisation of digested nutrients into account. Does it improve the predictability of bird performance and/or reduce feed costs? Furthermore, the potential of a net energy (NE) system in poultry analogous to swine needs to be evaluated, as it has been shown in swine nutrition that feed cost savings can be as high as 4.00 to 4.50 €/metric ton when a shift is made from a ME to a NE system, without any negative impact on production performance. The latter might even be improved due to lower dietary crude protein levels, which are generally linked to the introduction of a NE system. Of course feed cost savings depend on the feedstuffs available in different regions in the world and on the maximum dietary feedstuff inclusion levels for different animal species or categories. In principle, a NE system would have added value in poultry like in pigs, however, results in poultry are less conclusive. Differences between ME and NE in pigs are based on a higher efficiency of utilisation of energy from fat and a lower efficiency of utilisation of energy from protein compared to starch. In laying hens the extra caloric value of fat has been demonstrated (eg. Scheele et al., 1985 as cited by Van der Klis and Fledderus, 2007) and is well-accepted,

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but results in broilers are still contradictory. Nevertheless, in time several modified AME systems have been developed that take the efficiency of nutrient utilisation into account, for example:

(i) Effective Energy by Emmans (1994).

(ii) for egg-producing poultry using a 15% extra caloric value of digested fat and for meat-type poultry reducing the energy value of digested protein by approx. 15% (both modifications being used in commercial poultry feeding in the Netherlands (CVB, 2005).

Recently, the CVB in the Netherlands initiated the development of an ATP-based NE system for broilers. The added value of this new system over the currently used $AME_{broiler}$ was evaluated. In 2009, Schothorst Feed Research launched a new feeding table for laying hens, based on digestibility experiments with highly productive layers. In the current paper results are presented.

II. MODIFIED AME SYSTEM IN LAYING HEN DIETS

The basis of all energy evaluation systems lies in accurate nutrient digestibilities in feedstuffs. Until now, feeding values determined in adult roosters are used world-wide to calculate the dietary energy value for highly productive layer diets. Recently, nutrient digestibilities and AME values were determined using white LSL laying hens in peak production according to the European reference assay for poultry (Bourdillon et al, 1990), based on a three-day droppings collection period. Nineteen feedstuffs were evaluated so far and results were compared with current tabulated values from restrictedly fed adult roosters (CVB, 2005). Results are given in Figure 1.
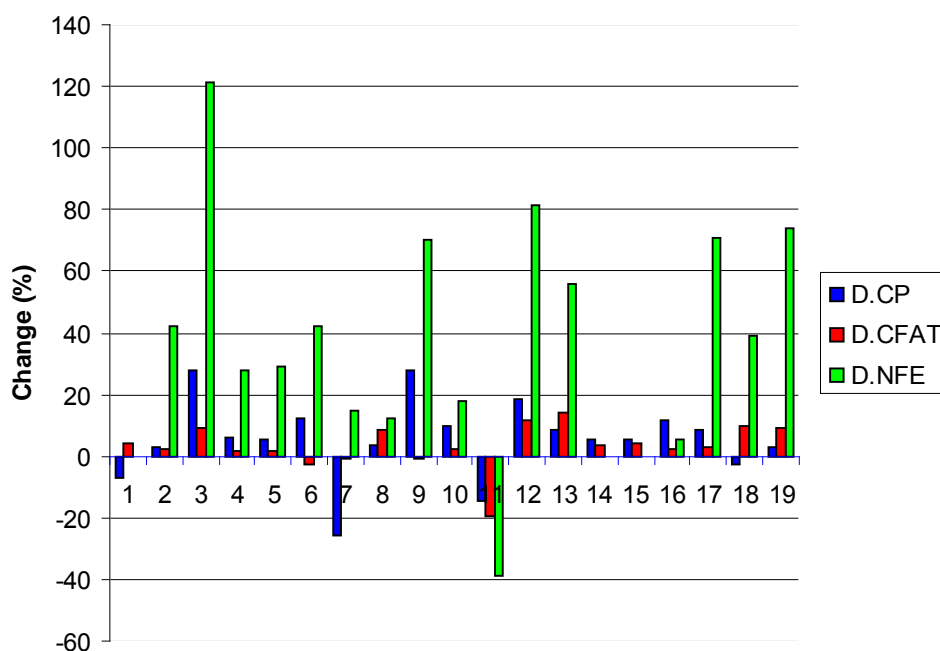
Figure 1.   Nutrient digestibilities (digestible crude protein (D.CP), digestible crude fat (D.CFAT) and digestible nitrogen free extract (D.NFE)) in feedstuffs using laying hens in peak production. Data are presented relative to current tabulated values (i.e. restrictedly fed adult roosters) as a reference (CVB, 2005).

It is shown in Figure 1 that especially the digestibility of carbohydrates was higher in *ad libitum* fed laying hens compared to restrictedly fed roosters. Differences in nutrient digestibilities resulted in absolute AME values in feedstuffs (as measured) that were -40 to 530 kcal/kg higher than current tabulated values (CVB, 2005). Although test feedstuffs in Figure 1 were not simultaneously evaluated in restrictedly fed roosters, it was concluded based on the large differences between laying hens and adult roosters that the latter have limited value to determine the energy value of feedstuffs for producing laying hens. Schothorst Feed Research therefore introduced in 2009 a new feedstuff table for layers. Currently, validation trials are being done and a new trial was started to evaluate twenty new feedstuffs and/or different qualities of feedstuffs already tested.

In the Netherlands a modified AME system for laying hens is being used, taking a higher efficiency of energy utilisation for fat compared to starch into account, as fatty acids and monoglycerides can be used as such to synthesize fat in body and eggs. Scheele et al. (1985) estimated the energetic efficiency of fat utilisation in comparison to starch using *ad libitum* fed laying hens from 24 to 36 weeks of age. Two sets of diets contained either 11.5 or 12.0 MJ AME_N/kg, each with three levels of dietary fat (3, 6 and 9%) that was isocalorically exchanged for carbohydrates and ash. Dietary AME, nutrient digestibilities and energy retention in body and eggs were measured. Results are given in Figure 2.
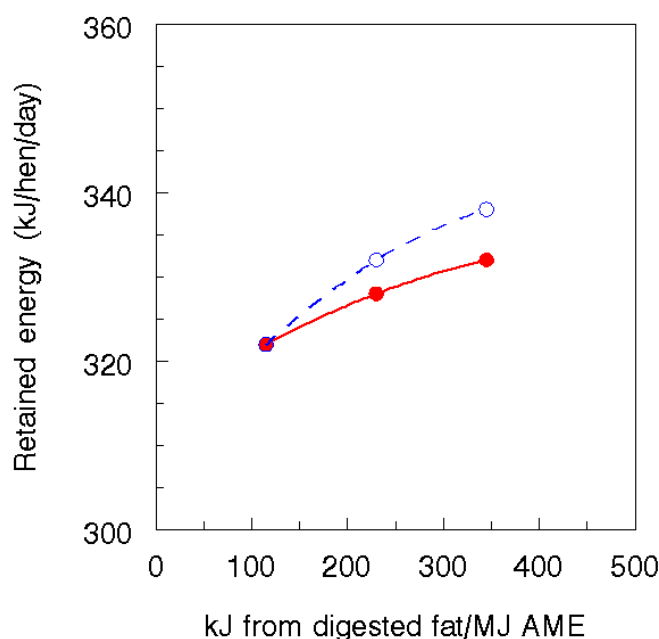
Figure 2.   The energy retention (in body and egg) in laying hens fed diets differing in AME_N content (11.5 MJ/kg (●) and 12.0 MJ/kg (○)), each containing three levels of dietary fat which were isocalorically exchanged for carbohydrates.

It is shown in Figure 2 that energy retention in laying hens increases with isocaloric dietary fat inclusion, indicating a higher energetic efficiency of fat utilisation over carbohydrates. From this experiment it was calculated that the extracaloric value of fat for layers was 15% to 19%. The CVB uses a 15% extra caloric value of fat in the Dutch modified AME system for layers to take this higher efficiency into account. In laying hens no adjustment has been made for a lower efficiency of protein utilisation, except for the energy correction for zero nitrogen balance, assuming that all nitrogen from feed will be excreted as uric acid.

III. DEVELOPMENT OF AN ATP-BASED NET ENERGY SYSTEM

In 2004 a NE formulae was derived based on the ATP yield of carbohydrates, amino acids, glycerol and fatty acids and volatile fatty acids (Jansman et al. 2004). The results of their calculations of the ATP yield of nutrients is given as mol ATP/g nutrient and recalculated to kJ/g in Table 1.

Table 1.   The ATP yield and ATP energy per g nutrient (absolute and relative to starch).

	mol ATP/g	ATP energy kJ/g	Relative to starch
Glucose	0.211	10.56	90
Starch	0.235	11.73	100
Protein[1]	0.194	9.70	83
Fat[2]	0.508	25.31	216
Volatile fatty acids	0.212	10.60	90
Fermentable carbohydrates	0.165	8.21	70

[1]Based on the composition of body protein; [2]Based on soya oil

From these coefficients the following equation to calculate the NE_{ATP} in feedstuffs was derived:

$$NE_{ATP}= 9.7 \times \text{dig. true protein} + 26.1 \times \text{dig. crude fat} + 11.7 \times \text{dig. starch} + 10.6 \times \text{dig. sugars} + 8.2 \times \text{dig. nitrogen free residue [1]}$$

Subsequently, an equation to calculate the NE_{ATP} requirement (kJ/day) was derived from comparative slaughter experiments based on regression analyses between the NE_{ATP} intake and protein and fat deposition, using male and female broilers from ten different pure and commercial lines (unpublished data), being:

$$NE_{ATP\,(req)}= 278 \text{ kJ } NE_{ATP}/BW^{0.75}/\text{day} \times BW^{0.75} + 3.058 \times \text{energy in protein deposition (kJ } NE_{ATP}/\text{day)} + 1.053 \times \text{energy in fat deposition (kJ } NE_{ATP}/\text{day) [2]}$$

Based on Equation 1 the NE_{ATP} content of feedstuffs has been calculated and compared to the AME equations, using maize as a reference (Table 2). It is suggested in Table 2 that the relative NE_{ATP} is of protein rich feedstuffs will be similar to the currently used $AME_{broiler}$.

Table 2. Relative feeding values of vegetable feedstuffs, using corn as a reference.

	$AME_{broiler}$[1] (growing)	AME poultry (adult)	NE_{ATP}
Maize	100	100	100
Wheat	91	94	84
Soybean meal	59	67	58
Sunflower seed meal	43	48	42

[1]AME broiler is based on an approx. 15% lowered energy value of digestible protein.

The NE_{ATP} system has been validated in Ross 308 broilers based on a set of five diets with a fixed $AME_{broiler}$ (12.2 MJ/kg) and variable NE_{ATP} (7.7 to 8.3 MJ/kg) and five diets with a fixed NE_{ATP} (8.0 MJ/kg) and variable $AME_{broiler}$ (11.8 to 12.6 MJ/kg) to test whether

the new NE_{ATP} equation has added value over the current $AME_{broiler}$ equation to predict the energy retention in broilers. Broilers were fed twice a day from day 8 onwards, and daily feed allowance from was standardised based on pairwise feeding. Nutrient digestibilities were measured from 13-17 days of age and from 27-31 days of age and energy retention was determined using the comparative slaughter technique from 8 to 24 days of age and from 24 to 36 days of age. Average feed intake from 8 to 36 days was 82.7 g/day (approx. 75% of *ad libitum* intake for Ross 308 in this period). Body weight gain during the experimental period was 1557 g, resulting in an average feed conversion efficiency of 1.491.

The energy retention from 8 to 36 days of age is given in Figure 3. AME and NE were related in this experiment, because the current modified $AME_{broiler}$ equation was used. Approx. 65.0% of the variation in energy deposition was explained by $AME_{broiler}$ intake and only 60.3% by NE_{ATP} intake. It was therefore concluded that in this experiment NE_{ATP} had no added value over $AME_{broiler}$ (Jansman and Van Diepen, 2008), in which broilers were restricted fed.
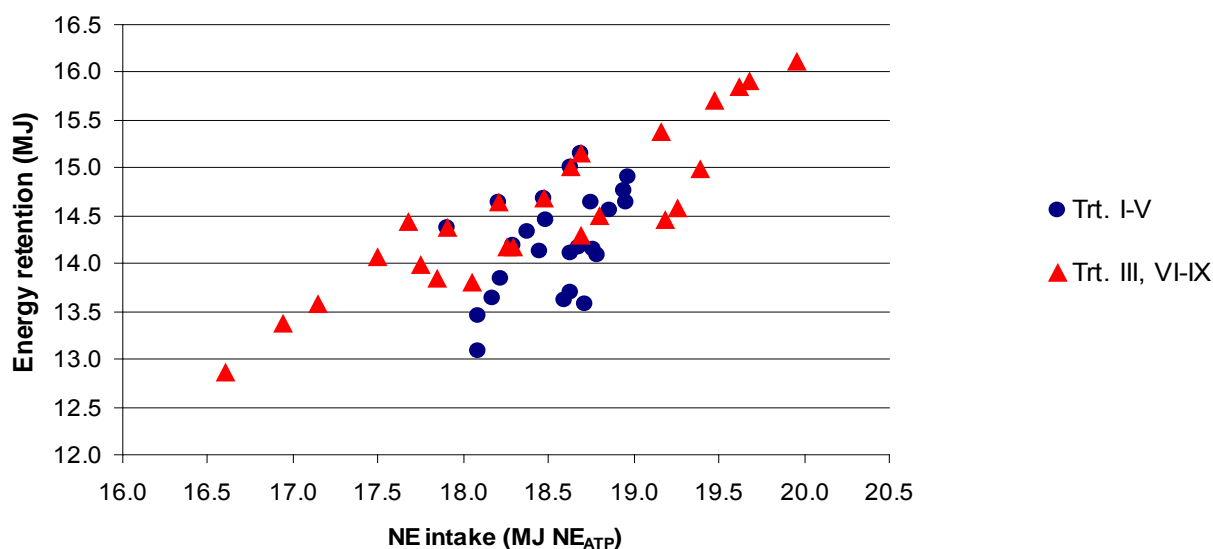
Figure 3.   The Energy retention (MJ) in broilers from 8 to 36 days of age as affected by NE_{ATP} intake. Diets I to V were formulated on variable $AME_{broiler}$ content and diet III and VI to IX on variable NE_{ATP} content (both in MJ/kg).

IV. NET ENERGY VERSUS METABOLISABLE ENERGY SYSTEM

Pirgozliev and Rose (1999) quantitatively reviewed the relationship between dietary AME, NE and Effective Energy on the one hand and energy deposition (or net energy for production: NEp) on the other hand, using a rather old dataset of 40 feedstuffs varying in AME value from 8.0 to 16.5 MJ/kg. NEp was measured as energy deposition in young growing chickens depositing mostly lean meat (Fraps 1946, as cited by Pirgozliev and Rose, 1999). Based on regression analyses, Pirgozliev and Rose (1999) concluded that the AME value (calculated as 22.4 x D.CP+ 39.2 x D.CFAT + 17.2 D.NFE (in MJ/kg)) overestimated NEp for high protein feedstuffs. The regression equations obtained (after we omitted two feedstuffs (dried butter milk and dried skimmed milk) with a high leverage from their dataset) were:

NEp= 0.711 x AME - 0.598 (r^2=0.92) for cereal and cereal by-products and
NEp= 0.545 x AME + 0.843 (r^2=0.81) for high protein feedstuffs

These authors concluded that correcting the AME for crude protein content gave an equally accurate prediction of NEp as Effective Energy according to Emmans (1994) and Net Energy according to Hoffmann and Schiemann (1980) and De Groote (1974) as cited by Pirgozliev and Rose (1999). This is more or less as expected as the dietary AME value was not yet corrected for zero nitrogen balance, using an energy value of 22.4 instead of 18.0 MJ/kg digested CP. Based on their regression analyses, the energy value of digested protein most probably needs to be corrected below 18.0 MJ/kg D.CP. Pirgozliev and Rose (1999) did not show any proof for an extracaloric value of fat in broilers, which can be attributed to the lack of feedstuffs with higher fat levels in their dataset. Nitsan et al. (1997) did show an extracaloric effect of 2.6% soybean oil (plus wheat bran) isocalorically exchanged for maize meal in broilers at 12.1 (comparing 2.6 and 5.3% fat) and 13.1 MJ/kg (comparing 6.9 to 9.5% fat). At the low dietary AME level heat production was reduced by 14% due to soybean oil inclusion, whereas effects at the high AME level were only small. Based on their calculations the extracaloric effect was 17% in the low, and 3% in the high AME diet. They suggested that the extracaloric effect is related to bird age (no effect expected in young birds having a low fat digestibility), dietary fat level and source: An excess of unsaturated fatty acids might even stimulate heat production. More recently, Noblet et al. (2009) were not able to show any effect of the dietary fat content in broiler diets on their heat production. The NE/ME ratio in their trial was approx. 75% irrespective of the dietary fat content (2.9% versus 9.6%). Noblet et al. (2007) did not demonstrate any increase in heat production either using high protein diets in 4-5 week old broilers. The NE/ME ratio was remained 68% although the dietary crude protein level was increased from 22.5 to 27.3%. It can therefore be questioned whether a net energy system would have any significance to broilers, despite a few references that might indicate some benefits of a NE system. A (modified) AME system most probably would be adequate to describe energy partitioning in broilers.

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NON-STARCH POLYSACCHARIDES AND ENZYME APPLICATION INFLUENCE THE NET ENERGY VALUE OF BROILER DIETS

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The apparent metabolisable energy (AME) assay is a default system of energy estimation in the poultry industry, but the system is not capable of accounting for losses of chemical energy in the solid, liquid and gaseous excreta or as heat (Pirgozliev and Rose, 1999). Measuring energy lost to account for heat increment (HI) and excreta volatile fatty acids (VFA), and thus calculating the net energy (NE) of the diet, should better explain the negative influence of non-starch polysaccharides (NSP) and benefits of their breakdown by xylanases. Four groups of day-old mixed broiler birds (Cobb strain), each consisting of 2 randomly selected birds of similar weights, were selected from 2 different batches. On d 17 birds were transferred to 4 closed circuit respiration chambers (2 per cage) in a climate-controlled room ($25 \pm 2^{\circ}C$) under continuous fluorescent lighting. On d 4 and 5, the birds were deprived of feed to establish their basal metabolism and fasting heat (FH). During d 6-9, they were fed a wheat-based diet containing 4% soluble NSP, and that diet with a xylanase product (250 mg Ronozyme WX), followed by collection of excreta collection for determination of AME, as well as measures for respiratory quotient (RQ), heat production (HP) and HI during d 10-14. On d 14 after the last collection, all the birds were sacrificed, and intestinal contents were collected for digesta viscosity (data not shown) and VFA analysis.

Table 1. The effect of soluble non-starch polysaccharides (NSP) and xylanase supplementation on energy utilisation

Measure	Wheat + NSP (- enzyme)		Wheat + NSP (+ enzyme)		P value
	Mean	SE	Mean	SE	
RQ	0.995	0.014	1.006	0.02	0.772
HP (MJ/kg wt/day)	0.91	0.013	0.81	0.02	0.103
HI (HP-FH) (MJ/kg wt/day)	0.20	0.01	0.13	0.01	0.004
HI (MJ/kg feed)	1.28	0.11	1.17	0.09	0.402
AME (MJ/kg feed)	10.5	0.06	14.8	0.07	0.003
AME-HP (NE: MJ/kg feed)	4.7	0.23	7.5	0.16	0.008

RQ: respiratory quotient; HP: heat production; HI: heat increment; FH: fasting heat; AME: apparent metabolisable energy (MJ/kg); NE: net energy (MJ/kg); wt: body weight; SE: standard error.

Enzyme supplementation did not affect RQ HP, but it numerically lowered HP by 11.0%. Enzyme reduced (P<0.01) HI based on per kg bodyweight/day but not on the basis of MJ/kg feed. AME and NE increased (P< 0.01) with enzyme supplementation (Table 1).On a 4-day average basis, energy loss as VFA was 101.5kJ/chamber/d for birds fed the control diet compared with 34.3kJ/chamber/d for those fed enzyme-supplemented diet. Overall, xylanase reduced HP by 11% and decreased energy lost to excreta VFA by 66.2% which resulted in enzyme increasing NE (37%) proportionally more than AME (29.1%). This further supports the argument to establish an NE-based feed formulation system.

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

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Summary

Previously reported near infrared (NIR) calibrations for predicting the apparent metabolisable energy (AME) content (MJ/kg as fed) and AME Intake Index of cereal grains have been updated with results from an additional 55 grains. The new calibrations have improved greatly the ability to predict values for unknown grains and the accuracy of prediction. The new calibrations are available to industry under license through the Pork CRC project, AusScan.

I. INTRODUCTION

The apparent metabolisable energy (AME) content (MJ/kg) of cereal grains and the amount eaten by broiler chickens when the grains are incorporated into diets are major determinants of the efficiency of feed use, time taken for birds to reach market weight and profitability of the broiler industry. The AME content of wheat, barley and triticale has been shown to range between samples by over 3 MJ/kg (Scott, 2004; Black et al., 2005). Similarly, the intake of energy (MJ/day) by broilers varies by approximately 34% when different samples of wheat are incorporated into diets (Black, 2008). This large variation in the energy value of cereal grains means that a rapid method is required for measuring AME and relative AME intake for individual batches of grain if profitability of broiler enterprises is to be maximised.

Black et al. (2009) described calibrations based on near infrared reflectance (NIR) spectroscopy for estimating the AME content and AME Intake Index of cereal grains for broiler chickens. The calibrations were developed from results obtained in the Premium Grains for Livestock Program (PGLP; Black, 2008). AME Intake Index (0-100) was used rather than AME intake (MJ/day) to estimate the relative effect of a grain sample on broiler feed intake, because AME intake changes each day as a bird grows.

The PGLP calibrations were established from results for just over 100 grain samples (Black et al., 2009). This number is regarded as being near the minimum needed for reliable and robust NIR calibrations for predicting accurately values for unknown samples. There were relatively few weather damaged and 'pinched' grains included in the PGLP samples. In addition, the ability of the calibrations to predict the energy value of unknown samples of grains was not tested. Consequently, two experiments, one including mainly weather damaged grains and the other with grains supplied by the Australian broiler industry, have been conducted to evaluate the ability of the NIR calibrations to predict the energy values for unknown grains. The results from these experiments were included with the PGLP results to upgrade the calibrations.

II. EXPERIMENTS

The experimental procedures used in PGLP (Black, 2008) were adopted in two experiments to measure the AME content (MJ/kg) and AME intake (MJ/day) of cereal grains incorporated

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into diets for broiler chickens. Experiment 1 included 30 weather damaged wheat (10), barley (11), triticale (3) and sorghum (6) grains, with a range in screenings content (< 2.0 mm) from 1% to 67%. Experiment 2 contained 25 grains (wheat, 9; barley, 7; triticale, 2; sorghum, 7) provided by the Australian broiler industry. Each experiment included approximately 30% additional grains that had been used in previous experiments to provide connectivity for statistical analysis needed to remove identified variation between experiments. Each grain sample was formulated into two diets, one containing xylanase and phytase enzymes and the other without enzymes. The results reported are for grains without enzymes.

A sample of each grain was scanned using a Foss 6500 instrument at the time the grains were first fed to the birds. Scans were conducted on whole and milled grain samples. Standard NIR technology was adopted to develop updated calibrations using WINISI software. For each experiment, the latest existing calibration was first used to predict the values for new grains. These predicted values were compared with the measured values and then included with existing values to update the calibrations. Results for calibrations based on whole grain scans only are presented.

III.   ACCURACY OF PREDICTIONS FOR NEW SAMPLES

The statistics for linear regression equations fitted to NIR predicted and measured values for AME and AME Intake Index for all grains in experiments 1 and 2 are given in Table 1. The ability of the calibrations established in PGLP to predict accurately the AME and AME Intake Index values for the weather damaged grains from experiment 1 was only fair (AME - R^2 = 0.63, slope = 0.89; AME Intake Index - R^2 = 0.68, slope = 0.93 for whole grain scans). This result was expected because the grains used to establish the PGLP calibrations contained a small range in screenings content. When the grains from experiment 1 were included with PGLP grains in a new calibration, there was a substantial improvement in ability to predict the values measured for experiment 1 grains, with R^2 for the regressions exceeding 0.8. The real test of the new calibrations was their ability to predict the values for a new set of unknown grains from experiment 2. The new calibrations predicted values for experiment 2 grains with R^2 values of approximately 0.8, which was a considerable improvement. Again, inclusion of experiment 2 grains with experiment 1 and PGLP grains in the development of third generation calibrations showed a further improvement in the ability to predict AME and AME Intake Index for experiment 2 grains, with R^2 values of approximately 0.9. In addition to improvements in the R^2 values, the slope of the regression between measured and predicted values improved from around 0.9 to near 1.0 and the intercepts were closer to zero. These values suggest that with the latest calibrations there is little bias in the ability to predict values for unknown samples.

IV.   IMPROVEMENTS IN CALIBRATIONS

Including results from experiments 1 and 2 with PGLP results produced a modest, but continuous improvement in the calibrations for predicting the AME content and AME Intake Index (Table 2). R^2 values for the calibration predicting AME content increased with each additional set of grains from 0.88 to 0.91, the robustness of the calibration (RPD) increased from 2.40 to 2.67 and the precision of prediction improved with a decrease from 0.48 to 0.45 MJ/kg. There were similar improvements in AME Intake Index calibrations. R^2 values increased from 0.84 to 0.87 and the precision of prediction improved from 4.85 to 4.22 units. The biggest improvement was in the calibration robustness, with RPD increasing from 1.82 to 2.38. The increase in RPD values for both AME and AME Intake Index indicates that the chances of obtaining spurious results are much diminished.

Table 1.   Linear regression coefficients for equations fitted to NIR predicted values for AME and AME Intake Index and measured values for grains in experiments 1 & 2

Calibration used for prediction	R^2	Slope	Intercept
AME (MJ/day as fed)			
Experiment 1			
PGLP calibration (1)	0.63	0.89	1.62
PGLP + Exp 1 calibration (2)	0.81	1.02	-0.22
Experiment 2			
PGLP + Exp 1 calibration (2)	0.81	1.09	-1.01
PGLP +Exp 1 + Exp 2 calibration (3)	0.91	1.05	-0.64
AME Intake Index (0-100)			
Experiment 1			
PGLP calibration (1)	0.68	0.93	6.61
PGLP + Exp 1 calibration (2)	0.80	0.96	2.84
Experiment 2			
PGLP + Exp 1 calibration (2)	0.79	0.95	6.83
PGLP + Exp 1 + Exp 2 calibration (3)	0.88	1.01	0.25

Table 2.   NIR calibrations information as the number of samples used is increased from PGLP by adding results from experiments 1 & 2

Calibration	N[1]	Mean[1]	SD[1]	RSQ[1]	SEC[1]	1-VR[1]	SECV[1]	RPD[1]
AME (MJ/kg as fed)								
PGLP (1)[2]	102	12.40	1.15	0.88	0.40	0.82	0.48	2.40
(1) + Exp 1 (2)	147	12.49	1.16	0.90	0.37	0.84	0.46	2.52
(2) + Exp 2 (3)	180	12.61	1.20	0.91	0.37	0.86	0.45	2.67
AME Intake Index (0-100)								
PGLP (1)[2]	104	64.8	8.84	0.84	3.57	0.70	4.85	1.82
(1) + Exp 1 (2)	147	67.1	8.98	0.85	3.54	0.77	4.31	2.08
(2) + Exp 2 (3)	184	69.3	10.0	0.87	3.64	0.82	4.22	2.38

[1] N, observations used in calibration; Mean of observations; SD, standard deviation of observations; RSQ, R^2 for predicted and observed relationship; SEC, standard error of calibrations; 1-VR, 1-Variance Ratio or fraction of variance accounted for when some observations are used for 'cross validation'; SECV, standard error of cross validation, RPD, ratio of Prediction to Deviation = SD/SECV an indication of the value of the calibration, < 1.5 calibration unsatisfactory; 1.5–2.0: calibration can distinguish between high & low values; 2.0-2.5: calibration is quantitative; 2.5-3.0: calibration predictions good; > 3.0: calibration predictions excellent.
[2] Values differ slightly from Black et al., (2009) because of calibration revision.

V.   DISCUSSION

Inclusion of additional grain samples in the calibrations has substantially improved the ability to predict AME content and AME Intake Index of unknown samples. Addition of new grains increased the R^2 for the calibration predicting AME content from 0.88 to 0.91, which is greater than 0.35 for an AME calibration derived from 94 whole grain scanned wheat samples developed by Owens et al. (2009). Similarly, the R^2 value was only 0.45 for predicting AME of wheat from a calibration based on milled grain scans of 160 samples by Garnsworthy et al. (2000).

RPD, which reflects the reliability for predicting values for unknown samples, of the calibration for predicting AME content of grains also increased from 2.40 to 2.67 as the grains from the new experiments were included. NIR experts regard calibrations with RPD values greater than 2.5 as being reliable for predicting values for unknown samples. However, SECV, which provides an indication of the likely precision of prediction, decreased only from

0.48 to 0.45. This result means that the latest calibration can predict with 95% confidence to within ± 0.45 MJ/kg as fed of the actual AME value. Whereas the addition of more grains to the calibration has increased its ability to predict values for a wider range of grains, it has not greatly increased the precision of the prediction.

The SECV value obtained for the AME content of cereal grains for broiler chickens is considerably higher than the value of 0.27 MJ/kg obtained for the digestible energy (DE) content of cereal grains for pigs when PGLP and Pork CRC experiments are combined. The numbers of grains used to establish the calibrations were similar for both species (180 broilers; 170 pigs), but the average standard error for measured AME values for broilers was 0.21 MJ/kg compared with 0.11 MJ/kg for DE in pigs. The lower variation in measurements of DE in pigs compared with AME in poultry may be due to the experimental designs and statistical correction of results because pigs were housed individually and broilers in cages of five birds. In addition, differences in gut microbial population between birds and variation in mean retention time of digesta in the gastrointestinal tract of broilers are known to have an effect on AME values (Torok et al., 2008; Hughes 2008). Nevertheless, there appears to be substantial opportunity for improving the precision of NIR calibrations for broilers through the inclusion of results from more grains.

Calibrations for AME Intake Index also improved as the grains from the new experiments were added to the data from PGLP, with R^2 increasing from 0.84 to 0.87 and RPD increasing from 1.82 to 2.38. The latter value suggests that the calibration is valuable, but could be improved further with the addition of more grains. The new calibrations are available under license to the animal and grains industries through the AusScan project of the Pork CRC.

VI.   ACKNOWLEDGEMENTS

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THE PERFORMANCE OF BROILERS OFFERED SORGHUM- COMPARED TO
WHEAT-BASED DIETS: A LARGE SACLE EXPERIMENT

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Summary

Sufficient evidence tended to indicate that at least four factors can negatively influence broiler performance when offered sorghum-based diets; in particular energy utilisation of sorghum in young birds. It was proposed that mainly CT would further influence sorghum grain AME values when consumed by young chicks (0-7 and 7-14 d old). Overall, birds consuming sorghum-based diets during the starter phase (0-21 d), did not match the performance of birds offered wheat-based diets. The use of phytase enzymes in sorghum-based diets tended to improve bird performance. However, reducing the obtained AME of sorghum grains by -0.8 MJ during the 0-21 d period appears to be a practical solution.

I. INTRODUCTION

There are at least four factors linked to reduced broiler performance and carcass quality when birds are offered sorghum-based diets. These factors are: (i) low cystine (52%) and tryptophan (63%) digestibility, (ii) the high link between condensed tannin (CT) found in sorghum and low tryptophan digestibility (r = -0.673), (iii) the high link between sorghum CT fractions lowering apparent metabolisable energy (AME) of sorghum in young birds and (iv) the high phytate-P content (76%) of sorghum. Regarding factor (iii), the reduced sorghum AME value has been calculated to be about -1.0 MJ/Kg DM in younger birds (14-21 d) when compared with AME values obtained in older birds (22-28 d). Normally in Australia, the AME values obtained with 22-29 d old birds are used to formulate diets for birds up to 21 days of age. Such an AME difference has a significant nutritionally negative outcome particularly during the starter phase (0-21 d old) where a poor feed conversion ratio (FCR) at 21 d has been observed. This poor energy utilization has been linked (r = 0.704) with total intakes of CT from sorghum grain during same period. When the sorghum grain AME adjustment value of -1 MJ was imposed to formulate new sorghum-based broiler diets, an excellent grower phase (22-42 d) performance and carcass quality were recorded. But during the starter period (0-21 d), the poor FCR in birds offered the sorghum-based diets with the adjusted sorghum AME was still present as compared with those on wheat-based diets (Perez-Maldonado and Rodrigues, 2009). Thus, the present experiment tests the hypothesis that, the observed detrimental effect of sorghum CT, would further reduce the AME value in younger chicks, 0-7 and 7-14 d old. Therefore, it is intended to confirm this assumption including an examination of other feeding alternatives that may provide information on improving the use of sorghum-based diets for broiler production.

II. MATERIALS AND METHODS

This experiment was conducted with 1800 (900 males and 900 females) Arbor Acres birds kept in floor pens from 0-42 days. The 64 pen building has artificial lighting, electric heating brooders with side shutters, cooling fans and sprinklers devices installed and electronically controlled. Each pen measured 7.5m^2 and was covered with wood shavings with feed and water available *ad libitum*. Each pen housed 30 chickens until 42 days of age, with a stocking density of 4 birds/mt^2. During the experiment, there was a 23 h/d lighting period from 1-42 d

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of age and the temperature was gradually reduced from 29-32 °C in accordance to industry practice for maximum bird comfort to 26-24 °C by the time they reached 21 days and to 22-21 °C (when possible) by the time they reach 42 days.

Day old chicks were received from a commercial hatchery; weighed in groups of 30 birds each and randomly allocated to pens and offered *ad libitum* the corresponding experimental starter diets. At day 21, pen feed residues and chick groups from each pen were weighed, with starter diet replaced with their equivalent grower/finisher dietary treatments which was offered *ad libitum* until day 42. Then again feed residue and birds from each pen were weighed. During the experiment chickens that died, or were culled during the first 72 h, were replaced by healthy birds and any bird dying thereafter was not replaced. Birds that died or were culled and not replaced were individually weighed at the time of removal from the pen and the pen feed residues recorded in affected pens.

The 10 dietary treatment were (1) control wheat-based diet, (2) sorghum H Pioneer Bonus, (3) sorghum M Pacific MR Buster, with reduced the obtained sorghum AME value by -0.8MJ in starter phase only to switch to sorghum M during finisher period using its normal grain AME value; (4) sorghum B Pacific Buster, (5) sorghum E Pacific Buster, with the obtained sorghum AME reduced by -0.8MJ in the starter phase only to switch to sorghum E during the finisher period using its normal AME value, (6) commercial Brisbane sorghum, (7) sorghum K Hylan Lyberty during starter phase and the commercial sorghum during finisher phase (insufficient sorghum K); (8) control wheat-based diet during starter period and switch to commercial sorghum during finisher period, (9) commercial sorghum with phytase added on top and (10) commercial sorghum with phytase added after reducing available phosphorous from 0.44 to 0.38% in the starter period and from 0.34 to 0.30% in the finisher period. Ca was reduced from 0.85 to 0.75% and from 0.68 to 0.60% in the starter and finisher periods, respectively.

Therefore, this trial examined whether the FCR can be improved during the starter phase (0-21 d) by changing sorghum AME specifications. Two sorghum-based, diets 3 and diet 5 were formulated with corresponding sorghums M and E in which its determined AME was lowered by -0.8 MJ/Kg but only during the starter phase. Then the diets used the actual measured grain AME value during the finisher phase. The responses to these formulations were then compared with the response to diets containing sorghums formulated on the actual measured sorghum AME value (Sorghum H, Sorghum B and commercial sorghum).

III. RESULTS AND DISCUSSIONS

The results (Table 1) indicate that during the 0-21 d period birds given treatments 2, 6, and 7 representing diets based on sorghums H, commercial and K, had a similar feed intake (FI) to birds consuming the wheat control diet. But these sorghums produced significantly ($P < 0.05$) poorer live weight gain (LWG) and FCR. Sorghum K of low CT content did not perform as expected in the present experiment and it was suspected that it had deteriorated before the diet formulation. Birds on sorghum treatments 3, 4, 5, 9 and 10 representing sorghum diets M, B, E and commercial sorghums with the phytase enzyme treatments added, exhibited a higher ($P < 0.05$) FI with a similar LWG when compared with the control birds, but resulted in a poorer FCR. It is noteworthy in this study, that birds consuming the sorghums M and E (diets 3 and 5) which had their AME reduced by -0.8MJ, exhibited a superior ($P < 0.05$) FCR among sorghums (1.398 and 1.391 respectively) and were only 3.2% and 2.7 % respectively less efficient than the control wheat diets (FCR 1.354). During the finisher period (22-42 d), except for the birds on diets 2 (sorghum H) and 4 (sorghum B), all birds on the sorghum-based diets had a similar ($P > 0.05$) FI to birds fed the control wheat diet with birds on all sorghum based diets exhibiting a significantly ($P < 0.05$) superior LWG and FCR than the control birds resulting in a superior overall LWG and FCR at 42 days. But the birds

consuming sorghum-based diets 3 and 5 that had their sorghums AME reduced by -0.8MJ during the starter phase, exhibited the largest LWG (1980 and 1923 g, respectively) of all treatments, with a similar FCR among sorghums cultivars at 42 d of age. In terms of bird energy intake and performance, the same diets 3 and 5, with reduced grain AME by -0.8 MJ, soy oil was added to be iso-caloric (12.5 MJ/kg diet) as with the other treatments assessed. The results show (Figure 1a) that during the 0-21 d period, birds offered these reduced sorghum energy based diets had a similar grain energy intake, producing similar LWG as birds in the wheat diets. Similarly, the FCR of birds fed on the sorghums based diets 3 and 5 (sorghums M and E) improved when compared with birds offered other sorghum diets (Figure 1b). It would appear that the addition of oil to these two starter diets was required, to compensate for the lower energy utilisation of the sorghum grain by young birds. Our results agree with Douglas et al. (1990) who indicated the need for adding animal fat to typical broiler diets, to compensate for the lower MEn of CT sorghum grains. It was suggested that the low energy efficiency with CT sorghum grains is caused, in part, by the cross-links between protein in sorghum grain, which decreases the digestibility of the protein and of the starch embedded in it. Another reduction on energy efficiency is due to undigested tannin-protein complexes. In the present experiment, the oil content of the sorghum diets 3 and 5 was raised 2.9% and 1.1%, respectively, to compensate for the -0.8 MJ AME reduction values in their respective sorghums during the diet formulation of the starter period. Therefore, both due to age of bird and the highly negative CT effect seen, it is necessary for this grain AME adjustment value to be applied in sorghum-based broiler diets in order to improve sorghum efficiency particularly during the early starter period 0-14 d.
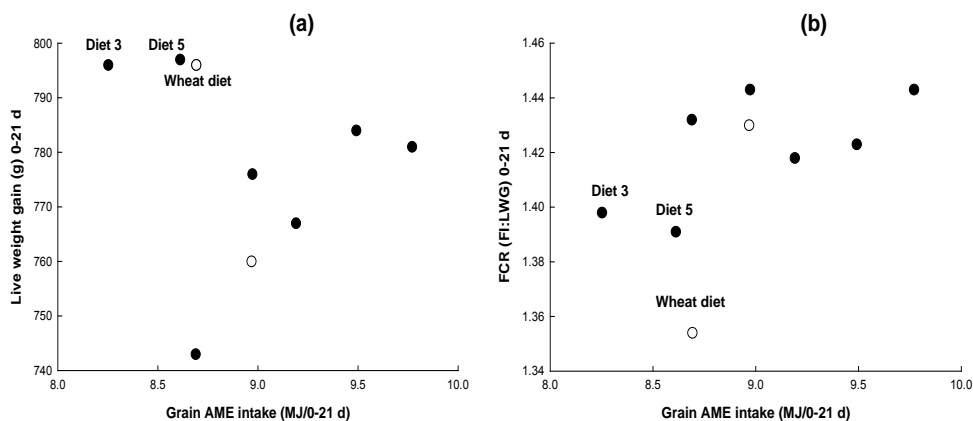
Figure 1.   Relationship between grain AME intake and live weight gain (a) and AME intake and feed conversion ratio (FCR) (b) of broiler during the starter period when given sorghum (●) or wheat (○) based diets. Each point is the mean value of six floor pens (30 birds/pen)

During the grower/finisher period, no extra oil was needed in these M and E sorghums diets which were formulated with the actually determined grain AME value. The results showed that during the grower/finisher period diets with sorghum grain M and E and all other sorghum diets produced excellent bird performance (Table 1) with no need to add extra oil during this period. Future research on sorghum grain to explain the reasons for this lower energy utilization during the starter period only is warranted.

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Table 1. Mean feed intake (FI), live weight gain (LWG), feed conversion ratio (FCR) of broilers in semi-commercial floor pens for starter (0-21 d) grower/finisher (22-42 d) and overall (0-42 d) periods when fed sorghum-based diets (2005 sorghum harvest) with various strategies applied or a wheat-based (control) diet

Diet	Treatment	FI (g/bird)			LWG (g/bird)			FCR (feed:lwg)		
No	Age in Days	0-21	22-42	0-42	0-21	22-42	0-42	0-21	22-42	0-42
1	Wheat control (both starter and grower)	1077^b	3567^{bc}	4637^{abc}	796^c	1803^d	2595^d	1.354^d	1.986^e	1.788^d
2	Sorghum H both starter & grower	1085^{abd}	3464^a	4549^{ab}	760^{bd}	1920^a	2681^{ab}	1.430^a	1.810^{bd}	1.702^b
3	Sorghum M reduced AME for starter & normal AME- grower	1114^{cd}	3607^b	4721^c	796^c	1980^c	2777^c	1.398^{bc}	1.855^{ac}	1.719^{ab}
4	Sorghum B (both starter & grower)	1116^{cd}	3466^a	4582^{ab}	784^{ac}	1894^{ab}	2679^{ab}	1.423^{ab}	1.847^{abc}	1.721^{ab}
5	Sorghum E reduced AME for starter & normal AME- grower	1110^{ac}	3605^b	4715^c	797^c	1923a	2720^{ac}	1.391^c	1.880^a	1.735^{ac}
6	Sorghum commercial both starter & grower	1079^{ab}	3535^{ab}	4614^{abc}	767^{ab}	1908^{ab}	2675^{ab}	1.418^{ab}	1.854^{abc}	1.728^{abc}
7	Sorghum K for starter & Sorghum Commercial for grower	1053^b	3473^{ac}	4526^b	743^d	1907^{ab}	2651^{bd}	1.432^a	1.831^{cd}	1.718^{ab}
8	Wheat for starter & sorghum commercial for grower	1077^b	3489^{ac}	4573^{ab}	796^c	1857^{bd}	2657^{bd}	1.354^d	1.882^a	1.722^{ab}
9	Sorghum commercial + Phytase both starter & grower	1117^{cd}	3532^{ab}	4649^{ac}	776^{abc}	1898^{ab}	2674^{ab}	1.443^a	1.863^{ac}	1.739^{ac}
10	Sorghum commercial + Phytase -AvP & -Ca starter & grower	1128^c	3517^{ab}	4645^{ac}	781^{abc}	1884^{ab}	2666^{ab}	1.443^a	1.878^a	1.749^c
	LSD (P=0.05)	33.6	94.8	117.4	22.5	56.52	62.2	0.025	0.041	0.0261

Different superscripts in columns indicate significantly (P<0.05) different means; comparing wheat *vs.* sorghum.
1= Control wheat-based diet; 2= Sorghum H Pioneer Bonus, western downs Qld; 3= Sorghum M Pacific MR Buster (Liverpool plains, NSW), with reduced AME by 0.8MJ in starter phase only to switch to sorghum M during finisher period using normal sorghum AME value; 4= Sorghum B (Pacific Buster, Lowood, Qld); 5= Sorghum E= Pacific Buster (Lockyer, Qld) AME reduced by 0.8MJ starter phase only; 6= Commercial sorghum, purchased in Brisbane; 7= Sorghum K= Hylan Lyberty (Northern Downs, Qld); 8= Control wheat-based diet during starter period to switch to commercial sorghum during finisher period; 9= Commercial sorghum + phytase added on top; 10= Commercial sorghum with phytase added after reducing available phosphorous (%) from 0.44 to 0.38 starter period and from 0.34 to 0.30 finisher period. Calcium was reduced (%) from 0.85 to 0.75 starter period and from 0.68 to 0.60 finisher period.

ENERGY UTILISATION AND DIGESTIBILITY OF FATS AS INFLUENCED BY THE AGE OF BROILERS

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A study was conducted to investigate the influence of age of broilers on the apparent metabolisable energy (AME) and total tract fat digestibility of different types of fats (tallow, soybean oil, 50:50 mixture of tallow and soybean oil, poultry fat and palm oil). The assay diets were developed by substituting the different fats for 4 % (w/w) of a maize-soybean meal basal diet. The diets were offered *ad libitum* in mash form to six replicate cages of broilers (6 birds/cage) from day 1 to day 35 post-hatching. Total excreta collection was made during the first, second, third and fifth weeks for the determination of AME. The influence of fat type and age of birds on the AME (MJ/kg dry matter) and total tract digestibility coefficient of fats is summarised in the table below.

	AME	Fat digestibility
Fat type		
Tallow	24.4^c	0.45^c
Soybean oil	32.4^{ab}	0.73^a
Tallow: soybean oil (50:50)	28.7^{bc}	0.62^b
Poultry fat	29.5^{ab}	0.64^b
Palm oil	33.7^a	0.67^b
SEM	1.18	0.015
Age of birds (week)		
1	17.4^b	0.39^c
2	31.6^a	0.66^b
3	35.5^a	0.71^a
5	34.4^a	0.72^a
SEM	1.06	0.013
Probabilities, P ≤		
Fat type	***	***
Age of birds	***	***
Fat type x age of birds	NS	NS

NS, not significant; *** P < 0.0001.
^{a,b}Within each main effect, means in a column not sharing a common superscript are significantly different (P < 0.05).

The results showed that these were no fat type x age interaction (P > 0.05), indicating that the effect of age on the AME and total tract fat digestibility was similar for all fat types. The AME of palm oil, soybean oil and poultry fat were determined to be high, whereas tallow AME was lower (P < 0.05). Age of broilers significantly affected the AME of fats. The AME was markedly lower (P < 0.05) during week 1, but improved during week 2. There were no further improvements (P > 0.05) in the AME after week 2. The patterns observed for total tract digestibility of fat were somewhat similar, highlighting the physiological limitation in young birds to effectively digest and utilise fats and also confirm the poor digestibility of tallow in poultry.

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TOOLS IN EARLY NUTRITION TO MAXIMISE GROWTH PERFORMANCE

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Summary

The effect of a commercial yeast extract was evaluated in a commercial broiler operation in Victoria. Yeast extract was added to a commercial starter diet at 1.66% and growth performance of birds was recorded over a 15 month period. The addition of yeast extract resulted in small numerical improvements in weight gain and feed conversion ratio. An economic analysis based on the actual performance data showed that despite the lack of statistically significant differences in the evaluation, there was an economic benefit of 5 cents per bird. In conclusion, this study showed that it is difficult to demonstrate statistically significant differences in broiler performance when testing specific feed additives under commercial conditions. However based on the findings in this study and comparable findings from the literature the addition of yeast extract in broiler starter diets can provide a tool to increase profitability for broiler producers.

I. INTRODUCTION

Genetic progress has enabled the broiler industry to grow broilers much faster and for birds to achieve market weights at a much younger age. A key factor in achieving this potential is the rapid and early development of the gastrointestinal tract, and in particular the development of functional and mature villi. The actual formation of villi starts before hatch and the most rapid growth of the intestine occurs in the last 3 days of incubation (Uni et al., 2003). Post hatch the weight and size of the intestine increases more rapidly in comparison to other organs. Within the first hours post hatch the crypts begin to form and intensive cryptogenesis occurs. At the same time villi length increases rapidly before reaching a plateau around 8 days in the duodenum or 10 days in the jejunum and ileum (Sklan, 2004).

In addition to the physical growth of the intestine, enterocytes in the small intestine mature and become fully functional. At hatch protein and lipids from yolk are the only nutrients available to the chick; however pancreatic enzymes and brush border enzymes are detected in the embryonic intestine before hatch (Uni et al., 2003) and pancreatic enzyme activities, brush border enzyme activities and transporter activities continue increasing post hatch (Sklan, 2004). Several authors reported a more rapid development of the intestine when birds were given access to feed immediately post hatch (Ao and Choct, 2004; Sklan, 2003).
Parallel to the morphological development of the intestine, colonisation of the intestine with microorganisms starts immediately after hatch. Comprehensive work by (Lee et al., 2006) showed that the composition of the microbial community depends very much on the digestibility of the diet and therefore the nutrients available to the bird and the microflora respectively. Bacterial colonisation is also related to the response of the gut-associated lymphoid system (GALT). The protection in the intestine is a combination of the innate immune response and an adaptive immune response through the secretion of antibodies (Sklan, 2004). Particularly in the early stages of development the GALT rapidly adapts to distinguish antigens as part of the diet and antigens derived from pathogens.
Considering the requirement of nutrients for the development of the intestine and growth, and the challenges in balancing the microflora and immune response, appropriate nutrition

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and diet composition during the first week are crucial to maximise overall broiler performance. It is proposed that using a pre-starter diet based on highly digestible nutrients results in bodyweights 15-20% higher in comparison to a conventional starter diet (Leeson and Summers, 2005). In addition to the supply of highly digestible nutrients it is important to provide dietary nucleotides to support the rapid cell and tissue multiplication that occurs post hatch. The chick has the ability to synthesise nucleotides, however under conditions of rapid growth and increased immune challenge *de novo* synthesis appears to be inadequate (Rutz et al.*,* 2008). A potential source of dietary nucleotides for pre-starter diets is yeast extract. Using a commercial source of yeast extract (NuPro), Zauk et al. (2006) found an improvement in histological traits of villi in the intestine, however this did not result in any significant improvements in broiler performance. A lack of environmental challenges is the most likely explanation for this observation. This paper reports the result of a study investigating the addition of yeast extract in the starter phase under commercial conditions in Australia.

II. MATERIALS AND METHODS

The current experiment was conducted on a commercial grow out farm (Hazeldene's Chicken Farm) near Bendigo, Victoria over a period of 15 months (August 2008 to October 2009). Birds were raised in houses with identical layout and environmental conditions, and normal bird management was applied across the entire farm. The control group received a commercial four phase feeding program (starter, grower, finisher and withdrawal), with change-over of feed at approx. 10 days, 26 days and 35 days of age. The experimental group was fed a commercial starter diet with 1.66% yeast extract (NuPro®, Alltech Inc). All of the other diets were the same as the control group.

The farm was divided into 10 commercial broiler grow out sheds with a capacity of 40,000 birds each. One feed silo was allocated to 2 sheds therefore each cycle comprised of 5 experimental units. A total of 6 cycles were included in the evaluation, as a result each treatment was replicated 15 times over the 15 month period. Day old chicks (AA, Cobb and Ross) were supplied from breeder flocks of different ages. Feed intake and weight gain was measured at the end of the experiment and mortality was recorded on a weekly basis. Feed conversion was standardised to 2.45kg bodyweight. At the end of the experiment an economic analysis was conducted using the Alltech Poultry i-solution program (Francis and Sacranie 2009). Data were analysed according to the GLM procedure for ANOVA. Duncan's multiple-range test was used to separate means when significant effects ($P < 0.05$) were detected by analysis of variance.

III.   RESULTS

Overall performance in the current trial was comparable to industry standards. The addition of yeast extract in the starter diet of broilers resulted in small numerical improvements in broiler growth performance (Table 1). Average bodyweight at the end of the 42day grow–out cycle was around 2.5kg in both groups, with numerical advantage of 35g (1.4%) for birds fed yeast compared to the control. Similarly, the adjusted feed conversion ratio (corrected to 2.45kg life weight) was slightly improved (+3 points, 1.5%) for birds receiving a modified starter diet, however these differences were not statistically significant.

An economic analysis based on the actual performance data clearly showed that despite the lack of significant differences in the evaluation, there was a substantial economic benefit for birds fed a yeast extract in the starter phase. Under current market conditions (October 2009), the value per broiler sold based on feed cost and sales price was 5 cents higher for birds fed yeast extract in the starter phase in comparison to birds fed a

conventional commercial starter diet. The return on extra outlay (REO) was calculated to be 2.4:1 when taking into account the cost of the additive.

Table 1.   Growth performance of broilers on commercial diets with or without yeast extract (NuPro) in the starter phase

	Number of birds	Final weight	FCR	FCR[1]	EPEF[2]
Control	1,160,015	2.544	1.995	1.972	277
Yeast Extract	1,154,561	2.579	1.985	1.942	281
STD		0.120	0.075	0.097	21.2
Treatment		NS	NS	NS	NS

[1]FCR corrected to 2.45kg live weight, [2]EPEF = European Poultry Efficiency Factor

Table 2.   Economic analysis of broilers on commercial diets with or without yeast extract (NuPro) in the starter phase

	Control	Yeast Extract
Number of birds	1,160,015	1,154,561
Cost of yeast extract per broiler[2]	$0.00	$0.02
Feed cost per broiler harvested	$2.03	$2.07
Margin over feed /broiler	$4.33	$4.38
Extra value per broiler sold	---	$0.05

[1]the price per kg live weight was set at $2.50 [2]yeast extract cost as supplied by the commercial operator

IV. DISCUSSION and CONCLUSIONS

The benefits of highly digestible pre-starter diets has been the subject of a number of scientific publications, however only a few authors looked specifically at the benefit of yeast extract on lifetime performance of broilers (Leeson, 2008; Nollet et al., 2008; Rutz et al., 2008; Santos et al., 2008; Zhang et al., 2005). Although it is generally recognised that the addition of yeast extract can result in better growth performance all of these studies failed to demonstrate a significant improvement in either weight gain or feed conversion ratio. For example, Leeson (2008) reported that feeding a pre-starter diet with yeast extract, organic minerals and Bio-Mos in the first 4 days resulted in a numerical improvement of 9% (2670g vs 24560g) at 42days. Similarly, Nollet et al. (2008) showed that adding 2% yeast extract in the first 2 weeks of age reduced the FCR by 3 points at 42days (1.632 vs 1.660). The findings in the current experiment under commercial conditions are in line with these reports. The lack of statistically significant differences in performance data is due to larger variation under commercial conditions compared to trials conducted in university or research settings and the limited number of replicates. Rutz et al. (2007) reported significant improvements in weight gain and feed efficiency at 42 days by inclusion of 2% and 1.5% of yeast extract in pre-starter diets under strictly controlled trial conditions.

Considering the number of studies in the literature which do show significant benefits when using yeast extract an economic analysis was conducted to determine the actual cost benefit of using yeast extract in the current experiment. Although the extract was only added in the starter phase (day 0-10) no actual figures on exact feed intake in this period were available, it is assumed that starter feed is 8% of the total feed consumed (Kenny and Kemp, 2003). Based on current market conditions (October, 2009) the benefit of adding yeast extract to a starter diet is 5 cents per bird.

In conclusion, this study shows that it is difficult to demonstrate significant differences in broiler performance when testing specific feed additives under commercial conditions. Nevertheless, based on the findings in this study and comparable findings from the literature the addition of yeast extract in broiler starter diets can provide a tool to increase profitability for broiler producers.

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DIETS HIGH IN LINOLEIC ACID REDUCE OMEGA-3 LONG CHAIN POLYUNSATURATED FATTY ACIDS IN CHICKEN TISSUES

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Summary

We have previously demonstrated that the level of omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) could be increased several fold by increasing the level of alpha-linolenic acid (ALA) in broiler feed. The lowest LA to ALA ratio of experimental diets resulted in the highest n-3 LCPUFA, EPA, DPA and DHA in both breast and thigh tissues. Because the n-6 PUFA, linoleic acid (LA), competes for the enzymes used to produce n-3 LCPUFA, the effect of dietary LA on n-3 LCPUFA accumulation in chicken meat was unclear. The objective of this study was to examine the effect of varying LA levels in diets on the conversion of ALA into EPA, DPA and DHA into chicken tissues. The level of ALA in the diets was held constant at 2.1% energy (% en) while the level of LA varied from 2.9 to 4.4% en. The ratio of LA to ALA of the experimental diets thus ranged from 1.4:1 to 2.1:1. The results indicated that the total n-3 LCPUFA levels in the breast meat of birds fed with the lowest LA content was 16% higher than the n-3 LCPUFA in the breast of birds fed with the highest LA content. In general, the decrease in n-3 LCPUFA due to inhibition by LA was less than the stimulatory effect of an equivalent level of ALA on n-3 LCPUFA accumulation. This study indicated that the strongest influence on n-3 LCPUFA accumulation in chicken tissues was the level of ALA in the diet. The experimental diets did not appear to affect the growth performance of chickens. We conclude that there was only a modest effect of dietary LA on omega-3 LCPUFA accumulations in chicken meat, but diets that are lower in LA will allow greater conversion of ALA into n-3 LCPUFA.

I. INTRODUCTION

There have been a number of reports of n-3 fatty acid enrichment of chicken meat through dietary enrichment with vegetable oils containing the n-3 precursor, ALA; however, the levels of n-3 LCPUFA found in meat have been low (Febel et al., 2008; Zelenka et al., 2008). Recently we have demonstrated that diets with high ALA content increased the incorporation of EPA and DHA into breast and thigh meat to levels 5 and 4-fold relative to birds fed low ALA (Kartikasari et al., 2009). The levels of n-3 LCPUFA achieved in our study were much higher than levels reported previously (Febel et al., 2008; Zelenka et al., 2008). The improvement in meat n-3 LCPUFA status was achieved by increasing the level of ALA in the diets and keeping a constant LA level in order to optimize the conversion of ALA into EPA and DHA. However, LA and ALA compete as substrate for desaturation and elongation enzymes in fatty acid metabolism (Liou et al., 2007). The results of this study indicated that the conversion of ALA was mainly driven by the level of ALA in the diet. Experimental diets with the lowest LA to ALA ratio resulted in the highest EPA, DPA and DHA in these tissues.

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These results indicated that increasing the ALA content of the diets was important but it was unclear whether keeping a constant LA level is also important in the regulation of EPA, DPA and DHA accumulation in chicken meat. The objective of the current study was to examine the effect of LA levels in diets on the conversion of ALA into EPA, DPA and DHA into chicken tissues.

II. MATERIALS AND METHODS

One-day-old mixed sex broiler chickens (48 Cobb 500) were used in this study. The birds were randomly housed in six pens (n=8 birds per pen) and distributed among three dietary treatments (2 pens for each dietary treatment) and reared for 28 days under controlled environmental conditions. The experiment was approved by the Animal Ethics Committees of the Department of Primary Industries and Resources South Australia and the University of Adelaide.

The three diets were based on a standard commercial starter diet, with a low level of fat. The dietary treatments had the same nutritional values as the basal diet except for the fatty acid composition of the fats. Pure or blended vegetable oils including macadamia, flaxseed and sunflower oils were included at a level of 2.8% in order to produce diets with the desired levels of LA and ALA. The LA level of control diet was 2.3% en with low ALA level (0.2% en). The experimental diets were formulated by varying the levels of LA, which ranged from 2.9 to 4.4% en, with ALA levels kept constant at 2.1% en. The total fat content was kept constant at approximately 5%. Each diet was provided *ad libitum* for the duration of the 28-d growth period. At 28 days post-hatch, six selected birds from each pen (12 birds per group) were weighed individually and breast and thigh tissues were collected. Total lipids in diet and tissue samples were extracted following the method of Folch et al. (1957) and the phospholipids were methylated as described by Blank et al. (2002). The fatty acid composition of tissue samples was determined and quantified by gas chromatography.

III. RESULTS

Elevating the level of dietary LA had an effect that was not quite significant on ALA levels in breast tissues and only reached significance in thigh tissue ($P < 0.05$). The diets caused a consistent reduction in EPA levels in all tissues ($P < 0.01$; Table 1 and 2), but DPA and DHA levels were unaffected by dietary LA. While increased levels of LA tended to decrease the level of total n-3 PUFA ($P < 0.1$), the level of total n-6 fatty acids increased ($P < 0.01$). The highest level of dietary LA (4.4% en) resulted in the highest level of total breast and thigh phospholipids n-6 fatty acids, which was 27.28 and 29.15% of total fatty acids, respectively. Elevating dietary LA increased the level of LA in all tissues but this was not translated to changes in AA levels. The increased levels of dietary LA from 2.9 to 4.4% en did not affect tissue fat content including breast and thigh and final weight of birds at 28 days of age.

IV. DISCUSSION

The results observed in this study indicated that increasing the dietary concentration of LA as energy from 2.9 to 4.4% whilst keeping a constant ALA level reduced tissue (breast and thigh) n-3 LCPUFA (EPA) accumulation; however DPA and DHA tended to not be affected. The decrease in n-3 LCPUFA levels might be due to competition between ALA, the precursor to n-3 LCPUFA, and LA for Δ6-desaturase. Thus, a high dietary level of LA might depress the conversion of ALA to n-3 LCPUFA (Arbuckle et al., 1992; Liou et al., 2007).

Table 1.   Fatty acid composition of breast phospholipids from chickens fed experimental diets varying in LA while holding ALA constant (2.1% en) for 28 days

	Experimental diets				
	Control (0.2% en ALA)	2.9 % en LA	4.4% en LA		
Fatty acids (%)				PSEM	P value
SFA	35.63	38.36[a]	39.40[b]	0.107	*
MUFA	38.08	26.51[b]	21.56[a]	0.825	*
18:2n-6	14.33	14.80[a]	18.31[b]	0.251	*
20:4n-6	3.48	3.62	4.78	0.255	NS
Total n-6	21.39	21.63[a]	27.28[b]	0.395	*
18:3n-3	0.40	1.52[b]	1.34[b]	0.061	NS
20:5n-3	0.62	2.63[b]	2.00[a]	0.054	*
22:5n-3	0.85	4.24	4.11	0.268	NS
22:6n-3	0.94	3.23	2.63	0.108	NS
Total n-3	3.09	12.66	11.09	0.366	NS
Total PUFA	24.48	34.29[b]	38.37[c]	0.750	NS

Values are means of twelve observations (n = 12) per treatment and their pooled standard error of the mean (PSEM). [a,b]Values in the same row with no common superscript are significantly different (P < 0.05). SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid. NS =not significant; *P < 0.05.

Table 2.   Fatty acid composition of thigh phospholipids from chickens fed experimental diets varying in LA while holding ALA constant (2.1% en) for 28 days

	Experimental diets				
	Control (0.2% en ALA)	2.9% en LA	4.4% en LA		
Fatty acids (%)				PSEM	P value
SFA	34.81	37.40[a]	38.52[b]	0.089	*
MUFA	39.10	27.32[b]	22.27[a]	0.692	*
18:2n-6	14.22	16.67[a]	20.42[b]	0.354	*
20:4n-6	4.49	4.27	5.37	0.214	NS
Total n-6	21.67	23.49[a]	29.15[b]	0.361	**
18:3n-3	0.32	1.48[b]	1.25[a]	0.023	*
20:5n-3	0.52	2.15[b]	1.46[a]	0.059	*
22:5n-3	0.98	4.12	3.93	0.172	NS
22:6n-3	0.83	2.63	2.11	0.100	NS
Total n-3	2.85	11.10	9.53	0.310	NS
Total PUFA	24.53	34.60[a]	38.69[b]	0.670	*

Values are means of twelve observations (n = 12) per treatment and their pooled standard error of the mean (PSEM). [a,b]Values in the same row with no common superscript are significantly different (P < 0.05); *P < 0.05, **P < 0.01.

Although increasing the proportion of LA in the diet appeared to decrease n-3 LCPUFA accumulation in tissue samples, the effect was similar in size to the increases in n-3 LCPUFA caused by increasing dietary ALA by a similar amount. The total n-3 LCPUFA levels in breast and thigh of birds fed with the lowest LA content (2.9% en) was 15.6 and 18.7%, respectively higher than the n-3 LCPUFA in the breast and thigh of birds fed the highest LA content (4.4% en). These findings indicate that the effect of LA content in the diets is important in regulating the n-3 LCPUFA accumulation in tissues. The changes found were in concordance with other authors (Marangoni et al., 1992; Liou et al., 2007; Zelenka et al., 2008). Zelenka et al. (2008) reported that there was no effect on the level of DPA and DHA both in breast and thigh meat by increasing dietary levels of LA in the diet of chickens from 14.2 to 58.4% of total fatty acids. Conversely, we found that with increasing LA content in the diet, the level of LA rose in tissue samples, but n-6 LCPUFA, AA, did not respond. These findings also agreed with studies reported previously (Marangoni et al., 1992; Hussein et al., 2005).

The increased levels of dietary LA did not affect tissue fat content. This result is in concordance with those of Zelenka et al. (2008) using increased levels of dietary LA. Their results show that there was no difference on fat content of breast by increased levels of dietary LA from 14.22 to 58.44 g/kg. The experimental diets did not influence significantly the final weight of birds. A number of investigators (Febel et al., 2008; Kavouridou et al., 2008) have reported similar findings regarding the final weight among broilers fed different types of fat sources involving vegetable oils.

In summary, increasing the levels of dietary LA reduced tissue n-3 LCPUFA (EPA and to a lesser extent DPA and DHA) concentration. In addition, the concentration of LA and total n-6 fatty acids appeared to be strongly influenced by the level of dietary LA while the accumulation of AA tissue samples did not change. We conclude that diets that are lower in LA will allow greater conversion of ALA into n-3 LCPUFA.

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THE VULNERABILITY OF SORGHUM TO 'MOIST-HEAT'

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Summary

A hydrothermal procedure involving microbial phytase and citric acid reduced the phytate content of sorghum by 89.8%. A slurry of equal parts finely-ground sorghum and distilled water was mixed for 2 hours at 45°C followed by drying at 60°C for 70 hours. However, both dephytinised and sham-treated sorghum significantly depressed growth performance and N retention to very similar extents. Collectively, the hydrothermal procedure reduced weight gain by 27.0%, feed intake by 20.3% and feed efficiency by 9.5% in broilers from 7-21 days post-hatch and N retention was reduced by 8.0% or 5.05 percentage units. The potential benefits of dephytinisation were presumably overwhelmed by the vulnerability of the nutritive value of sorghum to moist-heat as demonstrated in broiler chickens in this study, the implications of which are considered.

I. INTRODUCTION

A hydrothermal procedure was developed to dephytinise sorghum with microbial phytase and citric acid. An important prerequisite was that placebo or sham-treatment needed to be innocuous but an evaluation of sham-treated sorghum in broilers demonstrated that this exposure to moist-heat significantly depressed growth performance and nitrogen (N) retention. The unique vulnerability of the *in vitro* protein digestibility of sorghum to wet-cooking procedures, or 'moist-heat', has been extensively documented; however, few assessments of the impact of wet-cooked sorghum on growth performance and nutrient utilisation in broilers have been reported.

II. MATERIALS AND METHODS

The hydrothermal dephytinisation procedure involved mixing finely hammer-milled sorghum (1 mm sieve) with an equal quantity of distilled water plus 10,000 FTU/kg *Aspergillus niger* phytase and 3 g/kg citric acid. The slurry was constantly agitated for 2 hours at 45°C and then oven-dried at 60°C for 70 hours to a moisture content of approximately 5%. Sham-treatment followed the same procedure but without the addition of phytase and citric acid. Citric acid reduced the pH of the slurry from 6.24 to 4.89. Phytate concentrations were determined by a 'ferric chloride-precipitation' method described by Miller et al. (1980). The procedure reduced the phytate content of sorghum by 89.8%, from 10.04 to 1.03 g/kg on a dry matter basis.

Three sorghum-casein diets containing either control, sham-treated or dephytinised sorghum were prepared in which citric acid was either added as a dietary ingredient or incorporated into the dephytinised sorghum. Each of the dietary treatments was offered to 7 replicates (6 birds per cage) of male Cobb chicks from 7-21 days post-hatch to determine treatment effects on growth performance. Total excreta were collected over the final 72 hours to determine apparent metabolisable energy (AME) and N retention by standard procedures.

Experimental data was analysed by one-way analysis of variance using general linear model procedures (SPSS® Inc.). Least significant differences were calculated to separate

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mean values where the effect of treatment was significant at the 5% level of probability. The conduct of the experiment complied with specific guidelines set down by the Animal Ethics Committee of Sydney University.

III. RESULTS

As shown in Table 1, both dephytinised and sham-treated sorghum significantly depressed growth performance (P < 0.001) and N retention (P < 0.02) to very similar extents in comparison to untreated sorghum. Collectively, exposure of sorghum to moist-heat reduced weight gain by 27.0% (357.5 versus 490 g/bird), feed intake by 20.3% (659.5 versus 827 g/bird) and feed efficiency by 9.5% (1.85 versus 1.69) from 7-21 days post-hatch. Also, N retention was reduced by 5.05 percentage units or 8.0% (57.675 versus 62.72%), while AME was numerically depressed.

Table 1. Effects of moist-heated (sham-treated and dephytinised) sorghum on growth performance and nutrient utilisation of broilers.

Treatment	Growth performance			Nutrient utilisation	
	Weight gain (g/bird)	Feed intake (g/bird)	Feed efficiency (g/g)	AME (MJ/kg DM)	N retention (%)
Sorghum					
Untreated	490[a]	827[a]	1.69[a]	15.48	62.72[a]
Sham-treated	352[b]	650[b]	1.86[b]	15.28	57.36[b]
Dephytinised	363[b]	669[b]	1.84[b]	15.33	57.99[b]
SEM	15.75	21.38	0.0262	0.0689	1.246
Significance (P =)	0.000	0.000	0.000	0.128	0.013
LSD (P < 0.05)	46.8	63.5	0.077	-	3.701

[ab]Means with different superscripts within columns are significantly different (P < 0.05)

IV. DISCUSSION

Clearly, sham-treatment of sorghum under the conditions described was not innocuous and the hydro-thermal process is not suitable for the evaluation of dephytinised sorghum to define the anti-nutritive effects of phytate. In this context, the caution issued by Harland and Oberleas (1987) is instructive because they concluded that there is no practical way to remove phytate from feed ingredients without altering nutrient bioavailability. However, the addition of citric acid to the slurry facilitates the enzymic degradation of phytate (Selle, 2006), which is probably mainly achieved via the reduction in slurry pH from 6.24 to 4.89 pH. Phytate is mainly present in feedstuffs as a mineral-phytate complex involving magnesium (Mg) and potassium (K) as Mg_5-K_2-phytate (Lott et al., 2004). The pH reduction would substantially increase the solubility of magnesium-phytate complexes in sorghum (Cheryan et al., 1983) and facilitate the enzymic degradation of phytate. Nevertheless, the potential benefits of dephytinisation were presumably overwhelmed by the vulnerability of the nutritive value of sorghum to moist-heat under the conditions of this experiment.

Sorghum is perhaps uniquely vulnerable to moist-heat as Hamaker et al. (1987) reported that wet-cooking reduced the *in vitro* pepsin digestibility of sorghum by 24.5% (0.563 versus 0.808) in contrast to more modest reductions in maize (4.1%), wheat (5.4%),

rice (9.1%) and barley (13.0%). As reviewed by Duodu et al. (2003), exposure of sorghum to moist-heat induces disulphide cross-linkages and conformational changes particularly in β- and γ-kafirin and this, in turn, reduces the digestibility of α-kafirin which is centrally located in protein bodies in the endosperm. Recently, Emmambux and Taylor (2009) reported that boiling a 5:1 mixture of distilled water and sorghum for 30 minutes reduced *in vitro* pepsin digestibility of kafirin by 24% (0.576 versus 0.760); however, pepsin digestibility of sorghum protein was similarly reduced by 27% (0.599 versus 0.826). Interestingly, these results suggest that the 'non-kafirin' component of sorghum protein, which is predominantly glutelin, is equally vulnerable to moist-heat.

Few *in vivo* assessments of wet-cooked sorghum have been reported. However, moist-heating a tannin-free sorghum depressed protein digestibility in rat balance studies and reduced total tract digestibility of lysine and threonine by approximately 10% (Knudsen et al., 1988). In the Mitaru et al. (1985) study, moist-heat consisted of boiling a 3:1 slurry of distilled water and a low-tannin sorghum for 50 minutes and the impact of moist-heat on true ileal digestibility (TID) of sorghum amino acids in broilers was determined. This procedure reduced the average TID coefficients of 15 amino acids by 30.7% (0.629 versus 0.908) in sorghum.

Wet-cooking did not significantly influence the AME of sorghum in the present experiment, which suggests that starch digestibility was not altered. This is somewhat surprising as Ezeogu et al. (2008) concluded that disulphide cross-linkages in the protein matrix induced by wet-cooking limits expansion of starch granules and access of amylase to its substrate and compromises starch digestibility.

Steam-pelleting broiler diets at high temperatures may have detrimental effects on broiler performance (Raastad and Skrede, 2003; Creswell and Bedford, 2006). Moreover, the starch gelatinisation temperature for sorghum is higher than maize and wheat (Taylor and Dewar, 2001), which suggests that sorghum-based broiler diets are steam-pelleted at higher temperatures. Thus, there is the possibility that steam-pelleting sorghum-based broiler diets at high temperatures may constitute sufficient moist-heat to compromise the nutritive value of sorghum. However, as discussed by Taylor (2005), relevant investigations do not appear to have been completed.

It is noteworthy, however, that Zhuge et al. (1990) reported that increasing wet-extrusion temperatures of sorghum from 105 to 132.5ºC, prior to its dietary incorporation, significantly depressed weight gain by 17.0% (1235 versus 1488 g/bird) and feed efficiency by 11.7% (1.883 versus 1.686) in a 35-day broiler bioassay. The performance of birds offered sorghum extruded at 105ºC was comparable to the other processing methods (hammer-milling, roller-milling, flaking) evaluated. This could indicate that steam-pelleting sorghum-based diets at 90-95ºC may not be deleterious; nevertheless, the Zhuge et al. (1990) study demonstrates that exposing sorghum to sufficient moist-heat has the capacity to depress broiler growth performance. This was shown in the present experiment with the indication that protein utilisation was compromised, probably by the induction of disulphide cross-linkages, particularly in the kafirin protein fraction of sorghum.

In conclusion, an evaluation of the impact of steam-pelleting temperatures on growth performance and nutrient utilisation of broilers offered sorghum-based diets appears justified. Additional factors to be taken into consideration should include grain texture, grinding method and particle size, and pellet 'hardness'. Parsons et al. (2006) reported that broilers offered a hard pellet (1856 g pellet breaking force) performed significantly better than broilers on soft pellets (1662 g pellet breaking force). One possibility is that broilers may 'prefer' hard pellets (Nir, 1987) and the second is that hard pellets may stimulate gizzard function in an analogous manner to whole grains (Cumming, 1994).

V. ACKNOWLEDGEMENT

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THE NUTRITIVE VALUE OF HIGH-YIELDING TRITICALE VARIETIES AND THEIR POTENTIAL FOR INCLUSION IN POULTRY DIETS

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Triticale is a cereal grain that holds great promise as an alternative to wheat and other conventional grains used in poultry diets. Triticale generally has a higher yield than wheat and adapts to more difficult agronomic conditions than wheat (Korver *et al.,* 2004). A crop breeding group at the University of New England (UNE) has developed varieties that are even more high-yielding and more disease-resistant than the current commercial strains. These varieties will need further evaluation to establish their potential for animal, and particularly poultry feeding.

Eight varieties of triticale were obtained from the breeding group and subjected to detailed and proximate analysis, prior to feeding trials. Cultivar H116 contained more protein than the other cultivars (139 g/kg) while the lowest protein content was observed in cultivar H127 (Table 1). The cultivars were very similar in lysine content, containing between 4.3 g/kg in cultivar H127 and 4.9 g/kg in H249. Methionine content varied from 1.6 (cultivar H127) to 2.0 g/kg in cultivar H249. The gross energy content of the grains ranged from 18.2 (H127 and 128) to 18.5 MJ/kg (H55); the other five cultivars being iso-caloric at 18.4 MJ/kg. Crude fat was also highest (27.4 g/kg) in cultivar H55 and lowest in H128, and this may be the major cause of differences in energy values of between these two cultivars. Total starch varied from 578 g/kg in cultivar H157 to 657 g/kg in H249. Cultivar H55 was the highest in non-starch polysaccharides, 139 g/kg while H20 contained only 90 g/kg.

Calcium content varied from 0.3 to 0.5 g/kg, respectively in cultivars H426 and H55, while the phosphorus content was highest (4.4 g/kg) in cultivar H157 and lowest (3.5 g/kg) in H20. Phytate was quite high in all cultivars, generally close to half of the total P content. Cultivar H20 had the lowest level of phytate, 1.8 g/kg, while the highest amount, 2.2 g/kg, was found in H249.

Table 2 shows the digestibility of dry matter, starch and viscosity of samples during *in vitro* digestion. The *in vitro* method was adapted from Babynsky *et al* (1990), with slight modifications. The *in vitro* dry matter digestibility (IVDMD) varied between 71.1 % (H55) and 77.5 % (H128). Starch digestibility was between 19.5 % (H249) and 40.3 % (H157), and this low *in vitro* digestibility may be due to the high content of resistant starch. Cultivar H55 was the most viscous during digestion (1.2 cP) and this may be due to the high concentration of NSP in this cultivar.

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Tabel 1.   Chemical composition (g/kg, dry matter) of the different varieties of triticale

Component	Variety							
	H116	H127	H128	H157	H20	H249	H426	H55
Dry matter	879	873	873	873	872	875	871	873
Ash	19.2	17.4	18.1	18.7	18.1	17.5	17.5	16.7
Crude protein	139	125	135	136	135	136	132	126
Gross Energy (MJ/kg)	18.4	18.2	18.2	18.4	18.4	18.4	18.4	18.5
Crude fat	18.5	15.9	13.9	14.6	16.3	17.5	19.1	27.4
Ca	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.5
P	4.1	4.0	4.1	4.4	3.5	3.6	4.0	3.7
K	5.7	5.5	5.8	5.7	5.9	5.4	5.7	5.0
Mn (mg/kg)	45.7	59.6	44.1	59.7	53.5	49.4	45.9	47.3
Na (mg/kg)	26.5	16.6	33.1	35.0	17.7	38.8	13.8	30.1
Total Starch	612	654	639	578	612	657	626	592
Resistant starch	*442*	*478*	*484*	*463*	*455*	*509*	*453*	*453*
P-Phytate	2.0	2.1	2.1	2.2	1.8	2.2	2.0	1.9
Total NSP	102	104	100	119	90	96	100	139
Soluble NSP	*10*	*12*	*10*	*10*	*11*	*9*	*10*	*11*
Insoluble NSP	*92*	*91*	*89*	*110*	*79*	*87*	*90*	*128*
Free sugars	31	27	29	23	31	27	33	28
Amino Acids								
Arginine	7.5	6.6	7.6	7.4	7.3	7.9	7.6	7.4
Threonine	4.4	3.9	4.6	4.4	4.3	4.7	4.4	4.5
Alanine	5.9	5.1	5.7	5.5	5.8	6.0	5.6	5.4
Lysine	4.6	4.3	4.8	4.6	4.7	4.9	4.7	4.6
Methionine	1.9	1.6	1.8	1.9	1.9	2.0	2.0	1.8
Valine	6.5	5.5	6.5	6.4	6.4	6.8	6.4	6.1
Isoleucine	4.9	4.1	4.8	4.8	4.7	5.0	4.8	4.5
Leucine	9.1	8.0	9.3	9.2	8.9	9.5	9.0	8.8
Phenylalanine	6.2	5.6	6.6	6.4	6.0	6.5	6.4	6.1

Tabel 2.   *In vitro* digestibility of samples

Component	Variety							
	H116	H127	H128	H157	H20	H249	H426	H55
Dry matter digestibility (%)	75.8	76.3	77.5	74.0	75.4	77.3	77.4	71.1
Starch digestibility (%)	22.0	24.4	25.8	40.3	20.9	19.5	39.4	30.2
Viscosity during digestion (cP)	1.0	1.1	1.0	1.0	1.0	1.1	1.0	1.2

INFLUENCE OF HOUSING SYSTEMS ON THE BACTERIOLOGICAL QUALITY AND SAFETY OF TABLE EGGS

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Summary

With the introduction of alternative housing systems for laying hens in the EU, recently more research focused on the bacterial contamination of table eggs, e.g. eggshell and egg content contamination. Contamination of eggshells with aerobic bacteria is generally higher for nest eggs derived from non-cage systems compared to furnished cages or conventional cages. Studies indicate limited or no systematic differences in eggshell contamination with aerobic bacteria between eggs laid in the nest boxes of furnished cages and eggs laid in conventional cages. The major differences found in experimental studies between cage- and non-cage systems are less pronounced under commercial conditions. The effect of housing system on eggshell contamination with specific groups of bacteria is variable. Limited information is available on the influence of housing system on egg content contamination. Recent research does not indicate large differences in egg content contamination between eggs from cage- and non-cage systems (ignoring outside nest and floor eggs). The microflora of the eggshell is dominated by Gram-positive bacteria, whereas Gram-negative bacteria are best equipped to overcome the antimicrobial defences of the egg content. Much of the research on eggshell and egg content contamination focuses on *Salmonella,* since infection with *Salmonella* Enteritidis, resulting from the consumption of contaminated eggs or egg products, is still a major health problem. Observed *Salmonella* prevalence on the eggshell and in the egg content vary, depending on the fact whether investigations were based on randomly sampled table eggs or on eggs from naturally infected hens. Studies also show that it is highly unlikely that a move from conventional cages to alternative cage systems and non-cage housing systems will result in an increase in *Salmonella* infection and shedding, rather the opposite is expected.

I. INTRODUCTION

The shell can become contaminated when passing through the vent, but many researchers suggest that the main bacterial contamination occurs within a short period after laying due to contact with dirty surfaces (Quarles et al., 1970; Gentry and Quarles, 1972). Messens et al. (2005) and De Reu et al. (2006a; 2006b, 2006d) reported that increasing numbers of micro-organisms on the eggshell consequently increase the risk of microbial eggshell penetration and egg content contamination. Beside this horizontal route of bacterial infection of eggs, egg contamination also occurs through the vertical or transovarian route. In the transovarian route (vertical transmission), the yolk membrane (very infrequently the yolk itself), the albumen

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and/or the shell membranes are directly contaminated as a result of bacterial infection of the reproductive organs.

II. GLOBAL BACTERIAL EGGSHELL AND EGG CONTAMINATION

a) Effect of the housing system

In early studies, bacterial eggshell contamination has been compared in litter and wire floor houses. Quarles et al. (1970) reported that litter floor houses had on average nine times more bacteria in the air, and 20 to 30 times more aerobic bacteria on the shell than wire floor houses. Harry (1963) reported that the shells of eggs from deep litter systems had 15 times more bacteria and a higher proportion of potential spoilage organisms than eggs from battery cage systems.

Conventional cage housing for laying hens will be prohibited from 2012 in the European Union, following EU-directive 1999/74. From 2012 onwards, only furnished cages and non-cage systems (aviaries and floor housing) will be allowed. This has driven the recent attention towards the effect of housing system on the bacterial eggshell contamination of table eggs.

b) Conventional and furnished cages

De Reu et al. (2005b) compared the bacterial eggshell contamination of eggs laid in conventional cages with eggs laid in the nest boxes of furnished cages. No systematic difference in shell contamination with total counts of aerobic bacteria was found between these systems (ranging from 4.0 – 4.5 log CFU/eggshell). Also, for Gram-negative bacteria no difference was detected (both means ca. 3.0 log CFU/eggshell). The type of nest-floor material in the nest boxes of the furnished cages also did not systematically influence the bacterial eggshell contamination. Cepero et al. (2000; 2001) also found no differences in counts of aerobic mesophilic bacteria but reported a higher prevalence of coliforms on shells of eggs laid in furnished cages. Mallet et al. (2006) studied the hygienic aspects of eggs laid at different locations in furnished cages. A significant difference in total count of aerobic bacteria was observed on the eggshell of eggs collected from furnished cages (4.83 log CFU/eggshell) compared to conventional cages (4.56 log CFU/eggshell). This was mainly due to the eggs laid outside the nest in the litter area (4.96 log CFU/eggshell) or in the cage (4.94 log CFU/eggshell). The bacterial load on eggs laid in the nests was similar to those collected from the conventional cages. Similar conclusions were obtained for *Enterococcus.* Wall et al. (2008) also found a higher bacterial load on eggs from furnished cages compared to conventional cages. The bacterial counts were significantly (P < 0.001) higher in the furnished cages compared to the conventional cages as regards *Enterococcus* and total number of aerobic bacteria.

c) Cage- and non-cage systems

In further experimental studies, it was found that eggs from aviaries were contaminated with higher numbers of aerobic bacteria than eggs from cage systems (Protais et al., 2003a; De Reu et al., 2005b). The difference was more than 1 log unit (up to 5.1 – 6.0 log CFU/eggshell for eggs from aviaries), with much higher counts on those eggs laid on the floor of the aviaries (up to 7 log CFU/eggshell). For Gram-negative bacteria no systematic differences were found between cage and non-cage housing systems (De Reu et al., 2005b).

d) Experimental studies compared to on farm studies

De Reu et al. (2005a; 2006c) evaluated whether the differences in initial eggshell contamination, found in the experimental housing systems, were also applicable to

commercial conventional cage and non-cage housing systems. Two conventional cage systems, one organic aviary system and one floor housing system were included. On average, a higher (P < 0.001) initial eggshell contamination with total count of aerobic bacteria was found for eggs from non-cage systems compared to conventional cage systems; respectively 5.46 compared to 5.08 log CFU/eggshell. However, initial contamination with total count of Gram-negative bacteria on the eggshells was significantly lower (P < 0.001) in the non-cage systems; 3.31 compared to 3.85 log CFU/eggshell. This study showed that the major differences in eggshell contamination with total count of aerobic bacteria, found between conventional and non-cage systems in the experimental studies (>1 log) were less pronounced in the sampled commercial housing systems. The even lower initial contamination with Gram-negative bacteria in the commercial non-cage systems was remarkable.

e) On-farm studies

Six flocks of laying hens in furnished cages and seven flocks in non-cage systems (three aviaries and four floor systems) were compared in the international study of De Reu et al. (2009b). On average, eggshells from furnished cages were slightly, but significantly (P < 0.001), less contaminated with total count of aerobic bacteria compared to non-cage eggshells (4.75 versus 4.98 log CFU/eggshell). In the non-cage systems, no difference in average contamination between aviary and floor systems was found. Both within the groups of furnished cage- and non-cage systems, major differences between farms were obtained. Differences in farm management can possibly explain this. For Enterobacteriaceae no significant difference in average eggshell contamination was found between furnished and non-cage systems. Hunau-Salaun et al. (2009) also found a comparable higher eggshell contamination for eggs from non-cage systems (4,82 CFU/eggshell) compared to conventional cages (4,40 CFU/eggshell). On the other hand, no difference between furnished cages and non-cage systems was found.

f) Bacterial air contamination and its relationship with eggshell contamination

In some studies the total count of aerobic bacteria in the air of poultry houses was proven to be positively correlated with the initial bacterial eggshell contamination at the henhouse (Protais et al., 2003a; De Reu et al., 2005b). Averages of 4 log CFU/m^3 air for the conventional and furnished cages were found compared with a 100 times higher average (> 6 log CFU/m^3) for aviary housing systems.

g) Influence of housing system on quality of eggs and egg products

At this moment, it remains unknown whether the differences in bacterial counts on the shell of eggs produced in different housing systems have an impact on the quality of eggs and egg products. Harry (1963), De Reu et al. (2006b; 2006d) and many other researchers found a correlation between bacterial eggshell contamination and egg infection or egg content contamination. The higher prevalence of coliforms on the shells of eggs laid in furnished cages was not correlated with signs of coliform contamination in egg yolk or albumen (Cepero et al., 2000; Cepero et al., 2001). In a preliminary study of De Reu et al. (2007a; 2008), egg content contamination of nest eggs was 1.9% (5/269 eggs) for furnished cages compared to 2.3% (10/432 eggs) for non-cage systems.

III. EGGSHELL DIRT AND CRACKS IN DIFFERENT HOUSING SYSTEMS

a) Eggshell dirt

Beside bacterial eggshell contamination the occurrence of eggshell dirt may also be considered as a hygiene parameter. Additionally some types of eggshell dirt may give nutrients to bacteria present on the shell. In table 1 the occurrence of dirty eggs in different housing systems, found by different research groups, is summarized.

Table 1.   Occurrence of dirty eggs (in % of eggs) in different housing systems

Study	Type of study	CC (%)	FC (%)	Non-cage (%)	P
Tauson et al. 1999	Pilot	6.5	n.a.	5.7	
Mallet et al. 2006	Pilot	4.9 (4.9-4.9)	5.0 (3.0-7.1)	n.a.	< 0.001
De Reu et al. 2009b*	Comm.	n.a.	22	24	> 0.05
De Reu et al. 2009a	Comm. (shop)	17.2	n.a.	4.4	n.d.

CC = conventional cage, FC = furnished cage, Comm = commercial housing *= only nest eggs; n.a. = not analyzed; n.d. not determined

Both in the commercial studies of De Reu et al. (2009a, 2009b) as in the pilot study of Tauson et al. (1999) it was found that the frequency of dirty eggs in nests of non-cage systems was not higher than in cage systems. On the other hand the study of Mallet et al. (2006) showed that furnished cages can contain more dirty eggs compared to conventional cages. Two types of conventional cages and two types of furnished cages were compared. The study showed that a less optimal cage design of the furnished cages increased the number of outside nest eggs which contained more eggshell dirt compared to the nest eggs. As a result a significant difference was found in occurrence of dirty eggs between both types of furnished cage designs; 3.0 and 7.1% respectively (Table 1). Comparing both conventional cage designs with both furnished cage designs, on average both types of housing systems contained a comparable amount of dirty eggs (4.9% compared to 5.0%). To conclude the available research results indicate that nest eggs of non-cage systems are in normal circumstances not more susceptible for dirtiness compared to cage eggs.

An important aspect is also that 85 up to 98% of the floor eggs of non-cage systems have dirty eggshells (Abrahamsson and Tauson, 1998, De Reu et al. 2006a). This stresses the fact that floor eggs are unfit as table eggs.

b) Eggshell cracks

As eggshell cracks give the opportunity for bacteria to penetrate the eggshell and hence contaminate the egg content, the occurrence of cracks in eggs is also an important factor in comparing housing systems. In table 2 the results of different research groups studying the influence of housing systems on eggshell cracks are summarized.

The study of Guesdon et al. (2006) as well as the study of De Reu et al. (2009b) showed that furnished cages are most susceptible for cracks. Guesdon et al. (2006) found in pilot studies 15.4 to 19.6% of broken (visual observation) and hair-cracked (candling) eggs in furnished cages compared to only 8.1 to 12.2% in standard cages. This was mainly due to hair-cracked eggs at the narrow nests of the furnished cages without egg saver and a relatively low frequency of manual egg collection. The study of De Reu et al. (2009b) documented variations of cracked eggs from 0 up to 24% for the individual farms. A high percentage (24%) of cracks was found in a furnished cage flock and was probably caused by a bad adjustment of the egg saver and the accumulation of eggs next to the nest box on a

short part of the egg belt. In the study of Tauson et al. (1999) cracks varied from 2.2% to 7.7%, with no significant difference between the housing systems (conventional and floor housing systems) and an almost comparable mean % of cracked cage eggs (5.0% compared to 4.6%). In a market study on the quality characteristics of eggs from different housing systems, Hidalgo et al. (2008) found no significant difference ($P > 0.05$) in appearance of cracked eggs between cage (14%), free range (10%), barn (11%) and organic (5%) eggs. The comparison at the shop level of De Reu et al. (2009a) also showed that non-cage eggs do not contain more cracks compared to cage eggs (5.6 versus 7.8%). Of course eggs in those latter studies were already candled and sorted at the packaging station. In summary the different research results indicate that nest eggs of non-cage systems are in normal circumstances not more susceptible for cracks compared to cage eggs. In addition, results indicate that a good egg collection of the eggs from furnished cages is important to reduce cracks.

Table 2.   Occurrence of cracked eggs (in % of eggs) in different housing systems

Study	Type of study	CC (%)	FC (%)	Non-cage (%)	P
Tauson et al. 1999	Pilot	5.0	n.a.	4.6	
Guesdon et al. 2006	Pilot	8.1 - 12.2	15.4 - 19.6	n.a.	< 0.001
De Reu et al. 2009b	Comm.	n.a.	7.8%*	4.1	< 0.05
Hidalgo et al. 2008	Comm. (shop)	14	n.a.	8.7	> 0.05
De Reu et al. 2009a	Comm. (shop)	7.8	n.a.	5.6	n.d.

CC = conventional cage, FC = furnished cage, Comm = commercial housing *= only nest eggs; n.a. = not analyzed; n.d. not determined

IV. EFFECT OF HOUSING SYSTEM ON THE *SALMONELLA* CONTAMINATION

a) *Salmonella* contamination of eggs

Much of the research on eggshell and egg content contamination focuses on *Salmonella,* since infection with *Salmonella* Enteritidis, resulting from the consumption of contaminated eggs or egg products, is still a major health problem. Several EU member states have reported data from investigations of table eggs, and the overall EU prevalence in 2006 was 0.8% (EFSA, 2007). More than 90% of all egg-isolates were strains of the serotype Enteritidis.

Little research is done on the influence of housing system on eggshell and egg content contamination with *Salmonella.* In a study of Humphrey et al. (1991), over 5700 eggs from 15 naturally infected flocks were examined, of which 32 or 0.6% were contaminated. The prevalence of egg content contamination of eggs from battery or free-range were comparable; 0.73 and 0.64% respectively. A study of the UK Food Standards Agency in 2003 also did not found significant differences in *Salmonella* spp. contamination on the shell due to the production system (Anon., 2004). On a total of 4753 retail samples of boxes with six eggs, the eggshell of nine samples was contaminated. None of the 4753 pooled egg contents of retail samples were *Salmonella* positive. In a smaller study of De Reu et al. (2009a) 47 fresh egg samples from the Belgian market were sampled. Sixteen samples concerned cage-eggs, five floor housing eggs, 12 free range eggs, seven organic eggs, five samples from farm retail and two from private backyards. In none of the samples *Salmonella* was found.

b) Environmental *Salmonella* contamination

More research was focused on the influence of the housing system on environmental *Salmonella* contamination. The analysis of the existing data of an EU-wide baseline study on *Salmonella* in laying hens flocks performed in 2004-2005 (EFSA 2006) showed a significant

difference in *Salmonella* prevalence according to housing type (Anon. 2008). In cage systems the highest *Salmonella* prevalence was found followed by a intermediate prevalence in barn systems and the lowest prevalence in the free range systems. More than 51% of all *Salmonella* isolates were serotyped as *Salmonella* Enteritidis. Recent data collected in the large-scale cross-sectional and longitudinal field study of the EU Safehouse project confirm those findings (Anon. 2008). An overview by Dewulf et al. (2009), on published observational studies evaluating the effect of housing system on the prevalence of *Salmonella* Enteritidis infections, also clearly indicates that a cage system has an increased risk for being *Salmonella* positive in comparison to non-cage housing systems. However, there is not necessarily a causal relationship between the housing type and the *Salmonella* infection. It is more likely that the housing system is a proxy of many other production characteristics such as magnitude of the flock or herd, age of the building, probability of previous *Salmonella* infection on the farm. The authors summarized a number of important production characteristics that may be both related to the housing system and the probability of a *Salmonella* infection: herd and flock size, stocking density, stress, age of the building and carry-over infections, pests, vaccination.

V. CONCLUSIONS

It is clear that eggshell contamination with aerobic bacteria is on average significantly higher for nest eggs from non-cage systems compared to nest eggs from furnished cages or eggs from conventional cages. The major differences found in experimental studies between cage and non-cage systems are less pronounced under commercial circumstances. The scarce information available on the influence of the housing systems on the egg content contamination indicates no major differences in egg content contamination between cage eggs and non-cage eggs (ignoring outside nest and floor eggs).

Studies also show that it is highly unlikely that a move from conventional cages to alternative cage systems and non-cage housing systems will result in an increase in *Salmonella* infection and shedding, rather the opposite is expected.

VI. ACKNOWLEDGEMENT

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LITTER CONSUMPTION BY POULTRY AS AFFECTED BY DIET STRUCTURE

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Summary

Based on previous results indicating that poultry voluntarily consume litter material, two experiments were carried out to investigate the effect of access to litter for broiler chickens with diets with varying content of structural components. The results indicate that birds consume litter material, and that the amount consumed is inversely related to amount of structural components in the diet.

I.   INTRODUCTION

It has been shown in numerous experiments that structural components such as whole wheat and oat hulls will stimulate gizzard development, and that the resulting more muscular and voluminous gizzard may improve nutrient digestibility and performance (Svihus et al., 2004; Ravindran et al., 2006; Gabriel et al., 2008). Although the mechanism for the beneficial effect has not been fully revealed, data indicate that the beneficial effect is associated with a longer retention time and a lower pH of the gizzard, and a finer grinding of the diet ingredients (Gabriel et al., 2003; Hetland et al., 2002).

It has also been shown that birds will voluntarily consume fibrous components presented for the birds in a different trough than the feed, and that such a consumption of fibrous components is dependent on the coarseness of the diet (Hetland et al., 2005). This indicates that birds have a desire for structural components, and that birds will search for structural components which can be consumed in a situation where the diet lacks structure. Litter material used as bedding in floor systems may be such an alternative source for structural components. Thus, we have carried out two experiments to study litter consumption of broilers.

II.   MATERIALS AND METHODS

In study one, day-old male broiler chickens (Ross) were reared in cages with mesh-wire floor on commercial broiler starter feed till 11 days of age. From 6 days of age, 50 g/kg of whole wheat was mixed into the starter diet for half of the flock to allow the birds to get used to the whole wheat diet. At 11 days of age 180 chickens were randomly and equally distributed among 12 pens (75 cm x 150 cm). A constant daily temperature (28 C° from 11-14 days and 26 C° thereafter) and 23 hours light was provided. Half the pens were supplied with 1.5 kg litter, while the other half was supplied with a green rubber mesh mat. The litter was sieved through a 5 mm sieve, and only particles larger than 5 mm were used. All birds received the same wheat-based diet (779 g/kg), but half the birds received a pelleted diet with 500 g/kg whole wheat added before pelleting while the other half received a pelleted diet based on wheat ground through a 3 mm hammer mill before pelleting. The diets and water were given ad libitum. Chickens and feed were weighed at 14, 21 and 28 days of age. At termination of the experiment, all the litter material was soaked in water for 12 hours, followed by rinsing (10 litre per minute) through a 5 mm sieve and thereafter drying of the material remaining on the sieve for 3 days at 105 C°.

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In study two, a total of 360 broiler chickens (Ross 308) were placed in 24 pens with 15 birds per pen at 4 days of age. One half of the pens had rubber mat on the floor, and the other half had litter on the floor. One half of the pens on each floor type were fed on a pelleted wheat-based (709 g/kg) diet and the other half on the same diet but diluted with 5% coarse oat hulls before pelleting. This represents a 2 x 2 factorial design with the factors being dietary fibe (low and high) and litter (absent and present). Birds and feed were weighed at the start, day 19, and day 32.

Table 1.   Results from Experiment 1

	Wheat treatment	No litter	Litter	P-value for litter effect	P-value for diet effect	P-value for interaction
Weight gain 11-29 days, g	Ground	1175	1267	0.6836	0.3441	0.4464
	Whole	1405	1226			
Feed consumption 11-29 days, g	Ground	1794	1723	0.0391	0.1558	0.0960
	Whole	1922	1713			
Feed/gain	Ground	1.64	1.48	0.1054	0.7245	0.9812
	Whole	1.70	1.43			
Ileal starch digestibility, %	Ground	89	94	0.0333	0.1156	0.8946
	Whole	88	90			
Empty gizzard, % of live weight	Ground	1.4	1.5	0.0204	0.0004	0.7039
	Whole	1.6	1.7			
Gizzard content, % of live weight	Ground	0.3	0.6	0.0032	0.0001	0.2858
	Whole	0.7	0.8			
Fibre concentration in gizzard, %[1]	Ground	17.2	37.0	0.0001	0.0030	0.1826
	Whole	34.4	38.7			

[1]Based on the NDF analysis (Van Soest method).

III. RESULTS AND DISCUSSION

Weight gain was not significantly affected by either structural components in the diet or whether the birds were raised on wood shavings litter or not. The lack of bedding effect indicates that both environmental conditions were suitable for the birds. However, access to litter had a significant moderating effect on feed intake, which in turn resulted in a significantly improved feed/gain for birds with access to litter in experiment 2. As ileal starch digestibility was significantly improved with access to litter in experiment 1, this indicates that the cause for improved feed utilization was at least partly an improved nutrient digestibility.

Since gizzard size, gizzard content weight and fibre concentration increased for birds raised on litter, it is logical to conclude that birds consumed litter. In experiment one, weighing of the litter for each cage before and after the experiment indicated that birds were eating approximately one gram per bird per day. Since it has been shown in several experiments carried out previously that structural components in the diet stimulate gizzard development and starch digestibility, it can therefore be postulated that litter consumption is the cause for increased gizzard size, and thus the primary cause for increased starch digestibility and reduced feed/gain. Although addition of hulls to the diet resulted in a significantly reduced size of particles in the duodenum, in accordance with results observed before (Hetland et al., 2002), access to litter did not have the same effect. This could be due to a lower magnitude of litter structural effect caused by moderate consumption, as observed in experiment 1. The significant trend towards a larger increase in gizzard size for diets

without hulls in experiment 2 indicates that consumption of litter material interacts with access to structural components through the diet. This lends support to the hypothesis put forward earlier, that poultry eat litter material to compensate for lack of structural components in the diet (Hetland et al., 2004; Hetland et al., 2005). Thus, these experiments indicate that litter material, when used for birds given a modern broiler diet with a low content of structural material, should be regarded not only as a bedding material, but also as a dietary ingredient. This insight has bearing for the hygienic conditions of the digestive tract, and it has bearings for the choice of litter materials.

Table 2.   Results from Experiment 2

	Diet	No litter	Litter	P-value for litter effect	P-value for diet effect	P-value for interaction
Weight gain 6-32 days, g	No hulls	1957	1919	0.6802	0.6205	0.4486
	Hulls	1949	1960			
Feed consumption 6-32 days, g	No hulls	3181	2945	0.0136	0.1623	0.2376
	Hulls	3021	2931			
Feed/gain	No hulls	1.63	1.53	0.0006	0.0044	0.4469
	Hulls	1.55	1.50			
Starch content in ileum, %	No hulls	12.2	9.6	0.1042	<0.0001	0.0622
	Hulls	1.1	1.3			
Empty gizzard weight, g on day 19	No hulls	15.5	21.9	0.0037	<0.0001	0.0169
	Hulls	25.6	26.3			
Empty gizzard weight, g on day 32	No hulls	27.6	31.0	0.5303	<0.0001	0.0939
	Hulls	44.9	43.4			
Mean duodenal particle µm on day 19	No hulls	160	120	0.6821	0.0748	0.1809
	Hulls	89	110			
Mean duodenal particle µm on day 32	No hulls	237	264	0.0908	<0.0001	0.8169
	Hulls	136	172			

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THE EFFECTS OF INTERRUPTING A DUSTBATHING BOUT ON THE CHOICE BEHAVIOUR OF LAYING HENS IN A Y-MAZE TEST

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It has been suggested that interrupting the interaction of an animal with the reward provided in a choice test can be aversive and devalue the reward (e.g. Mason et al., 1998). Previous work of ours with hens given access to a dustbathing substrate for different lengths of time in a Y-maze preference test appeared to support this suggestion; hens allowed 20 minutes of interaction with peat moss appeared less motivated to choose peat moss on subsequent trials than hens given access for 45 minutes or 2 minutes (Laine et al., 2009). The average duration of a dustbathing bout of a laying hen is 27 minutes (Vestergaard, 1982), however, the hens receiving 20 minutes of dust reward in Laine et al. (2009) had an average bout duration of only 15 minutes. To determine if interrupting dustbathing mid-way through a bout is aversive, the preference of hens in a Y-maze was investigated when they were offered uninterrupted dustbathing vs. dustbathing interrupted after 15 minutes (unint vs. 15) and dustbathing interrupted after 15 minutes vs dustbathing interrupted after 2 minutes (15 vs. 2). For each hen the arm in which the options were presented were held constant and the arm visually cued (with a black or white board) at the choice point in the Y-maze. Sixteen hens were preference tested eight times, one trial per day on alternate days and were given 15 minutes in which to commence dustbathing after making a choice. The choice made determined whether dustbathing was interrupted and after what time. Each hen underwent both treatments over two periods (eight hens per treatment per period). Choice behaviour was compared to chance level (i.e. 50:50) by a Chi-square test and this revealed that the hens showed no preference on all trials (treatments unit vs. 15 and 15 vs. 2, P = 0.69 and P = 0.42 respectively) or on trials when dustbathing occurred (treatments unit vs. 15 and 15 vs. 2, P = 1.0 and P = 0.23 respectively). As a lack of preference was unexpected, we conducted a second experiment to determine whether the lack of preference was just that or an inability to learn the association between the visual cue, maze arm and duration of dustbathing. Thirteen hens were preference tested in a Y-maze for their choice between a dustbath filled with peat moss or an empty dustbath, which was visually cued with a black or white board as in experiment 1. Hens could not see the dustbath contents until they made their choice. As previously, for each hen the arms in which the rewards were presented were held constant. Hens were given eight trials (one per day) on alternate days. The hens chose the dust-filled dustbath on significantly more trials than the empty dustbath (P < 0.001) indicating that hens can learn a simple task of associating a visual cue and arm maze with access to a substrate. Even though the hens were successful at learning the task in experiment 2, it is possible that hens were unable to learn the complex task in the first experiment. Alternatively, contrary to our hypothesis, interruption of dustbathing mid-way through a bout was not aversive.

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EGG QUALITY IN THE AUSTRALIAN EGG INDUSTRY: AN UPDATE

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Summary

Eggs were sampled from commercial egg farms in NSW, QLD, VIC, SA and WA. A total of 6300 eggs were analysed on-site for egg weight, albumen height and Haugh Units. In addition, a total of 18,039 eggs were analysed in the laboratory for the full range of egg shell quality and egg internal quality measurements. Data were compared for state of Australia, strain of bird and production system. In general, egg shell quality and egg internal quality were relatively independent of state, strain of bird and egg production system although there was a range of values for all parameters measured.

I. INTRODUCTION

The egg is the final product of the Australian Egg Industry and its internal and external quality is of paramount importance to the industry and the consumer. Quality Assurance programs are an essential feature of all egg producing establishments. The current project conducted egg quality testing, further to the earlier AECL study (Roberts & Ball, 2004) to enable the continuing development of a data base of egg internal quality and egg shell quality within the Australian Egg Industry. In recent years, there have been some acute problems with poor albumen quality. The current project specifically targeted albumen quality of freshly-laid eggs taken straight from the cage front.

Australian per capita consumption of eggs (196 as at September, 2009) is about one third of egg consumption in the USA and Canada. Therefore, the potential exists for a substantial increase in egg consumption in Australia, particularly now that the AECL has been successful in achieving the Heart Foundation's Tick of Approval for eggs.

Losses of eggs owing to poor shell quality have been conservatively estimated at 10%. For the Australian industry (13 million hens x 27 dozen eggs at a farm-gate price of $1.60 per dozen) each 1% loss in saleable eggs is approximately $5 million annually. However, losses in saleable eggs would also include the input costs (pullet, feed) associated with producing that egg (analysis by TA Scott, 2006).

II. MATERIALS AND METHODS

The project involved egg quality testing from a range of poultry establishments for the purposes of updating the existing AECL egg quality database. Research and industry contacts in most states of Australia sampled eggs, 30 per flock, directly from the cage front for measurement of albumen quality. A sample of 90 eggs from the same source was sent by courier to the University of New England where they were subjected to the full range of egg quality tests: shell colour, shell breaking strength and deformation, shell weight, shell thickness, albumen height, Haugh Units, yolk colour score. Routine sampling was conducted across a range of flock ages, including early, mid and late lay. Sampling was largely opportunistic, depending on which producers agreed to participate in the study and were

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geographically accessible to the project sampling team. Egg internal quality was measured on-farm as egg weight and albumen height and Haugh Units calculated. In the laboratory, egg quality was measured as shell reflectivity, egg weight, deformation, breaking strength, shell weight (form 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

A total of 6300 eggs were analysed on-site for egg weight, albumen height and Haugh Units. In addition, a total of 18,039 eggs were analysed in the laboratory for the full range of egg shell quality and egg internal quality measurements. Eggs were collected from 210 flocks in total, 67 from NSW, 54 from VIC, 55 from QLD, 20 from SA, 14 from WA. Of these, 156 flocks were cage production, 46 free range and 8 barn. Strain distribution was HyLine 114 flocks, Isa 52, HiSex 44 flocks. Most flocks were single age and multi-age flocks were reported for only 29% of cage flocks. Reticulated town water was the water source for most of the flocks (93) followed by bore water (78) and dam (29). The method used to treat water coming from non-town sources was predominantly a combination of filtration and chlorination with some farms using reverse osmosis or iodine treatment. For cage production, most single age sheds had controlled ventilation and most multi-age cage flocks came from older style sheds with natural ventilation. Free range and barn flocks had shedding that was mainly naturally ventilated although some had some degree of ventilation control. Years of staff experience varied from less than one year to 60 years although the averages were similar for cage (22 yrs) and barn (23 years) which were higher than for free range (16 yrs). Most farms collected eggs at least daily although 17% of eggs from both cage and barn systems were collected only 6 days per week. The average temperature of egg storage rooms was 14°C for all production systems but varied between 9 and 17°C. Three quarters of cage flocks and approximately half of all free range and barn flocks were vaccinated for infectious bronchitis (IB) virus only during rearing. For flocks that were revaccinated regularly during lay, the frequency of revaccination generally ranged between 6 and 10 weeks. Relatively few cage and free range flocks were reported as having shown signs of IB infection but this proportion was higher for barn flocks. The majority of flocks were audited regularly, most by AECL Egg Corp Assured (ECA). Most farms used cardboard fillers. Age at first egg was similar for all production systems (17.5 weeks) with peak production occurring at 27-30 weeks of age and 90% at 44, 51 and 55 weeks of age for free range, cage and barn, respectively.

The effects of flock age were very similar to those which have been reported earlier (Roberts & Ball, 2004). Egg weight increased and then stabilised at between 60 and 70 grams. Shell reflectivity increased with hen age initially but then remained relatively stable. Shell deformation generally decreased with hen age and shell breaking strength decreased with hen age. Shell weight generally increased in a manner similar to egg weight but, for some flocks, tended to decrease later in lay. Shell thickness was relatively stable across a range of hen ages. Percentage shell was relatively constant until 60 weeks of age after which it tended to decrease. Albumen height and Haugh Unit generally decreased with increasing age of the flock and were more variable for the values measured in the laboratory, owing to varying lengths of time elapsing between egg collection and measurement. Yolk colour varied between 8 and 13 and was independent of hen age (although two barn flocks from VIC were well below the average).

There was generally no difference among states for the egg quality variables measured: egg weight; albumen height and Haugh Unit (both on-farm and laboratory); shell reflectivity (although three free range flocks from SA had significantly higher shell reflectivity); shell deformation; shell breaking strength; shell weight; shell thickness (although some individual flocks in NSW were below average); shell thickness; percentage shell; yolk colour.

The relationship between egg weight and hen age was similar for all strains. Albumen height measured on freshly collected eggs declined with hen age for all strains but was generally highest for the HyLine Brown and lowest for the HiSex birds, with Isa intermediate. Haugh Unit, which takes into account the size of the egg, declined with hen age but was more similar among strains of bird than was albumen height for freshly collected eggs. There was a greater variability in both albumen height and Haugh Unit measured in the laboratory owing to different ages of the eggs. There was considerable overlap among strains for shell reflectivity. Three free range flocks, two HyLine and one HiSex had significantly lighter shell colour. Shell breaking strength showed a considerable degree of overlap, but some flocks were noticeably below the average. Shell weight was generally highest for ISA and lowest for HyLine with HiSex intermediate. There was no consistent difference among strains for shell thickness and percentage shell was generally similar for all strains. Albumen height and Haugh Unit measured in the laboratory showed considerable variation because of the different amounts of time that had elapsed between the collection of the eggs and their analysis in the laboratory. For yolk colour, two barn flocks from VIC were well below the average.

There were no differences among production systems for egg weight; albumen height and Haugh Unit; shell breaking strength, shell thickness; percentage shell and yolk colour score. However, there were two barn flocks which had very low yolk colour score. Shell reflectivity was generally higher for free range flocks with several flocks well above average. Shell deformation was similar for all production systems. Shell weight was generally higher for ISA than for the other two strains. Yolk score was independent of hen age and production system.

Albumen height was higher when measured on-farm than when measured at varying time intervals later in the laboratory. Haugh Unit was also higher when measured on-farm than when measured later in the laboratory. When the loss in Haugh Units with time between on-farm analysis and analysis later in the laboratory are plotted on a graph, it can be seen that, for most flocks, there is a loss of Haugh Unit but, for other flocks, Haugh Unit was actually higher when measured in the laboratory. In general, the loss of Haugh Units was between 1 and 10 up to 15 days between analyses and between 10 and 20 for longer time delays between analyses.

IV. DISCUSSION

In general, egg shell quality and egg internal quality were relatively independent of state, strain of bird and egg production system although there was a range of values for all parameters measured. When flocks from different states were compared, several free range flocks from SA had lighter coloured shells, some QLD flocks had higher shell deformation and shell thickness and some NSW flocks were significantly below average for shell thickness. When the three strains of bird were compared, there were some differences. Albumen height of freshly measured eggs was generally highest for HyLine and lowest for HiSex although much of this could be explained by differing egg weights and there was less variation among strains for Haugh Unit. A small number of flocks, including three free range flocks, had lighter coloured shells. The cause of this reduced pigmentation is not

known athough suggestions include anaemia (Juergen Lohr, personal communication). The two barn flocks that had very low yolk colour appear not to have had pigment added to the feed. There were relatively few differences among production systems. As expected, albumen height and Haugh Unit measured later in the laboratory were generally lower than those measured directly at the cage front. However, watery whites were encountered only rarely and not consistently throughout a flock. This finding suggests that there is not a major problem with water albumen in Australian layer flocks. Comparison of these results with those obtained in 2003 reveal some general differences with egg weight being lower, egg shell colour darker, shell deformation lower and shell thickness higher in the 2009 study.

When the results of this study are compared with those of the study published in 2003 (Roberts & Ball, 2004), egg weight in the 2009 study is generally lower than for the 2003 study. Egg shell colour measured in the 2009 study was generally darker (lower reflectivity) than for the 2003 study, with the exception of the three free range flocks, mentioned earlier. Shell deformation measured in the 2009 study tended to be lower than for the 2003 study. Shell breaking strength and shell weight were very similar for the two studies. Shell thickness tended to be higher for the 2009 study. Percentage shell was very similar for the 2003 and 2009 studies. Albumen height measured in the laboratory in 2003 was generally higher than that measured in the laboratory in 2009. Albumen height measured in the laboratory in 2003 and on-farm in 2009 was very similar for the two studies. Haugh Unit measured in the laboratory in 2003 was similar to that measured in the laboratory in 2009. Haugh Unit measured on-farm in 2009 tended to be higher than that measured in the laboratory in 2003.

V. ACKNOWLEDGEMENTS

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OPTIMAL SULPHUR AMINO ACIDS TO LYSINE RATIO IN GROWER PHASE IN ROSS 308 BROILERS

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Summary

An experiment with growing male Ross 308 broilers (14-35 days) was performed to test the optimal ratio between true fecal digestible sulphur amino acids (DSAA) and true fecal digestible lysine (DSAA:DL). The ratio was increased from 63% to 82% with increments of approximately 5%. Live weight gain and feed conversion ratio (FCR) was measured for the 3 week period. At day 35 the broilers were slaughtered and carcass and breast meat yield were measured Results indicate that Ross 308 broilers respond positively to DSAA:DL ratio's higher than currently recommended and that optimum varies with performance parameter with the highest optimum for FCR, carcass yield, and breast meat yield. Based on these data it seems like the economic optimum will require a higher DSAA:DL ratio when the production goal is cut up chicken compared to whole birds.

I. INTRODUCTION

The Ideal Protein (IP) concept is a tool enabling quick and easy adjustments to changing production conditions. Thus, knowledge only of optimum dietary lysine levels for certain conditions are needed while the ratios between all essential amino acids and lysine are maintained the same. The optimal ratio between sulphur amino acids and lysine has been studied in a number of trials (Mack et al., 1999; Vieira et al., 2004). However, the optimum ratio is still being discussed Taherkhani et al. (2008). Recently Rostagno et al. (2007) reported optimal ratios well above the NRC (1994) recommendations of 72%. It has also been reported that requirements for sulphur amino acids depends on the parameters tested, e.g. growth, feed conversion ratio (FCR), and carcass composition (Rostagno et al., 2007; Lumkins et al., 2007). Therefore, an experiment with growing male Ross 308 broilers was performed to test the effect of increased ratio between digestible sulphur amino acids (DSAA) and digestible lysine (DSAA:DL) on growth, FCR, carcass yield and breast meat yield.

II. MATERIAL AND METHODS

The experiment was conducted with 720 male Ross 308 broilers from 14 to 35 days of age. The birds were all housed in 36 pens with raised floors at the Broiler Research Facility of Provimi's Research Centre in The Netherlands. The DSAA:DL ratio was increased from 63% to 82% with increments of approximately 5% by adding DL-Methionine to the sulphur amino acids deficient diet. An additional treatment was included in which the diet with highest DSAA:DL ratio was supplemented with extra L-Lysine HCl in order to provide evidence that digestible lysine was second limiting. Each of the 6 treatments was repeated six times and the birds had *ad libitum* access to feed and fresh drinking water. Up to the start of the trial at day 14 the broiler chicks were fed a commercial starter diet. The diet and nutrient composition of

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the 63 % DSAA:DL ratio diets and the positive control diet (high lysine) is given in Table 1. All diets were kept isocaloric and CP was also kept stable.

Live weight gain and FCR was measured for the 3 week period. At day 35 the broilers were slaughtered and carcass and breast meat yield were measured. Data were analysed by analysis of variance and the Tukey-Kramer test was applied to account for multiple comparisons between treatments. The optimal ratio between DSAA and digestible lysine (DL) was determined by exponential regression $(y = a + b * (1-e^{(-c * x)}))$ where the optimum were set at 95% of asymptotic response (see Figure 1).

Table 1.   Diet and nutrient composition for the diet with low digestible sulphur amino acids (MC) and digestible lysine ratio (low MC:L) and the positive control (PC) diet with adequate lysine content.

Diet composition,	Treatments		Nutrient	Treatments	
g/kg	Low MC:L	PC	composition, g/kg	Low MC:L*	PC
Maize	485.3	485.3	Crude protein	202	204
Wheat	50.0	50.0	Crude fat	104	103
Soybean meal	325.9	325.9	Crude fibre	24	24
Maize gluten	18.2	18.2	Crude ash	60	58
Soybeans	6.1	6.1	AMEn (kcal/kg)	3218	3220
Animal fat	35.9	35.7	TFD Lys	9.44	10.46
Soybean oil	35.9	34.8	TFD Met	3.02	4.86
Premix	10.0	10.0	TFD Met + Cys	5.94	7.78
Limestone	12.7	12.7	TFD Thr	7.32	7.32
MonoCaPO$_4$	10.9	10.9	TFD Trp	2.14	2.14
Salt	2.3	1.8	TFD Ile	8.29	8.29
Sodium bicarbonate	1.5	2.2	TFD Arg	12.99	12.99
DL-Methionine	-	1.86	TFD Val	9.13	9.13
L-Lysine HCL	-	1.31	Calcium	7.6	7.6
L-Threonine	0.21	0.21	Avail. Phosphorus	3.8	3.8
DIAMOL	5.00	2.94			

* 0.0 g/kg, 0.46 g/kg, 0.93 g/kg, 1.39 g/kg, and 1.85 g/kg DL-Methionine were added to the low MC:L diet to achieve MC:L ratios of 63, 68, 73, 78, and 82%, respectively, in the experimental diets

III. RESULTS AND DISCUSSION

All performance criteria improved significantly and nonlinearly with increasing dietary DSAA:DL (Table 2 and Figure 1). In most cases the treatment with extra Lys showed a better performance providing evidence that Lys was the second limiting amino acid. Thus, it can be concluded that the determined optimal ratios between true fecal digestible (TFD) sulphur amino acids and TFD lysine is not underestimated in this trial.

Looking at the exponential regressions for live weight gain, 95% of asymptotic response was reached at a DSAA:DL ratio of 75%. For FCR, carcass yield, and breast meat yield, DSAA:DL ratio required for achieving 95% of asymptotic response was higher than the higest tested ratio (82%) suggesting higher ratios to be optimal for these parameters in male Ross 308 broilers. This high optimum is in agreement with the results reported by Vieira et al. (2004) for both Ross 308 and Cobb 500 birds and Rostagno et al. (2007). In the study reported by Lumpkins et al. (2007) where they studied the optimal level for digestible sulphur amino acids, the ratio between total Methionine+Cysteine and Lysine at recommended level for sulphur amino acids would suggest 86%.

Table 2.   Live weight gain, feed conversion ratio (FCR), carcass yield, and breast meat yield in 35 days old male Ross 308 broilers fed diets with increasing levels of true fecal digestible (TFD) sulphur amino acids (DSAA) to TFD lysine (DL) ratios

	DSAA : DL ratio					Extra Lys
	63%	68%	73%	78%	82%	74%
TFD Lysine, g/kg	9.44	9.44	9.44	9.44	9.44	10.46
TFD Met+Cys, g/kg	5.94	6.40	6.86	7.32	7.78	7.78
Gain, g	1489[c]	1693[b]	1778[a]	1763[a]	1783[a]	1819 [a]
Feed intake, g	2723[c]	2888[ab]	2981[a]	2874[b]	2877[b]	2876 [b]
FCR, kg/kg	1.813[a]	1.706[b]	1.677[b]	1.631[c]	1.614[cd]	1.581 [d]
Carcass yield, % of LW	69.75[c]	70.65[b]	71.11[b]	71.23[b]	71.54[a]	71.35 [a]
Breast meat, % of CW	30.66[d]	31.65[cd]	32.39[bc]	33.02[b]	33.11[b]	34.34 [a]
Abdominal fat, % of CW	2.42[a]	1.99[b]	2.15[ab]	2.12[b]	1.95[bc]	1.71 [c]

Figures in rows with different superscript [a, b, c] differs significantly ($P<0.05$)
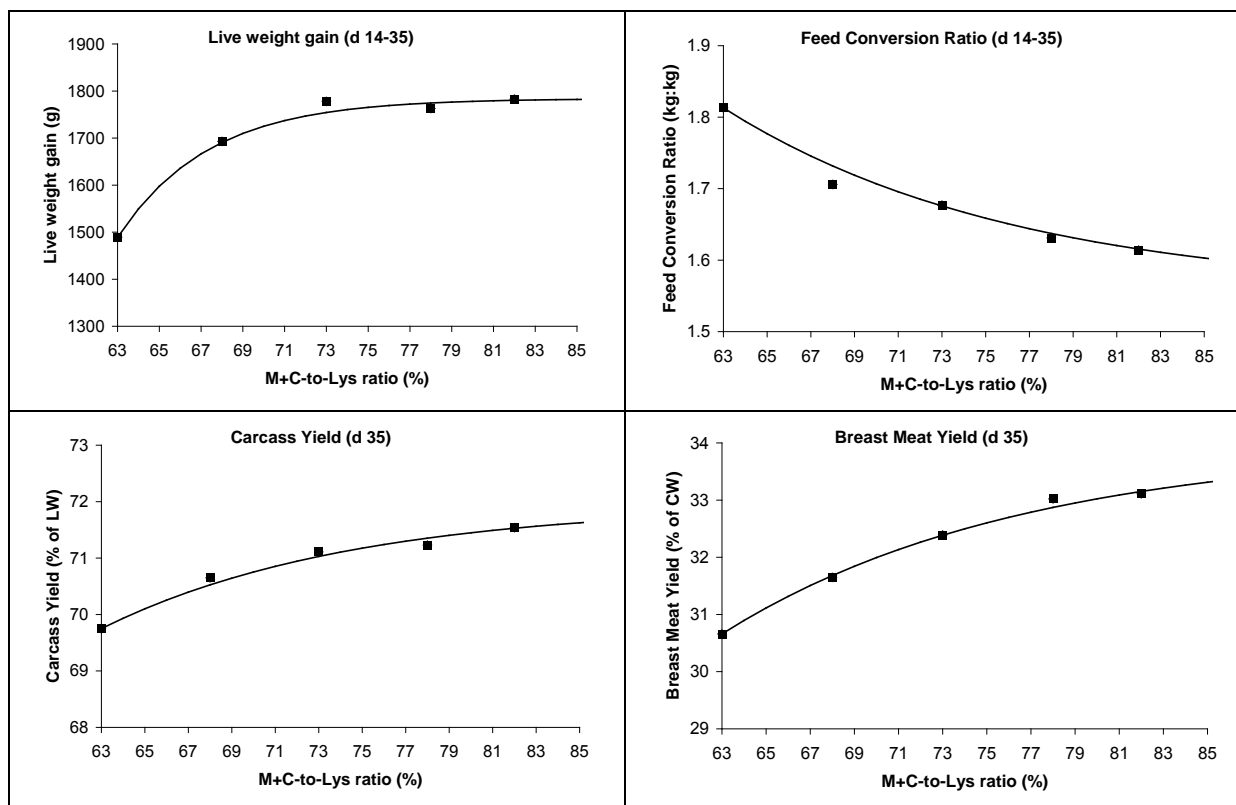
Figure 1.   Live weight gain, feed conversion ratio (FCR), carcass yield, and breast meat yield in 35 days old male Ross 308 broilers in response to increasing levels of Met+Cys:Lys ratio

In the present trial only the effect in males were studied but Rostagno et al. (2007) reported that for FCR the optimal DSAA:DL ratio is actually higher in females compared to male Cobb 500 broilers. In addition, Lumkins et al. (2007) found no difference in sulphur amino acid requirements for female and male Cobb 500 broilers when using growth and breast meat yield as parameter. However, they found higher sulphur amino acid requirements for males when looking at FCR.

IV. CONCLUSION

The present results indicate that Ross 308 broilers in the grower period respond positively to DSAA:DL ratios higher than currently recommended and that optimum varies with performance parameter. For weight gain the optimal DSAA:DL ratio was found to be 75% whereas for FCR, carcass yield and breast meat yield the optimum was even higher than the highest ratio tested in this trial, i.e. 82%. Based on these data it seems like the economic optimum will require a higher MC:L ratio especially if the production goal is processed chicken compared to whole birds.

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DIETARY ENZYMES ALTER SORGHUM PROTEIN DIGESTIBILITY AND AME CONTENT

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Composition and availability of nutrients in sorghum are variable, especially protein content and digestibility. Broilers fed sorghum based diets may under-perform, possibly reflecting low nutrient availability due to the presence in sorghum of antinutritional factors; polyphenols, phytate and kafirin (Bryden et al., 2009). The objective of this study was to assess the efficacy of different dietary enzymes on apparent ileal protein digestibility (IPD) and metabolisable energy (AME) content of sorghum. Eight mash diets were prepared with sorghum (918g sorghum/kg diet) as the sole protein source. Celite (20 g/kg) was added to allow acid insoluble ash (AIA) to be used as an indigestible marker. The treatments consisted of a control diet to which different enzymes were added (see Table 1). Broilers, 36-days-old, were housed (7 birds/cage) in an environmentally controlled shed and randomly assigned to replicated (n = 4) dietary treatments with free access to feed and water. On day-42, birds were euthanized and contents of the lower half of the ileum were pooled per pen, frozen and lyophilized. Nitrogen, gross energy and AIA content of all diet, ileal and faecal samples were determined using standard laboratory protocols and protein digestibility coefficients and AME values were calculated (Table 1).

Table 1.   Effect of dietary enzymes on AME and apparent ileal protein digestibility of sorghum in broilers.

Treatment	Ileal protein digestibility coefficient	AME (MJ/kg DM)
1 Control	0.780[bc]	14.07[e]
2 Xylanase	0.772[c]	14.37[d]
3 Phytase	0.808[ab]	14.62[c]
4 Protease	0.815[a]	14.81[bc]
5 Xylanase + Phytase	0.805[abc]	14.75[c]
6 Xylanase + Protease	0.797[abc]	14.66[c]
7 Phytase + Protease	0.825[a]	14.99[ab]
8 Xylanase + Phytase + Protease	0.813[ab]	15.18[a]
SEM	0.01	0.06

[abcde]Mean values within columns not sharing superscripts are significantly different (P < 0.05)

The apparent ileal protein digestibility coefficient (IPD) was significantly enhanced by 5.47% and 4.33% in treatments 7 and 4, respectively. The presence in the diet of all three enzymes resulted in an improvement of 4.02% in the IPD coefficient. All enzymes singly and in combination significantly improved AME. Maximum improvement (7.89%) in AME was seen in treatment 8, with all three enzymes in combination followed by treatments 7 (6.54%) and 4 (5.26%), respectively. These findings demonstrate that strategic application of enzymes to sorghum based poultry diet can reduce the negative influence of antinutritional factors, thus enhancing nutrient digestibility and bird performance.

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INFLUENCE OF ENERGY AND BALANCED PROTEIN LEVELS IN WHEAT-BASED DIETS ON ROSS 308 BROILER PERFORMANCE

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Summary

An experiment with male and female Ross 308 broilers was performed to investigate the interactions between graded levels of balanced protein (BP) and metabolizable energy (ME) level. At 42 days of age feed intake, live weight and feed conversion ratio (FCR) were significantly affected by BP, ME and sex of the birds (P<0.05). With increasing BP level feed intake declined and live weight and FCR improved independently of the dietary ME level. When ME was reduced the feed intake increased, FCR was impaired and live weight remained relatively stable. Breast meat yield increased with increasing BP level whereas increasing ME level had a negative effect on breast meat yield. The data do not indicate an optimal ME to BP ratio and economic optimum for both energy and balanced protein will therefore depend on the price ratio of protein and energy in feed ingredients.

I. INTRODUCTION

Prices for cereals and oils have fluctuated considerably over the last few years and have in general led to higher unit costs for the energy components of broiler diets. Thus, a reduction of the energy level of broiler diets seems attractive in order to control feed costs. It is commonly recognized that broilers adjust their feed intake in accordance with the dietary ME content; complete adjustment would keep ME intake constant (Leeson et al. 1996). Based on such findings it has been suggested that, as ME in varied, BP should be kept in a constant ratio to ME. However, Plumstead et al. (2007) found a positive effect of increasing BP which was independent of ME level and in this study they did not see an effect of dietary ME level on feed intake. Kamran et al. (2008) found that broilers given feed with constant ME:CP ratios were not able to fully compensate for a severe decrease in dietary ME level by increasing feed intake. Thus, it still remains unclear how BP should be adjusted when ME level is changed and therefore a trial was conducted to test the effect of graded levels of BP fed at two dietary ME levels on weight gain, feed conversion ratio, and carcass characteristics in broilers.

II. MATERIAL AND METHODS

A total of 4320 male and 4320 female day old Ross 308 broiler chicks (44 g) were housed in 96 floor pens (2.7 x 2.1 m; 90 chicks per pen) at the research facility of AVIAGEN Ltd., Newbridge, UK. The chicks received a common wheat based starter diet (sieved crumble, d 1 to 10). From d 11 a 2 x 4 factorial approach with 2 energy levels (95 and 100 % of Aviagen recommendation) and 4 levels of balanced protein (80, 90, 100, and 115 % of Aviagen recommendation) was applied resulting in 8 dietary treatments per sex and 6 replicate pens per treatment. Environmental conditions (temperature, humidity, light program) were in line with the recommendations given in the Ross Management Guide (Aviagen, 2002).

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The grower diets were fed from day 11 to 25 and finisher diets were fed from day 26 to 42. The pelleted grower and finisher feeds as well as water were offered *ad libitum*. Pellet quality was good due to the high inclusion levels of wheat. However, in the grower diets sieve analysis revealed a less stable pellet with increasing dietary fat content (increasing ME). This effect was not observed in the finisher diets.

Diets were mainly based on wheat and soybean meal and the ME level was reduced by decreasing fat and increasing wheatfeed (Table 1). At both ME levels the BP content was stepwise increased by increasing the levels of soybean meal, maize gluten meal and fish meal. The inclusion level of fat had to be increased at the high BP diets due to lower ME content of soybean meal compared to wheat.

Performance data were subjected to ANOVA analyses using Genstat (9th Edition) using the following statistical model:

$$Y_{ijkl} = \mu + R_i + B_j + F_k + R*B_{ij} + R*F_{ik} + B*F_{jk} + R*B*F_{ijk} + e_{ijkl},$$

where Y_{ijkl} is a specific trait per experimental unit (pen of birds), μ the overall mean, R_i is the ME effect ($i = 95$ or 100), B_j is the effect of BP level ($j = 80, 90, 100$ and 115), F_k is the effect of sex ($k =$ female or male), $R*B_{ij}$, $R*F_{ik}$, $B*F_{jk}$, and $R*B*F_{ijk}$ the interactions between the factors, and e_{ijkl} is the residual error term.

Table 1.   Ingredient and nutrient composition of experimental diets with lowest and highest balanced protein (BP) levels and the two metabolizable energy (ME) levels

	Grower (d 11 – 25)				Finisher (d. 26 – 42)			
ME[1]	100 %		95 %		100 %		95 %	
BP[1]	80%	115%	80%	115%	80%	115%	80%	115%
Diet composition, g/kg								
Wheat	696	470	659	437	731	528	695	497
Soybean meal	183	365	177	355	142	313	141	303
Wheat feed	20.0	20.0	80.0	80.0	20.0	20.0	80.0	80.0
Corn gluten meal	5.0	10.0	5.0	10.0	5.0	10.0	5.0	10.0
Fish meal	5.0	15.0	5.0	15.0	5.0	10.0	5.0	10.0
Vit. and Min.	39.2	35.6	38.9	35.3	37.6	35.1	37.3	34.8
Soya oil	20.0	20.0	10.0	10.0	20.0	20.0	10.0	10.0
Vegetable fat	25.7	57.2	18.9	49.8	28.3	57.5	21.0	49.4
Liq. L-Lys (50%)	3.5	2.6	3.5	2.7	3.3	2.4	3.2	2.5
DL-Methionine	1.8	3.5	1.8	3.4	1.5	3.1	1.5	3.1
L-Threonine	0.8	0.9	0.8	1.0	0.7	0.9	0.7	0.9
Calculated nutrient contents, g/kg								
Crude Protein	168	247	171	248	153	223	156	224
ME, MJ/kg	13.19	13.19	12.53	12.53	13.40	13.40	12.73	12.73
TFD[2] Lys	8.8	12.7	8.8	12.7	7.8	11.2	7.8	11.2
TFD Met+Cys	6.7	9.6	6.7	9.6	6.1	8.7	6.1	8.7
TFD Thr	5.8	8.3	5.8	8.3	5.2	7.5	5.2	7.5

[1]Energy and balanced protein target levels are given as % of Aviagen recommendation (Aviagen, 2002). [2]True Fecal Digestible

III. RESULTS AND DISCUSSION

Reducing the dietary ME level from 100 to 95 % significantly ($p < 0.001$) increased feed intake at all dietary protein levels. However, the increased feed intake did not entirely compensate for the reduced dietary ME level and thus the ME intake decreased on average by 2.2 %. This response is in contrast to former experiments where broilers fully compensated for a 5% reduction in dietary energy content in pelleted diets (Lemme 2004; Huang et al.

2008). At both energy levels, the feed intake decreased as the dietary content of BP increased and consequently the energy intake decreased as well. In contrast, the protein intake increased linearly as the reduced feed intake was more than compensated by the increased dietary protein content. Live weight increased significantly as the BP supply increased ($p < 0.05$), however differences between the two ME levels became smaller as the BP level increased (see Figure 1). Although 15 % above the Aviagen recommendations the highest level of BP led to a numeric increase in live weight gain at both ME levels. The FCR was impaired as the dietary ME level was reduced ($p < 0.01$). On the other hand, increasing BP supply improved FCR ($p < 0.05$) which is in line with previous trial results (Lemme et al. 2006) and the findings of Plumstead et al. (2007). Even the highest BP level caused a further improvement of the FCR although it was above the recommended level.

Table 2.   Effect of energy and balanced protein (BP) level on live weight (LW), feed conversion ratio (FCR), carcass yield and breast meat yield in female (F) and male (M) Ross 308 broilers at day 42

Sex	Energy[1] (%)	BP[1] (%)	LW (g)	FCR[2] (g)	Carcass (% of LW)	Breast (% Carcass)
M	95	80	3159	1.89	65.8	24.9
		90	3357	1.82	65.9	25.5
		100	3346	1.76	66.0	26.7
		115	3433	1.69	66.7	27.3
M	100	80	3301	1.79	65.9	24.6
		90	3330	1.75	65.9	25.1
		100	3397	1.70	66.4	26.0
		115	3446	1.63	67.0	27.3
F	95	80	2696	1.90	66.6	25.5
		90	2745	1.84	67.2	26.6
		100	2802	1.80	67.4	27.3
		115	2774	1.76	67.5	27.8
F	100	80	2727	1.83	66.5	25.4
		90	2759	1.79	66.8	25.9
		100	2761	1.77	67.3	27.6
		115	2805	1.72	67.2	27.9
P values	ME		0.006	0.001	0.947	0.044
	BP		0.001	0.001	0.001	0.001
	Sex		0.001	0.001	0.001	0.001
	ME*BP		0.004	0.157	0.809	0.362
	ME*Sex		0.058	0.015	0.202	0.334
	BP*Sex		0.001	0.010	0.402	0.302
	ME*BP*Sex		0.010	0.756	0.976	0.299

[1]ME and BP levels are given in % of Aviagen recommendation. [2] corrected for mortality

The dietary level of BP significantly increased carcass yield ($p < 0.001$) whereas the ME level had no effect ($p > 0.05$). Furthermore, reducing the ME level as well as increasing the dietary BP supply decreased the abdominal fat and increased breast meat significantly ($p < 0.05$). This is in line with the findings of Lemme (2004) and suggests that the genetic

potential of the broilers was not fully expressed by feeding the 100 % BP diet.
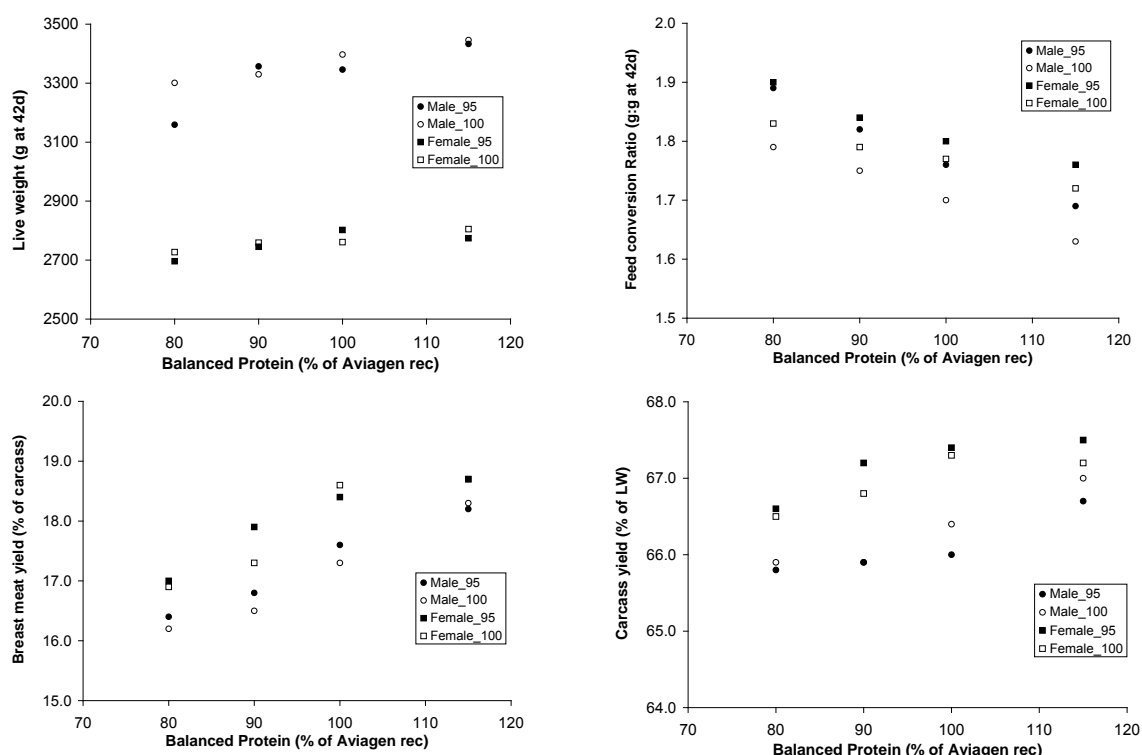
Figure 1.   Live weight (top left), mortality corrected feed conversion ratio (top right), breast meat yield (bottom left), and carcass yield (bottom right) in 42 days old female (■, □) and male (●, ○) Ross 308 broilers in response to increasing levels of balanced protein at two energy levels (95% (■, ●) and 100% (□, ○))

IV. CONCLUSION

The present data show that broilers did not completely compensate ME intake by increased feed intake when dietary content of ME was reduced by 5% compared to recommended levels. Moreover, increasing dietary BP improved weight gain and particularly feed conversion ratio independently from the dietary ME level. Thus, economic optimum for both ME and BP will depend on the price ratio of protein and ME in feed ingredients and one optimum can not be given.

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VARIATION IN NUTRIENT COMPOSITION AND STRUCTURE OF HIGH-MOISTURE MAIZE DRIED AT DIFFERENT TEMPERATURES

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Summary

The chemical composition and structure of high moisture maize grains were investigated after drying at different temperatures and compared to sun-dried maize. The chemical composition was affected by artificial drying of high-moisture maize grain. It is thought that this may have some ramification for the nutritive value of the grain when fed to chickens.

I.INTRODUCTION

In many parts of the world maize (*Zea mays*) is harvested at relatively high moisture (HM) content, with a view to minimizing damage in the field when left to dry naturally. The grain is then subjected to artificial drying, which may result in loss of quality such as increase in retrograde starch content (Brown, 1996). Heat processing may also anneal the starch as the grain cools down. The digestibility of cereal grains is influenced by the starch component, especially the ratio between amylose and amylopectin (McDonald et al., 1995) Due to its amorphous nature, amylopectin is more readily digested than the amylose. The normal structure (spherical, 10-16 microns across) of starch granules with protein bodies and matrix may be influenced easily and can create a favorable environment for enzymatic digestion (Taylor and Belton, 2002). The present study was carried out with a view to investigate the variation in chemical composition and structure of the HM maize grains and how this affects on the nutritional value subsequently.

II. MATERIALS AND METHODS

Maize grain, from the 2009 planting year, was obtained from Inverrel in northern NSW at around 23% moisture. After collection, maize cobs were split into four groups and dried under the sun or artificially in an oven at 80, 90 or $100^{o}C$ for 24 hours. The proximate nutrient composition of the maize samples were determined using standard methods (AOAC, 2002). The gross energy (GE) contents of the samples were determined using an IKA® - WERKE bomb calorimeter at UNE. Metabolizable energy (ME) was calculated according to the equation [ME (Kcal/kg) = 53+38 × (%CP +2.25 × %EE +1.1×%Starch +%Sugar)] developed by Carpenter and Clegg (1956).

Total and resistant starch contents were determined according to the method of McCleary et al. (1994). Amylose/amylopectin ratio was also determined with Megazyme amylose/amylopectin assay kit using the selective quantitative precipitation reaction of concanavalin A (ConA) with amylopectin according to Gibson et al. (1996).

The concentrations of amino acids were determined at the Australian Proteome Analysis facility at Macquarie University using pre-column derivatisation of amino acid with 6-aminoquinoly-N-hydroxysuccinimidyl carbamate (AQC) followed by separation of the derivatives and quantification by reversed phase high performance liquid chromatography. Mineral elements were analyzed by inductively coupled plasma (ICP) method (Vista MPX-radial) and the sealed digest chamber digest (SCD) at UNE on the basis of Anderson and Henderson (1986).

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Non-starch polysaccharide (NSP) contents were measured by gas chromatography (VARIAN, CP-3800, USA) at UNE as outlined by Englyst and Hudson (1993). The *in vitro* digestibility of dry matter and starch was determined by the method of Babinszky et al. (1990) and viscosity according to Bedford and Classen (1993). The grains were scanned on a NeoScope, JCM-5000 table-top SEM (JEOL Ltd, Tokyo, Japan). Whole grain samples were scoured around the edges and cut in sections, then mounted on the machine for assessment with a magnification of x1000. All the collected data were subjected to non-parametric analyses using SPSS (17th Version) followed by calculations of coefficient of variation (CV).

III. RESULTS AND DISCUSSION

The proximate composition of the different batches of maize is shown in Table 1. The DM and ash contents were increased by artificial drying of the grains at all temperature, compared to sun drying. However, the CP, CF and phytate contents were decreased by up to 4.7, 8.2 and 32.8%, respectively, as a result of artificial drying of the grains. The GE content was decreased by 2.2% but ME content, estimated from nutrient composition, was increased by 2.1%. Artificial drying also increased the concentrations of most amino acids.

Table 1.   Chemical composition of maize batches observed under different drying temperatures

A. Dry matter, crude protein, crude fat, ash, phytate-P (g/kg DM) GE and ME (MJ/kg DM)

	DM	CP	CF	Ash	Phytate-P	GE	ME[1]
Sun-dried	870.0	98.4	45.0	1.23	1.80	18.86	15.42
80 °C	950.0	93.4	42.0	1.29	1.36	18.36	15.58
90 °C	963.0	92.2	42.1	1.30	1.35	18.41	15.50
100 °C	980.0	93.8	41.3	1.32	1.21	18.45	15.74
CV	*0.05*	*0.03*	*0.04*	*0.03*	*0.18*	*0.01*	*0.01*

B. Amino acids (g/kg DM)

	Met	Lys	Thr	Ala	Phe	Arg	Leu	Isoleu	Val
Sun-dried	1.2	2.2	3.0	6.4	4.3	4.2	10.3	3.3	4.1
80 °C	1.4	2.0	3.1	6.8	4.4	4.3	11.2	3.7	4.3
90 °C	1.4	1.9	3.2	6.9	4.6	4.2	11.7	3.6	4.4
100 °C	1.3	1.9	3.3	7.0	4.7	4.3	11.9	3.8	4.4
CV	*0.08*	*0.08*	*0.03*	*0.04*	*0.04*	*0.01*	*0.07*	*0.06*	*0.03*

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

	Starch	Resistant starch	Amylopectin	Amylose	Amylose: Amylopectin
Sun-dried	670.1	316.6	506.7	280.3	0.55
80 °C	691.3	362.7	424.1	303.7	0.72
90 °C	687.8	366.3	369.0	308.0	0.83
100 °C	684.0	415.7	306.6	313.8	1.02
CV	*0.01*	*0.11*	*0.21*	*0.05*	*0.25*

D. Minerals

	Macro-elements (g/kg DM)					Micro-elements (mg/g DM)				
	Ca	P	Na	K	Mg	Mn	Zn	Fe	Cu	Mo
Sun-dried	0.05	2.9	7.9	3.5	1.6	6.2	17.0	37.4	1.0	0.43
80 °C	0.05	2.6	7.3	3.3	1.4	8.7	15.4	27.9	1.1	0.43
90 °C	0.04	2.5	7.1	3.1	1.3	5.1	14.0	25.2	1.1	0.39
100 °C	0.03	2.6	7.0	3.2	1.4	5.6	14.9	18.7	1.5	0.39
CV	*0.23*	*0.08*	*0.05*	*0.05*	*0.08*	*0.25*	*0.08*	*0.28*	*0.19*	*0.06*

CV = Coefficient of variation, [1]According to chemical analysis (Carpenter and Clegg, 1956).

Total starch, resistant starch and amylose contents were increased by 2.1, 31.3 and 12.0%, respectively for the grain dried at 100 $^{\circ}$C, compared to the sun-dried grain. Conversely, the content of amylopectin was decreased by 39.5%.

Mineral content was adversely affected by artificial drying of the grain. For example, the concentrations of Ca, P, Na, K and Mg were reduced by 40.0, 10.3, 11.4, 8.6 and 12.5%, respectively as a result of drying at 100 $^{\circ}$C, when compared to sun drying.

Total soluble NSP content varied from 3.29 to 3.79 g/kg, being generally higher in the artificially dried grains than in the sun-dried samples (Table 2). Ribose and arabinose were the most variable of the component sugars, in terms of the CV. There was very little variation in the concentrations of insoluble NSP, with CV generally lower than 0.05.

Table 2.   Non- Starch Polysaccharide (NSP) components of maize batches observed under varying drying temperatures

A. Soluble NSP content and components (g/kg DM)

	Rib	Ara	Xyl	Man	Gal	Glu	NSP
Sun-dried	0.05	0.55	0.35	1.89	0.34	0.50	3.29
80 $^{\circ}$C	0.08	0.77	0.46	1.84	0.43	0.60	3.87
90 $^{\circ}$C	0.07	0.76	0.46	1.86	0.40	0.58	3.69
100 $^{\circ}$C	0.08	0.85	0.49	1.83	0.39	0.61	3.79
CV	*0.19*	*0.17*	*0.14*	*0.05*	*0.10*	*0.09*	*0.07*

B. Insoluble NSP content and components (g/kg DM)

	Ara	Xyl	Man	Gal	Glu	NSP
Sun-dried	20.08	25.34	1.00	5.71	22.34	66.11
80 $^{\circ}$C	19.42	24.68	0.94	5.29	21.98	64.21
90 $^{\circ}$C	19.56	25.27	0.93	5.50	22.93	65.87
100 $^{\circ}$C	19.38	25.29	0.93	5.38	22.60	65.33
CV	0.02	0.01	0.04	0.03	0.02	0.01

In vitro digestibility indicated (Table 3) that digestibility of DM was improved by artificially drying the HM maize but the starch digestibility was reduced (Table 3). There was no effect of treatment on *in vitro* digesta viscosity.

Table 3.   *In vitro* digestibility of dry matter and starch as well as viscosity of maize batches observed under varying drying temperatures

Treatment	Digestibility		Viscocity (cp)[1]
	Dry matter	Starch	
Sun-dried	48.0	63.0	0.92
80 $^{\circ}$C	52.4	58.2	0.91
90 $^{\circ}$C	52.3	57.6	0.91
100 $^{\circ}$C	52.5	58.4	0.90
CV	0.04	0.04	0.009

[1] cp (centipoise) = 1/100 dyne second per centimeter^2

The scanning micrographs of the different grains show variations in the morphology of the starch granules (Plate 1). The granules shrunk in size as a result of artificial drying of the grains. The internal matrix of the grains, which may reflect the linkage of starch and protein bodies, was also different.
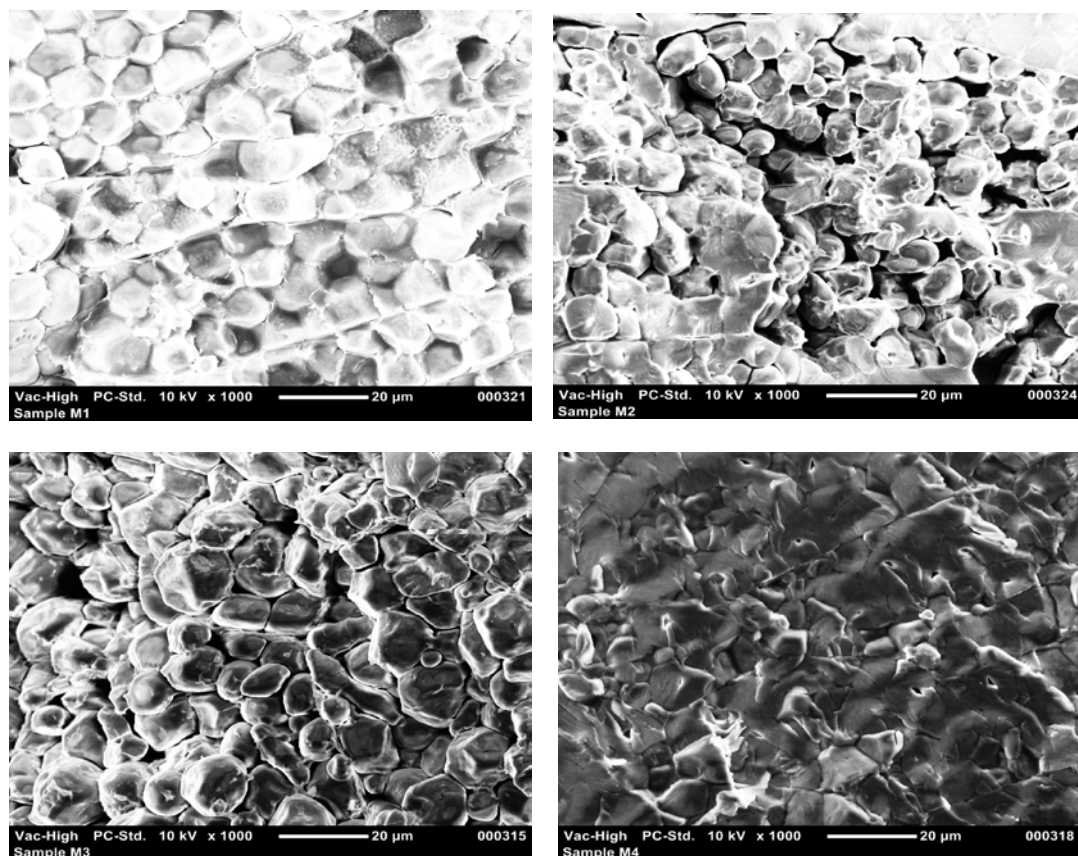
Plate 1.   Electronic microscope images (x1000) of sun-dried (top left) or grain dried at 80°C (top right), 90°C (bottom left) and 100°C (bottom right).

IV. CONCLUSIONS

It can be concluded that artificial drying of HM maize alters the composition of the grain. Further study can be conducted to ascertain if the nutritive value of the grain is affected, or if it could be improved through nutritional intervention.

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INFLUENCE OF FEED FORM ON INTAKE PREFERENCE AND PERFORMANCE OF YOUNG BROILERS

K.H. HUANG[1] and M. DE BEER[1]

Summary

A series of experiments was conducted to investigate feed particle size preference and the effect of feed form on performance of young broilers. Feed particle size significantly influenced feed intake preference of modern young broilers, and affected their ability to achieve genetic potential weight gain at both 7days and at market age. In experiment 1 with broilers aged between 1 and 14 days, the birds preferred a particle size between 0.86mm and 2.00mm. As birds grew, they tended to prefer bigger particle sizes. Birds did not select fine particles (< 0.86 mm) even at 3 days of age. Experiment 2 studied the performance of broilers between 0 and 9 days of age. Feeds of different particle sizes significantly (P < 0.05) influenced performance over this period. In experiment 3, comparing crumble feed with cold mash, the crumble significantly increased body weight and feed intake, while feed conversion was significantly better in crumble, compared coarse mash, but similar to find mash. It was concluded that young broilers have preferences for feed particles of a certain size to maximize feed intake and subsequently this behaviour influenced their growth performance. With broilers reaching market weight at younger ages, the improved early growth as affected by particle size and feed form would be of commercial significance.

I. INTRODUCTION

Feed physical quality has a major influence on modern broiler performance. Published literature shows that broilers fed with good-quality pellets have better growth performance and feed conversion than those fed with mash, reground pellets, or pellets with more fines (Huang et al., 2008; Kenny and Flemming, 2006; Lemme et al., 2006). However, there is less clarity about how feed particle size influences broiler feeding behaviour and about how responses to feed physical quality in the early stages of growth, up to 14 days, are reflected in performance at later ages. In general 7-day body weight is strongly correlated with body weight at market age. The work reported here investigated the effect of feed form on young broiler feed choice, and on biological performance.

II. FEED PREPARATION

Corn-soybean meal based broiler starter and grower feeds, formulated to Aviagen Broiler Nutrition Specifications (2007), were pelleted (3 mm die) and then crumbled. The crumbled feed was then passed through a series of sieves in experiment 1 and 2 in order to separate different size fractions. The feeds for experiment 1 and 2 were mixed to the same specifications and separated by the same methods. After sieving there were four feed fractions in experiment 1 and three fractions in experiment 2 as shown in Table 1. In experiment 3, the particle size analysis of crumble and mash feeds was as shown in Table 2. In experiment 1 and 2, the different fractions were tested for moisture, crude protein, fat, fibre, calcium and phosphorous. In experiment 3, both feeds were tested for moisture, crude protein, fat, calcium and sodium. There were no significant differences in the contents of any major nutrients between the separated fractions or feed forms (data not shown).

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Table 1.   The fractions of feed particle size for Experiment 1 and 2.

Experiment	Feed particle size (mm)	
	Starter (1-10 days)	Grower (11-14 days)
Experiment 1	< 0.86	< 2.00
	0.86 - 2.00	2.00 – 3.18
	2.00 - 3.18	3.18 – 4.76
	> 3.18	> 4.76
Experiment 2	< 0.86	
	0.86 – 2.00	
	2.00 – 3.18	

Table 2.   The distribution of feed particle size in Experiment 3 (starter 1-10days).

Feed particle size (mm)	Crumble (%)	Fine mash (%)	Coarse mash (%)
< 1.00	22.73	96.00	41.44
1.00 – 2.00	32.25	4.00	24.74
2.00 – 3.00	21.18	0.00	17.36
> 3.00	24.84	0.00	16.46

III. FEED PARTICILE SIZE PREFREENCE OF YOUNG BROILERS

Experiment 1, utilized a total of six pens with 50 straight run birds per pen. All pens had identical nipple drinkers and brood lights. Each pen contained four feeders with one each of the four different size fractions of starter or grower feeds. All feed fractions and water were offered *ad libitum.* The feeders were placed equidistant from the brood light and randomly rotated so that no feeder was in the same location for two days running. This prevented chicks from "learning" where their preferred particle size was located. The results are shown in Table 3, expressed as relative percentage of total intake for each period. At each age there were significant differences in the amount of each fraction consumed. and, as birds got older, they increased consumption of bigger particle sizes. Birds were eating significantly more feed of particle size between 0.86 to 3.18 mm for each age studied.

IV. FEED FORM AND BROILER PERFORMANCE

In experiment 2, a total of 12 pens with 50 straight run birds per pen were utilized. Three separated feed fractions were compared (Table 1), with all material over 3.18 mm being discarded. Each fraction was fed separately to 4 replicate pens. All feed fractions and water were offered *ad libitum.* Feed intake, body weight and feed conversion ratio (FCR) were determined for each pen at 5 and 9 days of age. The results were shown in Table 4. Feed particle size significantly ($P < 0.05$) affected live weight, feed intake and FCR at 9 days, but not at 5 days ($P > 0.05$). The particle size between 2-3.18 mm achieved the best body weight and FCR among the treatments, while particle size less than 0.86 mm gave the poorest performance.

Table 3.   Feed particle size preference of broiler chicks between days 1 to 14 (Experiment 1).

Feed particle size (mm)	Starter (day 1-10)			Feed particle size (mm)	Grower (day 11-14)
	Feed intake day1-3 (%)	Feed intake day 4-6 (%)	Feed intake day 7-10 (%)		Feed intake day 11-14 (%)
< 0.86	25.19[b]	21.87[b]	9.68[c]	< 2.00	22.02[c]
0.86 - 2.00	38.90[a]	49.57[a]	53.97[a]	2.00 - 3.18	42.12[a]
2.00 - 3.18	21.60[c]	16.82[bc]	20.59[b]	3.18 - 4.76	33.91[b]
> 3.18	14.32[d]	11.74[c]	15.76[bc]	> 4.76	1.95[d]
SEM	1.634	1.813	2.301	SEM	1.95
P	0.001	0.001	0.001	P	0.001

[abc]values without common superscript differ significantly ($P < 0.05$).

Table 4.   Effect of feed particle size on broiler performance at 5-days and 9-days (Experiment 2)

Feed particle size	5 Days				9 Days			
	Live Weight (g)	Feed Intake (g)	FCR (g/g)	Livability (%)	Live weight (g)	Feed Intake (g)	FCR (g/g)	Livability (%)
2.0 - 3.18mm	112.5	62.55	0.555	99.5	233.8[a]	140.3[a]	0.869[c]	99.0
0.86 - 2.0mm	108.5	61.675	0.566	99	215.0[b]	132.9[b]	0.902[b]	98.0
< 0.86mm	104.75	61	0.583	99.5	194.0[c]	126.6[c]	0.967[a]	99.5
SEM	2.727	0.859	0.0172	0.726	2.827	1.683	0.00916	0.500
P	0.213	0.485	0.543	0.857	0.001	0.004	0.001	0.178

[abc]values without common superscript differ significantly ($P < 0.05$).

V. FEED FORM AND BROILER FEEDING BEHAVIOR

Experiment 3, utilized a total of 24 pens with 16 male birds per pen to give 8 replicates per treatment in a randomised block design. Three treatments consisted of crumble, coarse and fine mash. The particle size distribution is shown in Table 2. All feeds and water were offered *ad libitum.* Feed intake, body weight, FCR, and liveability were determined for each pen at 10 days of age. The results are presented in Table 5. Live weight, feed intake and FCR were significantly ($P < 0.05$) affected by feed form, with best performance on the crumble treatment. FCR was significantly ($P < 0.05$) better in crumble, compared coarse mash, but similar ($P > 0.05$) to fine mash.

Table 5.   Effect of feed form on broiler performance at 10days (Experiment 3)

Treatment	Live Weight (g)	Feed Intake (g)	FCR (g/g)	Liveability (%)
Crumble	327.5[a]	300.4[a]	1.064[b]	100
Coarse Mash	289.0[b]	284.1[b]	1.165[a]	99.22
Fine Mash	289.6[b]	264.4[c]	1.079[b]	100
SEM	2.981	2.785	0.0105	0.451
P	0.001	0.001	0.001	0.393

[abc]values without common superscript differ significantly ($P < 0.05$).

VI. CONCLUSION

The present results demonstrate that young broilers do show preference for feed particle size and this preference tends to change to bigger particle size as the birds age. Birds rejected fine crumbles (< 0.86 mm) even at 3 days of age. When the birds were provided with the preferred crumble size, their performance was significantly ($P < 0.05$) improved at 9 days. Feed form is important for modern broilers to achieve maximum growth, even at a very young age.

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DIFFERENTIATION BETWEEN PATHOGENIC SEROTYPE 1 ISOLATES OF MAREK'S DISEASE VIRUS (MDV1) AND THE RISPENS VACCINE IN AUSTRALIA USING REAL-TIME PCR.

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Summary

Two real-time PCR assays were developed which enable quantification and differentiation between pathogenic Australian isolates of MDV serotype 1 and the serotype 1 vaccine strain Rispens/CVI988. The assays are based on a DNA sequence variation in the *meq* gene between pathogenic and vaccinal MDV1 which has been confirmed by sequencing of 20 Australian field strains of MDV. Complete specificity has been demonstrated in samples containing pathogenic MDV (n=20), Rispens (3 commercial vaccine strains), or both. The limit of detection of both the Rispens-specific and the pathogenic MDV1-specific assays was 10 viral copies/reaction.

I. INTRODUCTION

Marek's disease virus (MDV) is classified as an Alphaherpesvirus and isolates of MDV can be classified into three serotypes, namely serotypes 1 (MDV1), 2 (MDV2) and 3 (herpesvirus of turkeys or HVT) of which only MDV1 is pathogenic and oncogenic (Schat and Calnek, 1978). Since the detection of the aetiological agent in the late 1960s (Churchill and Biggs, 1967), MD has been controlled to a large extent using vaccines which consist either of attenuated isolates of oncogenic MDVs or the apathogenic HVT or MDV2 serotypes (Witter et al., 1970; Schat and Calnek, 1978).

Vaccination against Marek's disease using live vaccines provides protection against clinical MD but not against co-infection with wild-type pathogenic MDVs which continue to multiply in the host and be shed in feather dander at very high levels (Islam and Walkden-Brown 2007). Thus vaccinated chickens may harbour mixed populations of MDVs with considerable implications for diagnosis of infection. In Australia broiler chickens either remain unvaccinated or are vaccinated *in ovo* with HVT while layers and broiler breeders usually are vaccinated with the MDV1 Rispens/CVI988 vaccine for long-term protection. Rispens/CVI988 is the dominant attenuated MDV1 vaccine in use worldwide.

Several molecular tests using the real-time quantitative PCR (qPCR) technique are available in order to differentiate between the three MDV serotypes (eg. Islam et al., 2006, Renz et al., 2006), but there is no quantitative test which will differentiate between pathogenic MDV1 and Rispens/CVI988. Previous tests to differentiate Rispens from pathogenic MDV were based on the number of 132 bp repeats (Becker et al. 1992). However these assays were not able to quantify virus and it has subsequently been shown that this marker is lost in as little as one back passage in chickens (Young and Gravel 1996). There is a preliminary report of a qPCR test to differentiate wild type MDV1 from Rispens vaccine based on the pp38 and ICP4 genes (Zelnik et al., 2008) but it is unclear that assay development is complete.

This paper describes the first molecular assay using the qPCR technique to differentiate between Australian field isolates of MDV1 and the Rispens vaccine.

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II. MATERIAL AND METHODS

DNA from six Australian isolates of MDV1, namely MPF57, Woodlands1, MPF132/5, 02LAR, 04KAL, FT158 was obtained from spleen samples collected at 13 days post infection (dpi) from experimentally infected unvaccinated specific pathogen free (SPF) chickens involved in a pathotyping experiment (Walkden-Brown et al., 2006). The isolates induced gross MD lesions in 52.9-94.4 % of unvaccinated chickens and the protective index provided by HVT vaccination varied from 38.2 % to 100 %.

The spleens were stored in sterile 1.5 ml Eppendorff tubes and kept at -20 °C until DNA was extracted from infected spleens using the QIAamp DNA Kit (Qiagen, Clifton Hill, Australia) according to the manufacturer's instructions.

The three commercially available Rispens vaccines in Australia (Vaxsafe® RIS, Poulvac® CVI988 and Nobilis® Rismavac) were obtained from the manufacturers (Bioproperties, Fort Dodge, Intervet). Prior to phenol-chloroform extraction of DNA, samples were treated with Proteinase K. All vaccinal samples were subject to sequence analysis of a fraction of the *meq* gene to confirm the presence of the targeted polymorphism in *meq* as described by Renz (2008). Sequencing of purified DNA was conducted by Macquarie University, Sydney, Australia using an ABI 377 sequencer (Applied Biosystems Inc., Foster City, CA, USA). All DNA samples were quantified using a NanoDrop® ND-1000 UV-Vis spectrophotometer (NanoDrop® Technologies Wilmington, USA). In addition to the six initial pathogenic MDV1 isolates, the target region of MDV from 14 field samples positive for MDV1 were sequenced and tested in the two assays.

Extracted DNA was used as template for two standard PCR tests, designed to either amplify only pathogenic MDV1 or Rispens/CVI988. Primers and the probe for the qPCR assays were designed using Beacon designer 6.00 (PREMIER Biosoft International, Palo Alto, USA). The standard PCR was performed in a 25 µl reaction mixture containing 1 µmol of each primer, 1.8 mM $MgCl_2$, 0.2 mM dNTP's, 10x reaction buffer (Fisher Biotec, Perth, Australia), 1 unit of Taq DNA polymerase and approx. 1 ng of template DNA. Amplification was carried out over 35 cycles each consisting of 1.5 min at 94°C, 1 min at 60°C and 2 min at 72°C, except for the initial 2 cycles in which the period at 94°C was extended to 5 min. After the final cycle, the elongation phase at 72°C was extended to 10 min with a consecutive step at 4°C for 5 min. The amplified fragments were separated on an agarose gel (1%) and visualized by staining with ethidium bromide.

After the primer sets were confirmed to only amplify either pathogenic or Rispens/CVI988 in several standard PCR assays, TaqMan® real-time qPCR assays were set up using a RotorGene 3000 real-time PCR machine (Corbett Research, Sydney, Australia). The qPCR cycling parameters used for the assays were described by Islam et al. (2006). However, the annealing time was extended from 45 sec to 60 sec.

The sensitivity of the assays was determined by running tenfold serial dilutions of plasmid DNA with known copy numbers for both pathogenic and vaccinal MDV1. The lowest dilution in the tenfold dilution series which amplified reliably was defined as the detection limit. The reproducibility of the qPCR assays with the new plasmid-derived standard curves was measured by calculating the intra-assay coefficient of variation (CV) by taking the mean CV for duplicate Ct and calculated copy number for plasmid standards in all runs of the same assay. The inter-assay CV was determined by comparing the mean C_t and calculated copy number for each standard in three separate but identical assay runs and determining the CV for each across assays. Each individual assay was performed on separate days and serial dilutions of plasmid as well as the reference standards were prepared freshly on each day. After parallelism with the plasmid standard curves had been confirmed, the

reference standards derived either from spleen tissue or Rispens vaccine were quantified in terms of viral copy number in three independent identical assays.

III. RESULTS

The product size with both primer sets matched the expected 130 bp (Figure 1). None of the three Rispens vaccines amplified with the pathogenic specific primer set whereas all six pathogenic MDV1 isolates amplified (Figure 1, upper lanes). Conversely, none of the six pathogenic MDV1 isolates amplified with the Rispens specific primer set whereas all three Rispens isolates did (Figure 1, lower lanes). All 14 additional field isolates which were tested with both assays only amplified in the pathogenic MDV1 specific assay, but not in the Rispens specific assay which was expected as all field isolates were derived from flocks which had not been vaccinated with Rispens (data not shown).
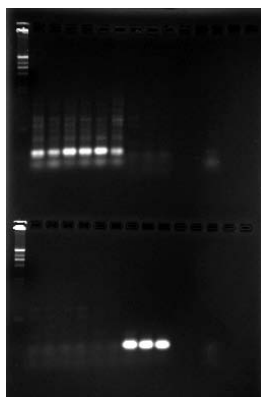
Figure 1:   1% Agarose gel. Upper lanes: pathogenic MDV1 specific primers. From left to right: λ-HindIII standard, six pathogenic MDV1 isolates, three Rispens isolates, blank, negative control. Lower lanes: Rispens/CVI988 specific primers. Same alignment as above.

For the real-time quantitative PCR assay none of the three Rispens vaccines amplified in the pathogenic MDV assay, but all samples containing pathogenic MDV1 or both Rispens and pathogenic MDV1 amplified. For the Rispens/CVI988 specific assay pathogenic MDV1 samples did not amplify whereas the 3 Rispens vaccines did.

For both assays, the qPCR conditions were optimised (increased annealing time from 45 to 60 sec) in order to provide sensitivity similar to that of our generic, non-differentiating MDV1 assay (Islam et al., 2006) which is 2.7 viral copy numbers (VCN) per reaction. With these settings, standard curves of both tests amplified reliably 10 VCN/reaction. Based on three individual runs for each assay with samples run in duplicate, intra-assay C_t- values had a CV of 1.58 % (pathogenic MDV assay) and 1.37 % (Rispens assay), while the mean inter-assay C_t- values had a CV of 22.34 % and 19.87 % respectively.

IV. DISCUSSION

This is the first set of molecular assays which reliably detect, quantify and differentiate Australian pathogenic strains of MDV and Rispens, a tool which the poultry industry has long sought. The tests are highly sensitive and have shown 100% specificity when screened against the 3 Rispens vaccines on the market and 20 Australian MDVs. They now enable flock testing for vaccine take, and for monitoring of pathogenic MDV infections in vaccinated layer and breeder chickens, probably via pooled feather or dust samples. It is now also possible to test MDV1 isolates for Rispens contamination, and to study the replication and spread of MDV in breeder and layer populations. To determine the stability of the target area in our tests over time, the three available Rispens vaccines are currently serially back-passaged five times in chickens and will be subject to DNA sequencing after the last passage.

However it should be noted that these assays are specific for Australian strains of MDV only. Based on the DNA sequences available online from pathogenic strains of MDV1, American, Asian or European strains do not differ from Rispens at this position in the *meq* gene. We are currently evaluating potential sites of other candidate genes such as pp38, ICP4 or vIL-8 in order to develop assays which will differentiate between both Australian and international pathogenic MDV1 isolates and Rispens vaccine.

V. ACKNOWLEDGEMENTS

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A QUANTITATIVE PROFILE OF INFECTIOUS BRONCHITIS VIRUS IN FAECES OF LAYING HENS

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Summary

Two independent real time PCR assays were designed to detect and quantify T and N1/88 strains of infectious bronchitis virus (IBV) from faeces of experimentally-infected unvaccinated and unvaccinated laying hens. Vaccination VicS-A3-VicS during rearing can reduce the viral load in the faeces of vaccinated and T or N1/88 challenged hens. Both vaccinated and unvaccinated hens can shed T strain of IBV in faeces up to 9 weeks p.i. It is now possible to detect and differentiate N1/88 strain of IBV from vaccine strains. It is also possible to detect and quantify T and N1/88 strain of infectious bronchitis virus directly from faeces.

I. INTRODUCTION

Infectious bronchitis virus (IBV) is a highly infectious and contagious pathogen of chickens worldwide. IBV spreads rapidly amongst the chickens in a flock. The frequency of isolation varies with the time frame but Alexander and Gough (1977) isolated virus from caecal tonsils and faeces up to 14wk and 20wks, respectively. Jones and Ambali (1987) isolated the G strain of IBV up to 28 wks of age in tracheal and cloacal swabs from birds infected at day-old. Currently, reverse transcriptase polymerase chain reaction (RT-PCR) is commonly used for diagnosis of IBV. Using RT-PCR, IBV has been detected directly from clinical samples such as the trachea, kidney or cloacal swabs (Mardani et al., 2006). Previously, we have reported use of the real time PCR assay for the detection and quantification of Australian strains of IBV from the oviduct (Chousalkar et al., 2009). There are no reports of IBV detection and quantitation from faeces using molecular diagnostic tests such as real time PCR. In the current study, the real time PCR test was designed for the rapid detection of IBV strains from the faeces of IBV infected unvaccinated and vaccinated laying hens.

II. MATERIALS AND METHODS

Day old chickens (n= 190) were obtained from the Baiada Hatchery at Marsden Park. At day-old, all the chickens received Rispens vaccine against Marek's disease but no other vaccinations at the hatchery. The chickens were raised on the floor in isolation sheds at the University of New England. Half 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, birds were exposed to A3 at the dose rate of $10^{3.9}$ E.I.D.$_{50}$. At 13 weeks of age, birds were again vaccinated with Vic S at the dose rate of $10^{4.5}$ E.I.D.$_{50}$. Vaccines were obtained from Fort Dodge, Australia. The other 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 T (T), vaccinated challenged with T (VT), unvaccinated challenged with N1/88 (N) and vaccinated challenged with N1/88 (VN). All the birds were moved into cages at the age of 25 weeks. At 30 weeks of age, each bird from groups T, VT,

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N and VN were challenged with one of two different strains of IBV, T and N1/88 (obtained from Dr. Jagoda Ignatovic, CSIRO, Geelong) and the control birds were sham inoculated with normal saline. Faeces were collected in individual sterile plastic containers from five hens from each group at weekly intervals from 1 to 9 weeks post infection (p.i.). RNA from faeces was extracted as described previously by Culver et al. (2008) using QIAamp DNA stool mini kit (Qiagen) with some modifications. 0.2 gm faecal samples, collected from 1 to 9 weeks p.i. from five hens of each group, were weighed and dispensed into microcentrifuge tubes containing 2 mL ASL buffer. The samples were vortexed and heated in a $70^{\circ}C$ water bath for five minutes. The samples were centrifuged at 4800 x g for 10 min and 120 µL of the superannuant was transferred to another clean microcentrifuge tube containing an inhibitex tablet. The samples were vortexed and stored at room temperature for 1 min. The samples were then centrifuged at 4800 x g for 10 min and 200µL of resulting supernatant was treated with 15µl of proteniase K and 200 µL of AL buffer. The mixture was reheated at$70^{\circ}C$ and transferred to a spin column. Washing and elution was performed according to the manufacturer's instructions. The elution volume was 100 µL. Extracted RNA was quantified using Nanodrop and stored at $-70^{\circ}C$ until used for real time RT-PCR. 50 ng of faecal RNA was used during the real time PCR reaction. Nucleocapsid sequences from N1/88, A3, Vic S and T strains of IBV were retrieved from GenBank accession numbers U52599, DQ490205, U52594 and U52596. All the sequences were aligned using Clustal W2 (EBI sequence analysis tool, UK). The sequence alignment indicated that the nucleocapsid gene sequences of A3 and Vic S were dissimilar to N1/88. The primers (Forward primer 5'-AGATGGCTGAGCGTAAGTAC-3', Reverse primer 5'-CCTCCTCAATCATCTTGTCATC-3') and LNA probe (5'-FAM aaaGgcCaaGctCcaaattt BHQ-2-3') required for the real time RT-PCR were generated to amplify the 123 bp sequence within the nucleocapsid region of the N1/88 strain of IBV. The primers were manufactured by Geneworks (Adelaide, Australia) and the LNA probe was synthesised by Exiqon (Denmark). The primers and LNA probe for the real time PCR for the detection of T strain of virus were used as described earlier (Chousalkar *et al*., 2009). 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

In the N1/88 infected group (N), virus was detected from the faeces of three hens in the first two weeks, and two hens during the third and fourth weeks. In the VN group, virus was detected in the faeces of two hens during first week and then in one hen during second and third weeks. Virus was not detected in either the unvaccinated or vaccinated group from five weeks p.i.

Amongst the T strain infected group, virus was detected from the faecal samples of three out of five in the first week and then consistently two out of five hens from two to nine weeks p.i. Amongst the VT infected group, virus was consistently detected from the faecal samples of two out of five hens from one to nine weeks p.i. The virus load was low in vaccinated hens compared to the unvaccinated and challenged hens. The details regarding the IBV viral load in the faeces are presented in Table 1.

The faecal samples of the hens from control groups were negative throughout the experiment.

Table 1.   N1/88 and T Viral RNA load from faecal samples of vaccinated and unvaccinated hen

Weeks Post inoculation	Hen	N	VN	T	VT
		Mean Viral RNA	Mean Viral RNA	Mean Viral RNA	Mean Viral RNA
1	1	11274	0	886634	269089
	2	9676	0	14477	8790
	3	123	1115	758	0
	4	0	136	0	0
	5	0	0	0	0
2	1	9633	0	216694	16694
	2	10112	0	1144	5279
	3	193	6639	0	0
	4	0	0	0	0
	5	0	0	0	0
3	1	676	0	12800	13462
	2	715	0	1769	133
	3	0	115	0	0
	4	0	0	0	0
	5	0	0	0	0
4	1	133	0	8963	1973
	2	192	0	16698	1822
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
5	1	0	0	1852	338
	2	0	0	7703	432
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
6	1	0	0	2870	237
	2	0	0	3338	875
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
7	1	0	0	928	494
	2	0	0	726	655
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
8	1	0	0	910	16
	2	0	0	109	20
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
9	1	0	0	147	57
	2	0	0	166	31
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0

IV. DISCUSSION

The real time PCR assay was based on the nucleocapsid region of the N1/88 virus genome (Sapats et al., 1996). Primers and probes designed within the nucleocapsid region of N1/88 strain of IBV did not give a positive reaction to T, VicS and A3 strains of IBV. Hence it is now possible to differentiate the N1/88 strain of IBV from vaccine strains. Real-time RT-PCR offers both speed and accurate quantification of viral load, and a real time PCR test has been developed recently using a Taqman probe for the detection of American strains of IBV from tracheal swabs (Callison et al., 2007) and using LNA probe for the detection of other Australian IBV strains (Chousalkar et al., 2009). The sequence analysis suggests that the assay developed earlier by Chousalkar et al. (2009) and Callison et al. (2007) would not detect the N1/88 strain of IBV used in this study. The current test will be useful to differentiate and monitor the spread of N1/88 strain of IBV amongst vaccinated chicken populations. Detection and quantitation of N1/88 and T strains of IBV from the faeces using real time PCR assay has not been reported previously. The T strain of IBV was isolated from the faecal samples of T strain infected hens up to 225 days p.i. (Alexander and Gough, 1977). Massachusetts serotype vaccine virus has been isolated also from cloacal swabs at 63 days p.i. (Naqui *et al.*, 2003). Excretion of virus and its subsequent isolation from faeces could be due to the urates present in the faeces. Further studies are required to study the virus detection simultaneously from kidney, gut contents and faeces.

Virus was consistently isolated from the faecal samples of two hens in the T infected group from 1 to 9 weeks p.i. Such hens can be persistent virus shedders and these hens appeared healthy throughout the experiment. Shedding of virus in such fashion by apparently healthy birds could be an important means of introducing virus into new premises (Jones and Ambali, 1987). Based on the real time PCR results shown above in the report it could be concluded that vaccination can reduce the shedding of wild strains (T and N1/88 strains of IBV) through faeces.

V. ACKNOWLEDGEMENTS

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THE STRATEGIC USE OF ORGANIC ACIDS TO IMPROVE GUT HEALTH IN POULTRY

L. LI[1]

Summary

Gut health presents significant challenges to modern poultry production. The outcome of poor gut health, often is costly and devastating to poultry producers. Up to date, numerous effective solutions have been adopted by producers, in order to improve the gut health. These actions include: (i) minimising pathogen exposure via bio-security efforts; (ii) improving host resistance through genetic selection; and (iii) strategic use of feed additives, such as organic acids, to manipulate intestinal biochemistry to either directly kill or inhibit pathogenic bacteria colonization and to support the growth of protective bacteria. For decades, organic acids and salts have been used, as a direct replacement for antibiotic growth promoters (AGPs) or in combination with AGPs to improve poultry productivity. However, only some organic acid based products are most likely to make a significant, positive impact on the poultry industry, as they are developed not only to address current needs, but also to solve the predictable problems of tomorrow. This paper will focus on the strategic use of organic acids to improve gut health in poultry.

I. GUT HEALTH SIGNIFICANTLY CHALLENGES MODERN POULTRY PRODUCTION

Not only does the gut perform such basic functions as digestion of food and absorption of nutrients, but the gut also functions as a physical barrier to prevent opportunistic pathogens from colonising and invading host tissues. The gut microflora is a significant part of the gut mucosal barrier. It is, therefore, not surprising that their activities have a major impact on the gut health and *vice versa*. This mutual influence also affects the host, beyond the gut. Pathogenic bacteria, such as Salmonella, E. coli, Campylobacter and Clostridium, are commonly found in the gastro-intestinal (GI) tract of healthy birds. Under normal conditions (good nutrition and adequate immune defence), if a small number of pathogens are present within the GI tract, the beneficial bacteria can overrule this small number of bad bacteria; therefore, they may not cause severe clinical infections. The real challenge is when birds experience stresses, such as changing diet, intestinal disease (virus attach and parasites infestation) and feed withdrawal, the conditions in the gut might change and then the opportunistic pathogens will thrive and produce toxins, which in turn, may cause serious clinic enteric disorders. Generally speaking, birds with clinic enteric disorders have poor efficiency of feed utilisation, wet droppings, poor uniformity and high mortality. The cost of any additional treatments will certainly result in more economic loss for the producer.

To prevent economic loss from enteric diseases, AGPs, such as virginiamycin, tylosin and penicillin etc, have been used extensively in those countries where antibiotics are allowed to be included in poultry diets. In recent years, with the restrictions on the use of regular antibiotics, poultry producers have adopted a few effective intervention strategies to reduce the productivity losses. These strategies include vaccination, possible dietary alternatives to antibiotics, i.e. organic acids and salts, probiotics and prebiotics. Due to limited space, this paper will only focus on organic acids and salts, mode of actions and their application concepts for poultry production.

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II. POSSIBILITY TO USE ORGANIC ACIDS TO IMPROVE GUT HEALTH

From a clinical view point, it is fair to say, that all diseases start in the gut. A healthy gut is also the key to maximise the nutrient utilisation from feed (Yegani and Korver, 2008). For decades, poultry feed producers have been aware of organic acid feed supplements as a means of improving bird health and food safety, but only in a limited capacity. Reluctance for their widespread use in poultry is mainly due to a limited amount of consistent trial data, failure to understand their mode of action, and other misguided fallacies.

First of all, short chain fatty acids (SCFAs, $C \leq 4$), especially propionic and butyric acid, are essential for the normal structure and function of the gut epithelium. SCFAs are readily absorbed by the normal intestinal epithelium, and they maintain the integrity of the intestinal mucosa, stimulate proliferation of normal colonocyte, and stimulate water and electrolyte absorption from the colonic lumen (Cummings et al., 1987; Velazquez et al., 1996). The antibacterial effect of dietary SCFAs in poultry takes place mainly in the upper part of the digestive tract. The reason being that SCFAs are rapidly metabolised and absorbed from the fore-gut (crop, proventriculus, gizzard) of birds, which will then reduce their antibacterial activity in the small and large intestines, impacting on growth performance (Waldroup et al., 1995). Recently, the use of a combination of SCFAs and medium chain triglycerides (MCTs) as a potential antimicrobial agent has been investigated. This combination was postulated to be an efficient antimicrobial agent in acidic (gastric-) environment, as well as in a neutral (intestinal-) environment (Velazquez et al., 1996; Decuypere and Dierick, 2003; Skrivanova et al., 2005; Van Immerseel et al., 2004). The mode of action of the synergetic antimicrobial effect can be summarised as: Most pH sensitive bacteria do not grow in acidic environment as acid tolerance is a major factor that limits how well certain types of bacteria survive in an ecosystem. SCFAs reduce fore-gut pH, hence increase barrier function for harmful microbes and improve nutrients digestion. Simultaneously, MCFAs from medium chain triglycerides are liberated in the intestines by intestinal lipases, providing a natural "slow release" antimicrobial function. The final outcome is improved gut health (low wet dropping score) and enhanced bird performance. Furthermore, the reduced microbial growth in the intestines, allows the birds to more efficiently utilise available nutrients.

Numerous studies have shown that SCFAs and medium chain fatty acids (MCFAs) and medium chain triglycerides (MCTs) are effective in either directly killing or inhibiting Salmonella, Campylobacter and Clostridium perfringens colonization and supporting the growth of protective Bifidobacteria and Lactobacilli bacteria in poultry (Dibner and Buttin 2002; Owens et al., 2008; Solis de los Santos et al., 2009). More importantly, studies demonstrated that supplementation of feed or drinking water with organic acids could be used as potential additives for the control of necrotic enteritis (Gornowicz and Dziadek, 2002; McDevitt et al., 2006). Therefore, organic acids not only control pathogenic bacteria in the feed and GI tract of the host, they also help the bird to achieve maximum nutrient digestion and absorption, hence, improved productivity.

Formulating a diet with a low acid binding capacity (ABC) diet to reduce diarrhoea incidence is a practical consideration. Most pH sensitive pathogens, such as Salmonella, E. coli and Campylobacter, stop growing in the environment with a pH lower than 4.5. It is generally established that the gastric acid secretion is proportional to the buffering capacity of the meal. On the other hand, the secreted acids must be neutralised in the duodenum by the bicarbonates from the bile. This will dilute the digestive enzymes secreted by the pancreas and increase the feed flow, leading to malabsorption and diarrhoea (Decuypere et al., 1997). It is, however, neither practical, nor economical to achieve this low feed pH by adding large quantities of acids. In practice, feeds with low ABC value (meq/kg) can be formulated by: (i)

lowering the calcium levels to the minimum required, (ii) replacing inorganic calcium with organic calcium sources, (iii) restricting the inclusion of inorganic phosphates using phytase enzymes and (iv) formulating feeds with lower crude protein levels.

III. CONCLUSION

Successful use of organic acids in poultry production requires knowledge of their mode of action and their numerous potential benefits. One of the most important benefits is to lower feed and gastric pH, thereby, reducing the buffering capacity of feeds and enhancing nutrient utilisation. Furthermore, organic acids reduce intestinal colonisation of pathogens; affect the composition of intestinal microflora and mucosal morphology. In practice, a combination of short chain fatty acids and medium chain fatty acids can be applied along with nutritional, management and biosecurity measures in order to improve gut health in poultry.

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INACTIVATION OF VIRUSES AND COCCIDIA IN BROILER LITTER FOLLOWING HEAPING OR WINDROWING AT THE END OF THE BATCH

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Summary

Two on-farm experiments were conducted to determine the effectiveness of windrowing or heaping end of batch broiler litter for up to 10 days on inactivation of pathogenic viruses and coccidia. In Expt 1 (Sydney) litter treatments were heaping, heaping with turning at day 4 or windrowing. In Experiment 2 (Brisbane) litter treatments were no heaping (simply turning litter *in situ*), windrowing, or windrowing with turning at day 4. There were two replicates of both treatments in each experiment. On 4 occasions (days 0, 3, 6, 9 in Expt. 1 and days 0, 4, 7 and 10 in Expt. 2) representative litter samples from each treatment were subjected to a chick bioassay to measure litter infectivity for a number of key viral diseases as determined by seroconversion at 35 days post exposure to the litter. Coccidial oocyst counts in faeces were also conducted in Expt 1. Heaping or windrowing litter led to marked reduction in the proportion of bioassay chicks positive for chicken anaemia virus (CAV) and fowl adenovirus (FAV) but there was no clear effect on infectious bursal disease virus (IBDV) for which initial litter infectivity was low. Turning provided no additional benefit overall and heaping appeared to provide a greater level of inactivation than windrowing. Litter infectivity was very low or absent for pathogens such as infectious bronchitis virus (IBV) and Marek's Disease virus (MDV) so inferences could not be made about these. Serology for additional viral pathogens is ongoing. Coccidial oocysts were completely inactivated in the treated litter by day 6. These preliminary data suggest that heaping or windrowing of litter is beneficial in reducing viral pathogen load in litter, that most (but not all) inactivation is completed by days 6-7, that large heaps inactivate more effectively than windrows, and that turning of heaps or windrows does not provide an additional benefit.

I.   INTRODUCTION

Re-use of litter by broiler chickens can reduce the environmental impact and cost of chicken production but uptake of the practice is limited by risks of pathogen carryover. Unlike some other major poultry producing countries, only a small proportion of Australian broiler farms reuse litter in the shed (East, 2007). The reluctance to reuse litter in the Australian broiler industry is primarily based upon concerns on the animal health and productivity issues (Groves, 2003) particularly due to carry-over infection with pathogens, particularly viruses.
A small proportion of Australian broiler growers do reuse litter in the shed following partial composting by heaping or windrowing for 4-10 days, where a temperature of ~60 °C can be reached inside the litter heap or windrow. The aim of this study was to assess the effectiveness of such litter treatments over time on the inactivation of selected poultry viruses and coccidia under field situations. Unlike bacteria, viruses are not easy to detect and quantify from poultry litter, as viruses do not grow in non-living media. Therefore, little or no data are available on viral survival rate in poultry litter following different litter treatments. Recently we developed and validated a bioassay to detect and quantify viral pathogens from

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poultry litter (Islam et al., 2009) and this tool was used to measure change in litter infectivity following treatment in the present study.

II. MATERIALS AND METHODS

Two field experiments were conducted. Experiment 1 had three litter treatments in duplicate in the three sheds of a Sydney region farm. Each shed was divided into half to make six experimental units. Three litter treatments were applied in the six units in duplicate for nine days. The treatments involved forming half of the litter in the shed into windrows (W), a long row of litter approx. 1.0–1.2 m tall, 2 m wide and 40 m long along the centre of the shed, heaps (approx 2.5 m in height) without turning (H) of with mechanical turning on day 3 (HT). Representative litter were collected and transported to UNE at days 0, 3, 6 and 9 for bioassay of litter infectivity as described by Islam et al. (2009). Experiment 2 was conducted in a Brisbane region farm, where three treatments; windrow (W), windrow with turn (WT) and no heaping (NH) with litter left alone and mechanically turned at day 4, were applied in six different sheds in duplicate. Litter was collected at days 0, 4, 7 and 10 for bioassay.

The experimental bioassay utilized a 3×4 factorial design with three treatments and four time periods as described above for each field experiment. Each cell was replicated in 2 isolators, and there were also 2 negative control isolators. In each isolator 10 specific pathogen free chickens were exposed to 8 L of litter. At day 35 post-exposure serum samples were collected and chickens held until a common age across treatments was reached (42 days old) after which they were humanely killed, weighed and sampled for spleen and faeces.

Sera were tested for antibodies directed against IBV (ELISA), CAV (ELISA), IBDV (ELISA), Infectious laryngotracheitis virus (ILTV, ELISA) and FAV (ELISA). MDV was detected using real-time quantitative PCR of DNA extracted from spleen. MDV was quantified in spleen samples using real-time quantitative PCR and oocysts counted using standard methods. Data were analysed using appropriate models in JMP Version 7.

III. RESULTS

Negative control chickens were negative for all the pathogens tested in both experiments. Serological data for experiments 1 and 2 are summarised in Tables 1 and 3 respectively. The field litter collected prior to the application of treatments (day 0) was highly positive for CAV and FAV in both experiments. Positive samples for IBDV (both experiments) and IBV (experiment 2) were also observed in some treatment groups, but at much lower levels (Tables 1 and 2). None of the chickens was positive for ILTV or MDV in experiment 1 (data currently not available for experiment 2).

Table 1.   Experiment 1. Proportion and % (in brackets) of SPF chickens serologically positive for CAV, FAV IBDV and IBV 35 days after exposure to treated litter.

Treatment	Day post-treatment	CAV	FAV	IBDV	IBV
Neg. control		0/17 (0)	0/17 (0)	0/17 (0)	0/17 (0)
Pre treat (d0)	0	29/30 (97)	20/27 (74)	7/33 (21)	0/30 (0)
Heap No Turn	3	7/19 (36)	0/19 (0)	2/19 (11)	0/22 (0)
(H)	6	1/19 (5)	0/20 (0)	11/20 (55)	0/20 (0)
	9	0/19 (0)	0/18 (0)	0/18 (0)	0/18 (0)
Heap Turn	3	19/19 (100)	0/19 (0)	1/21 (5)	0/19 (0)
(HT)	6	8/20 (40)	0/19 (0)	5/20 (25)	0/19 (0)
	9	0/20 (0)	8/21 (38)	2/20 (10)	0/20 (0)
Windrow (W)	3	14/20 (70)	0/20 (0)	1/20 (5)	0/18 (0)
	6	10/18 (55)	1/19 (5)	1/20 (5)	0/20 (0)
	9	4/20 (20)	0/21 (0)	0/21 (0)	0/21 (0)

Table 2.   Experiment 2. Proportion and % (in brackets) of SPF chickens serologically positive for CAV, FAV IBDV and IBV 42 days after exposure to treated litter.

Treatment	Day post-treatment	CAV	FAV	IBDV	IBV
Neg. control		0/17 (0)	0/17 (0)	0/17 (0)	0/20 (0)
Windrow No	0	13/20 (65)	15/20 (75)	1/20 (5)	0/20 (0)
Turn (W)	4	1/18 (6)	8/18 (44)	1/19 (5)	0/20 (0)
	7	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)
	10	0/20 (0)	11/20 (55)	0/20 (0)	0/20 (0)
Windrow turn	0	19/20 (95)	19/20 (95)	5/20 (25)	0/20 (0)
(WT)	4	3/18 (17)	9/18 (50)	1/19 (5)	0/20 (0)
	7	1/20 (5)	0/20 (0)	0/20 (0)	2/20 (10)
	10	1/20 (5)	0/20 (0)	0/20 (0)	0/20 (0)
No heaping	0	19/20 (95)	19/20 (95)	0/20 (0)	0/20 (0)
	4	15/18 (83)	17/18 (94)	0/18 (0)	0/20 (0)
	7	8/20 (40)	10/20 (50)	2/20 (10)	2/20 (10)
	10	7/20 (35)	9/20 (45)	0/20 (0)	0/20 (0)

Heaping or windrowing litter led to marked reduction in the proportion of bioassay chicks positive for chicken anaemia virus (CAV) but infectivity remained after 9-10 days in the W (but not H) treatments (Tables 1 and 2). For FAV there was also a marked reduction, particularly in Expt 1, but there was unexpected reappearance of the pathogen in samples following a clear test and in both experiments there were some positive samples at days 9-10. The results for IBDV are inconclusive as initial litter infectivity was low but again positive samples were observed as late as day 9. Overall, turning provided no additional benefit and heaping appeared to provide a greater level of inactivation than windrowing. In Expt. 1 coccidial oocyst counts were dramatically reduced by litter treatment and no chicken exposed to day 6 and 9 litters was positive for coccidia (Table 3).

Table 3.   Experiment 1. Coccidial oocyst count (per gram of faeces) from pooled faecal samples collected at 42 days post exposure to litter.

Shed	Treatment	Oocyst count (g/faeces)			
		Day 0	Day 3	Day 6	Day 9
A	Heap Turn	241,000	0	0	0
	Windrow		0	0	0
B	Heap NT	42,500	0	0	0
	Heap Turn		1,000	0	0
4	Heap NT	16,000	0	0	0
	Windrow		0	0	0
Neg Control	Nil	0			

IV. DISCUSSION

The results demonstrate clear reductions in the infectivity of litter for CAV, FAV and coccidia with heaping and windrowing treatments. Coccidial oocysts were inactivated quickly, with no evidence of litter transmission at day 6 onward. This is consistent with the report that high temperature ($\sim 65^{\circ}$C) in the presence of ammonia inactivates coccidial oocysts within hours (McDougald and Fitz-Coy, 2008).

CAV and FAV are relatively environmentally resistant viruses (Schat and Woods, 2008; Adair and Fitzgerald, 2008) and the reduction in infectivity was greatest for the heap treatment without turning in Expt 1. This treatment resulted in no detectable infectivity by day 9. Turning the heaps appeared to reduce the rate of decline in infectivity and resulted in some infectivity appearing at day 9 for FAV. Windrow treatments clearly reduced CAV and FAV infectivity, in some cases removing infectivity as early as day 7 (Expt 2). Turning the windrow in Expt 2 reduced its efficacy against CAV, but not FAV for which the turned windrow was superior. Overall detectable infectivity remained at days 9-10 in 2 of 3 windrow treatments for CAV and 1 of 3 windrow treatments for FAV.

The results for IBDV are inconclusive as initial litter infectivity was low but again positive samples were observed as late as day 9 demonstrating the well-documented environmental resistance of this virus. The appearance of IBV in two samples at day 7 post-treatment is unexpected as the virus is fragile and we have previously found that it does not transmit effectively on litter particularly after transportation (Islam et al., 2009).

Two limitations to this study are apparent. The first is that the outcome is dependant on the range of pathogens present on the site being tested. Thus we have limited or no data on the effectiveness of the litter treatments against MDV, ILTV and IBDV due to the low level of initial pathogen load in the samples. In future it may be preferable to work with litter that has been deliberately contaminated by chickens harbouring a wider range of pathogens. A second limitation is the appearance of unexpected increases in infectivity of samples over time. This occurred in some cases with FAV, IBDV and IBV but and were mostly small. It probably reflects sampling variation despite the rigorous sampling procedure used (10 sites and 4 depths per windrow or heap). A localised source of surviving virus in the litter may only be sampled by chance on one of the sampling days. A small number of weak positives following a series of negative results may also represent false positives. Expressing the data in terms of mean titre, rather than ratio of positive to negative may reduce this effect.

Despite this, the study has provided a clear demonstration of the efficacy of litter heaping and windrow treatments in reducing litter transmission of CAV, FAV and coccidia.

V. ACKNOWLEDGEMENTS

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SPATIAL AND TEMPORAL VARIATION IN AMMONIA CONCENTRATIONS IN BROILER SHEDS: EFFECTS OF CHICKEN AGE AND SHED TYPE

A.F.M.F. ISLAM[1], M. DUNLOP[2], B. WELLS[3], and S.W. WALKDEN-BROWN[1]

Summary

For studies monitoring ammonia concentrations in chicken sheds, determining a suitable position and height of the monitoring equipment in a shed is important. To investigate this an experiment was conducted in which aerial ammonia concentrations were measured at 10 positions within the shed and three heights (5, 30 and 150 cm above the litter) in conventional and tunnel ventilated sheds at various chicken ages throughout the batch on 8 farms in the Mangrove Mountain and Sydney regions during autumn. All sheds contained chickens on new litter following full cleanout. Position of measurement within the shed had no significant effect on ammonia concentrations, but height above the litter did, with significantly higher ammonia at 5 cm than other heights. Ammonia concentrations remained within acceptable limits (< 25 ppm) throughout, increasing rapidly with chicken age up to week 3 then holding and reducing after week 5. Slightly higher ammonia concentrations were observed in conventional than tunnel ventilated sheds.

I. INTRODUCTION

Ammonia (NH_3) is a colourless irritant alkaline gas produced during the decomposition of organic matter by bacterial de-amination of nitrogenous substances in the poultry shed. High NH_3 is detrimental to chicken welfare and productivity, causing reduced feed intake and growth and increased susceptibility to pathogens (Becker et al., 2002; Miles et al., 2004; Ritz et al., 2004). The reported concentrations at which adverse affects occur vary but an exposure limit of 25 ppm has generally been set by industry and welfare bodies of different countries on human safety and animal welfare grounds (Kristensen and Wathes, 2000; Wathes et al., 1983). Ammonia concentrations in broiler sheds depend on many factors including litter moisture level, pH, ventilation rate and chicken age. In-shed NH_3 could also vary depending on location within a shed and this effect may differ between conventional and tunnel ventilated sheds. We plan to conduct several longitudinal studies of ammonia production in the field and the issue of where to locate the monitoring equipment within the shed is important. This study was therefore conducted to determine the spatial and temporal variation of NH_3 in both types of broiler sheds at various chicken ages to resolve this issue.

II. MATERIALS AND METHODS

The experiment was conducted on 8 co-operating broiler farms in Mangrove Mountain and north-western Sydney basin area between 26 March and 4 April 2009 and details are given in Table 1. Aerial ammonia concentrations in ppm were measured using VRAE7800 Hand Held Gas Surveyors (Geotech Environmental Equipment, Inc., Colorado, USA) calibrated against a 50 ppm NH_3 standard sample. Wind speed, humidity and temperature were recorded with

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Kestrel Weather Meter K4000 (Nielsen-Kellerman, Inc., PA, USA). All equipment had a data logging function.

The experiment was a 2×4 factorial design, with two types of broiler shed (conventional and tunnel ventilated) and four ages of chickens (Weeks 1, 2-3, 5 and 7). All selected sheds were on different farms and all used fresh wood shavings as litter material, following full cleanout. Within the overall design there were 3 separate studies viz:

1. Position within shed. Between 1130 and 1400 h, measurements were taken at a fixed height of 30 cm from 10 fixed positions within the shed in both the morning (between 900 and 1130 h) and afternoon (between 1400 and 1600 h). Ammonia was measured for at least 15 minutes at 3-minute intervals at each location with temperature and wind speed also averaged over this period. The order of measurement of each location was selected at random each time.

2. Height above the litter. Measurements were taken from nine positions at heights of 5, 30 and 150 cm above floor level. Again measurements were for 15 minute periods or more.

3. Overnight fluctuation. Between approximately 1700 and 0700 h continuous measurements were recorded at two positions and two heights (30 and 150 cm).

Table 1.   Details of the experimental sheds and chickens.

Sample date	Shed type	Shed size (m)	Ventilation system	Chicken age (days)	Sex	Number of birds
27/03	Conv.	70×14	Blinds down	7	Mixed	21,000
28/03	Tunnel	160×14	1 x low speed fan	6	Male	40,000
30/03	Conv.	120×16	Blinds down	14	Mixed	29,400
31/03	Tunnel	100×14	Fans on auto	22	Mixed	21,800
01/04	Tunnel	120×15	Fans on auto	33	Mixed	29,500
02/04	Conv.	100×13	Completely open	34	Mixed	17,600
03/04	Tunnel	138×16	Fans on auto	45	Male	4,000 (17,000)
04/04	Conv.	112×13	Completely open	46	Mixed	17,000 (22,000)

For studies 1 and 2, data were averaged for each position and height to provide a single estimate for analysis. Position data (Study 1) were analysed by ANOVA for the fixed effects of shed type, position, period (morning and afternoon) and chicken age. Height data (Study 2) were analysed by ANOVA and for the fixed effects of shed type, chicken age, position in the shed and height above the litter. Overnight data (Study 3) were not analysed statistically. Data for each time point were plotted against time to show the NH_3 level in each shed.

III. RESULTS

In study 1 (Position) there was a significant effect of age ($P < 0.0001$) on the ammonia concentration at 30 cm above the floor without a significant effect of shed type ($P = 0.08$), position ($P = 0.78$) or period ($P = 0.09$). There were no significant interactions between these effects. The mean NH_3 concentrations in both types of sheds at various ages of chickens are shown in Table 2.

In study 2 (Height) there were significant effects of height ($P < 0.0001$), shed type ($P < 0.001$) and age ($P < 0.0001$) but not position on NH_3 concentrations. There were also significant interactions between the effects of age and height ($P < 0.0001$) but not height by shed type ($P < 0.061$) or other interactions. Ammonia concentrations were higher in conventional than tunnel ventilated sheds (9.5 vs 8.4 ppm). No height effect was evident up to week 3, after which NH_3 were significantly higher at 5 cm than any other heights (Figure 1).

Table 2.   Study 1. Mean ammonia concentration, temperature, relative humidity and wind speed in tunnel ventilated and conventional broiler sheds containing chickens of various ages.

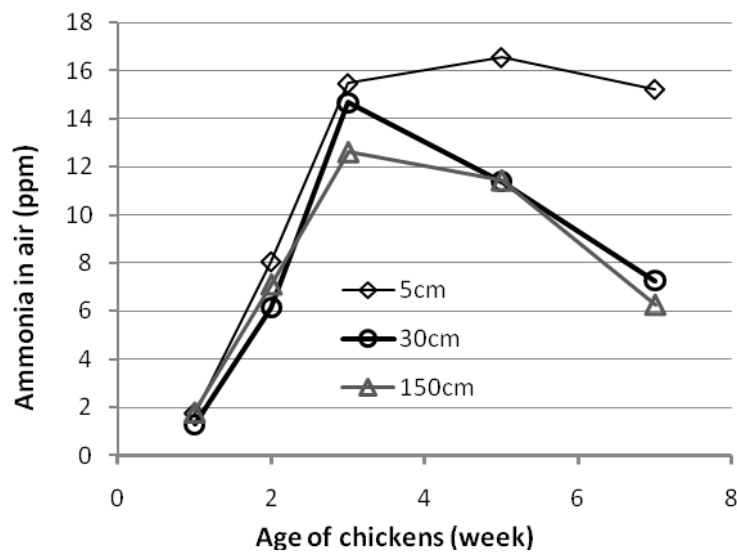| Tunnel ventilated shed					Conventional shed				
Age (d)	NH_3 (ppm)	Temp (°C)	RH (%)	Max wind sp. (km/h)	Age (d)	NH_3 (ppm)	Temp (°C)	RH (%)	Max wind sp. (km/h)
6	0.81	19-32	42-73	1.3	7	2.17	19-32	49-93	1.8
22	12.16	20-26	74-100	1.8	14	6.31	19-28	56-94	1.4
33	12.25	19-27	60-93	3.3	34	12.57	18-25	54-83	3.0
45	9.26	20-25	92-100	6.0	46	9.82	15-24	74-95	4.4

Figure 1.   Study 2. Interaction between the effects of height of measurement above the floor and chicken age on air ammonia concentrations.

The overnight data (Study 3) were plotted against time for shed types (Fig 2). There was not much difference in ammonia concentration in conventional and tunnel ventilated sheds, however, the variability of air ammonia in the conventional sheds appeared to be greater than in tunnel ventilated sheds. There was also no discernable systematic variation in ammonia concentrations over the period of measurement.

IV. DISCUSSION

The main purpose of this study was to evaluate spatial variation of NH_3 concentrations in sheds to determine whether single point monitoring in longitudinal studies would be valid.

There was no systematic difference in ammonia concentrations for the chosen 10 positions within the shed indicating that that monitoring of NH_3 concentrations from any of these positions within the shed is representative of the whole shed. However, there was a significant effect of height above the litter, with ammonia concentrations being higher near the surface of the litter (5 cm). The height at which sampling occurs should be selected to represent the air being inhaled by the target population (chickens or people).
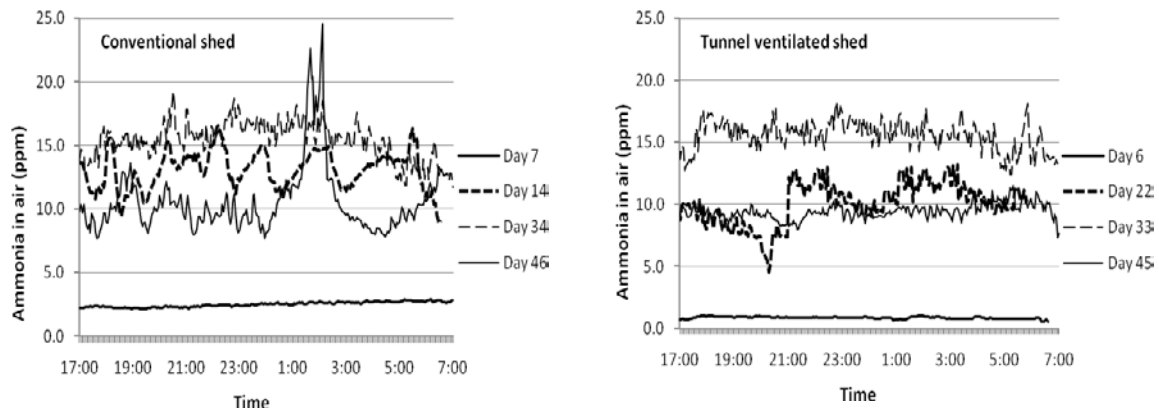

Figure 2.   Study 3. Overnight air ammonia concentrations in conventional and tunnel ventilated broiler sheds containing chickens of various ages. Mean of two heights (30 and 150 cm).

The ambient NH_3 concentrations in the eight studied broiler farms were relatively low compared to some overseas studies (eg. Wathes et al., 1997), where in-shed NH_3 concentrations often exceed 50 ppm. Mean HN_3 concentrations always remained below the threshold value of 25 ppm, with slightly higher NH_3 concentrations in conventional than tunnel ventilated sheds. This may reflect the use of new litter in this study as NH_3 could be expected to be higher on reused litter (Miles et al., 2004).

Air NH_3 concentrations increased with increasing age of the chickens to week 3 then plateaued to week 5 before declining. The plateauing after week 3 is likely due to increased ventilation rates after brooding. The decline after week 5 is likely due to both increased ventilation rates and decreased stocking densities following partial removal of chickens. Overall, we conclude that NH_3 concentrations in Australian broiler production systems based on full cleanout and single use litter are relatively low and the distribution within a shed is homogenous in regard to position, but not height.

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EGGSHELL FACTORS INFLUENCING EGGSHELL PENETRATION AND WHOLE EGG CONTAMINATION BY DIFFERENT BACTERIA, INCLUDING *SALMONELLA* ENTERITIDIS

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Summary

Trans-shell infection routes and whole egg contamination of seven phylogenetically diverse bacterial strains were studied. Eggshell penetration was correlated with various eggshell characteristics and the identity of strains. Contrary to the cuticle deposition, the shell surface area, shell thickness and number of pores did not influence the eggshell penetration. The results indicate that Gram-negative, motile and non-clustering bacteria penetrated the eggshell most frequently; *Pseudomonas* sp. (60%) and *Alcaligenes* sp. (58%) were primary invaders followed by *Salmonella* Enteritidis (43%). In comparison with the non-*Salmonella* strains, *Salmonella* Enteritidis was a primary invader of whole eggs (32%). Egg related *Salmonella* Enteritidis strains had no special capacity to contaminate whole eggs. Penetrated eggshells and contaminated whole eggs showed a significant higher bacterial eggshell contamination.

I. INTRODUCTION

Transovarian or "vertical" transmission of micro-organisms occurs when eggs are infected during their formation in the hen's ovaries. Horizontal transmission occurs when eggs are subsequently exposed to a contaminated environment and micro-organisms penetrate the eggshell. Studies conducted by Barrow and Lovell (1991) suggest that most of the contamination is due to horizontal transmission, although others do not agree (Humphrey, 1994). Contents contamination of whole intact eggs with *Salmonella* Enteritidis should be mainly the result of infection of the reproductive tissue (Humphrey, 1994). In this study the influence of eggshell characteristics on the eggshell penetration on the one hand and the egg content contamination on the other hand was investigated. To study more in detail the potential of *Salmonella* to contaminate whole eggs by the horizontal infection route, whole egg contamination with different *Salmonella* strains was determined.

II. MATERIALS AND METHODS

Intact eggs (no cracks, pin-holes) were filled with agar and inoculated (agar approach) or directly inoculated (intact egg approach). Seven phylogenetically diverse bacterial strains; *Staphylococcus warneri*, *Acinetobacter baumannii*, *Alcaligenes* sp., *Serratia marcescens*, *Carnobacterium* sp., *Pseudomonas* sp. And *Salmonella* Enteritidis, all own isolates from egg contents, were inoculated in the first study. In the second study four different *Salmonella* Enteritidis strains and one *Salmonella* Typhimurium strain were used. The four *Salmonella* Enteritidis strains were originally respectively isolated from two different egg contents, from a deer and a lizard; the *Salmonella* Typhimurium strain was isolated from overshoes taken at

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the outside environment of a pig farm. Strains were selected for resistance to streptomycin. An agar method (agar approach) described by Berrang et al. (1998) was adapted to study and visualize the bacterial eggshell penetration. The used method is described in detail by De Reu et al. (2006). Where bacterial penetration occurred, organisms grew on the agar and reduced the indicator triphenyl tetrazolium chloride to formazan which is red in color. Penetration was recorded when red colonies on the agar were visible by candling. Agar-filled (agar approach) and whole eggs (intact egg approach) were inoculated by immersion for 1 min in phosphate buffered saline containing 10^5–10^6 CFU/ml of a streptomycin resistant strain of one of the selected species. This resulted in 10^3–10^4 CFU of the selected bacterium on the eggshell. After drying, the eggs were stored at 20°C and 60% relative humidity for up to 21 days. At day 0 and day 21 the eggshell contamination with the selected strains was determined. To remove the egg contents of whole eggs aseptically (intact egg approach), a modification of the method described by Himathongkham et al. (1999) was used. During the eggshell penetration and egg content contamination experiment of the first study, different eggshell characteristics were determined. The shell surface area, the shell thickness, the number of pores, and the cuticle score were studied in the penetration experiment. In the whole egg contamination experiment only shell surface area and loss of weight at the pores were measured. Details on the used methods are outlined by De Reu et al. (2006).

III. RESULTS

No significant difference between area eggshell, shell thickness and number of pores and the presence or absence of bacterial eggshell penetration was found. The mean cuticle deposition was lower for penetrated compared to non-penetrated eggshells (individual strain and all strains). For the individual strain *Carnobacterium* sp. And for the general result of all strains this difference was significant ($P < 0.001$). The whole egg contamination was not influenced by either the area of the eggshell or by the porosity of the eggshell.

The individual data per selected strain and the general data (all bacterial strains) obtained with the agar approach, showed a higher count of the inoculated strain on the eggshell at day 21 for penetrated eggshells compared to non-penetrated eggshells. This higher count was even significant for the general data ($P < 0.001$) and for six of the seven selected strains; respectively for *S. warneri*, *Alcaligenes* sp., *A. baumannii*, *Pseudomonas* sp., *Salmonella* Enteritidis and *S. marcescens* (respectively $P = 0.011$, < 0.001, 0.0018, < 0.001, 0.0016 and 0.0038). The count of bacteria on the entire shell of whole eggs was on average 0.6 log CFU lower compared to agar-filled shells; respectively 1.7 versus 2.3 log CFU/shell. For 5 of the 7 selected strains the contaminated whole eggs had a (slightly) higher count of the inoculated strain on the eggshell at day 21; for none of the individual strains this was significant. The overall data of all strains showed that the count on the eggshell of the contaminated whole eggs was significantly higher ($P = 0.0029$); 1.89 log CFU/shell versus 1.66 log CFU/shell for the non-contaminated whole eggs.

Figure 1a shows the percentage of eggshell penetration (agar approach) for all strains tested, after 21 days of incubation. *Pseudomonas* sp. and *Alcaligenes* sp followed by *Salmonella* Enteritidis penetrated the eggshell most frequently. They accounted for 60, 58 and 43% of the agar-filled eggs penetration, respectively. Figure 1b shows the percentages of whole egg contamination (intact egg approach). The egg contents of whole eggs were most frequently contaminated by *Salmonella* Enteritidis (33%) followed by *Carnobacterium* sp. (17.5%). All strains were able to penetrate in agar-filled eggs (eggshell penetration) as well as to contaminate whole eggs (whole egg contamination). Of the 403 agar-filled eggs, 131 (33%) were penetrated by the selected strains compared to a content contamination of 16% (60 on 385) whole eggs.

For the second study; contamination percentages of 18%, 6%, 14% and 26% for *Salmonella* Enteritidis isolated respectively from two different egg contents, a deer and a lizard; and of 24% for *Salmonella* Typhimurium isolated from overshoe of a pig house were found. Fifty intact whole eggs were used in each case. Average eggshell contaminations on day 21 were comparable.
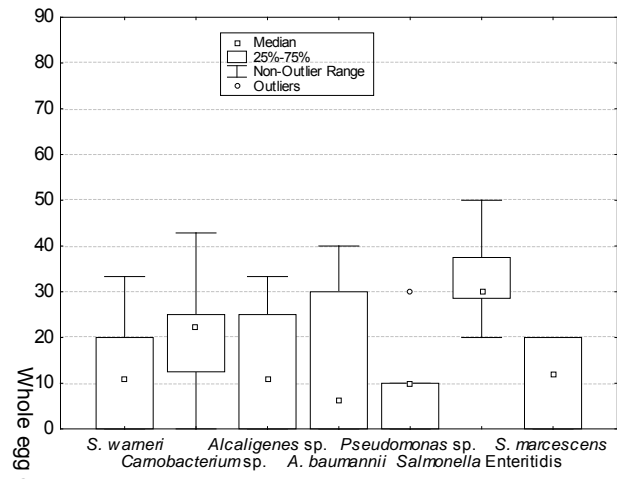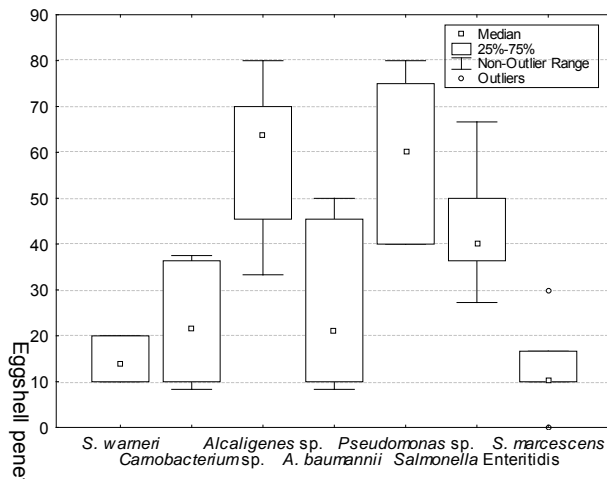

Figure 1a: Percentage of eggshell penetration for each individual bacterial strain.
Figure 1b: Percentage whole egg contamination for each individual bacterial strain.

IV. DISCUSSION

In our study a significant lower cuticle deposition was found on penetrated eggshells compared to non-penetrated eggshells. Alls et al. (1964) found that cuticle removal increased microbial contamination from 20 to 60%. Drysdale (1985) found also a significantly higher bacterial contamination in eggs which had a poor cuticle (40%) compared to eggs with a medium or good quality cuticle (26%). The defence of the cuticular layer has on the other hand been questioned by Nascimento et al. (1992) and Messens et al. (2005) using agar-filled eggs.

A positive correlation was found between bacterial eggshell contamination with the inoculated strain(s) on day 21 and shell penetration and whole egg contamination with the strain(s). This corresponds with ample evidence in the literature that eggs with highly contaminated eggshells suffer more from bacterial spoilage or whole egg contamination. Messens et al. (2005) also showed that the probability of eggshell penetration was higher with higher counts of *Salmonella* Enteritidis on the eggshell.

Using the agar approach *Pseudomonas* sp., *Alcaligenes* sp and *Salmonella* Enteritidis penetrated most frequently. The higher shell contamination (on day 21) with *Pseudomonas* sp. and *Alcaligenes* sp. can explain the higher fraction of penetrated eggshells. Notwithstanding the comparable eggshell contamination with *Salmonella* Enteritidis and *S. warneri* (both 2.5 log CFU/eggshell) on day 21, penetration prevalence with *Salmonella* was higher (43% versus 18%). It is likely that the motile, non-clustering properties of *Salmonella* favour the eggshell penetration.

Using the intact egg approach in the first study; *Salmonella* Enteritidis followed by *Carnobacterium* sp. seemed to penetrate, survive and eventually grow most frequently; respectively 33% and 17.5% of the inoculated eggs. Sauter and Petersen (1974) found a contamination average of 47.5% for various salmonellae using whole eggs of poor shell

quality and 21.4% and 10.0% for whole eggs of intermediate and excellent shell quality. Sauter and Petersen (1969) also challenged eggs with high, medium and low levels of shell quality and found an incidence of spoilage with *P. fluorescens* of 6.3, 19.4 and 29.1%, respectively. In our study 10.5% of the whole eggs were contaminated with *Pseudomonas* sp.

The second study showed no higher resistance of *Salmonella enteritidis* to egg albumen compared to other salmonellae like *Salmonella* Typhimurium. *Salmonella* Enteritidis strains originally isolated from the egg content were also not the primary invaders of the egg content. Knowing that *Salmonella* Enteritidis is the most frequently isolated *Salmonella* serovar in eggs, our results do not contra-indicate that the frequent egg content contamination with *Salmonella* Enteritidis would be mainly due to the transovarian or vertical route.

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THE EFFECTS OF TWO LIGHT-DARK SCHEDULES ON EGG LAYING TIME AND SYNCHRONY, THE INCIDENCE OF LAYING IN THE DARK AND HEN WELFARE

G.M. CRONIN[1,2], S.S. BORG[2], T.H. STOREY[3], J.A. DOWNING[2] and J.L. BARNETT[3]

Summary

We investigated the effects of inserting a light period during the night on the shift of egg laying times and inducing hens to lay in the dark, which would minimise egg laying at the cage edges. Two methods of introducing light during the night were compared. A light period was introduced either gradually, commencing with 0.5 h/night at 18 wks and increasing incrementally to reach 3 h/night at 23 wks, or abruptly, by inserting 3 h/night at 23 wks. The time and synchrony of egg laying, incidence of egg laying in the dark and stress response of hens were measured. Regardless of the method of introducing the light period, median egg laying time was shifted forward by about 1.5 h and about half the eggs were laid in darkness (between 0300-0600 h). There were no effects on synchrony of laying or stress response, measured via egg corticosterone. Photoperiod manipulation therefore offers a practical method of reducing the high incidence of eggs laid in preferred sites such as the cage edges.

I. INTRODUCTION

The trend for modern layer cages to accommodate larger group sizes compared to earlier cage designs, introduces potential egg quality problems if hens lay simultaneously in the preferred sites in cages, which are the nest box (if present) and the edges of the cage (Cronin and Barnett, 2008). Photoperiod strongly influences the timing of ovulation and oviposition in laying hens (Morris, 2004). In environmentally controlled sheds with large group cages, synchrony of egg laying may result in more eggs laid in preferred sites, increasing the incidence of cracked shells as eggs roll into the collection tray and collide with other eggs. Synchrony of egg laying may also result in increased stress on hens, if the preferred nest site is not available due to coincidental occupation by cage-mates. A potential strategy to avoid this problem is to decrease the proportion of hens that choose the 'preferred' egg laying sites.

Laying hens are inactive in the dark (Tanaka and Hurnik, 1991), including during the pre-laying period if it coincides with darkness. Also, hens that lay in the dark tend to lay in their current location, rather than in their 'preferred' egg laying site (Sherwin and Nicol, 1993). Shifting oviposition time, so that some hens lay in the dark, offers a method of decreasing competition for 'preferred' laying sites and reducing the risk of egg breakage. Manipulating light-dark schedules, and in particular the time of actual lights-off and 'expected sunset' (*viz.* the time birds expect darkness, which may be under the control of an internal time clock) affect the time of ovulation and thus oviposition in hens (Lewis *et al.*, 2007a and b). We investigated the effects of two photoperiod schedules that involved introducing a period of light to the birds during the night time, on the timing and synchrony of egg laying, incidence of egg laying in the dark and hen welfare assessed by egg corticosterone concentrations.

II. MATERIALS AND METHODS

The experiment involved 96 Hy-Line Brown hens and was conducted in two adjoining controlled environment rooms each containing six experimental cages. The hens were reared

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from day old to 13 weeks in group cages in a controlled environment shed. Pullets were not beak trimmed and they were reared using conventional commercial lighting programs and nutrition. At 13 weeks the birds were placed in groups of eight in Victorsson 8-bird cages measuring 1.2 m wide and 0.5 m deep. All cages contained a nest box located on the right side (viewed from the front), which measured 0.24 m wide and 0.5 m deep and the floor area was covered with astro-turf. Nest box entrances were closed until 15 weeks. The same photoperiod (i.e. total number of hours of light per day) was applied to both rooms, increasing from 12 h at 15 weeks to 16 h at 23 weeks in 30 min increments weekly.

The experiment involved exposing birds to a period of light during the night, inserted either gradually or abruptly. The treatments were assigned at random to one of two available rooms. Thus, treatment was unavoidably confounded with room. Lighting was independently controlled in each room and provided 20 lux at the level of the experimental cages. The Gradual Light Introduction Treatment (Gradual) commenced at 18 weeks, when birds were exposed to 30 min of light during the night (Table 1). The time of lights off for the inserted light period was 0300 h daily and the duration of the light period was increased by 30 min per week until 23 weeks of age, when the birds received 3 h of light at night within their overall photoperiod of 16 h light to 8 h dark. The Abrupt Light Introduction Treatment (Abrupt) commenced at 23 weeks, when birds were exposed to 3 h light inserted from midnight to 0300 h within their photoperiod of 16 h light to 8 h dark. Prior to 23 weeks however, the Abrupt treatment served as a control for the Gradual treatment.

Low-light video cameras positioned below each cage and inside each nest box provided a continuous view of the birds and enabled the time and location of egg laying to be collated later from the digital video record (Cronin et al., 2007). While the number of eggs laid in 'preferred' sites may be relevant to incidence of cracked shells, the present experiment involved 8-bird cages as a model for cages with larger group sizes. Although the incidence of cracked/broken eggs was recorded, the occurrence was insignificant and is not reported here. All eggs laid each Friday were collected, numbered and taken to the laboratory where each egg was broken to separate the albumen from the yolk. The albumen was weighed then frozen for later analysis of corticosterone concentrations using the method developed by Downing and Bryden (2008). Total corticosterone concentrations were assayed using a commercial diagnostic kit (ICN ImmuChem Double antibody RIA, Seven Hills, NSW).

Table 1. Clock times (h) when the room lights were switched on and off each day over progressive weeks of the experiment, and the median times of egg laying per week for birds in the Gradual and Abrupt treatments.

Hen age (wks)	Gradual treatment				Abrupt treatment				Median egg laying times		
	On	Off	On	Off	On	Off	On	Off	Gradual	Abrupt	SED
17	0600	1900			0600	1900			0740	0740	0.553
18†	0600	1900	0230	0300	0600	1930			0620	0730	0.601
19	0600	1900	0200	0300	0600	2000			0545 [a]	0740 [b]	0.473
20	0600	1900	0130	0300	0600	2030			0630 [a]	0730 [b]	0.316
21	0600	1900	0100	0300	0600	2100			0540 [a]	0720 [b]	0.298
22	0600	1900	0030	0300	0600	2130			0650 [a]	0830 [b]	0.511
23‡	0600	1900	0000	0300	0600	1900	0300	0600	0620 [a]	0805 [b]	0.620
24	0600	1900	0000	0300	0600	1900	0300	0600	0650	0725	0.659
25	0600	1900	0000	0300	0600	1900	0300	0600	0720	0715	0.554
26	0600	1900	0000	0300	0600	1900	0300	0600	0730	0735	0.625

Within hen age pairs, values with different superscripts differ a,b: $P<0.05$
SED: standard error of difference between the means
Start of Gradual and Abrupt Light Introduction Treatments indicated by † and ‡, respectively

Light schedule changes occurred on Monday and the number of eggs laid per hour were collated from the video record of the next four days each week. Time of egg laying was estimated as the median value per cage per 4-day period and synchrony was estimated as the duration of the second plus third quartiles, that is, the time taken for the middle 50% of eggs to be laid per day. Analysis of variance (GenStat 10.1, Lawes Agricultural Trust) was used to test differences due to the treatments on egg-laying parameters and egg albumen corticosterone concentrations and the experimental unit was the cage of birds.

III. RESULTS

Maximum hen day egg production was reached by both treatments at 20 weeks of age and there was no effect of treatment on egg production. As shown in Table 1, the median egg laying times in weeks 19-23 combined were about 1.5 h earlier each day in the Gradual compared to Abrupt treatment. After 23 weeks, when both treatments received 3 h of introduced light during the night, the median times did not differ. Although median egg laying times were shifted earlier, there were no differences in the synchrony of egg laying, which ranged from about 1.5-3.0 h. The introduction of light during the 'normal' night time in the Gradual treatment, increased the proportion of eggs laid during darkness (Figure 1). Between 18-23 weeks combined, 55.7% compared to 28.5% of eggs were laid in darkness in the Gradual and Abrupt treatments, respectively (sed 16.98, $P<0.01$). In weeks 24-26 combined, following the introduction of the light period in the Abrupt treatment, there was no difference in the proportion of eggs laid in darkness (38.4% and 51.2%, respectively for the Gradual and Abrupt treatments, sed 8.87, $P=0.18$). Egg albumen corticosterone concentrations did not differ between the treatments in weeks 18-23 combined (0.86 and 0.80 ng/g, sed 0.030) or weeks 24-26 (0.77 and 0.76 ng/g, sed 0.034).
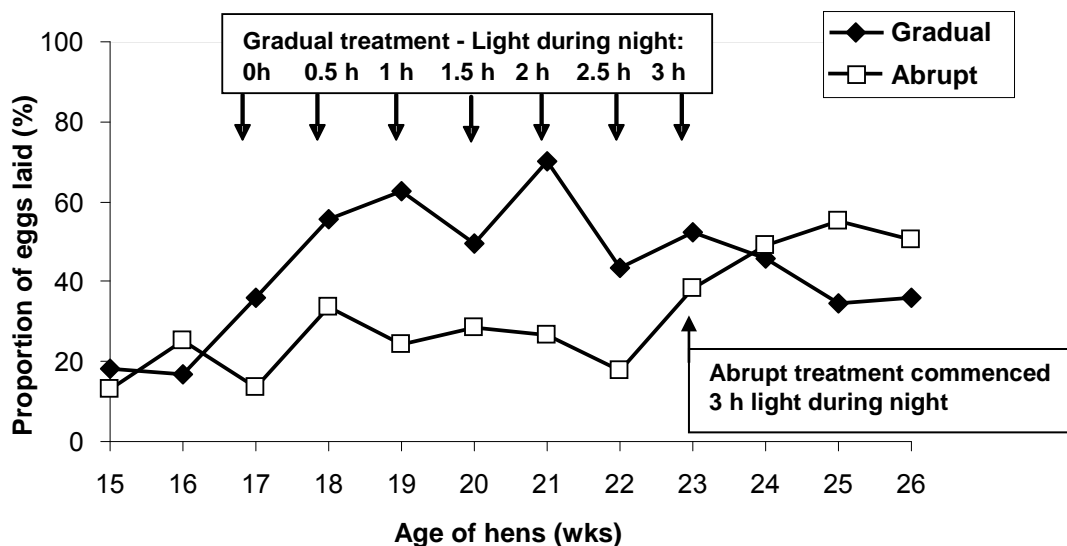

Figure 1. Eggs laid in the dark by birds in the Gradual and Abrupt Light Introduction treatments.

IV. DISCUSSION

Manipulation of the light schedule to insert of a period of light after midnight and before 0300 h, resulted in more eggs laid earlier in the day. Importantly, this coincided with a planned period of darkness between 0300 and 0600 h. This was predicted, since the times of

lights on and lights off are two key factors influencing the time of ovulation and thus oviposition in the laying hen (Lewis *et al*. 2007a). Neither the synchrony of egg laying nor hen day egg production were altered by the treatments. Regardless of the light-dark schedule manipulation, all birds were exposed to an increasing photoperiod, culminating in a total of 16 h of light per day by 23 weeks of age, which is the recommended photoperiod to ensure good egg production in modern laying strains (Morris, 2004). Many studies are reported in which manipulation of the light-dark cycle of laying hens has been investigated, including comparisons of various intermittent light programs, including asymmetrical patterns, symmetrical patterns with full light and symmetrical patterns with restricted light (van Tienhoven and Ostrander, 1976; Morris and Bhatti, 1978; Morris and Butler, 1995; Morris, 2004). Provided birds received a minimum of 14 h light per 24 h, interruption of the night by introducing a period of light did not affect shell breaking strength, egg size, hen day production or hen mortality. Although we did not measure all these parameters in the present experiment, as previously stated no egg quality problems were noted. An objective of this research was to model the effects of light manipulation on egg laying patterns that could be interpolated for use where larger cages housing larger groups of hens were used. The method of achieving the light insertion during the dark period, that is whether it occurred gradually in 30 min increments per week over six weeks, or abruptly, produced a similar incidence of egg laying in the dark and did not result in a stress response in the birds, measured via elevated corticosterone concentrations in eggs.

In conclusion, the experiment demonstrated that a shift in egg laying time could be achieved by inserting a period of light during the night. For about one-half of the hens, oviposition could then coincide with darkness. One potential benefit of inducing a proportion of birds to lay in the dark is that the number of eggs laid in 'preferred' egg-laying sites is reduced, which could reduce the risk of cracked or broken eggs. No evidence of increased stress response was detected, suggesting there were no adverse effects on bird welfare.

ACKNOWLEDGEMENTS

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ATTRACTING LAYING HENS INTO RANGE AREAS USING SHELTERBELTS

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In free range layer systems there is only a small percentage (9%) of birds that use the outdoor range (Hegelund et al., 2005). This depends on the prevailing weather, season, age of birds, flock size, time of day and the type of outdoor structures birds are provided (Hegelund et al., 2005; Zeltner and Maurer, 2009). The birds that do range stay close to the house increasing the stocking density (Hegelund et al., 2005) which may contribute to feather pecking.

A trial was undertaken to examine the role of shelterbelts in reducing feather pecking behaviour by attracting hens into the range. A total of 120 Hyline Brown hens were housed at 17 weeks in an eco-shelter which had 6 internal pens (20 birds/pen) of equal size (2 m x 3 m) and a free range area (726 m^2) adjoining each pen. There were feeders, drinkers, nest boxes and perches in each pen but no artificial light. Two treatments were examined; a shelterbelt vs. no shelterbelt replicated 3 times. The treatment hens were provided a shelterbelt 10 m and 20 m from the poultry house while the control hens only had access to a bare paddock. The shelterbelt consisted of a range of sizes of trees in pots [small (1 m high), medium (2 m high) and large (3 m high)] as well as shrubs 1 m in height. Shelterbelts were established two weeks before the trial commenced. From 24 to 31 weeks (May-July 2009) the birds were allowed access to the range from 0800-1700 h. Measurements were made daily for egg production, weekly for egg weight and fortnightly for feather score and body weight. On 5 occasions per week spot observations as well as weekly video recordings were made to assess the behaviour of the birds in the range. The behavioural measures included the number and distribution of birds <10 m from house and >10 m from house as well as feather pecking, aggressive behaviours, dust-bathing, foraging, running, and comfort behaviours. Behavioural recordings were analysed using Interact 8 software (Mangold, Germany).

There was a significantly (P = 0.019) higher percentage of birds in (67.4%) in the range when provided a shelterbelt compared to 58.5% with no shelterbelt. In addition there was a higher percentage (52.3%) of birds provided a shelter belt observed in areas >10m from the house compared to the no shelterbelt (46.2%) treatment. Feather pecking was not observed in either treatment throughout the experiment and other aggressive behaviours were only observed once and therefore were not considered in the statistical analysis. Foraging and running behaviour occurred significantly (P < 0.05) more in the shelterbelt treatment. There was no significant effect on production and feather score of hens whether they were provided shelterbelts or no shelterbelts in the range. The trial suggests that shelterbelts result in a significant improvement in the percentage of birds using the range during the day.

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ATTRACTING LAYING HENS INTO RANGE AREAS USING SHADE AND FORAGE

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A concern for the free-range layer system is that only 9% of birds use the range area. The factors which influence use of the range include weather (temperature, wind and rain), season, age, flock size, time of day, shade and variety of overhead structures (Hegelund et al., 2005, Zeltner and Maurer 2009).

Two trials were undertaken, one examined the role of shade areas in attracting laying hens into the range and the other examined the role of forage. In the first trial a total of 120 laying hens (Hyline Brown) were housed at 18 weeks. The eco-shelter had 6 internal pens of equal size (2m x 3m) with a free range area ($726m^2$) adjoining the shelter. Hens were provided feeders, drinkers, nest boxes and perches in each pen but no artificial light. There were 2 treatments provided in the range, shade vs. no shade, with each treatment comprising 20 birds replicated 3 times. The control hens were not provided outdoor shade while the treatment hens were provided a shaded area (3m x 2m x 1m= l x b x h) fitted with shade cloth located 10 m and 20 m from the shed. From 32-44 weeks (March-May, 2008) hens were allowed access to the range and measurements were made daily for egg production, weekly for egg weight and four weekly for feather score. Video records were made of hens from each of the replicates using the shade or in the range.

Shaded areas were visited by 18% of the hens with a tendency (P = 0.07) for more hens to be in the paddock; 43% for paddocks with shade compared to 25% for the paddocks with no shade provided. There was no significant effect on production and feather score of hens whether they were provided shade or no shade in the range. The provision of shaded areas in the free range attracted some additional hens into the range but other attractants are needed.

In the second trial a total of 120 chickens (Hyline Brown) were randomly allocated into 6 groups of 20 birds. There were 3 treatments provided in the range, no pasture (control); vetch pasture and wheat pasture. Over the period 58-70 weeks of age (Aug–Oct 2008) birds were allowed access to the range and measurements were made on production, feather score and percentage of birds foraging. No difference in feather cover of the treatment groups were observed but there was a significant interaction (treatment x time of day) for percentage of birds foraging. The percentage of control birds in range was greater (55%) in morning than the afternoon (30%) while for the pasture treatments about 45% if the birds were in the range both during the morning and afternoon. The trial suggests that forage availability has an impact on the percentage of birds using the range during the day.

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OPTIMUM LEVEL OF CASSAVA PULP IN DIETS FOR LAYERS

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Summary

An experiment was conducted to determine the optimum level of cassava pulp that can be included in layer diets without adverse effect on egg production and quality. Cassava pulp replaced maize at 0, 5, 10, 15, 20 or 30 %, with equal metabolisable energy and crude protein contents. The 30 % diet resulted in the lowest bulk density (610 g/litre). As the cassava pulp level increased in the diet, the weight of the small intestine and bursa increased (P<0.001). Inclusion of cassava pulp at 20 and 30 % in the diet was found to increase (P<0.001) feed intake per dozen eggs and egg production was reduced (P<0.001) between 25 and 26 weeks of age. Yolk colour score and shell thickness were adversely affected as cassava pulp level was increased. It can be concluded that cassava pulp can be used at up to 15 % in layer diet without detriment to egg production.

I. INTRODUCTION

Cassava is a cultivated crop that has been used widely as an energy source for livestock. The principal characteristic of cassava flour is the high soluble carbohydrate (starch) compared to other crops. It has been reported that cassava contains 40 and 25 % more carbohydrate than rice and maize, respectively (Tonukari, 2004). Some studies have reported that whole cassava can be used as a replacement of corn up to 60 % without adverse effects on egg production in layers (Enriquez and Ross, 1972; Hamid and Jalaludin, 1972). Less research has been conducted on cassava pulp, which is a residue obtained after extraction of starch from cassava root (Sriroth et al., 2000). Chotineeranat et al. (2004) reported that cassava pulp still contains up to 66 % starch. A large amount of this pulp is produced and is a potentially inexpensive feed ingredient to replace corn, and other grains for livestock. However, there is lack of information on its value as a feedstuff for poultry. Thus, the objective of this study was to evaluate the potential value of pulp for laying hens, in terms of egg production and egg quality.

II. MATERIAL AND METHOD

One thousand and two hundred Hisex layers were used in the experiment, between 16 and 28 weeks of age. The experiment was conducted in cages, each holding 2 birds. Twenty cages (40 birds) constituted one replicate, with 5 replicates per treatment and there were 6 treatments in total, varying in cassava pulp content; 0, 5, 10, 15, 20 or 30 %. The cassava pulp was obtained from Sonitch Starch Technology Co., Ltd, Thailand and the study was conducted at a facility in Thailand. The diets were similar in energy and protein contents. Birds had access to feed and water *ad libitum*. Temperature and ventilation were controlled with an evaporative cooling system. Birds received a total of 17 hours of light per day. Room temperature was controlled at 20-28 ˚C, and the relative humidity maintained between 60 and 70 %. Egg production and egg quality were assessed at 5 and 50 % lay, and at peak production.

Ovary development was measured at 20 weeks of age; two birds per replicate were randomly selected and slaughtered through cervical dislocation. The birds were dissected and the weight of various visceral organs (ovary, proventriculus/gizzard, small intestine,

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pancreas, reproductive tract, spleen and bursa) and number of primary follicles were recorded. Body weight was also recorded at the start and thereafter, every 4 weeks along with feed intake. Data were analysed with Minitab statistical package. Results presented in this study are mean values and differences between means were determined by the one-way ANOVA.

III. RESULTS

(a) Bulk density

The bulk density of the diets was reduced as the level of cassava pulp in the diet was increased in both phases (16-21 and 22-28 weeks of age). At 30 % cassava pulp in the diet, bulk density was 610 grams/litre, compared to 775 and 750 g/litre for the control diets fed at 16-21 and 22-28 weeks, respectively.

(b) Ovary development and organ weight

The relative weight of the proventriculus and gizzard was found to be highest at 10 % cassava pulp in the diet. Small intestinal weight increased (P<0.001) as cassava pulp level increased in the diet. The weight of bursa also increased (P<0.05) in line with the level of cassava pulp in the diet. However, the weight of other organs (ovary, reproductive tract, pancreas and spleen) and number of primary follicles at 20 weeks of age were not affected by the level of cassava pulp in the diets (Table 1).

Table 1.   Effect of cassava pulp level in the diet on ovary development at 20 weeks of age (g/kg body weight)

Cassava Pulp (g/kg)	NF	OW	P+G	SI	Pancreas	RT	Spleen	Bursa
0	1.08	6.7	29.7^{ab}	49.3^a	1.95	9.5	2.72	1.72^a
50	0.22	2.4	29.0^{ab}	52.5^a	2.48	7.3	2.54	2.76^b
100	0.26	3.0	32.0^a	52.1^a	2.29	7.7	2.43	2.36^{ab}
150	0.33	3.0	27.4^b	56.6^{ab}	2.56	8.8	2.62	2.71^b
200	0.40	2.2	26.1^b	65.8^{bc}	2.51	13.3	2.15	2.27^{ab}
300	0.39	2.8	28.7^{ab}	68.5^c	2.07	9.1	2.61	2.88^b
SEM	0.14	0.71	0.52	1.31	0.08	1.5	0.09	0.10

NF - Number of primary follicles, OW - Ovary weight (g) P+G - Proventriculus+gizzard weight (g), RT - Reproductive tract weight (g). ^{a,b,c}Mean values in the same column not sharing a superscript are significantly different. SEM is standard error of difference between mean values.

(c) Feed intake and body weight

Feed intake per day and feed intake per dozen eggs were not affected by level of cassava pulp in the diet in the early lay period. However, as laying commenced inclusion of cassava pulp at 20 and 30 % in the diet increased (P<0.001) feed intake per dozen eggs, while feed intake was not affected at any level of cassava pulp in the diet. At the beginning, body weight was not different between the treatment diets. However, at 20 weeks the hens fed with 30 % of cassava pulp in the diet were found to be lighter and this was significantly different (P< 0.05) from the control group. At 24 weeks of age, the lowest body weight was observed in hens on the 30 % cassava pulp diet and this was significantly lower (P< 0.05) than on the 5 % diet.

However, at 28 weeks of age hens fed on the control (corn) diet had lower (P< 0.05) body weight than the 5 % treatment group. At levels of cassava pulp higher than 5%, body weight tended to be negatively affected.

(d) Egg production

Hen-day egg production during early lay (16-22 weeks) was not affected by level of cassava pulp in the diet. At 23 and 24 weeks of age egg production tended to be reduced as the level of cassava pulp was increased. The inclusion of cassava pulp in the diet at 20 and 30 % significantly reduced (P<0.001) egg production between 25 and 26 weeks of age. However, at 27 and 28 weeks, the inclusion of cassava pulp at 5 to 15 % in the diet seemed to improve egg production compared to the control diet (Figure 1). However, at 20% and 30 % of cassava pulp egg production was reduced to the same level as on the control diet.
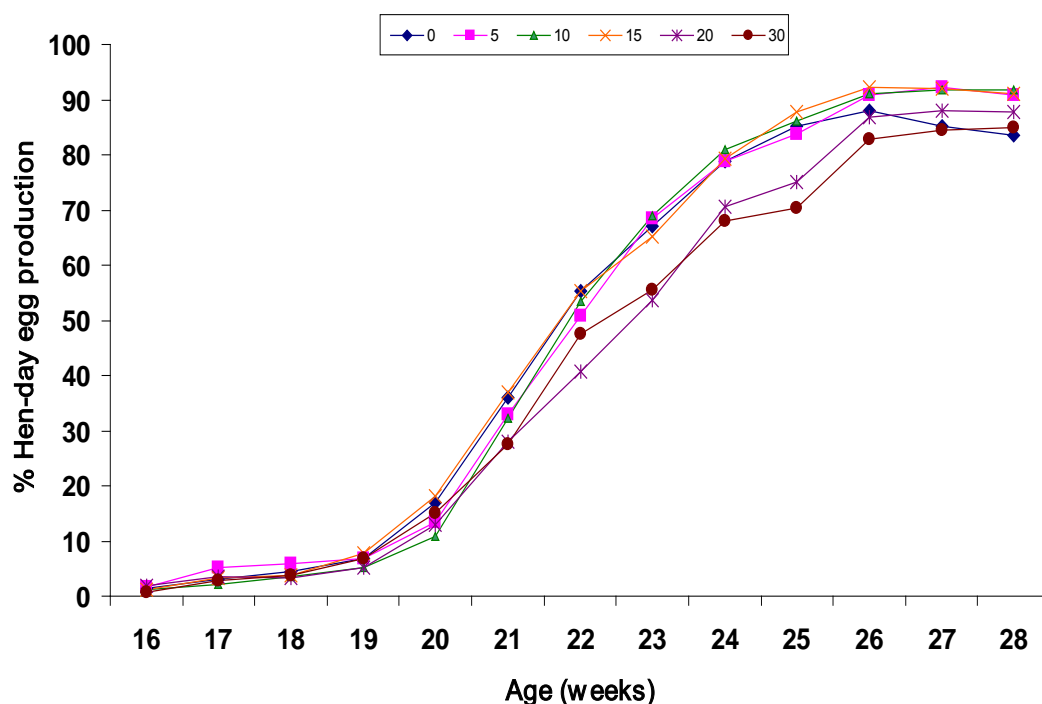

Figure 1.   Effect of cassava pulp level in the diet on egg production at 16–28 weeks of age.

(e) Egg quality

At about 5 % lay, egg quality was not affected by any treatment diets. Yolk colour at 22 and 26 weeks of age was significantly reduced (P<0.001) with an increase in cassava pulp level in the diet. Similarly shell thickness was reduced (P<0.05) at 22 weeks when the level of cassava pulp increased to 30 %. However, shell thickness was better at 5% of inclusion than on the control diet.

IV. DISSCUSION

The results showed that feed intake per dozen eggs was increased at 20 and 30 % of cassava pulp in the diets. This may be due to the bulk density of the feed. Feed flow could be reduced at lower bulk density, and thus affect feed consumption and egg production (Fairfield, 2003). The inclusion of cassava pulp at 5-15% in the diet seemed to improve egg production when compared to the control diet. This could be related to the increase in weight of small intestine

as the cassava pulp level was increased. In the experiment of Nestor et al. (1981), the weight of the small intestine was affected by high-fibre diet but this had no effect on egg production or egg weight. The reduction in yolk color with increase in the level of cassava pulp may be due to the absence of pigments in the ingredient. Shell thickness was also found to decline with cassava pulp level, in line with the report by Tani et al. (2005) that high fibre reduces the absorption of calcium. It can be concluded that cassava pulp can be used as an alternative ingredient to maize at up to 15 % without negative effects on egg production. However, artificial pigments might be needed to maintain yolk colour. Further studies are planned to examine the benefits of supplementation with microbial enzyme.

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IMPLICATIONS ON THE USE OF CANOLA MEAL AND RECOMMENDATIONS TO REDUCE FISHY TAINT IN EGGS FROM BROWN HENS

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Canola meal (CM) is a source of highly digestible protein (360-420 g/kg DM) with low anti-nutritive factors making it an ideal feed ingredient for intensive livestock. But, the use of high levels of CM in brown layer diets leads to the production of a "fishy" taint in eggs. The smell is caused by the presence of trimethylamine (TMA) which is generated by the high level of sinapine (10-15 g/kg DM) found in Australian CM. Sinapine passes to the gut and is converted to choline and further metabolised to TMA. In white layers TMA is effectively metabolised and excreted. But a significant percentage of brown hens have low enzyme levels and when the amount of TMA exceeds the capacity of the hen to metabolise and excrete it, some TMA is diverted to the developing ova, producing a "fishy" taint in the egg.

The effects of sinapine, choline, and CM on the generation of fishy taint were studied using populations of brown layers known to lay taint affected eggs and layers that produced eggs without fishy taint (non-responders). The amount of supplemented choline in the diet significantly increased the occurrence and severity of the fishy taint when the total level of choline in the diet reached 2600 mg/kg (twice the daily recommended allowance). The effect of CM inclusion with known endogenous sinapine content, show that a small amount of taint was present in all of the hens groups, including the group that was fed zero CM and also the group of non-responders hens that was fed a maximum inclusion level of 15%. There was no significant difference in either the occurrence or severity of taint up to an inclusion of 12% CM (containing 1.46 g/kg endogenous sinapine). Increasing the level of CM to 15% (containing 1.83 g/kg endogenous sinapine) led to a significant increase in the level of taint (see Table).

Treatment	Sinapine (g/kg)	Choline (mg/kg)	Set 1 Affected Eggs	Set 2 Affected Eggs	Set 3 Affected Eggs	Overall Affected Eggs (%)
A 0% CM	trace	1082	1/10	2/10	1/10	13.3%
B 6% CM	0.73	1236	1/10	1/10	2/10	13.3%
C 9% CM	1.10	1355	1/10	1/9	2/10	13.8%
D 12% CM	1.46	1490	2/10	2/10	1/10	16.7%
E 15% CM	1.83	1595	3/10	4/9	3/9	35.7%
F 15% CM*	1.83	1595	1/10	0/10	0/9	3.4%

*Note: Treatment F contain layers not enzyme deficient, effectively degrading TMA

The total endogenous choline level in the diets increased from 1082 mg/kg (diet without CM), to 1595 mg/kg in the diet with 15% CM. While our work have shown that this level of choline (when using supplemental choline chloride) did not result in an increase in taint by itself, it is possible that such an increase in the amount of choline (endogenous choline from CM) may have influenced the observed levels of taint in the presence of sinapine. As CM is high in both, choline and sinapine (endogenous source), the combined effect of these two compounds on the generation of taint is an important factor in the usefulness of CM in layer diets and requires more attention.

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EFFECT OF A COMPLEX ENZYME ON THE DIGESTIBILITY OF LAYER DIETS CONTAINING PALM KERNEL MEAL

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Summary

The effects of a broad range activity enzyme complex produced by solid state fermentation (Allzyme SSF) was tested using a commercial peak layer diet and an experimental diet containing palm kernel meal (25% w/w substitution). The addition of a solid state fermentation enzyme resulted in improvements in energy in all diets. In addition diets containing 25% PKM plus enzyme had a significantly better fat digestibility as well as numerically improved crude protein and amino acid digestibility. This study demonstrated that the use of a broad range activity enzyme complex produced by solid state fermentation leads to increased energy and nutrient release in laying hen diets with increased levels of palm kernel meal.

I. INTRODUCTION

Last year, feed prices reached record highs due to the global demand of corn and soyabean and partly because of climatic conditions and poor harvests in same parts of the world. Many producers faced a huge challenge to balance nutrient requirements for their animals and the cost of diets without negatively impacting on overall animal performance. The use of by-products from the oilseed industry (rapeseed, coconut or palm kernel) and the use of endogenous feed enzymes have become paramount in maintaining poultry performance at affordable levels.

The two largest producer of palm nuts and palm kernel meal (PKM) or palm kernel cake (PKC) are Malaysia and Indonesia (FAOSTAT, 2007). The rising cost of transport and the cost of conventional feed material make PKM a product of increasing importance for the local poultry industry in Asia. PKM is the residue of the palm kernel after the solvent extraction of oil. The nutritive value will depend on the species of palm nut, the oil extraction process and the shell residue in the meal. Although PKM is considered a to be a reasonable source of protein for poultry, the inclusion level is limited due to its high content of insoluble NSP and its low amino acid content (Sundu et al., 2006). The majority of non-starch polysaccharides (NSP) are in the form of insoluble linear mannan (78%) with low substitution of galactose (Düsterhöft et al., 1992). The lack of endogenous enzymes to depolymerise these mannans manifests itself in poor growth and feed utilisation when PKM is included at levels above 10% in broiler diets or layer diets (Chong et al. 2008; Soltan, 2009). On the other hand, reports by Perez et al. (2000) and Sundu et al. (2005) indicated that bird performance was not affected with 40% of PKM in the diet. It has to be noted that in both these studies the diets were balanced for amino acids. The availability of amino acids in PKM is generally high; however, levels of lysine and methionine are significantly lower in comparison to soyabean meal. Furthermore, PKM has a wide ratio (3.7 to 3.9) of argentine to lysine (Sundu et al., 2006), since the nutritional requirement of lysine, methionine and argentine are interrelated, diets with high levels of PKM need to be balanced for these three amino acids (Chamruspollert et al., 2002).

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A number of studies have shown that the use of endogenous feed enzymes can improve digestibility of diets with levels of PKM above 20% (Chong et al., 2008; Sundu et al., 2005). In these studies the actual bird performance was not significantly improved in enzyme supplemented diets. Many commercially available feed enzymes are a focusing on the depolymerisation of the insoluble carbohydrates in PKM, little attention has been given to the changes in amino acid digestibility. This paper reports the result of a study investigating the effect of an exogenous enzyme complex on the apparent metabolisable energy and ileal amino acid digestibility of laying hen diets.

II. MATERIALS AND METHODS

The effects of a commercially available feed enzyme (Allzyme SSF, Alltech Inc, USA; included at 150g/t) was tested using a commercial peak layer diets (Mainland Poultry, NZ) and an experimental diet containing palm kernel meal (25% w/w substitution). Titanium oxide was added to all diets as a digesta marker (3 g/kg). A total of 64 layers (Hi Line; 30-week old) of uniform body weight, were allocated to individual cages housed in an environmentally-controlled shed. Four diets were fed for 10days. During the last 4 days, feed intake and excreta output were measured quantitatively per cage for the determination of AME. On the last day, all birds were euthanased by an intracardial injection and the contents of the lower half of the ileum were collected. Diet and ileal digesta samples were analysed for dry matter, titanium, fat, protein and amino acids, including methionine and cystine, but not tryptophan.

III. RESULTS

Table 1.   Influence of an enzyme complex on the apparent metabolisable energy and ileal digestibility coefficients of fat and protein

	AME MJ/kg DM	Fat	Crude protein
Control	12.99[b]	0.864	0.780
Control + enzyme.	13.14[a]	0.901	0.811
Probability	0.05	0.16	0.07
Pooled SEM	0.039	0.016	0.010
PKM diet	11.41[b]	0.893[x]	0.650
PKM diet+enzyme.	11.64[a]	0.928[y]	0.693
Probability	0.001	0.05	0.12
Pooled SEM	0.023	0.007	0.017
PKM	6.69[b]	0.980	0.259
PKM + enzyme	7.13[a]	1.008	0.342
Probability	0.05	0.53	0.42
Pooled SEM	0.091	0.029	0.068

[a,b] Means bearing different superscript in a row are statistically different (P<0.05).

The addition of enzyme resulted in small, but significant (P < 0.05), improvements in the AME of the control diet (Table 1). Numerical increases were seen in the ileal digestibility of fat (P > 0.05) and protein (P = 0.07) when enzyme was added to the diet. Furthermore, addition of enzyme resulted in significant (P < 0.05) improvements in the digestibility of alanine and histidine, and tended (P < 0.10) to improve those of proline, valine and leucine (Table 2). The addition of the enzyme complex significantly increased (P < 0.05) the AME of

PKM diet by 0.23 MJ/kg. Ileal fat digestibility was significantly improved (P<0.05) by the enzyme. A 4% unit improvement in ileal protein digestibility was observed with the addition of the enzyme complex, but this difference was not significant (P = 0.12). Digestibility of several amino acids tended to be increased with the addition of SSF and these included aspartic acid (P = 0.07), threonine (P = 0.11), serine (P = 0.07), glutamic acid (P = 0.08), alanine (P = 0.09), valine (P = 0.08), leucine (P = 0.08), phenylalanine (P = 0.07),histidine (P = 0.06), lysine (P = 0.06) and arginine (P = 0.08). The average digestibility of amino acids tended (P = 0.08) to be greater when enzyme was added to the diet. In calculating the values for the AME and ileal digestibility of fat, protein and amino acids in PKM and the effects of enzyme it was assumed that there were no interactions between the basal diet, PKM and SSF. The values calculated for PKM were found to be very low – especially ileal digestibility coefficients of protein and amino acids. It is likely that the assumption of no interaction may not have been applicable for a high-fibre ingredient such as PKM and this may be responsible for the apparently spurious results. The only notable observation is that the calculated AME of PKM was increased (P<0.05) from 6.69 to 7.13 MJ/kg – an increase of 0.44 MJ/kg (105 kcal/kg).

Table 2.   Influence of an enzyme complex on the ileal digestibility of amino acids

Treatment	Lys.	Met.	Arg.	Pro.	Ala.	Val.	Leu.	His.	Mean
Control	0.870	0.830	0.834	0.771	0.842[b]	0.813	0.856	0.815[b]	0.798
Cont+enzyme.	0.881	0.831	0.846	0.810	0.888[a]	0.843	0.881	0.857[a]	0.823
Probability	0.46	0.95	0.52	0.08	0.05	0.09	0.08	0.05	0.16
Pooled SEM	0.010	0.015	0.013	0.013	0.012	0.011	0.009	0.012	0.011
PKM diet	0.752	0.731	0.718	0.633	0.753	0.691	0.761	0.633	0.669
PKMdiet+enz.	0.807	0.758	0.762	0.651	0.791	0.731	0.798	0.690	0.710
Probability	0.06	0.33	0.08	0.48	0.09	0.08	0.08	0.06	0.08
Pooled SEM	0.017	0.018	0.15	0.016	0.013	0.013	0.012	0.017	0.013
PKM	0.396	0.436	0.369	0.221	0.487	0.323	0.475	0.088	0.282
PKM+enzyme	0.583	0.540	0.511	0.173	0.497	0.396	0.549	0.188	0.372
Probability	0.10	0.35	0.15	0.62	0.38	0.38	0.33	0.35	0.28
Pooled SEM	0.068	0.072	0.060	0.065	0.053	0.054	0.049	0.069	0.054

[a,b]Means bearing different superscript in a row are statistically different (P<0.05). Average is the mean of 17 amino acids.

IV. DISCUSSION and CONCLUSIONS

The use of exogenous feed enzymes is a common tool in the poultry industry to increase nutrient availability in cereal based diet. The addition of a multi activity enzyme complex to either a commercial diet or a diet with 25% PKM resulted in a significant increase in AME of 1.1% in the control diet 2% in a diet with 25% PKM or 6.66% on the basis of PKM alone. The higher AME values in the present of the same enzyme preparation are consistent with previous work, (Wu et al., 2004) reported improvements between 1.9% (sorghum), 2.1% (wheat), 2.6% (maize) and 7.8% (barley) in the presence of enzyme and (Chong et al., 2008) showed a 6.0% improvement in TMEn in diets containing 25% palm kernel cake.

        The improvement in nutrient digestibility in the presence of exogenous feed enzymes is the result of depolymerising soluble NSP causing high intestinal viscosity and reduced nutrient digestibility but it also can be a direct result of the enzymatic breakdown of the cell

walls and the release of entrapped nutrients (Choct, 2006). Industrial enzymes used in the animal feed industry are produced either by submerged liquid fermentation or by solid substrate fermentation (SSF). Enzyme production using SSF technology, which is characterised by growing a selected strain of yeast, bacteria or fungi on a water insoluble substrate, results in a wider range of enzymatic activities which are not produced by submerged liquid fermentation (Filer, 2008).

The addition of a solid state fermentation enzyme resulted in improvements in energy in all diets, in addition diets containing 25% PKM plus the enzyme complex had a significantly better fat digestibility as well as numerically improved crude protein and amino acid digestibility. It is likely that the board range of enzyme activities produced in the solid state fermentation process was able to release some of the nutrients entrapped in the rectangular honeycomb-like cell wall of the palm kernel (Chong, 1999) resulting in increased digestibility of nutrients. This study demonstrated that the use of an broad range activity enzyme complex produced by solid state fermentation lead to increased energy and nutrient release in laying hen diets with increased levels of palm kernel meal.

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OPPORTUNITIES AND SUSTAINABILITY OF SMALLHOLDER POULTRY PRODUCTION IN THE SOUTH PACIFIC REGION

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Summary

Village poultry provides a source of income, improves human nutrition and helps meet family and social obligations. Local feed resources are available in the Pacific that could be utilized more effectively for feeding poultry. Balanced rations for village birds can be devised based on the variety of potential feeds. An education program based on new feeding systems can then be extended to farmers. New crops and pasture species with higher nutritional value for poultry could also be introduced. Where smallholders use imported commercial feeds the viability of these operations has been threatened by rising costs of imported feeds. To overcome this problem there is potential to use a concentrate diet that can be blended with cheap local feed ingredients or to dilute a commercial diet with cheap locally available ingredients with little impact on production and sale of birds and eggs. Alternatively mini mills could be established to make diets in areas where appropriate local feed ingredients are available and cost competitive. Adoption of such feeding systems is considered a solution for the viability of village broiler farming in Papua New Guinea (PNG) and is a potential method of supporting smallholder poultry operations in other areas of the Pacific.

I. INTRODUCTION

Smallholder poultry farming makes an important contribution to the livelihoods of rural households in the Pacific. Under the traditional "village bird" system on smallholder farms poultry are allowed to scavenge for feed, have little shelter and are free to wander around the village. This system is cheap and the farmers have little work to do. There are minimal provisions for shelter, feed and water and few management skills are required. However there are considerable problems with this system including slow growth and poor productivity due to energy and protein deficiencies, poor bird genetics, losses due to predators and theft and damage to village gardens. However, many smallholders have changed from producing poultry solely for household purposes to producing meat birds and eggs for sale in local markets. They have adopted improved housing and nutrition and use modern genetic strains fed on commercial feeds. However the viability of these semi commercial village operations has been threatened by the rising costs of imported ingredients and feeds. In Pacific countries this problem is primarily due to the reduction in the value of the local currencies and consequent increases in the cost of all imports. This has contributed to the demise of some smallholder operations relying on imported feed. When transport problems are added (and these are also made worse by the rising real cost of fuel), the smallholder poultry industries that rely on imported ingredients are in difficulty. The viability of the smallholder operations

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will be substantially improved if feeding regimes based at least in part on local ingredients can be developed as an alternative to imported complete feed or feed ingredients from overseas.

II. OPPORTUNITIES

Local feed resources are available in the Pacific that could be utilized more effectively for feeding poultry (ALFID, 2002). Farmers could also introduce new crops and use pasture species with higher nutritional value for poultry (Glatz, 2008). Effective rations for village poultry can be developed based on feed resources available. Four feeding strategies for poultry could be adopted by smallholder farmers; a) complete ration formulation using local feed ingredients; b) free choice of feed ingredients; c) mixing a concentrated diet with local feed ingredients and d) dilution of a commercial diet with locally available food products.

(a) Development of village poultry rations for village farmers in the Solomon Islands
There is a wide variety of local feed resources available that could be utilized more effectively such as root crops, fruit, forages, bush plants and vines. Farmers in remote areas can introduce new crops (sorghum, mung bean, pigeon pea, sunflower, amaranth and others) with higher nutritional value for poultry and provided they have the appropriate tools and growing technologies. For example in the Solomon Islands three feeding trials with village hens were conducted using local feed resources.

Composition of diet 1: Corn (45%), fresh grated cassava (6.31%), ripe paw paw (5%), mung beans (30%), fishmeal (5%), lime (8%), premix 0.25%, lysine 0.09%, methionine 0.05% and salt (0.3%). Specifications of diet; AME 10.8 MJ/kg; CP 147 g/kg; fat 26 g/kg; fibre 22g/kg, Ca 30 g/kg and P 5 g/kg (calculated values).

Composition of diet 2: Corn (25%), pigeon pea (15%), ripe paw paw (5%), mung beans (30%), fresh grated coconut (5.81%), fresh grated cassava (10%), lime (8%), dicalcium phosphate (0.5%), premix 0.25%, lysine 0.09%, methionine 0.05% and salt (0.3%). Specifications of diet; AME 9.3 MJ/kg; CP 136 g/kg; fat 33 g/kg; fibre 30g/kg, Ca 30 g/kg and P 5.2 g/kg (calculated values).

Composition of diet 3: Pigeon pea (25), sorghum (45%), ripe paw paw (8%), fresh grated cassava (8.31%), fishmeal (5%), lime (8%), premix 0.25%, lysine 0.09%, methionine 0.05% and salt (0.3%). Specifications of diet; AME 10.2 MJ/kg; CP 125 g/kg; fat 27 g/kg; fibre 36g/kg, Ca 30 g/kg and P 5.5 g/kg (calculated values).

The trials compared the performance of birds fed the local home mix layer ration with an imported commercial layer feed. The feeding trials were conducted in a naturally ventilated barn at the Solomon Islands College of Higher Education comprising 16 pens (1.5 x 1.5m) each with perches, nest boxes, drinkers and feeders. A total of 64 local hens were used for each trial which included 4 replicates of a control commercial layer diet (as the gold standard) and 4 replicates of the local feed diet. The birds were of a mixed age and obtained from local farmers. Corn and mung beans were included as whole grain in the rations. Cassava and paw paw were chopped, weighed, mixed and fed fresh twice daily. Numbers of eggs laid were recorded daily. Egg production was significantly lower in birds fed the local mix ration compared to the commercial ration (Figure 1). However the cost of using the imported commercial feed is too expensive for village farmers to consider purchasing (Glatz, unpublished).
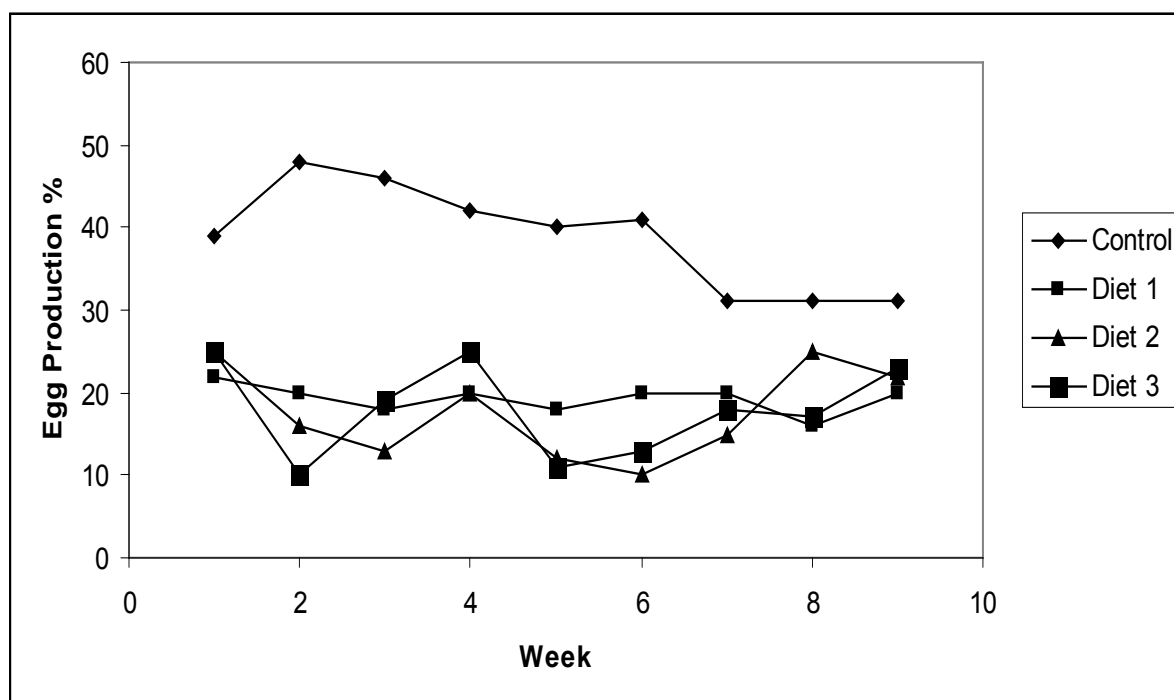
Figure 1.   Egg production % of village hens in the Solomon Islands fed a commercial ration vs. three local diets.

In Tonga the composition of the local ration was maize (45%), cassava meal (15%), copra meal (16.56%), fish meal (15%), crushed sea shells (7%), salt (0.3%), dicalcium phosphate (1%), lysine (0.09%), methionine (0.05%). Specifications of diet; AME 12.2 MJ/kg; CP 181 g/kg; fat 53 g/kg; fibre 7 g/kg, Ca 32 g/kg and P 6.8 g/kg (calculated values). A total of 36 commercial hens (34-37 weeks) were used for the poultry trial, with two birds/cage (30cm wide x 45 high and 30 cm deep) located in the middle row of naturally ventilated single tier cage shed. The diets tested were; 1) commercial imported layer diet, 2) commercial imported layer diet diluted with 30% copra meal and the local ration. A commercial layer diet diluted with 30% of copra meal resulted in similar egg production compared with the imported commercial layer diet. However, layers fed on the diet formulated with local feed produced the lowest (P<0.05) numbers of eggs. This was due to the batch of copra meal that was added to the local diet being rancid.

Table 1.   Egg production % of birds in Tonga fed a commercial ration, a commercial ration diluted with 30% copra meal and the local diet.

Treatment	Production (%)
Commercial	94.3a
70% Commercial+30% copra meal	95.7a
Local feed	27.1b
P	0.026

Means with a common letter are not significantly different ($P > 0.05$)

(b) <u>Free choice feeding in village poultry</u>

In village layers, Grayson and Campbell (2004) reported that free choice feeding system can be used successfully for village poultry in the Solomon Islands. Feed from the three food groups; protein, carbohydrate and minerals and vitamins are provided separately every day so the birds can choose feed according to their requirements.

(c) <u>Development of a concentrated diet that can be blended with local feed ingredients</u>

Glatz (2006) reported on a feeding strategy whereby PNG fishmeal and copra meal (plus minerals and vitamins) were used to produce a concentrate that can be supplemented with 50-80% of local ingredients (e.g. sweet potato) to make up the whole ration. The promising results from an on-station evaluation of the concentrate were followed by an on-farm evaluation in the Eastern Highlands Province in PNG. The trial with 60 smallholder village farms (each growing 50 meat birds) compared the performance of birds fed a commercial diet vs. a diet comprising the poultry concentrate fed with cooked sweet potato. While the higher moisture content of the treatment diet (containing 70% sweet potato) resulted in a higher feed intake (4.26 vs. 4.0 kg/bird) and a lower live sale weight (2.0 vs. 2.4 kg), the income from sale of live birds was only about 5% lower for the concentrate plus sweet potato diet.

Pandi and Ayalew (unpublished) subsequently tested four diets in a large broiler grow out trial at the National Agriculture Research Institute (NARI) in Lae. The diets were; 1) 50% sweet potato plus 50% low energy concentrate; 2) 70% sweet potato plus 30% low energy concentrate; 3) 50% cassava plus 50% high energy concentrate and 4) 70% cassava plus 30% low energy concentrate.

The composition and calculated nutrient specifications of high energy and low energy concentrate were as follows:

(a) High energy concentrate (11.6MJ/kg); Composition (g/kg), sorghum 118; soya 485.9; meat & bone meal 286; tallow 65; L-lysine 12.5; DL-methionine 11.8; L-threonine 1.3; salt 5; mycocurb 1; choline cloride 4.5; boiler premix 9; Specification (g/kg); Protein (419.75), Arg (28.59), Isoleu (15.95), Lys (32.89), Meth (17.41), M+C (23.25), Threo (16.58), Fibre (23.62), Ca (27.56), Ptot (16.73), AvP (9.4), NA (3.58), K (10.48), CI (6.08)

(b)Low energy concentrate (9.4MJ/kg); Composition (g/kg) mill run 246; soya 389.45; meat & bone meal 309; + tallow 8; L-lysine 13.8; DL-methionine 12.4; L-threonine 1.7; salt 5; mycocurb 1; choline chloride 4.5; ronozyme phytase 0.15; broiler premix 9; Specification (g/kg); Protein (417.87), Arg (28.19), Isoleu (14.91), Lys (33.01), Meth (18.01), M+C (24.03), Threo (16.45), Fibre (38.47), Ca (30.29), P tot (20.18), AvP (11.44), Na (3.7), K (10.5), CI (6.43).

Each diet was replicated 4 times in 16 floor pens in a naturally ventilated shed. Results indicated favorable bird performance with all the feeding options assessed. However, the better performing feeds when assessed for overall weight gain, feed intake and feed conversion were; 50% sweet potato plus 50% low energy concentrate; 70%; sweet potato plus 30% low energy concentrate and 50% cassava plus 50% high energy concentrate (Table 3).

Table 3.   Weekly body weights (kg) of meat chickens grown at NARI on combinations of concentrate, sweet potato and cassava compared to a commercial grower ration.

Age (weeks)	Commercial Grower	SP50 LEC	C50 HEC	SP70 LEC	C70 HEC	LSD	P value
3	0.90a	0.91a	0.91a	0.90a	0.91a	0.012	0.838
4	1.27a	1.23b	1.24ab	1.08c	1.09c	0.041	<.001
5	1.86a	1.59b	1.53c	1.49c	1.24d	0.055	<.001

Means with a common letter within a row are not significantly different ($P > 0.05$). SP50=50% sweet potato; SP70=70% sweet potato; C50=50% Cassava; LEC=low energy concentrate; HEC=high energy concentrate

The two most promising feeding options that were tested based on the results in Table 3 were diets comprising 50% cassava mixed with a high energy concentrate and 50% sweet potato mixed with a low energy concentrate. It was considered that the best bet feeding options that required further evaluation in lowland and highland areas of PNG were 50% sweet potato and 50% LEC; 50% Cassava and 50% HEC and 70% sweet potato and 30% LEC. These diets were tested at 3 different sites in PNG; Christian Leaders Training Centre (CLTC), Lutheran Development Service (LDS) and OK Tedi Development Foundation (OTDF). The grow out facilities at each of these sites comprised 8 floor pens in a naturally ventilated shed with two replicates of each diet.

Table 4a.   CLTC site; weekly body weights of meat chickens fed on combinations of concentrate, sweet potato and cassava.

Experimental Diet	Day 21	Day 28	Day 35	Day 42
Commercial finisher	0.810	1.371[a]	1.988[a]	2.724[a]
50SP + 50 LEC	0.813	1.276[a]	1.735[b]	2.283[b]
50C + 50 HEC	0.813	1.221[a]	1.566[b]	2.082[c]
70SP + 30 LEC	0.810	0.906[b]	1.157[c]	1.514[d]
P-value	0.803	0.004	<0.001	<0.001
LSD	0.009	0.147	0.174	0.151

Means with a common letter within a row are not significantly different ($P > 0.05$)

Table 4b.   LDS site; weekly body weights of meat chickens fed on combinations of concentrate, sweet potato and cassava.

Experimental diets	Day 21	Day 28	Day 35	Day 42
Commercial finisher	0.801	1.477[a]	2.011[a]	2.724[a]
50SP + 50 LEC	0.800	1.318[b]	1.802[b]	2.342[b]
50C + 50 HEC	0.801	1.327[b]	1.860[b]	2.377[b]
70SP + 30 LEC	0.801	1.154[c]	1.454[c]	1.785[c]
P-value	0.651	<0.001	<0.001	<0.001
LSD	0.002	0.067	0.099	0.200

Table 4c.   OTDF site; weekly body weights of meat chickens fed on combinations of a concentrate, sweet potato potato and cassava.

Experimental diets	Day 21	Day 28	Day 35	Day 42
Commercial finisher	0.870	1.536[a]	2.181[a]	2.570[a]
50SP + 50 LEC	0.878	1.365[b]	1.916[b]	2.463[a]
50C + 50 HEC	0.870	1.339[b]	1.919[b]	2.457[a]
70SP + 30 LEC	0.880	1.174[c]	1.627[c]	2.010[b]
P-value	0.598	0.012	<.001	0.008
LSD	0.024	0.149	0.111	0.224

The results (Tables 4a, 4b, 4c) from the three climatic zones in PNG confirmed findings in grow-out trials at NARI that birds fed the 50% sweet potato with 50% low energy concentrate and 50% cassava with 50% high energy concentrate diets were able to reach market weight of 2 kilograms or more at 42 days of age. However birds fed the 70% sweet potato with 30% low energy concentrate did not reach market weight for both the CLTC and LDS sites. At the OTDF site birds were able to reach market weight when fed higher amounts of sweet potato, possibly due to a more suitable environment for the birds.

(d) <u>Dilution of a commercial diet with locally available food products</u>

In some Pacific countries considerable quantities of copra meal are available for use in poultry feeds. Pandi (2005) examined the growth rate of village broilers that were fed with a commercial finisher feed diluted with 20-80 % of copra meal. In figure 2 diet 1 was 100% broiler finisher (BF); diet 2, 80% BF + 20% copra meal (CM); diet 3, 60% BF + 40% CM; diet 4, 40% BF + 60% CM; diet 5, 20% BF + 80% CM and diet 6, 100% CM. Diluting a broiler finisher diet with 20-40% copra meal result in similar growth as the control diet and inclusion of 60% copra meal resulted in acceptable growth (Figure 2). The extent of dilution that is practiced depends on the availability and cost of copra meal.
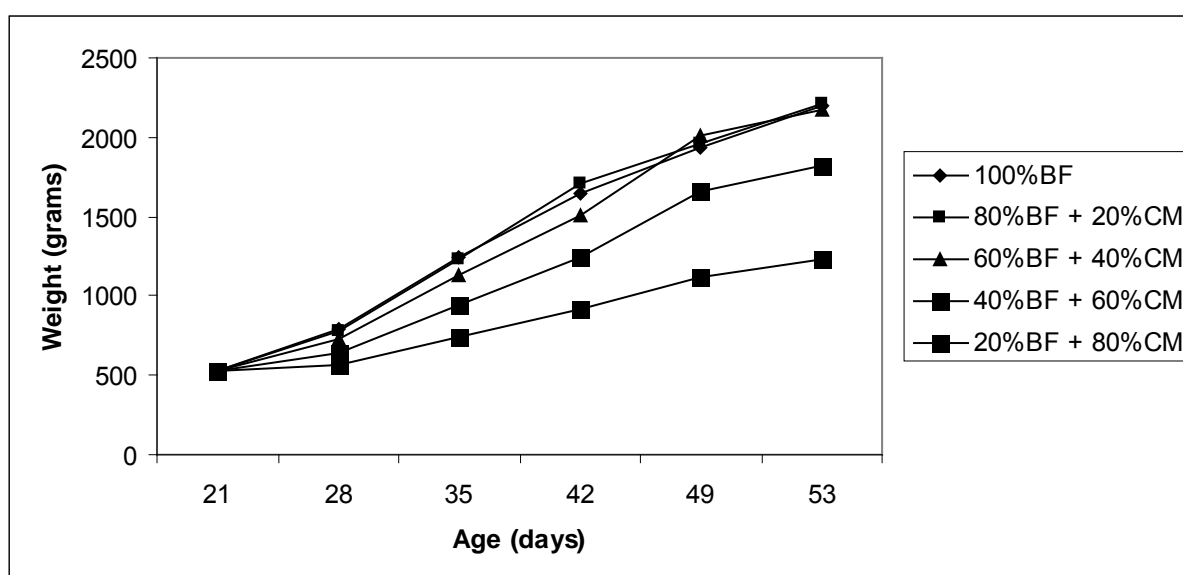

Figure 2.   Weekly average weights of meat birds fed various ratios of copra meal and commercial broiler finisher over the period 21-53 days (from Pandi, 2005).

III. SUSTAINABILITY

(a) Cost of imported feed

The major issue restraining the development of the smallholder poultry sector in the Pacific is perceived to be the lack of regional small scale feed manufacturing plants and the high cost of imported feed as well as cheap imports. Despite this there are adequate supplies of fishmeal, cassava, sweet potato, fresh coconut and maize, which could form the basis of the feed industry throughout the Islands. Poultry production is an important smallholder industry. However the growth and expansion of the smallholder industry is dependent on reducing the reliance on imported complete feed or feed ingredients from overseas. Smallholder poultry production has been hampered or abandoned in many parts of the Pacific due to increased feed costs. Cheap diets based on locally available ingredients and agricultural by-products will encourage new poultry farmers into the industry and encourage others back to poultry production. Use of local feeds will provide an opportunity to develop a sustainable system that will support smallholders who have not benefited from such production systems. Knowledge and skills in poultry feed diet formulation based on locally available ingredients and agricultural by-products have improved rapidly in many Asian countries. Smallholder poultry farmers in several locations around the Pacific appear to have demonstrated that they can make a profit from these farming systems using imported complete feeds supplemented with local feed ingredients or in some cases, using only local formulated diets.

(b) Milling equipment

In the Pacific Islands most of the commercial diets used to feed poultry have been imported from New Zealand, Australia or the United States. Where feed mills have been established in the Pacific they have relied on the use of imported feed ingredients, but many commercial mills have closed due to the escalating costs of imports. Currently only small amounts of local feed resources are used in commercial rations by the larger feed mills in the Pacific due to the high cost of transport, storage difficulties, reliability of supply and constraints in feed formulation because of variable quality. However the establishment of small scale regional feed manufacturing centres (producing 5-10 tonne/week) in areas where local feed supply is plentiful may overcome some of these issues. The lack of suitable feed making equipment to produce diets is the major constraint limiting the development of the small-scale feed industry in the Pacific. However such equipment is becoming progressively more available. In PNG and Tonga progress is being made in establishing small scale feed manufacturing centres which use local feed ingredients to produce lower cost pig and poultry diets. Relatively cheap (approx. A$8000-10000) small scale feed mixing equipment can be purchased to mill diets.

(c) Cost benefits of small-scale feed mills

Smallholder broiler farmers in PNG purchase day-old broilers usually in lots of 52 and grow them out for 6 weeks using commercial feed and then sell them as live birds in local markets. The cheapest carbohydrate source to feed broilers in the highlands is sweet potato while in the lowlands cassava is preferred. Comparisons were made of the costs of the following feeding options; 1) lowlands mini-mill HEC (50%) supplemented with cassava (50%); 2) lowlands commercial HEC (50%) supplemented with cassava (50%); 3) lowlands commercial feed (50%) diluted with copra meal (50%) and 4) lowlands commercial feed. In the highlands the costs of the same feeding options were determined except sweet potato was used instead of cassava. The lowland broiler farmers are close to the commercial mills while

the highland farmers have extra costs associated with transport of commercial feed. The feeding costs in both the highlands and lowlands show advantages when concentrates (HEC and LEC) are fed with sweet potato or cassava compared to use of commercial finisher broiler feed based on imported ingredients (Black 2009, unpublished) – Table 5.

Table 5.   PNG village feeding costs in Kina (A$0.43) per batch of 52 meat chickens grown to 6 weeks of age. (1Kina=AUD$0.43)

PNG feeding system	Kina
Lowlands mini mill HEC + cassava	276
Lowlands commercial concentrate + cassava	318
Lowlands diluted commercial feed	334
Lowlands commercial feed	431
Highlands mini mill LEC + sweet potato	311
Highlands commercial concentrate + sweet potato	350
Highlands diluted commercial feed	359
Highlands commercial feed	481

The proportion of the costs of the concentrate and dilution feeding system relative to using commercial feeds alone show that there is a strong cost reduction incentive at the village level to adopt alternative feeding strategies based partly or wholly on local feed ingredients (Table 6) .

Table 6.   Alternative PNG village feeding systems costs as a proportion of the commercial feeding costs

PNG Feeding system	Proportion
Lowlands mini mill HEC + cassava/lowlands commercial feed	0.64
Lowlands commercial concentrate + cassava/lowlands commercial feed	0.74
Lowlands diluted commercial feed/lowlands commercial feed	0.77
Highlands mini mill LEC + sweet potato/highlands commercial feed	0.65
Highlands commercial concentrate + sweet potato/highlands commercial feed	0.73
Highlands diluted commercial feed/highlands commercial feed	0.75

It may be possible to achieve feed cost savings of up to 35%, with the mini-mill concept being the most advantageous. The mini-mill concentrate was based on copra, fishmeal and coconut oil.

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THE ROLE OF THE WORLD'S POULTRY SCIENCE ASSOCIATION (WPSA) IN SUPPORT OF POULTRY PRODUCTION IN DEVELOPING COUNTRIES

R.A.E. PYM[1]

Summary

Over the past thirty years WPSA has played an increasingly active role in support of poultry production in the developing countries. The recent and projected dramatic increases in poultry production and consumption in many of the developing countries argues for the establishment of sound scientific and technical support to backstop and facilitate an efficient and sustainable poultry industry in these countries. Poultry production from all sectors has the potential to impact positively and meaningfully upon the United Nations' Millennium Development Goals of alleviation of extreme poverty and hunger in the developing world, despite the mitigating effects of climate change, biofuel production and HPAI. Through its involvement in the organisation globally of meetings on poultry science and technology and on all manner of industry-related issues, the establishment of WPSA branches in developing countries, its close cooperation with FAO, its support for the establishment of poultry networks in developing countries, and its involvement in poultry development projects, WPSA has played a meaningful role in support of poultry production in many of the developing countries of the world.

I. INTRODUCTION

In light of the recent global economic downturn, it is difficult to predict future global production and consumption of commodities generally and of poultry meat and eggs in particular. Projections (Farrell, 2008; Speedy, 2003) based on FAO statistics from 2000 to 2006, suggest that chicken meat production will increase by 2.3 and 4.0% per year respectively in developed and developing countries between 2006 and 2016, and egg production will increase by 0.1 and 3.5% respectively. Much of this increase will be seen in the more affluent, larger population developing countries. Similarly, estimates were made of the increase in feed required for poultry meat and egg production in developed and developing countries over the same period. The increase in feed required for poultry meat production was 2.5 and 18.0% per year respectively and for egg production was 2.0 and 12.3% respectively. The potential impact of biofuels on the significantly greater increase in feed requirements in developing countries over the next 8 years, gives considerable cause for concern. Farrell (2008) estimated that some 250 million hectares of arable land will be required globally by 2016 to annually grow the 1100 million tonnes of grain and protein crops to meet the need for feedstuffs for compounded feeds for all livestock.

Although world human population growth has slowed down to about 1.1% per year, there will still be another 700 million mouths to feed by 2016, the large majority of these in developing countries. Africa's population is expected to increase by about 2.4% from about 890 million at present, to 1050 million by 2016. Growth in the developed world will be almost zero; estimates for the population increase in the EU to 2016 are only about 0.1% (Anonymous, 2007).

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There is no doubt that affluence is the force driving livestock production; as developing countries become more wealthy, the populace demands more animal products and poultry production is often the first livestock industry to respond to that demand. The technology is universal and portable, easily transferable, and eggs and chicken meat are generally free from cultural taboos. Demand in China and India for livestock products has been increasing at a rapid rate in concert with rapid economic growth (at least up until the recent economic downturn). Other countries with large populations, such as Indonesia, Vietnam and Brazil have recently experienced rapid economic growth and have a large expanding middle class. It is thus not surprising that the future demand for animal products will come from developing countries and comparatively little from developed countries. However, many of the very poor countries such as those in sub-Saharan Africa, West Asia and North Africa, even prior to the global economic woes, have experienced a decline in energy and protein from animal products.

II. POULTRY PRODUCTION SYSTEMS IN DEVELOPING COUNTRIES

FAO has categorised the different poultry production systems which operate in most developing countries into four sectors. These range from sector 1 large scale commercial operations, often integrated with hatcheries, feed mills and processing plants, and utilising sophisticated housing and equipment, with confinement rearing/housing and feeding of commercial layer or broiler genotypes, through to sector 4 small-scale family semi-scavenging flocks of indigenous genotype birds. The former sector is essentially identical to production systems seen in most of the developed countries and the feed used is often compounded from imported feedstuff ingredients. Sector 2 systems tend to be similar to sector 1, but are generally smaller, not integrated to the same extent with other poultry facilities, and make greater use of local materials in shed construction and equipment. They also tend to employ manual rather than automated systems of feeding and egg collection. For both sectors 1 and 2 broiler operations, chickens are usually processed centrally before distribution and sale.

Sector 3 production units are essentially small-scale commercial units of from 100 to 500 commercial broilers or layers with confinement housing and feeding using commercially compounded feed. Houses and equipment are often constructed from local materials and eggs and live birds are usually sold at local markets, rather than through distributors or directly into regional markets. Whilst sector 3 production can be profitable in the short-term in niche markets, competition with the larger commercial operators for chickens, feed, pharmaceuticals and markets, puts considerable strain on the long-term viability of such operations.

By way of contrast with the above commercial systems, sector 4 is largely comprised of very small flocks of 10 to 50 indigenous breed birds kept by a large proportion of families in villages in the rural regions of many countries. These birds receive household scraps and scavenge around the village during the day; as such feed costs are minimal. The hens hatch their own eggs and rear the chicks to an age when they can fend for themselves. These small poultry flocks make a meaningful contribution towards poverty alleviation and household food security in rural communities (Gueye, 2000). They also account for a significant proportion of poultry meat consumed in many of the developing countries (Pym *et al.* 2006)

Productivity in sector 4 production systems is low, but so too are inputs. The hen usually lays only 40 to 60 eggs per year over 3 to 4 clutches, but spends much of her time hatching the eggs and rearing the chicks. Probably the greatest constraint to productivity and profitability of this system, is the usually very high attrition rate in young chicks (often more than 60%) due to predation, disease, malnutrition and climatic stress. In most countries,

there tends to be a preference for chicken meat than for eggs, and consequently, most of the eggs tend to get set under the hen to produce chicks. Whilst hatchability is normally quite good, the high attrition rate means that only a few of these chicks survive to an age when they can be eaten, or in the case of females, to sexual maturity.

There are opportunities for improving productivity and sustainability in all four production systems, but it is important to understand the constraints under which each system operates. For poor people living in rural regions of many countries, the only poultry meat and eggs they are ever likely to eat are those they produce themselves from their small family flocks of indigenous chickens. However, because of limited availability of a scavenging feed resource base, and concerns about the transmission of diseases (particularly HPAI) to people and to commercial poultry flocks, there are relatively few remaining sector 4 scavenging flocks in urban or peri-urban communities in most countries. There is no question that the most efficient and effective means of providing poultry meat and eggs to people in urban and peri-urban areas, is from commercial production from broilers or layers, whether it be from sector 1, 2 or 3 production systems. Household income in these communities is for the most part significantly higher than in rural communities and poultry meat and eggs are moderately affordable by many.

III. THE ROLE OF WPSA AND ITS CONTRIBUTION TOWARDS POULTRY PRODUCTION GLOBALLY.

The World's Poultry Science Association (WPSA) has over seven thousand members in 75 countries around the world. The objectives of the association are to promote the advancement of knowledge of all aspects of poultry science and the poultry industry world wide, principally by facilitating exchange of information through the organisation of group meetings, regional conferences and World Congresses. To promote membership of the organisation in developing countries, the cost of belonging to the world Association in those branches, is only half that of the membership of branches in the developed countries. All members receive copies of the World's Poultry Science Journal, published quarterly and now in its sixty-first year of publication.

The main international forums organized by WPSA are the World's Poultry Congresses, held every four years. World Congresses include a wide ranging scientific and technical program and the meeting also incorporates a large and comprehensive poultry industry trade exhibition. The meetings are very well attended by a broad cross section of people from the scientific community, poultry industry, educational institutions and government. The last meeting (the 23rd Congress) was held in Brisbane, Australia in July 2008 and the 24th Congress will be held in Salvador, Brazil in 2012.

There are presently two Federations of the Association: viz the European and Asian Pacific Federations. Each Federation stages a conference every four years, approximately midway between the World Congresses. Additionally to this, the European Federation, has some 11 working groups on *Economics and Marketing; Nutrition; Breeding and Genetics; Egg Quality; Poultry Meat Quality; Reproduction; Poultry Welfare and Management; Turkey Production; Education and Information; Physiology;* and *Ratites*, whilst the Asian Pacific Federation, established somewhat later, has two working groups on: *Small-scale Family Poultry Farming;* and *Waterfowl*. Each of the working groups has the responsibility for organizing regular symposia in the relevant area. These symposia are recognized internationally as the main global forums for scientific and technical discussion in the respective areas and are for the most part very well attended by industry.

The nature and scope of the above meetings and the close involvement of the poultry industry, means that they have had, and will continue to have, a significant impact on improvements in the efficiency and sustainability of the global commercial poultry industry. The extent to which this impacts upon the wellbeing of the poor in developing countries, is influenced by many factors. An important area of involvement with potential to impact more directly upon poverty and household food security, is the work in support of small-scale family poultry farming.

IV. WPSA SUPPORT FOR SMALL-SCALE FAMILY POULTRY FARMING

The commitment to improving the efficiency and sustainability of small-scale family poultry farming was very much in evidence in the scientific and technical programme at the 19th World's Poultry Congress in Amsterdam in 1992; this commitment has continued in subsequent Congresses.

A meeting was held in the family poultry farming session at the 7th Asian Pacific Federation Conference at the Gold Coast, Australia in October 2002 where a proposal to establish a Working Group on Family Poultry Farming was approved by the assembled membership of WPSA. The inaugural meeting of the Working group was subsequently held in March 2005 in conjunction with the 4th International Poultry Show and Seminar in Dhaka, Bangladesh. At that meeting WPSA funded the attendance of two delegates each from Sri Lanka and Indonesia to provide accounts of the impact of the 2004 Boxing Day tsunami on the poultry industry in the respective regions, with the aim of developing proposals for assistance.

In keeping with the commitment to support small-scale family poultry development, at the 11th European Poultry Conference in Bremen in October 2002, the WPSA Board endorsed the proposal to include the International Network on Family Poultry Development (INFPD) as a global working group within WPSA. Discussions regarding the most effective and efficient structural arrangements for this cooperative arrangement are on-going, and will be a focus of the WPSA presidency of the writer.

In March 2007, the WPSA Asian Pacific Federation working group on Small-scale Family Poultry Farming convened a symposium on *The impact of Avian Influenza on Small-scale Family Poultry Farming in Developing Countries* in conjunction with the 8th Asia Pacific Poultry Conference in Bangkok, to which a large number of internationally recognized family poultry researchers and promoters were invited. The meeting was convened with the express purpose of providing objective information about the impact of HPAI on small-scale family poultry farming (SSFPF) production and the role of SSFPF in transmission of the disease, following the demonization of SSFPF by a number of governments. The other important aim was to identify appropriate measures that need to be taken by SSFP keepers to minimize the effects and transmission of the disease. Attendance of the 30 or so speakers was jointly sponsored by FAO, CTA and WPSA. Additional to papers dealing with a wide range of technical and procedural issues, there were regional reports on the then current status of HPAI and small-scale poultry farming in Lao PDR, Thailand, Bhutan, Burma, Papua New Guinea, The Philippines, Malaysia, Cambodia, Indonesia, India, Sri Lanka, Bangladesh, Vietnam, Mozambique and Africa (generally).

At the 23rd World's Poultry Congress held in Brisbane, Australia in July 2008, one of the eight main concurrent streams was on *Poultry Production in Developing Countries.* There were some 40 papers presented in the stream and the final session within the stream involved a workshop aimed at facilitating cooperation and collaboration between all stakeholders involved in support of small-scale family poultry production. The report from the workshop and the 15 invited papers in the stream was published in the June 2009 edition of the World's

Poultry Science Journal. A recommendation from the workshop report was that an over-arching entity *"Poulet Sans Frontiers",* mooted by the writer in an AusAID-sponsored conference in Tanzania in 2005 and with membership from all agencies and organizations supporting family poultry farming, be established. The collective desire is that this body will impact meaningfully on the level and nature of global support for small-scale family poultry farming.

V. POULTRY PRODUCTION IN DEVELOPING COUNTRIES AND THE MILLENNIUM DEVELOPMENT GOALS

The relatively recent problems of climate change, biofuel production (Lyons, 2007) and the global economic uncertainties with their attendant effects upon global agricultural production, have made the United Nations' Millennium Development Goals, announced in 2000 with a range of targets set for 2015, considerably more difficult to attain. The Millennium Development Goals are broad and multi-faceted and address all elements of desirable development. Although not fully comprehensive, improvement in the efficiency and sustainability of poultry production in developing countries has the potential to have a significant impact upon a number of the Goals, particularly the primary goal of eradication of extreme poverty and hunger. An additional mitigating factor here relating specifically to poultry production, is the effect of HPAI.

The mechanisms by which improvements in poultry production efficiency can impact upon poverty alleviation, are quite different for urban dwellers, who are largely dependent upon purchased poultry meat and eggs, and rural villagers who mostly produce their own meat and eggs from their small scavenging flocks of indigenous birds. In the former case, the price of meat and eggs is very much in the hands of private enterprise and is influenced by many external factors including government regulation. Since feed accounts for about 70% of production costs, the impact of feed prices, as influenced by global markets, climate change, biofuel competition etc, on the price of meat and eggs from commercial broilers and layers, is profound. High feed prices present real challenges to the governments in question and to donor agencies if the negative impact of this on the attainment of the MDGs is to be averted.

The absence of the need to provide compounded feed to small-scale rural scavenging poultry flocks, means that they are significantly more buffered to the effects of the factors influencing feed prices. The critical limitation here, however, is the generally low level of productivity of the indigenous birds, the risk-averse attitude of the farmers to adoption of improved technologies, and as a consequence, the commonly high levels of attrition in young birds and mortality in the older birds. It has been demonstrated (Pym, 2005) that appropriate and simple cost-effective interventions can have a significantly positive impact on the profitability of small-scale family poultry production resulting in the alleviation of poverty, improved household food security and a reduction in malnutrition, improved educational opportunities for the children, and empowerment of women as the principal poultry keepers in most communities. Small-scale poultry production offers the opportunity for the first step out of poverty; to allow choices to be made as to further avenues of income generation (Alders and Pym, 2008). The aim is certainly not to turn all rural families into small or medium-scale commercial poultry producers; this would be counter-productive. A small percentage may choose to go down this track.

There is thus the opportunity for improvement in the efficiency and sustainability of poultry production to have a beneficial impact upon a number of the Millennium development Goals including: the eradication of extreme poverty and hunger; education of the children; malnutrition and hence childhood mortality; and the empowerment of women. In households where there is a lack of able-bodied workers, such as households affected by

HIV/AIDS or those that have a disabled family member, village poultry provide a source of high quality nutrition and income without requiring much in the way of labour or financial inputs (Alders and Pym, 2008). Poultry keeping is an easy activity that can contribute to household food security and income. Given that women are the main carers of sick people and that the poultry are usually under women's control, poultry can play an important role providing them with additional resources to carry out their important task of supporting people living with HIV/AIDS (IRPC, 2005).

VI. ACROSS-SECTOR SUPPORT FROM WPSA FOR POULTRY PRODUCTION IN DEVELOPING COUNTRIES

Following on from the FAO sponsored "Poultry in the 21st Century" meeting in Bangkok in November 2007, it became apparent that there was a need for a broad thrust to facilitate efficient and sustainable poultry production from all sectors of the industry in developing countries, not just small-scale sectors 3 and 4. WPSA has worked in close cooperation with FAO in this endeavour. In the preparation of the FAO's *State of Food and Agriculture 2009* report, the writer was asked in 2008 to coordinate a review on *Technological Change and its Impact on Poultry Development,* covering genetics and breeding, disease prevention, housing and environmental control, processing of poultry meat and eggs, with input from five Australian and overseas WPSA members.

More recently, the writer was asked by FAO to assist them by coordinating a series of reviews to include in their revised AGA "Poultry Production in Developing Countries" website. The focus of the site is to be broadened from just small-scale poultry production, to all aspects of poultry production across all sectors of the industry in developing countries. Most of the contributors to the twelve component reviews, are members of WPSA. The reviews are at the time of writing, in an advanced stage of preparation and the website should be operational by the time of the 2010 Australian Poultry Science Symposium.

(a) WPSA Mediterranean and African poultry networks

In recognition of the need to establish structures to facilitate the advancement of poultry science and the development of the poultry industry in developing countries, WPSA has been involved in two notable developments over the past twelve months. In June 2009 the Board of WPSA endorsed the establishment of the Mediterranean Poultry Network which will focus on the promotion of poultry science and the development of the poultry industry throughout the Mediterranean region in Southern Europe, the Middle East and Northern Africa. The Network will operate initially under Working Group 11 *(Education and Information)* of the WPSA European Federation. One of the particular aims of the Network is to provide support to the poultry industry in the countries of Northern Africa and the Middle East, that are not members of the European Federation or of its working groups. The main forum for scientific and technical discussion between members of the Network will be at the biennial Mediterranean Poultry Summit meetings, already established. The most recent Summit was held in Antalya, Turkey in October 2009 and the next meeting will be held in Egypt early in 2012.

There have been ongoing discussions for a number of years about the possibility of forming an African Federation of WPSA. It has proven to be a difficult task to bring this to fruition, largely because of the size of the continent, difficulties in identifying suitable working group areas/titles, and the difficulty and cost of bringing people together at one location. To address some of these problems, a proposal was made recently for a virtual (web-based) African Federation of WPSA. Further discussion by the Board of WPSA has resulted in the Board's endorsement of the development of a sub-Saharan African Poultry

Network whose focus would be on the promotion of poultry science and the development of the poultry industry in the region, and whose main mode of communication and interaction between members, would be via the Internet. To initiate the formation of the Network and bring the key players together, a workshop is to be held in France, immediately following the European Poultry Convention in Tours in late August 2010. The plan is that delegates to the workshop will attend EPC prior to their meeting. A steering committee has been established and the program for the workshop is in preparation. FAO have provided considerable encouragement, both verbal and material, for the conduct of the workshop and the establishment of the Network.

It is important to note that the proposal is NOT focused only on small-scale village poultry farming; the focus is on giving the African members of WPSA (scientists and industry personnel) an opportunity to meet at a first-class, broad-based poultry science and technology meeting in Europe to (apart from attending this) spend some time discussing the constraints to the efficiency of commercial and "village" poultry meat and egg production in Africa, and how poultry can play a bigger role in meeting the protein needs of the burgeoning population in the developing countries of Africa.

(b) Poultry Think Tank Meeting.

A "think-tank" meeting was held in Freising, Germany on 19th June 2009 to discuss social equity and sustainability issues relating to the present systems employed globally in the production of poultry meat and eggs. Participation at the meeting was by invitation and included representatives from the poultry industry, the poultry research community, FAO and WPSA. Representatives from WPSA were Roel Mulder (Secretary), Piet Simons (Past Secretary) and the author.

The meeting arose from an invitation issued by Dr John Hodges, an agricultural researcher with interests in ethical issues related to animal production, during the presentation of his keynote address "*Emerging boundaries for poultry production: Challenges, opportunities and dangers*" at the 23rd World's Poultry Congress in Brisbane on 30 June 2008. He challenged the industry to examine its practices from social equity and sustainability perspectives. Dr Dietmar Flock, Poultry breeder and Past President of the European Federation of WPSA, subsequently initiated discussions with Dr Hodges which led to the organization of the think tank meeting in Freising. A full report on the outcomes of the meeting will shortly be published in WPSJ. The draft report is presently with the meeting participants for their comments and suggestions.

One of the recommendations from the Think Tank was that better use should be made of the technical expertise in WPSA, FAO and industry as advisers to development projects involving poultry in developing countries. Poultry often form part of agricultural development projects, but not infrequently poultry production expertise in the NGO team is quite limited, and poor advice is not uncommon. It should be noted that this is not restricted to the smaller NGOs. WPSA needs to be proactive in establishing links with development project funders globally.

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THE EVOLVING ROLE OF FAO IN POULTRY DEVELOPMENT

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Summary

Both the global poultry sector and FAO's involvement have changed significantly over the last 50 years. Enormous advances in poultry productivity have occurred and with modern husbandry chicken has become the most effective terrestrial animal in converting feed grain into meat. Yet the poultry sector still remains highly diverse with a growing dichotomy between the industrial, commercial production and extensive back-yard poultry systems. Small-scale poultry keeping continues to make a significant contribution to the livelihoods of some of the world's poorest communities and, as such, remains an important tool in rural poverty reduction programmes. The growth of intensive poultry production has given rise to a range of issues concerning food safety, environmental pollution, biodiversity and animal welfare. FAO meanwhile has moved away from direct technical assistance and traditional development projects, with the exception of its emergency and disaster response programme, to a more supporting and informing role. The current focus is on the interaction and impact of poultry production with the international public goods such as poverty reduction, public health and natural resource management. This paper reviews the link between poultry production and these international public goods. It then provides an overview of the range of activities that FAO undertakes to support the global poultry sector and draws heavily on material represented at an FAO sponsored conference in Bangkok in November 2007 (http://www.fao.org/AG/againfo/home/events/bangkok2007/en/index.html).

I. INTRODUCTION

Within the lifetime of FAO, the global poultry sector has undergone unprecedented change. Poultry meat has moved from a luxury food item to an abundant, affordable and accessible source of quality protein in the diet. Whilst the most visible structural changes have been seen in the developed economies, the consequences on how poultry products are produced, processed, marketed and consumed are evident across the globe. The poultry sector has evolved into the fastest growing, most efficient and indeed the most flexible of all the livestock sectors (Upton, 2008).

The rapid growth in the poultry sector has been driven by both supply and demand factors. Demand has been driven by population growth; increases in per capita incomes; urbanization, and changing consumer preferences (Narrod et al., 2008). There remains a positive relationship between income and the consumption of food with a high income elasticity, such as poultry products, and this has traditionally involved a shift from starches to meat.

On the supply side, major technological advances have resulted in a dramatic shift from labour intensive to capital intensive systems, resulting in specialization, greater biological efficiency, economies of scale and vertical integration within the supply chain. Advances in animal breeding have resulted in major increases in production efficiency. In commercial broiler production feed conversion ratios (FCR) have reached 1.6:1.0 making the modern hybrid broiler the most efficient terrestrial farm animal to utilize feed grain. Likewise, the modern hybrid layer is capable of producing 330 eggs per year. Genetic improvement has

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gone hand-in-hand with a greater understanding of poultry nutrition and the pathology of poultry diseases. Modern husbandry practices (feeding systems, animal health care, housing and control of the micro-environment) have been developed that utilize these and resulted in a strong, integrated and international poultry industry.

The poultry sector is however far more diverse than just the large-scale commercial operations that provide affordable poultry products to the growing urban communities. In the rural areas of developing countries, the ubiquitous free-ranging chicken represents a widespread, extensive, 'low-input, low output' production system which provide supplementary income and food to the household, an asset and a means to meet social obligations (Dolberg, 2008). There are also many small to medium-scale poultry producers have also exploited the wide availability of commercial hybrids and compound feeds to develop successful enterprises. There is a concern over the longer-term future of these small to medium-scale enterprises. Unlike the backyard systems, they are dependent on purchased inputs but do not have the economies of scale of the industrial poultry sector. Furthermore it is expected that their transaction costs will increase as they have to abide with more and more public health and trading standards.

II. POULTRY AND INTERNATIONAL PUBLIC GOODS

The FAO Animal Production and Health Division (AGA) anchors it programme on three, interrelated, global international public goods and how they relate to livestock. These include (i) social equity (poverty alleviation), (ii) veterinary public health (animal health in its broader context) and (iii) sustainable natural resources (animals and land, water, air, biodiversity, ecology).

a) Poultry and social equity

There is a growing recognition amongst the development community of the contribution of small-scale poultry production in reducing poverty, enhancing household food security and in promoting of gender equality (McLeod, 2008). Traditional back-yard poultry production systems remain essentially 'low-input, low output' systems that utilize family labour (women and children) and characterised by rudimentary supply chains. Products are either consumed in the household or sold locally and poultry are also an important asset to meet social obligations. With extremely high losses of chicks before they reach a productive age from disease (notably Newcastle Disease) and predation, there is ample scope for increasing productivity through loss reduction. Simple housing to protect chicks from predators and the elements, supplementary feeding, and vaccination against Newcastle Disease can have a substantial impact. Reducing losses also reduces the requirement for hatching eggs to replace the lost birds which immediately release eggs to be sold or consumed (Sonaiya, 2008). It has to be said that, even with the advent of heat stable Newcastle Disease vaccines and numerous project interventions, relatively little impact has been made in increasing the productivity of these systems. These systems while important in supplementing and maintaining livelihoods, are unlikely in themselves to provide a viable mechanism lift households out of poverty.

There are a number of notable exceptions. The FAO Village Poultry Groups in Afghanistan (see III i) and the Bangladesh Model. The Bangladesh Model consists of an integrated system of production, marketing, input-supply and service provision supporting a network of individual, specialised producers and entrepreneurs (Dolberg, 2008). First initiated in 1983 it has attracted substantial donor support and driven by the Department of Livestock Services and BRAC (Bangladesh Rural Advancement Committee). Evaluation studies have demonstrated that the approach has a strong 'pro poor' bias and had a significant impact of the economic and nutritional status of the poor – especially women and children. Despite

concerns over the sustainability of the programme after the withdrawal of donor support; there is little doubt that the programme has demonstrated the potential of small-scale poultry as a tool for improving rural livelihoods.

Semi-commercial production systems depend far more on a functioning, if informal, supply chain for reliable access to inputs (day-old-chicks, feed, drugs etc.) to exploit local markets and market preferences where traditional products may command a premium. Growing rural populations, improve infrastructure (roads, transport and communications) are opening up new market opportunities. There are many examples, especially in Asia, of successful small to medium-scale poultry entrepreneurs. While the back-yard and the large-scale commercial operations are largely segmented and are likely to remain so, there is an increasing overlap and competition between the modern large-scale commercial sector and the medium to small-scale producers. This happened in Thailand where, over two decades, the share of small-scale production has fallen from 95 to 10 percent (Vinod and Arindam, 2008).

b) Poultry and public health

The poultry sector is associated with a range of public health risks arising from zoonotic diseases notably, but not exclusively, Highly Pathogenic Avian Influenza (HPAI) and product contamination. The poultry industry is dependent on maintaining the trust of the consumer that it provides a safe and wholesome product. The dramatic, albeit short-lived, crash of the poultry market in the aftermath of HPAI due to the loss of consumer confidence illustrates the point.

Risks associated with food poisoning, notably from *Salmonella* and *Campylobacter* spps. have also focused public attention on the safety of poultry meat and eggs. In addition to the microbiological risks, chemical contamination of the feed (dioxins, heavy metals, pesticide residues) can also enter into the food chain and the misuse of antibiotics in the feed can result in increasing drug resistance. International guidelines and standards, such as *Codex Alimentarius,* exist in addition to a plethora of public and private sector standards. In developed countries, such food safety regulations are largely enforced and the poultry industry is well motivated to minimize risk and HACCP procedures are well established and mainstreamed. This is not always the case in developing countries were regulations, if present, are extremely difficult to enforce. Consequently, food-borne illness remains a serious public health issue in these countries and a major concern to international agencies such as the World Health Organization (WHO) and FAO.

c) Poultry and sustainable resource management

The growth of the commercial poultry sector has resulted in geographical concentration and intensification of production in 'landless' systems. Intensive production favors those areas with access to cheap inputs and services (feed processors, slaughter houses, transport and utilities) - such conditions are found in the vicinity of urban conurbations. This separation of poultry production from its agricultural base and its move to areas requiring little or no agricultural land can have a high environmental impact (Gerber et al., 2008).

At the production level there are local issues with smell, flies and a waste disposal. Birds raised in intensive conditions consume high protein and high energy diets which give rise to a manure high in nutrients such as nitrogen, phosphorus, and other excreted substances (heavy metals, drug residues) introduced through the feed. Generally poultry manure is regarded as a valuable commodity and is recycled as an organic fertilizer or used in animal feed. It can however lead to local cases of nutrient based pollution which can result in eutrophication of surface water, pollution of the ground water (nitrate leaching) and the emission of nitrous oxide. Some metals (arsenic, cobalt, copper, iron, manganese, selenium and zinc) that may be used as growth promoters or as prophylactic feed additives, and manure

can be a significant source of environmental contamination of these metals. There are also issues with drugs and hormones entering into various food chains (Kiilholma, 2008).

The poultry sector is a major consumer of cereals (estimated at eight percent) and soyabean meal (Hinrichs and Steinfeld, 2008; FAOSTAT). There is therefore an indirect global dimension to the environmental impact of poultry production. The environmental impact of feed production include those associated with intensive agriculture (misuse of fertilizer, pesticides and herbicides), inappropriate crop expansion (deforestation), biodiversity (including poultry breeds), associated greenhouse gas (carbon dioxide and nitrous oxide) emissions and overexploitation of natural resources, notably fish stocks. The carbon footprint of intensive poultry also has to take into account the relatively high level of energy use associated with production, slaughtering, processing as well as those associated with national and international trade of poultry products and inputs.

Concern has also been expressed regarding the erosion of biodiversity within the poultry sector. Highly selected hybrid broilers and layers, controlled by a handful of breeding companies, now dominate commercial production. Indigenous breeds represent the majority of the poultry genetic biodiversity, they are well adapted to local conditions, especially those found in some of the most resources poor communities in the world; yet many are under the threat of extinction.

III. FAO SUPPORT TO POULTRY DEVELOPMENT

FAO addresses these public good issues through a) its field programme which provides direct development support to its member countries, and b) its normative programme which examines and informs on the broader issues associated with livestock and poultry production.

a) Field Programme

FAO has, since its inception, implemented projects that directly assist farmers and institutions in developing countries. Projects can be classified according to their funding source. Trust Fund projects are those implemented with external donor funding. The Technical Cooperation Programme (TCP) funds projects from FAO's core funds and aim to plug a specific technical gap; they are catalytic in nature and with a short duration (maximum two years). Telefood is a programme funded by public support (annual charity events) and provides small grants to farmer groups to assist them establish or expand an enterprise – poultry keeping is a population choice.

The nature of such assistance continues to change. Up until 15 years ago, every technical division in FAO managed a large portfolio of development projects. The situation began to change when the United Nations Development Programme (UNDP) strategy changed to have its projects managed nationally rather than by the UN specialized agencies such as FAO. This lead to a decline in the FAO field programme, since UNDP was a major source of funding, and it is now dominated by TCP and other donor funded projects. There has also been a major shift away from longer-term development assistance to shorter-term, input focused emergency assistance programmes. Of the current portfolio of 481 projects (TCP, Trust Fund and Telefood), where AGA is the Lead Technical Unit, 166 (35 percent or 60 percent excluding Telefood projects) are classified as emergency projects.

Poultry are a component in many FAO projects; however it is difficult to disaggregate the information since poultry activities are often subsumed within broader development or emergency assistance projects. The electronic database of FAO projects currently goes back to 1980. Table 1 indicates the number and type of projects with a) poultry in the title and b) livestock in the title. This is purely indicative and underestimates the actual number of projects in which poultry activities can be found.

Table 1   FAO projects with 'poultry', 'livestock' or 'avian' in the title by funding
               source 1980-2009

	TCP	Trust Fund	Telefood	UNDP
'Poultry 'in the title	35	30	150	7
'Livestock' in the title	109	131	31	35
'Avian' in the title	20	109	-	4

Source: FAO internal data

Poultry figure strongly in the portfolio of emergency projects. Projects associated with avian influenza (HPAI) feature significantly in the project portfolio since the late 2000's. However, many non avian influenza emergency response programmes also contain a poultry component, usually in the form of restocking households who have lost stock, providing supplementary feed or in some cases the provision of shelter. Poultry activities are also found in some of the longer-term rehabilitation programmes in countries such as Afghanistan, Iraq, Sudan, Gaza and the West Bank.

Indeed, one of FAO's most successful poultry programmes has been in Afghanistan. FAO started work in developing Village Poultry Producers Groups in 1999 in Faizabad working with local women's groups. The approach identified potential groups of women and gave them practical training over a six month period including some limited inputs (chicks and a small amount of mixed feed). The group leaders took on a service provider role giving vaccinations, basic health care, selling feed and facilitating the marketing of eggs. The FAO project continued to provide support to the group leaders for a year after the end of the initial training period. Apart from the economic benefits and improvement in the household nutrition, the programme had an important social impact in empowering women in their communities. The programme has been expanded and today FAO is still supporting village poultry production throughout Afghanistan with World Bank and IFAD support.

FAO support to its member countries is delivered primarily through its network of 136 country offices, five regional, 10 sub-regional offices and four FAO/OIE Animal Health Officers. Each sub-regional office has a multi-disciplinary technical team which, in most cases, includes a livestock specialist who is in turn supported by a livestock officer in the regional office. At the Rome headquarters, AGA has around 30 professional officers at any one time.

b) Normative Programme

All normative activities related to poultry provide directly and indirectly some degree of policy support. AGA aims to inform decision makers (at all levels) on the important issues they face, the options available to them and, importantly, their implications. In addition, it is recognized that an enabling policy environment is essential for the development of a robust and equitable poultry sector that provides producers with a fair return, the consumers with a safe and affordable product, the private sector with a fair return on their investment, and the public with a clean and safe environment. AGA, with support from DFID, has established a Pro Poor Livestock Policy Initiative (PPLPI) with the aim to support the formulation and implementation of livestock-related policies and institutional changes that have a positive effect in the world's poor. (http://www.fao.org/ag/againfo/programmes/en/pplpi/home.html) Since keeping poultry is an important livelihood strategy for the world's rural poor, the PPLPI is of direct relevance. The PPLI approach compiles information and conducts analysis to enhance pro-poor decision making within the livestock development community. Since poultry keeping is an important livelihood strategy for the rural poor the PPLPI is of direct relevance.

Given the importance and attention attached to livestock-related environmental issues it is not surprising that AGA is working in this area. Notable was the publication of the *Livestock's Long Shadow - Environmental Issues and Options* (FAO, 2007a) which successfully raised awareness to some of the negative environmental impacts caused by livestock, and to gather the political will to address the issues. AGA also hosts the Livestock Environment and Development Initiative (LEAD) (http://www.fao.org/agriculture/lead/en/) which focuses on livestock issues related to deforestation, soil and water pollution, land degradation and desertification. LEAD offers a number of decision support tools including the Livestock and the Environment Toolbox that assists in assessing livestock environmental interactions and models to predict nutrient balance and the direct and indirect consumption of fossil fuels in various livestock production systems.

The erosion of genetic diversity in domesticated farm animals, including poultry, especially in developing countries, is a serious concern to FAO. Its programme on managing of animal genetic resources addresses policy and institutional concerns as well as technical issues (http://www.fao.org/ag/againfo/programmes/en/A5.html). *The State of the World's Animal Genetic Resources for Food and Agriculture* (FAO, 2007b) identified significant gaps in capacity to manage animal genetic resources. The international community responded in 2007 when it endorsed the *Global Plan of Action for Animal Genetic Resources* which contains 23 strategic priority areas covering characterization and monitoring; sustainable use and development; conservation; and policies, institutions and capacity building. AGA hosts the Domestic Animal Diversity Information Systems (DAD-IS) as a definitive source of information on the world's species and breeds of farm animals (http://dad.fao.org/).

Poultry are commonly included in the international response to emergencies and disasters. Poultry are seen as a quick and acceptable means of rebuilding the assets and livelihoods of affected communities. However well meaning, not all interventions are appropriate or have their desired impact. Hybrid birds distributed to village locations where the management and inputs are inadequate inevitably do not survive. Distributing free feed, drugs etc can also seriously disrupt the livelihoods of the local service providers. To address the concerns, AGA working together with colleagues from the African Union, the International Committee of the Red Cross (ICRC), the Feinstein Center (Tufts University) and Veterinaries sans Frontier (VSF) developed and launched the *Livestock Emergency Guidelines and Standards* (LEGS) (http://www.livestock-emergency.net/). LEGS takes a livelihoods approach and provides basic standards, guidance, check lists and electronic decision support tools for each of the major interventions: animal health, destocking, supplementary feeding and water, shelter and restocking.

Information and knowledge are at the core of FAO's work. The majority of AGA's publications and information are downloadable free, along with specialized pages, from its website (http://www.fao.org/ag/againfo/home/en/index.htm). Over the last two years, AGA has completed country profiles (http://www.fao.org/avianflu/en/poultryproduction.html) for poultry in 27 countries which provide overviews of the main poultry systems, policies statistics, reports and contact points. In early 2010, a new series of discussion and issues papers on poultry development will be released on its poultry web page with the aim of moving to a more interactive and participatory web portal later in 2010. Animal welfare is an issue of interest to the poultry industry and in 2009 AGA launched the Gateway to Animal Welfare gateway which is supported by 13 collaborating organizations (agencies and NGOs) (http://www.fao.org/ag/againfo/programmes/animal-welfare/en/). The *Gridded Livestock of the World* provides global maps for the distribution of the main species (http://www.fao.org/ag/againfo/resources/en/glw/home.html) of livestock including poultry. FAOSTAT (http://faostat.fao.org/) provides time-series data on trade, production and consumption of poultry production and products for some 200 countries.

As an intergovernmental agency, FAO has close relations with the agricultural departments in its 192 member countries. AGA also works closely with the private sector, including the International Poultry Council (IPC), the International Federation of Feed Industries (IFIF) and the International Federation for Animal Health; scientific organizations, including the World Poultry Science Association (WPSA), as well as NGOs and sister UN organizations. AGA was instrumental in establishing the International Network for Family Poultry Development (INFPD) (http://www.fao.org/ag/againfo/themes/en/infpd/home.html) which is an information exchange network to promote sustainable productivity within the family poultry sub-sector.

Capacity building and training has always been central to FAO's programme. With financial support from IFAD, AGA is assisting the INFPD embark on a three year programme to train young poultry professionals to give them experience and exposure to international work. Each candidate will spend four to six weeks assigned as professionals in the FAO and IFAD headquarters in Rome before they join an on-going project for six to nine months practical experience where they will also carry out a specific assignment.

c) Animal Health

Protecting livestock against diseases and preventing disease spread is fundamental to AGA's programme in support of poverty reduction and veterinary public health. AGA's animal health programme, more than others, blurs the distinction between field and normative activities. Responding to major epizootic diseases requires immediate direct assistance but needs to be backed up by longer strategic planning, early warning and information systems, and capacity building.

Of international concern is the transboundary nature of many animal diseases, including zoonotics, which can quickly spread from the farm, through markets, to the entire country and beyond. Highly Pathogenic Avian Influenza is a notable, but not the only, example. AGA is responsible for the animal health component of the Emergency Prevention System for Transboundary Animal and Plant Diseases (EMPRES) which was established by FAO in 1994 http://www.fao.org/ag/againfo/programmes/en/empres.html, EMPRES provides information, guidance and training to prevent, contain and control major disease outbreaks. It is also responsible for the surveillance of newly emerging pathogens, which history has shown often to originate from the poultry sector. The EMPRES approach incorporates early warning, early detection, early reaction, enabling research, co-ordination and communication. A Crisis Management Centre (CMC) has been established to ensure an effective and prompt response to new disease outbreaks. It provides immediate technical and operational assistance to governments facing a major disease outbreak http://www.fao.org/emergencies/home0/emergency-relief-and-rehabilitation/cmc/en/ .

Responding to Avian Influenza (H5N1 HPAI) has been the greatest challenge facing EMPRES. It was also a major economic shock and a wake-up call for the poultry industry and attracted considerable public and media attention. Outbreaks have been recorded in 62 countries (five countries having reported only a single outbreak) in Europe, Asia, Africa and the Near East, with the main foci being in Egypt, Indonesia and Vietnam. To date there have been 258 human deaths from 442 reported cases (FAO AIDE News Update 62).

FAO, through EMPRES and with major donor support and in close collaboration with OIE, has worked with all the affected and at-risk countries to strengthen national disease intelligence, emergency preparedness, field surveillance and laboratory capacities, and has developed and supported public awareness campaigns. Major studies and analysis have been, and continue to be, undertaken on the epidemiology, economic and social consequences of the disease and its control. Increasing biosecurity across all production systems and value chains is an important focus within the programme primarily to control the spread of HPAI but equally important for other important infectious poultry diseases (Sims, 2008). Numerous national and international meetings of experts and stakeholders have been initiated or facilitated by FAO since the first outbreak, which have resulted in important recommendations and guidelines. In October 2008, as a result of a consultative process, FAO and OIE in collaboration with WHO published *The Global Strategy for the Prevention and Control of H5N1 Highly Pathogenic Avian Influenza* (FAO, 2008).

Early warning and the ability to predict the spread of a disease is essential for effective containment and control of disease. The Global Early Warning and Response System for Major Animal Diseases including zoonoses (GLEWS) (http://www.glews.net/) is a collaborative effort between FAO, OIE and WHO to combine their early warning and response mechanisms into an efficient tool.

IV. CONCLUSIONS

Growth in the scale, efficiency, market access and production of commercial poultry production over the last 50 years is nothing short of phenomenal. As a result, poultry products are now available and affordable. Yet, despite these advances, the poultry sector as a whole is even more diverse than before. There is remains a large, primarily unchanged back-yard 'low-input low output, poultry system commonly found in poor rural households irrespective of where they are. In addition, there is now also a significant small-medium scale poultry sector benefiting from many of the technologies and improved breeding stock, feeds etc to meet local and national demand. Given access to a reliable supply of inputs and a market for their products these can be successful. They will however become increasing squeezed when having to meet increasingly stringent and enforceable health and safety regulations but without the benefits of the economies of scale and vertical integration of the industrial sector. There is expected to be an increasing dichotomy between the industry and the back-yard systems at the expense of the middle-ground. As long as there is rural poverty, back-yard systems will survive and continue to make an important socio-economic contribution.

The growth of the poultry sector has not been without its negative consequences. A number of serious or potentially serious human diseases have their origins in the poultry sector. Intensive production systems, high poultry densities often associated with high human population densities and exacerbated in many cases by ineffective standards and non-existent biosecurity, have proven to be an ideal breeding ground for emerging new diseases. There are also issues of pollution, greenhouse gas emission, erosion of biodiversity and the food versus feed grain issues that remain largely unresolved.

FAO originally supported a major field programme providing direct assistance to poultry producers in the developing world. As the technical capacity in many countries continues to grow, as well as the parallel growth in the scale, scope and expertise of the NGO operations, FAO's traditional field programme has declined. With a well established programme of decentralization, direct support to member countries is the responsibility of country offices and technical teams based at the sub-regional level. The exception is the emergency and rehabilitation programme which continues to grow and coordination remains at headquarter level.

Technical divisions at FAO headquarters have taken on a more informative and analytical role. AGA anchors its livestock programme around the three international public goods: poverty reduction; public health and sustainable resource use. This has proved an effective way to examine some the major issues and challenges affecting the poultry sector. As long as there are rural poor, back-yard 'low-input - low-output' systems will continue to make an important socio-economic contribution to improving livelihoods. Major epizootic and zoonotic diseases are associated with poultry production, and the poultry sector has historically been a major source of emerging diseases. Given the volume of fresh and highly perishable poultry products traded each year, food safety is not surprisingly an issue, especially in countries and markets where standards are either not available or enforced. The impact of the poultry sector on the environment includes potential pollution, direct and indirect greenhouse gas emissions and the food-feed issue. These are all areas in which, to some extent, AGA has on-going activities, for example: poultry production, animal health, policy support, management of animal genetic resources, animal welfare and livestock and the environment.

The poultry sector will continue to adapt and change. FAO will continue to have an important role in analyzing, guiding, advising, informing and warning to ensure the sector makes a safe, secure and equitable contribution to human and economic development. The 2009 FAO *State of the World's Agriculture* (SOFA) is entitled *Livestock in the Balance* (to be published in early 2010) examines the major issues associated with the livestock sector including poultry. As the title suggests, it reviews its positive contribution to human development as well as the more negative consequences and, importantly, how these can be mitigated. The 2009 SOFA is a good example of the way in which AGA now works to support the livestock sector.

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IMPROVED VILLAGE POULTRY PRODUCTION IN THE SOLOMON ISLANDS

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Summary

Village poultry keeping is a crucial activity in the Solomon Islands because over 80% of the 500,000 plus population lives in a rural subsistence existence generally without supplementary wages, pensions or other income. Providing the family's daily food requirement is often impossible, particularly as a balanced diet. Most rural households have a food garden but despite the lush tropical environment more than 30% of the children are malnourished. Protein crops in the gardens are limited and the population generally is lacking protein. Traditional wildlife sources of protein are depleted either from past hunting or environmental damage by logging or commercial fishing. Increased consumption of chicken meat and eggs through improved village chicken keeping is a quick and simple solution to these problems as well as a relief response to disasters like famine, storm, tsunami and earthquake. It has been recognised from the Prime Minister down that improved village chicken keeping is one of the two key development activities which are necessary for the country. Unfortunately the Agriculture Department does not yet have the capacity to provide the training and support needs for this activity. The author's training and development program, Kai Kokorako Perma-Poultry is attempting to meet the needs of the whole country.

I. INTRODUCTION

Many rural villages have a few chickens wandering freely without any formal care or attention. As breeding is not structured there is often and imbalance of roosters over hens causing uncertain fertility and breeding results. Eggs are laid in the bush and sitting hens or their eggs are often lost to predators. A broody hen can hatch up to 15 chicks however she is often left with one or two chickens to raise to maturity because of lack of feeding and is therefore left out of production for a longer period than necessary. The use of commercial feeds is usually cost prohibitive and distribution networks of this feed is often unreliable or difficult.

II. IMPROVEMENT PROPOSAL

Commencing in 1994 it was recognised by the author that the simple backyard poultry keeping hobby practiced by him and many other Australians was an ideal improvement method for Solomon Island households. He then commenced writing a training manual to suit Solomon Island conditions and needs. It was decided to address four main husbandry issues: (i) breeding, (ii) feeding, (iii) housing as well as (iv) general care and management.

     The training principles are presented as "Kai Kokorako Perma-Poultry" because Kai Kokorako means "eat chicken" in Pidgin English and Perma-Poultry refers to the permaculture and sustainable nature of the husbandry methods. Village chicken housing is built from the same

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climate friendly bush materials as the peoples' own houses and the chicken feed is provided in the form of a balanced diet from the peoples' own food gardens. Each chicken flock is stocked with a crossbred mixture of existing village chickens, hybrid layers and broilers which have naturally become a hardy, self reliant local breed able to survive the difficult village lifestyle. This improvement program is meeting the development needs in two major ways.

III. TRAINING

The training manual was first published as a general village training book in 2004 in association with the Rural Youth Livelihoods Project resulting from Aid responses to the needs of displaced youth from the Ethic Conflict. Some 250 rural youth and villagers benefited from exposure to training workshops conducted using this manual with the result of several substantial village farms being successfully established.

Later it was further recognised that many more competent trainers were needed if the whole country was to benefit. The manual was therefore rewritten and republished in 2007 in the format of "Train the Trainer " and made available online through the nine Distance Learning Centres (DLC) across the country as well as by CD Rom and hardcopy.

Training workshops are currently delivered both onsite and online by the author through the various DLCs with the assistance of local tutors seconded from the Department of Agriculture and various schools or colleges as well as Rural Training Centres.

A further development program focusing on the construction of small chicken breeding farms alongside each DLC has commenced so that practical experience can also be offered at the time of the written training. Many of these DLCs are constructed within close proximity to schools or colleges so these practical farms can also complement each school's curriculum and as many are boarding schools, the larger farms also contribute to the food needs of the students.

IV. STRUCTURED BREEDING

The existing village chicken is usually a small bird with a mixture of bloodlines including imported pure breeds, hybrid layer and broiler. The small size of the village chicken results not only from the semi-feral lifestyle but also from an almost accidental infusion of bloodlines from the wild chicken of St Cruz Island. This closely resembles the red jungle fowl in appearance and size. These village chickens are hardy, good scavengers and because of their various mixed bloodlines respond quickly to better feeding and care.

No hatchery currently exists in the country specifically for village chickens so there is limited stock available to meet the peoples' needs. Only day old layer and broiler chickens are commercially available however they need commercial feeds to survive and produce well. By crossing the hybrid layers and broilers with village and wild chickens their progeny are more able to cope with garden based feed supplies. There is a further need for larger regional farms to be built to produce suitable replacement stock using natural methods because of lack of access to electricity and cost- prohibitive incubators and brooders.

The increased importation restrictions imposed since the Bird Flu outbreaks around the world have made it impossible to import the necessary improved breeds of chicken suited to village life. This has meant that any breeding improvement activities within the country need to be carried out with what is already available.

Fortunately on the small far northern atoll of Ontong Java the chicken population is dominated by a previous importation of valuable Naked Neck chickens. Two years ago a breeding program was put in place to increase the numbers of this valuable breed so that they can be conserved and be distributed more widely across the country to various breeding upgrade programs. Early in 2009 this breeding project was placed under threat by flood tides so conservation and wider distribution was made even more urgent. Whilst Ontong Java was not within the 2007 tsunami affected area of the Solomons it is still constantly at risk because it is low lying and unprotected. Additional breeding improvement projects are planned for wild chicken conservation and captive breeding to provide the necessary breeding upgrade stock for the local chicken hatcheries.

V. CONCLUSION

Whilst funding for these various improvement and developmental activities is limited it is believed that by combining the continuing training program and establishment of breeding centres across the country a huge improvement can not only be made to human nutrition and health but also unique wildlife can be conserved. The planned development of both a central hatchery as well as several regional hatcheries will help provide the necessary breeding and replacement stock to ensure success of the whole program. It is also recognised that the more successful breeding farms, hatcheries and training centres can provide employment opportunities and small incomes for necessary personal needs like school fees, kerosene for lighting and supplementary food items or developmental needs like rural electrification and water supplies.

HEAVY METAL CONTAMINATION IN MINERAL SOURCES FOR MONOGASTRIC FEED IN ASIA PACIFIC

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Summary

A survey of mineral supplements for pig and poultry feeds was conducted in Asia-Pacific to determine the presence and extent of heavy metal contamination across the region. Types of materials sampled included inorganic mineral premixes, zinc oxide (ZnO), zinc sulfate ($ZnSO_4$), copper sulfate ($CuSO_4$), ferrous sulfate ($FeSO_4$), manganous oxide (MnO), manganous sulfate ($MnSO_4$), sodium selenite, chelated minerals/premixes, and complete feeds with inorganic minerals. Countries surveyed were Australia, China, India, Malaysia, Pakistan, Philippines, Taiwan, Thailand and Vietnam. It was found that of 275 samples submitted, 17% were found to be contaminated at least one heavy metal in excess of EU limits was present.

I. INTRODUCTION

Mining is the most common source of inorganic minerals used in the feed industry. Without careful processing and filtration, heavy metal contamination of inorganic minerals can find its way into the feed chain. Detection of the contamination is dependent on adequate monitoring systems. Too often, the contamination is not found until the inorganic mineral is already made into pre-mixtures or complete feeds.

The European Union has implemented a Rapid Alert System for Food and Feed (RASFF) to detect and alert member countries when risk products have made it into the market place. In 2007, China was the leading source of alerts for contaminates such as heavy metals (RASFF, 2007). A Chinese origin contamination scandal occurred in Australia in 2008. Zinc oxide containing high levels of lead found its way into the Australian feed industry and excessive lead levels were detected in the livers and kidneys of pigs fed the zinc oxide (Spragg, 2008).

High cadmium contamination in zinc sulfate lead to infertile eggs being laid by breeder hens in South Africa in 2007 (Johns, 2007). The young chickens had severely reduced growth. Organic minerals are also at risk of contamination. The need for a wide and frequent screening system for all minerals is required by suppliers to ensure excessive levels are detected before they enter the feed chain.

The effects of heavy metal exposure in poultry are not widely reported. Lysenko (2005) found that adding 100 ppm of lead to a broiler diet rendered all edible parts of the chicken unfit for human consumption. Exposing laying hens to lead in the diet will result in increases in lead levels in the tissues, organs and egg yolk (Trampel et al., 2003).

Human effects of heavy metal exposure are varied. Detrimental effects to a number of vital systems and organs have been seen. Cadmium can have serious negative effects on the renal and respiratory systems in humans and is not easily eliminated (Palachy et al., 1998). Järup (2003) reports that long-term exposure to arsenic in drinking-water has been associated with increased risks of skin cancer.

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[4] Alltech Center for Animal Nutrigenomics and Applied Animal Nutrition, Nicholasville, KY, USA

It was theorised by the authors that the occurrence of heavy metal contamination in the Asia-Pacific region was more wide-spread than previously thought. Therefore, the objective of the current survey was to gather and analyise a range of mineral types and sources from Asia-Pacific countries and evaluate for heavy metals.

II. MATERIAL AND METHODS

A total of 275 100 g samples were collected in the southern hemisphere autumn of 2009. Types of materials sampled included inorganic mineral premixes, zinc oxide (ZnO), zinc sulfate ($ZnSO_4$), copper sulfate ($CuSO_4$), ferrous sulfate ($FeSO_4$), manganous oxide (MnO), manganous sulfate ($MnSO_4$), sodium selenite, chelated minerals/premixes, and complete feeds with inorganic minerals. Countries surveyed were Australia, China, India, Malaysia, Pakistan, Philippines, Taiwan, Thailand and Vietnam. All samples were analyzed for lead, arsenic, mercury, and cadmium using inductively coupled plasma mass spectrometry (ICP-MS) at the Alltech Center for Animal Nutrigenomics and Applied Animal Nutrition in Kentucky. Metal concentrations were compared against EU contamination limits for animal feed. Maximum allowable EU limits for lead, arsenic, mercury and cadmium are 100 ppm, 15 ppm, 0.05 ppm and 10 ppm respectively for feed additives and 30 ppm, 50 ppm, two ppm and 0.5 ppm for complete feed.

III. RESULTS

By country, the percentage of feed material samples with levels of at least one heavy metal above allowable EU limits is as follows: Australia (27%), China (3%), India (43%), Malaysia (32%), Pakistan (33%), Philippines (18%), Taiwan (11%), Thailand (11%), and Vietnam (12%). Overall, 17% of samples analysed, tested high for at least heavy metal in the Asia-Pacific region.
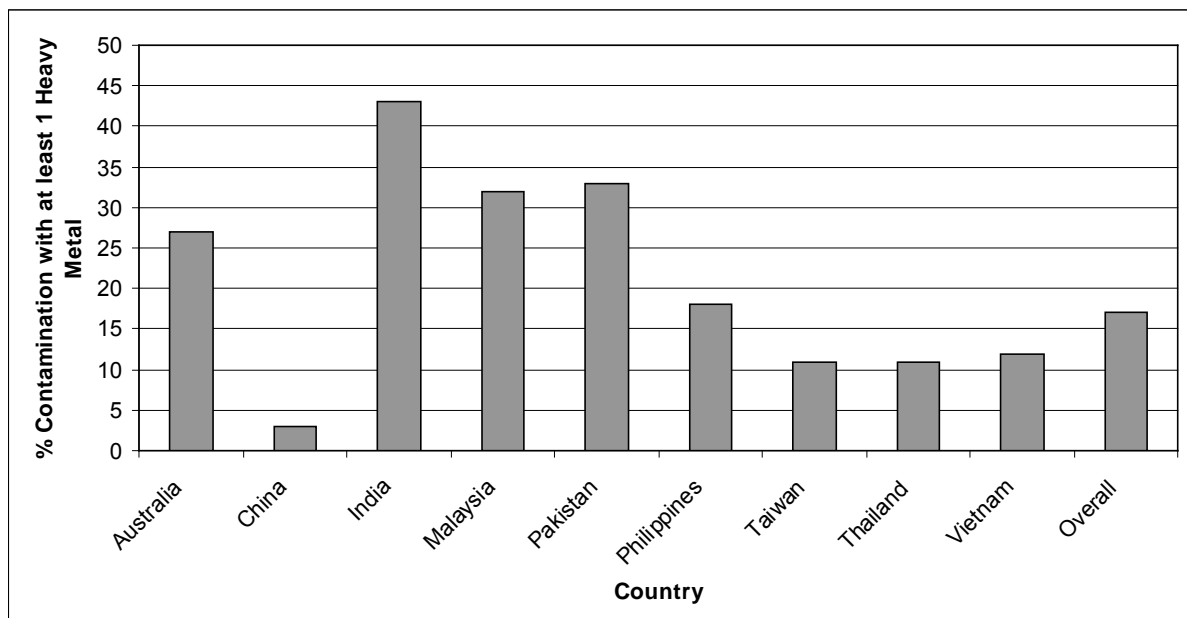
Figure 1   Percentage of samples contaminated with at least 1 heavy metal by country.

Of 25 Poultry pre-mixes containing inorganic minerals, 48% were found contaminated with at least 1 heavy metal. The highest level of lead, arsenic, mercury and cadmium was 697.12 ppm, 90.46 ppm, 0.14 ppm and 111.01 ppm respectively. All are above EU allowable limits for feed materials.

Individual minerals were analysed and found that the following were contaminated with at least one heavy metal in excess of EU limits: ZnO (12 samples) 25%; $ZnSO_4$ (12 samples) 25%; $CuSO_4$ (21 samples) 19%; $FeSO_4$ (13 samples) 15%; MnO and $MnSO_4$ (15 samples) 20%; sodium selenite (six samples) 60% and Non-Alltech organic mineral pre-mix (29 samples) 10%. The highest level of lead, arsenic, mercury and cadmium was 632.19 ppm, 659.52 ppm, 1.63 ppm and 3982.67 ppm respectively. All are above EU allowable limits for feed materials.

Of 30 complete feeds containing inorganic minerals, 7% was found contaminated with at least 1 heavy metal. The highest level of lead, arsenic and cadmium was 277 ppm, 8.05 ppm, and 0.84 ppm respectively. All are above EU allowable limits for feed materials.

Table 1.   Heavy metal levels of inorganic poultry pre-mixes (n = 25).

Heavy metal	Maximum limit (ppm)	Samples over max. limit (%)	Levels (ppm)		
			Lowest	Highest	Mean
Lead	< 100	8	0.21	697.12	49.73
Arsenic	< 15	20	0.09	90.46	12.3
Mercury	< 0.05	28	ND	0.14	0.02
Cadmium	< 10	8	0.06	111.01	0.08

Contaminated with at least 1 heavy metal: 48%

Table 2.   Heavy metal levels of individual inorganic minerals and Non-Alltech organic minerals.

Item	Lead (ppm)		Arsenic (ppm)		Mercury (ppm)		Cadmium (ppm)		Sample numbers
	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	
$ZnSO_4$	50.93	5.58	149.36	19.02	ND	ND	3982.6	369.6	12
ZnO	632.19	83.96	17.94	2.75	0.07	2.42	12.02	2.42	12
$CuSO_4$	20.51	2.73	659.52	38.74	0.07	0.07	1.33	0.14	21
$FeSO_4$	0.09	0.62	1.04	0.53	0.51	0.05	75.92	6.95	13
$MnO/MnSO_4$	68.42	11.9	26.99	4.44	1.63	0.11	19.43	1.6	15
Na_2SeO_3	0.81	0.44	65.55	15.75	1.21	0.24	0.08	0.02	6
Mineral px.	87.86	6.05	65.92	3.78	0.22	0.01	12.35	1.14	29

Table 3.   Heavy metal levels of complete feeds

Heavy metal	Maximum limit (ppm)	Levels (ppm)		
		Lowest	Highest	Mean
Lead	< 30	0.06	277	6.69
Arsenic	< 50	0.36	8.05	1.56
Mercury	< 2	ND	ND	ND
Cadmium	< 0.5	0.02	0.84	0.06

IV. CONCLUSION

In conclusion, contamination with at least one heavy metal in excess of EU limits was present in feed materials from all Asia-Pacific countries sampled. Of the 275 samples analysed, 17% tested high for at least one heavy metal. Excessive exposure to heavy metals in the feed will have a negative affect on animal health and performance.

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DUCK PRODUCTION IN AUSTRALIA

P. R. BROWN[1]

I. INTRODUCTION

Up until the 1970's duck production in Australia was confined mostly to small scale operations producing ducks from stock that took up to 12 to 14 weeks to grow to a marketable size. Over the past three decades the industry has undergone rapid growth due to increasing demand for product and this has led to the emergence of two large scale vertically integrated businesses, Pepe's Ducks based at South Windsor, New South Wales and Luv a Duck based at Nhill, Victoria. This growth has seen one business expand from a backyard situation with twenty two ducks to an annual production exceeding 3.5 million ducks. Overall production in Australia is around 8 million ducks per year. Duck production in Australia today is valued at about $100 million per annum. The domestic market is based mostly on the supply of ducks to Asian communities throughout Australia. Approximately 75% of all ducks processed are supplied to Chinese BBQ shops, Asian restaurants and butchers. The remaining 25% is supplied to fine dining restaurants, delicatessens, major retail outlets and export markets. Over the past five years industry sales have grown by about 60% and future growth appears to be exceptionally good throughout all market sectors.

II. DUCK BREED AND PERFORMANCE

The Pekin duck is the breed of choice for duck meat production as it can be reproduced all year round and is a large sized meaty duck that performs well in commercial farming operations under varying conditions. The importation of genetic stock in recent times from Grimaud in France by Pepe's Ducks and from Cherry Valley in the United Kingdom by Luv a Duck has significantly improved production performances and meat yields. The performance for Pekin ducks varies depending on the genetic stock but in general the following standards apply for duck production in Australia. Breeding ducks commence egg production at 24 weeks of age and their peak egg production is at 32 weeks with 88 to 92% production. The length of their laying period is 40 to 45 weeks with a production of 200 to 220 eggs with a hatchability of 80-85%. Meat ducks are processed at 42 days of age at a 2.85 kg live-weight with a feed conversion ratio of 2.20 and water consumption of 18.5 litres. The mortality rate is in the order of 3.0%.

Throughout other parts of the world it has been more common practice to grow ducks to a processing age of 47 days and a live-weight of 3.40 kg. However, in the past year and a half financial pressures have required that the processing age be reduced to 42 days and with a live-weight of 3.0 kg but still maintain the same meat yield percentages as of a 47 day duck. With increasing prices for cereal grain and other essential raw ingredients the lowering of the age and weight is a solution to reducing feed costs which represents 58% of the cost of producing a duck. To meet this challenge duck breeders in the northern hemisphere have bred stock to achieve these requirements. To keep up with the rest of the world, supply of this new genetic stock will be imported to Australia starting as of 2010.

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III. BROODING AND GROWING DUCKS

The brooding and growing of ducks is mostly conducted in deep litter conventional shedding comprising typically of insulated roofing, enclosed end walls, wired side walls with adjustable curtains, nipple drinkers, automatic pan feeders, hot spot gas fired brooders, lighting, fans and foggers. Controlled environment shedding for duck production in Australia is still in its infancy. Brooding and growing ducks is very similar to other poultry species with the exception of water. Since ducks are a waterfowl species they require a plentiful supply of water not only for consumption but also for preening. Contrary to common belief ducks are very clean birds and perform best when they can rest on fresh dry litter material. The fact that ducks require more water and have web feet makes litter management a critical component of husbandry. It is important that fresh litter material is added regularly throughout the growing cycle to maintain dry fresh litter conditions. Litter requirements also impact directly on stocking densities which can vary from 5 to 6 ducks per square metre in winter and 4 to 5 ducks per square metre in summer. Meat duck production is mostly conducted on company operated farms and independent operated farms with a growing agreement to supply a processor.

IV. NUTRITION

The feeding programme for meat ducks is usually a combination of starter and grower feeds. For the first two weeks of production ducks are commonly feed a coarse starter crumble with a protein level of 21% to 22% and energy level of 12.2MJ/kg. After two weeks of age a grower pellet consisting of 17% to 18% protein and energy level of 12.7MJ/kg is fed to the ducks up until the time of processing. Breeding ducks are fed a program of starter, grower and developer feed during the rearing phase and breeder feed during egg production. Crumble and pellet durability is of critical concern for duck production. Ducks tend to shovel feed into the bill rather than peck at feed. Due to this action, ducks prefer solid crumbles and pellets and avoid fines wherever possible. It is therefore important that the integrity of the crumble and pellet structure is maintained to minimise fines and production losses. Wheat is the preferred grain to feed to ducks due to better performances achieved with this grain. Wheat also has good glutinous characteristics and this can assist with feed binding and can minimise the percentage of fines in the finished product.

V. PROCESSING

Processing ducks is not as straight forward as for other species of poultry due to the thicker layering of feathering that ducks produce. Removal of feathering is a challenge and requires a three stage process of scalding, plucking and waxing. The presence of pin feathers on ducks can cause feather removal issues and this is a worldwide problem within the industry. In the past it was common practice for duck producers to process ducks using modified equipment that was designed and manufactured for processing broiler chickens. Today equipment is designed and built specifically for the purpose of processing ducks. The scalder, plucker, wax system, eviscerator and packing equipment are controlled individually by a Program Logical Control (PLC) system which can be programmed to set parameters for age, weight and strain of duck in order to maximise processing efficiency. This purpose designed and built equipment can be found today in some Australian duck processing plants and these plants are equal to any in the world. The meat, fat, skin and bone percentages for a 42 day processed duck with head off and minus wings varies depending on the genetic stock. In general, meat comprises 23-24% of the carcass, fat and skin 33-34% and bone 31-32%. The fat content is

an essential component of the duck and this gives the meat a tender and favoured appeal which is most important to the Asian community.

VI. HEALTH

Ducks are very robust and are not subjected to any significant viral or bacterial related diseases in Australia and therefore do not require routine vaccination or treatment programmes. The most severe disease to affect ducks in Australia is *Rimerella (Pasteurella) anatipestifer*. Conditions such as ascites, leg weakness and other physical conditions are of minor consequence and concern.

VII. BIOSECURITY

The two major duck producers are committed to biosecurity and have implemented standards and procedures that comply with the Australian Government Department of Agriculture, Fisheries and Forestry National Farm Biosecurity for poultry production. Maintaining these biosecurity standards is important to prevent diseases and pests from infecting poultry premises and may be the mechanism that continues to protect the poultry industry from the threat of imported poultry products.

VIII. RESEARCH AND DEVELOPMENT

The Australian duck industry has been neglected in the past in respect to domestic based research and development work. The industry has relied heavily on research and technical information developed primarily for the production of ducks in the northern hemisphere and has adapted this information to Australian conditions. This situation has turned around in the last few years with a partnership forged with a major duck producer, Rural Industries Research and Development Corporation and Sydney University. This has led to extensive trial work being conducted to establish the strain effect on growth rate, feed consumption, water usage, processing and cooking. This partnership is set to continue with further trials to be conducted to investigate the effects of stocking density, transportation, summer heat and behaviour in relation to performance.

IX. AUSTRALIAN DUCK MEAT ASSOCIATION

The Australian Duck Meat Association (ADMA) was formed and incorporated in 2008 as an initiative of the two major duck producers. The association was formed for the following objectives:

(i) To provide a forum whereby duck meat processors can be represented by a common association for the long term being of the industry. (ii) To offer protection of financial interests for members and to ensure a reasonable financial return as the industry grows. (iii) To promote duck meat as an alternative food base and gain a greater acceptance by consumers. (iv) To establish and maintain compliant growing and processing standards to further enhance the industry. (v) To work closely with state and federal bodies and other representative bodies to maintain a high standard of operation for the benefit of the entire industry. (vi) To maintain and pursue practices that will help ensure a disease free status giving consumers confidence in a wholesome meat product. (vii) To develop and monitor strict biosecurity standards resulting in optimum disease control and to help reduce the likelihood of serious disease spreading.

In 2008, the Australian Duck Meat Association became a member of Animal Health Australia (AHA). Since becoming a member, the ADMA has been actively involved in AHA matters participating at forums, workshops and staff training events.

X. CHALLENGES AND THE FUTURE OF THE DUCK INDUSTRY

There are a number of challenges facing the industry, which must be countered in the future. These include (i) sourcing long term adequate supplies of suitable litter material at affordable prices or the development of viable alternative products or systems. (ii) The threat of duck meat products being imported into Australia from countries such as China, Thailand and Vietnam. (iii) Long term provision of a Quarantine facility for the importation of genetic stock. (iv) Increasing prices for cereal grains and other raw ingredients for the production of feed. (v) Development of value added products and introduction of new product lines to meet the requirements of customers. (vi) Urban encroachment on poultry farming areas. (vii) Recruitment of staff.

The demand for duck meat continues to grow from year to year in Australia and the industry is also aware that to the north of the country lays the largest number of duck consumers in the world, that being Asia. China, Thailand, Vietnam, Taiwan, Korea, Hong Kong and Malaysia have the potential to develop into large export markets for Australian duck meat products. As much as there is a huge amount of duck grown and processed in Asia their greatest threat is from diseases especially highly pathogenic avian influenza. With modern growing and processing facilities, world class genetic stock, high standards of biosecurity and focus on animal welfare this will hold the Australian industry in good stead to supply duck product to the world's largest markets.

THE EFFECTS OF STRAIN AND SEASON ON THE PERFORMANCE OF COMMERCIAL DUCKS UNDER AUSTRALIAN CONDITIONS

J.A. DOWNING[1] and W. TAYLOR[1]

Summary

The Australian duck industry has a very specific market requirement, this being for a 2.85 kg bird at 6 weeks of age. The strains of Pekin duck presently used in Australia, the Cherry Valley and Grimaud Frères, have different growth characteristics but both have difficultly meeting this target weight especially in summer. It was considered that by crossing these two strains, hybrid vigour might allow advantages to be gained in growth performance. The present study investigated the performance of the two main strains of Pekin ducks and their reciprocal crosses grown to 6 weeks of age in summer and winter. Ducks were reared following industry practices. The strains and their crosses were bred by PE'S Ducks Pty Ltd, and reared in single sex groups or as mixed sex groups. In summer only one strain reached market weight by 41 days of age. In winter all strains reached market weight by 41 days but the FCR was higher in winter than summer. Males grew to heavier weights than females in both summer and winter but there was no advantage gained by rearing ducks as single sex groups.

I. INTRODUCTION

Because it is a relatively new industry, the amount of information specific to duck production under Australian conditions is limited. At present, two different strains of Pekin duck are used by the Australian industry, the Grimaud Frères (GF) and the Cherry Valley (CV). Both have distinct growth characteristics but because they have been developed in Europe to meet needs of the local market, individually they fail in some respects to meet the very stringent requirements of the Australian market. In Australia the current market specifications are for a bird grown to 2.85 kg at 6 weeks of age to meet the needs of the whole bird restaurant trade. Another issue of industry concern is the late breast muscle development in Pekin ducks. So getting a strain that grows rapidly to market weight with a high yield of breast muscle is a major contradiction faced by local producers. The GF grows to larger size than the CV but matures later and is considered to produce meat of lower eating quality. One consideration given to obtaining a better breeding outcome to meet local needs is to cross the GF and CV strains and achieve improved performance from hybrid vigour.

A primary objective of the present studies was is to evaluate the performance of the CV and GF strains and the reciprocal crosses of these strains reared under Australian summer and winter conditions. A further objective was to evaluate the performance of different sexes and the affect of rearing ducks as single or mixed sexes. One study was carried out in February/March and the other in June/July 2007.

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II. METHODS

Studies using the same experimental design were undertaken in summer (Feb-Mar) and in winter (Jun-Jul). Birds were reared in a tunnel ventilated shed with 48 floor pens (1.5m x 3.0m) with four replicate pens allocated to each treatment. The ducks were raised on deep litter, consisting of wood shavings. The four strains, GF, CV and the reciprocal crosses, GF male x CV female and CV male X GF female, were breed by the industry partners, PEPE'S Ducks Pty Ltd. Eggs were incubated at their commercial hatchery and at hatch ducklings were vent sexed and transported as day olds to The University of Sydney, Camden. On arrival 36 ducklings of a specific strain were allocated at random to the appropriate treatment pens as single sex groups (male or female) or mixed sex groups (equal numbers of males and females). On day six wing tags were inserted into the right wing of all birds. Birds were fed a commercial starter crumble diet on days 1-14 formulated to supply 12.14 MJ/kg ME and 22% protein, and pelleted grower diet from days 15-41 formulated to provide 12.77 MJ/kg ME and 19% protein. The ducks were provided with feed *ad libitum*. Each pen had its own water supply which ducks accessed via a row of 4 nipple drinkers. Birds were weighed individually at the end of each week, except in week 6 when they were weighed at day 41. Both feed intake and water intake were determined at the end of each week on a pen basis. Throughout the study birds were removed from the pens for carcass measurements and a metabolism study. At the start of week six the bird density was 22 birds per pen in summer and 24 birds per pen in winter.

Data were analysed using the REML linear mixed model function of Genstat® 11th edition. Data was $\log_e$ transformed where necessary. The fixed model included the effects of strain, sex, pen-sex and week and the random model included the effects of block, pen and bird identification. Significance testing of fixed effects was conducted using Wald chi-square tests with a significance threshold of P < 0.05. Any non-significant interactions were removed from the model and for significant effects the least significant difference (LSD) procedure was used to make pair-wise comparisons of means.

III. RESULTS

For reasons of 'commercial-in-confidence' the strains will be identified as A, B, A X B and B x A. The final mean (± SEM) six week liveweight (LW), FCR and water to feed ratio for the effects of strain and sex are given in table 1. In summer and winter there were highly significant effects of strain, sex and pen-sex on LW but these effects changed over time, as indicated by the significant interactions with week (all *P*< 0.001). Mean LW were lower in summer than winter. At the end of the six week production period, ducks of strain B (P < 0.05) were heavier than other strains in both seasons, followed by strain A X B (P < 0.05) being heavier than strains A and B x A again in both seasons. In summer strain A and A X B had similar weights but in winter strain B X A (P < 0.05) was heavier than strain A. In summer, males were heavier than females at all weeks (P < 0.05) and similarly in winter, except for week 1, males were again heavier than females (P < 0.05). The differential between males and females was similar for both seasons, approximately 200g. In both seasons the pen-sex x week interaction (P < 0.001) was significant but on no individual week were the differences significant suggesting the effect is questionable.

Table 1.   The mean (± SEM) six week liveweight (LW), FCR and water to feed ratio for two strains (A and B) of commercial Pekin ducks and their reciprocal crosses (A X B and B x A). For these measures the effect of sex is also given.

Strain	Six week Liveweight (g)	FCR	Water to feed ratio	Six week Liveweight (g)	FCR	Water to feed ratio
Strain		Summer			Winter	
A	2705 ± 33[c]	1.95 ± 0.02[b]	3.34 ± 0.05[a]	2847 ± 20[d]	2.09 ± 0.01	2.70 ± 0.05
A X B	2844 ± 34[b]	2.00 ± 0.02[a]	3.43 ± 0.05[a]	3106 ± 22[b]	2.10 ± 0.01	2.75 ± 0.05
B	2963 ± 39[a]	1.96 ± 0.02[ab]	3.33 ± 0.05[a]	3262 ± 23[a]	2.10 ± 0.01	2.68 ± 0.05
B X A	2724 ± 33[c]	1.89 ± 0.02[c]	3.23 ± 0.05[b]	2951 ± 21[c]	2.06 ± 0.01	2.70 ± 0.05
Sex		Summer			Winter	
Female	2708 ± 34[b]	2.01 ± 0.01[a]	3.36 ± 0.04	2942 ± 18[b]	2.14 ± 0.01[a]	2.76 ± 0.04[a]
Male	2910 ± 32[a]	1.89 ± 0.01[b]	3.29 ± 0.04	3137 ± 19[a]	2.03 ± 0.01[b]	2.68 ± 0.04[b]

Within columns for strain and sex values with different superscripts are significantly different.

In summer, the mean (± SEM) LW at six weeks for birds reared in single pens (2821 ± 34 g) and mixed pens (2793 ± 31 g) was not different. In winter, the mean (± SEM) LW of birds reared in single pens (3035 ± 21 g) and mixed pens (3038 ± 18 g) and again was not different. In summer, strain had significant effect on FCR (P < 0.001). Strain B X A had a better FCR than other strains (P < 0.05). Strain A had a lower FCR than strain A X B (P < 0.05) with the FCR for strains B and A X B not different. In winter strain had no effect on the FCR (P=0.18). In both summer and winter (P < 0.001) sex had a significant effect of FCR with males in both seasons having a better FCR than females (P < 0.05). Strain influenced water to feed ratio in summer (P=0.02) but not in winter (P=0.23). In winter, sex (P=0.04) had significant effect on the water to feed ratio with males having a lower ratio than females. However, in summer sex had no influence on water to feed ration (P=0.33).

IV. DISCUSSION

In Australia the market specification for ducks is for a liveweight of 2.85 kg at slaughter. Processing ducks at 2.85 kg limits the yield of breast muscle and this represents a major limitation faced by the Australian meat duck industry. It was considered that by forming reciprocal crosses from matings of the main strains, a production advantage might be gained from hybrid vigour and that the crosses may better meet the needs of the Australian market. However, the crosses provided no advantage above the parent strains. Wawro et al. (2004) reported that crossbred ducks did not demonstrate heterosis for liveweight. While on the surface it appears that rearing ducks in summer is more efficient with a lower FCR, it needs to be remembered that in commercial practice the rearing age would need to be increased to get ducks

to correct market weight in summer and that this would negate any benefit in FCR and actually increased production costs because slaughter age would be increased.

In the Australian meat duck industry it is common practice to raise ducks in mixed sex pens. This is adopted because it is widely believed that the sexual dimorphism in Pekin ducks is small until 6 weeks of age (Farhat and Chavez, 2000). The current study supports the present practice, as little advantage is achieved by rearing ducks as single sex groups. Rearing ducks in mixed sex pens avoids the necessity of vent sexing, which is both an invasive and expensive procedure. The differences between sexes of the same strain, in the current study, ranged from 142 g for Strain A to 272 g for Strain A X B. Differences in LW between sexes increased with age and the strains with the highest growth rates showed the greatest levels of sexual dimorphism at six weeks. Therefore, sexual dimorphism could, in part, be responsible for the large variation of body weights observed at Australian processing plants. Cross breeding of the present strains of Pekin ducks available in Australia will not solve the dilemma facing the industry, this being the poor breast muscle yield at the desired market weight of 2.85 kg. The Australian industry needs to find either management, nutritional or genetic solutions to alter the ducks' growth allometry so breast muscle development occurs at an earlier age.

V. ACKNOWLEDGEMENTS

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THE PERFORMANCE OF COMMERCIAL DUCKS FED DIFFERENT DIETARY PROTEIN
CONCENTRATIONS DURING THE FINISHER PHASE

J.A. DOWNING[1] and J. ISCHAN[1]

Summary

The objective of the present study was to investigate the dietary protein requirements of three commercial duck strains needed to obtain best performance and growth under Australian summer conditions. Three lines of Pekin duck, one Grimaud Frère line and two Cherry Valley lines were fed a commercial starter (days 1-14) and grower (days 15-28) diets and then 4 diets containing the same metabolisable energy content (12.5 MJ/kg) but differing in protein content (diet A, 16.8%; diet B; 16.1%; diet C, 15.4% and diet D 17.5%) from days 29-41. Individual ducks were weight weekly and feed intake was measured weekly on a pen basis. No differences in live-weight gain (LG) were seen in weeks 1-4. In week 5, LG was lower for birds fed diets B and C compared to those fed diets A and D ($P < 0.05$). In week 6, the effects were reversed, with LG for birds fed diets B and C being greater than for birds fed diets A and D ($P<0.05$). Except for week 1, the live-weight (LW) for strain X was less than for both strains Y and Z ($P < 0.05$). For the first 4 weeks there was no difference in the LW for strains Y and Z. However, in weeks five and six the LW for strain Y was greater than for strain Z ($P < 0.05$). The data indicate that producers can reduce costs by introducing a low protein finisher diet into the feeding program of commercial ducks.

I.   INTRODUCTION

There is a lack of information on the dietary requirements of ducks grown under Australian conditions with little having been done since the work of Siregar et al. (1982a, b). These researchers reported that ducks needed a crude protein content of 18.7% in the starter diet (fed days 1-14) and 16% (fed days 15-42) in the grower diet. Presently, diets fed to modern strains of Pekin ducks contain more protein than these recommendations being around 22 and 19%, respectively. Providing excess protein is wasteful and expensive, with the surplus protein metabolised and used as a source of energy (Siregar, et al., 1982a). Evidence from a study in 2007, funded by RIRDC, indicated that ducks fed 19% protein in the grower diet had low nitrogen retention, suggesting that this concentration was too high. In 2008, a further study looked at the performance of commercial ducks fed a starter ration containing 18.1% and grower ration with 15.7% protein. This was 3% less protein in both rations, than was being used by industry at the time. In the first four weeks, ducks on the low-protein diets had significantly less weight gain than ducks on the commercial control diet but remarkably, in week five, ducks on the low-protein diet gained as much weight as ducks on the commercial control grower diet and in week six gained more weight. These findings suggested that less protein than presently used in commercial diets could be fed in weeks five and six of the production period and possibly warrants the introduction of a finisher diet in commercial production.

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Due to genetic selection occurring in different countries, there has been diversification of the Pekin breed and development of specific commercial strains. Strains of particular importance to Australian duck-meat production are the Cherry Valley (CV) and the Grimaud Frère (GF). These strains have different growth characteristics but really neither meets the very tight specification required by the Australian market which is for a 2.85-2.90 kg at six weeks of age.

The objective of the present study was to investigate the dietary protein requirements of three current duck strains, during weeks 5 and 6 of age, needed to obtain best performance and growth under Australian summer conditions.

II. MATERIAL AND METHODS

One line of GF and two lines of CV Pekin duck strains were used in the study. For 'commercial-in-confidence' reasons, the strains are identified as X, Y, and Z. The three lines were bred and eggs incubated at a commercial breeder farm owned by our industry partners PEPE'S Duck's Pty Ltd. At hatch, ducklings were vent sexed and delivered to the University of Sydney's Poultry Research Unit in Camden. On arrival, eighteen males and eighteen females of the same strain were randomly selected and weighed as a group prior to placement in their designated pen. Birds were maintained in 48 floor pens (3m x 1.5 m), four replicate pens/treatment, in a tunnel ventilated shed. Ducks were maintained in continuous lighting for the duration of the growth trial. They were raised on deep litter, consisting of dry wood shavings. At placement, the brooding temperature was 32°C and this was gradually reduced to 20°C at four weeks of age. On day six, identification tags were inserted into the right wing of all ducks. During the six week production period birds were removed at various times for growth and carcass measurements. At the start of week six, 22 ducks remained in the pen (2045 cm^2/bird).

Ducks had continuous access to both food and water. Diets were formulated and supplied by Inghams Pty Ltd. A starter crumble containing 12.6 MJ/kg ME and 21.5% protein was fed to all ducks from day 1 to day 14. The grower diet was fed as a pellet and contained 12.5 MJ/kg ME and 17.5% protein and fed to all ducks from day 15 to day 28. On days 29 to 41, four treatment finisher pelleted diets were fed which varied in protein content. Finisher diets contained 12.5 MJ/kg ME with 16.8. 16.1, 15.4 and 17.5 % protein and were identified as diets A, B, C and D, respectively. Diet D was considered the control as it had the same protein content as the diet fed in the grower period.

Data were analysed using the REML linear mixed model function of Genstat® 11th edition. Data was log$_e$ transformed where necessary. The fixed model included the effects of diet, sex, strain and week and the interactions while the random model included the effects of block, pen and bird identification. Significance testing of fixed effects was conducted using Wald chi-square tests with a significance threshold of $P < 0.05$. Any non-significant interactions were removed from the model and for significant effects, the least significant difference (LSD) was used to make pair-wise comparisons of means.

III. RESULTS

The effect of diet on liveweight gain (LG) and strain on liveweight (LW) are given in table 1. There was a significant interaction between diet and week, ($P < 0.001$). No differences in LG were seen in weeks 1 to 4. In week 5, LG was lower for birds fed diets B and C compared to those fed diets A and D ($P < 0.05$). Interestingly, in week 6 these effects were reversed, with the gain for birds fed diets B and C being greater than for birds fed diets A and D ($P < 0.05$). At the end of the 6 week the only difference in LW was where birds on diet D were heavier than those on diet C ($P < 0.05$).

Table 1.   The mean ($\pm$ SEM) weekly LG and weekly LW for three strains (X, Y and Z) of commercial Pekin ducks fed the same diets during the first 4 weeks and then diets differing in protein content (diets A-D) during weeks five and six.

Liveweight gain (LG) (g/week)						
Diet	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
A	216 ± 4	482 ± 9	610 ± 11	611 ± 12	587 $\pm 11^a$	407 $\pm 8^b$
B	217 ± 4	483 ± 9	627 ± 12	626 ± 12	507 $\pm 10^b$	439 $\pm 8^a$
C	214 ± 4	489 ± 9	610 ± 12	609 ± 11	525 $\pm 10^b$	440 $\pm 8^a$
D	216 ± 4	478 ± 9	614 ± 12	626 ± 12	605 $\pm 11^a$	410 $\pm 8^b$
Liveweight (LW) (g)						
Strain	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
X	262 $\pm 2^b$	723 $\pm 5^b$	1308 $\pm 9^b$	1878 $\pm 13^b$	2394 $\pm 17^c$	2799 $\pm 20^c$
Y	270 $\pm 2^a$	756 $\pm 6^a$	1390 $\pm 11^a$	2030 $\pm 16^a$	2617 $\pm 21^a$	3077 $\pm 25^a$
Z	269 $\pm 2^a$	767 $\pm 5^a$	1390 $\pm 10^a$	2026 $\pm 14^a$	2594 $\pm 18^b$	3022 $\pm 21^b$

There was a significant interaction between strain and week, ($P < 0.001$). Except for week 1, the LW of strain X was less than for other strains ($P < 0.05$). For the first 4 weeks there was no difference in LW of strains Y and Z. However, in weeks 5 and 6 the LW of strain Y was greater than for strain Z ($P < 0.05$).

Diet had no effect ($P = 0.18$) on the feed conversion efficiency (FCR). For diets A, B, C and D the FCR was 2.08 ± 0.02, 2.03 ± 0.02, 2.00 ± 0.02 and 2.00 ± 0.02, respectively. Strain had a significant effect on FCR ($P < 0.001$) with strain X (2.06 ± 0.01) having lower efficiency ($P < 0.05$) than strains Y (1.99 ± 0.01) and Z (1.99 ± 0.01).

IV. DISCUSSION

A major challenge for the duck-meat industry at the moment is formulating diets that meet duck requirements for optimal growth in a cost-effective manner. While there were dietary effects influencing weight gain during week five and six these were not consistent and tended to balance out over the two week period such that the total weight gain over weeks five and six were similar for all diets. This suggests that there is no benefit in feeding more than 16% protein in the diet during weeks five and six. This would have economic benefits as feed makes up 70% of total production costs in the duck-meat industry and protein is an expensive component of the feed (Cherry and Morris, 2008). As there were no dietary effects on FCR, the data indicate that producers can reduce costs by introducing a low protein finisher diet into the feeding program of commercial ducks.

Previous studies in ducks have revealed that genotype has a significant effect on growth rates (Maruyama, et al., 1999; Cherry and Morris, 2008). Ducks of strain X grew slower than the other strains and were smaller at market age. In fact they failed to meet the market specification of 2.9 kg and in commercial practice would need to be grown to an older age before processing. Strain Y reached the heaviest weight at market age but the difference with strain Z was not great, on average this being 55g. While it is not part of the results discussed here, strain Z had a significantly higher breast muscle yield at six weeks than did strain Y (average: 359 Vs 286 g). This is a major advantage for strain Z over strain Y and indicates that it is possible to select for improved meat yield at an earlier age.

V. ACKNOWLEDGEMENTS

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TWO-DAY-OLD DUCKLINGS INTERACT MORE WITH A BELL DRINKER THAN A NIPPLE DRINKER SUSPENDED ABOVE A TROUGH

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Farming ducks for meat production is increasing in Australia. In Europe, the welfare issues associated with intensification of meat duck production were reviewed by Rodenburg et al. (2005), who identified the manner in which water was provided was a potential welfare issue. Specifically, concerns were raised whether ducks require access to 'open water' for their welfare, since open water stimulated the performance of preening, dabbling, head-dipping, bathing and swimming (Rodenburg et al., 2005). However, a consequence of water-related behaviours was that more water may be used, resulting in increased spillage and reduced litter quality. Cooper et al. (2002) investigated the behaviour of young ducks provided open water via bell drinkers compared to nipple drinkers. Young ducks had a clear preference for bell drinkers and placed a higher value on wider, deeper drinkers that allowed a greater range of drinker-related activities than nipple drinkers alone. The objective of the present experiment was to investigate the preference of 2-day-old ducks for two water presentation systems, which provided different levels of open water but which, in principle, were constructed using similar water-holding structures that permitted the ducks to sit in a trough.

Six pens of 36 ducklings (Cherry Valley and Grimaud Freres) were continuously video recorded from the time of placement in pens at day-old. The ducklings were restricted to an area of ~3.1 m^2 within pens measuring 3.0 m x 1.5 m in an environment controlled shed. Lighting was continuous and heating was provided in each pen by an infra-red globe heater suspended 0.6 m above the floor, which was 50 mm deep wood shavings. Feed was available *ad libitum* from a 40 cm diameter tray and a circular feeder. Water was provided by a bell drinker positioned in the middle of the pen (Multiquip Pty Ltd, Austral, NSW, 13 l water capacity, 35 cm diam) and three nipple drinkers with water catching 'cups' about 0.5 m apart, suspended above a trough on one side of the pen. The number of ducklings interacting with the bell drinker and the trough was collated from the digital video record at 5-min intervals for 24 h commencing at 1200 h on the second day of life. Interaction with the water facility was defined as ducklings having their head adjacent to (within 2 cm) or over the bell drinker or trough. The number of ducklings sitting in the bell drinker or trough was also recorded. The data were analysed using a two-sample T-test (paired) in Genstat (Release 11.1 (2008) VSN International Ltd., UK) and the experimental unit was the pen of ducklings.

The likelihood that ducklings were observed at the bell drinker was twice that for the nipple drinker/trough system (17.9 vs. 8.6% of observations, respectively; t = 2.50, 5 df, P = 0.027), supporting the findings of Cooper et al. (2002) that open water may be a visual stimulus increasing ducks' level of interaction with the water supply apparatus. However, there was no difference in the incidence of ducklings sitting in the bell drinker (i.e. in the water) compared to in the trough beneath the line of nipple drinkers where some spillage may collect (1.6 vs. 1.5% of observations, respectively; t = 0.30, 5 df, P = 0.39). Further research is required to investigate the relative stimulus values of the bell drinker and the trough, and the relationship between the performance of water-related behaviours and welfare.

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MICROBIAL PROFILES IN THE GASTRO-INTESTINAL TRACT OF BROILERS AND ITS RELATION TO FEED EFFICIENCY

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Summary

In a field survey and in a controlled experiment the gut microbiota of broilers was studied using fragment lengths of DNA from genes encoding 16S rRNA to reveal the relation between the composition of the microbiota in the gut and the performance of broilers. The relation between feed utilisation and microbiota in the proximal part of the intestine of the young broiler is more pronounced than on a later age in the more distal parts. The relative quantities of DNA from *L. acidophilus* and probably *L. salivarius* in the crop and ileum, expressed as percentage of the total microbial DNA, are negatively correlated with feed efficiency.

I. INTRODUCTION

The ban on antibiotic growth promoters (AGP's) since 2006 in the European Union has increased the interest in gut microbiota composition of broilers and its effect on animal performance. Many research projects have started to find alternatives for these AGP's. Knowledge about the effect of AGP's on microbiota in the gastro-intestinal tract (GIT) however is limited, and the relationship between the microbiota in the GIT and animal performance has not been clarified very well.

Recently, new DNA-techniques to study the microbiota have been developed. In the current study, the Terminal Restriction Fragment Length Polymorphism (T-RFLP) technique described by Liu *et al* (1997) was used to study the microbiota in the GIT. The objective of this research was to improve our understanding about the microbiota in the different segments of the intestinal tract at different ages in broilers and how this relates to feed utilisation.

The Dutch Ministry of Economic Affairs has supported this research financially.

II. MATERIALS AND METHODS

To reveal the microbiota in the different parts of the GIT of broilers two studies were performed: one big field survey on 23 broiler farms with either a good or moderate performance and one controlled experiment with different feeds on a test farm. On the 23 farms, samples were taken from the content of the crop, ileum and caeca at 7, 27 and 35 days of age. Per farm, segment and age, 10 samples were taken. Thus, in total 2070 samples from the field experiment were analysed.

In addition, in a controlled experiment with 1200 birds divided over 48 pens and eight treatments with different starter and grower diets, samples were taken from 6 birds per pen from the content of the crop and the ileum at day 9 and 29. Subsequently 1152 samples from the controlled experiment were analysed on DNA fragment lengths.

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The 16S rRNA genes of the micro-organisms present in the sample were amplified by PCR using blue fluorescently labelled primer 8f (5'-AGA GTT TGA TCC TGG CTC AG-3') and yellow fluorescently labelled primer 926r (5'-ACC GCT TGT GCG GGC CC-3') and were subsequently digested with restriction endonucleases *Msp*I and *Hinp*I (both from New England Biolabs, Ipswich, MA, USA). This technique provides two outcomes per sample: one from the blue and one from the yellow primer. The results were expressed as percentage of the total DNA, per fragment length (FL). The FL varies from 50 to 600 nucleotides or base pairs (BP). Databases were available to determine which lengths, depending on the used primers and restriction enzymes, correspond with certain bacterial species. Because the data from the blue primer were more discriminating for the bacterial species than the data from the yellow primer, only the results of the blue primer are presented in this paper. For identification of the bacterial species, the data from both primers were used and some samples were sequenced with the kind help of M. Lee at the lab of Poultry Science from the University of Georgia, USA.

III. RESULTS

(a) Field survey

Only a few big peaks with 10–20% of the total DNA, most probably representing *Lactobacillus* species, are observed in the microbial DNA from the crop and ileum. In the caeca many small peaks are observed with no peak reaching more than 7% of the total DNA. The resemblance in microbial composition between the crop and ileum is large, whereas the composition in the caeca differs very much from that in the crop and ileum. The interaction between the effect of farm type and age on the relative quantity of DNA with 181 BP is significant ($P < 0.05$) as is shown in figure 1.
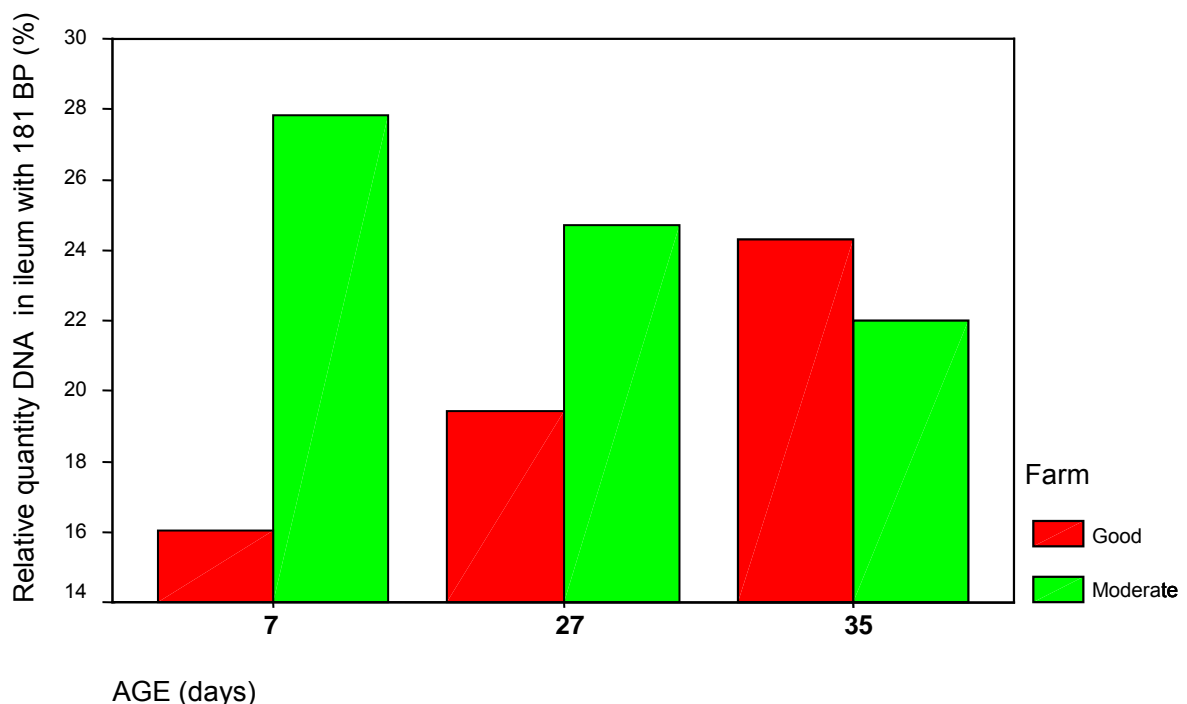
Figure 1.   Relative quantity of DNA in the ileum with 181 BP on good and moderate farms at different age

(b) Controlled experiment

In this experiment, the focus was on the relationship between feed efficiency (FE) and the relative abundance of DNA with a certain FL. The relationship between FE in the starter period and the relative DNA quantities of certain fragment lengths at day 9 were weak. The relationship between FE and DNA abundance was more pronounced when the FCR during the starter and grower period was related to the T-RFLP data on day 29. The relative quantity of DNA with a FL of 188 BP in the crop had a positive relationship with FE, while fragments with approximately 571 -573 BP in the crop showed a negative relationship with FE (Figure 2).
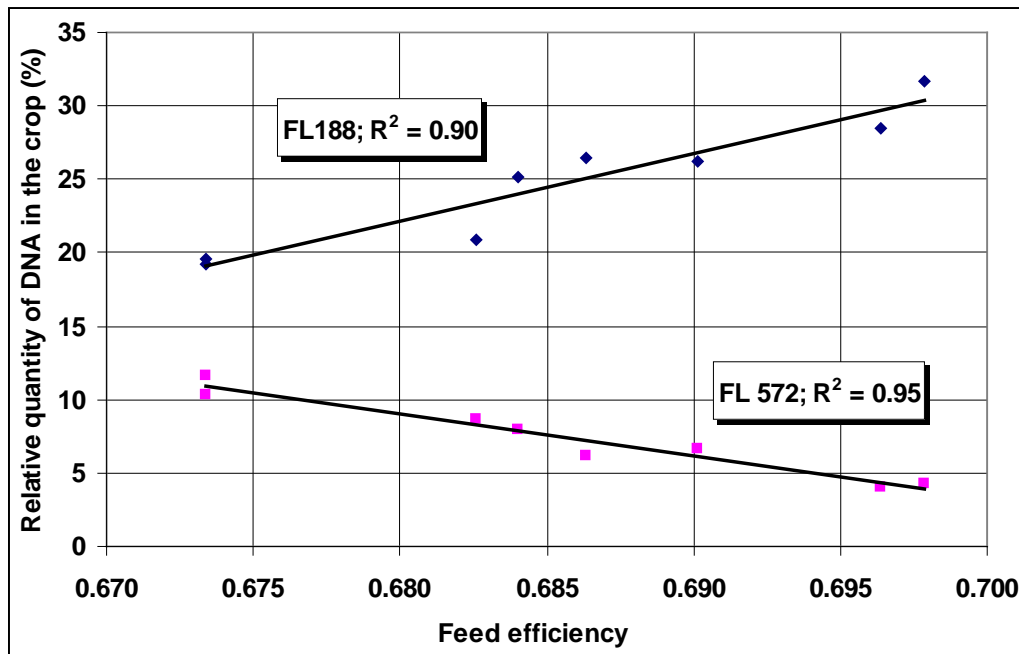
Figure 2.   Relation between relative abundance of DNA with certain fragment lengths with FE

(c) Identification

The peak with a FL of 181 BP with the blue primer corresponded well with the peak with a FL of 345 BP with the yellow primer. Based on databases including the above-mentioned primers and restriction enzymes, the sequencing of some samples and the literature review by Lu *et al.* (2003), this peak most likely represents the species *Lactobacillus acidophilus*. It is not clear which bacterial species represents the peak with a FL of 188 BP. It might be *L. crispatus, L. johnsonii, L. gasseri* or a mixture of these species. Most probably, the peak with circa 572 BP represents *L. salivarius.*

IV. DISCUSSION

One should realise that the used primers do not cover all bacterial species and that identification with T-RFLP-technique with only two primers is not 100% reliable. Firstly, is difficult to determine the exact length of especially long fragments. Secondly, more than one species can have the same primed FL. Also the studied fragments can occur more than once on the DNA of one bacterial species. Sequencing more samples will provide more reliable information about identification. With good reviews about the main species that are common

in the GIT of broilers, the T-RFLP-technique can give a good view on the development of the microbial population in the different segments of the gut of broilers.

From this research it cannot be concluded that the bacterial species *L. acidophilus* and *L. salivarius* have caused the negative effect on the feed utilisation, although the relative abundance of these species was clearly associated with FE. *L. acidophilus* is a bacterial species, which can multiply very fast and might be an indicator for bacterial overgrowth. All microbes compete with the host for nutrients, and also Lactobacilli trigger the immune system with as possible consequence a decreased feed efficiency.

IV. CONCLUSIONS

The T-RFLP technique provides good information about the development of the microbial composition in the gastro-intestinal tract during the lifetime of a broiler. The relation between feed utilisation and microbiota in the proximal part of the intestine of the young broiler is more pronounced than on a later age in the more distal parts. The relative quantities of DNA from *L. acidophilus* and probably *L. salivarius*, expressed as percentage of the total microbial DNA in the GIT, are negatively correlated with feed utilisation.

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CHARACTERISATION OF GUT BACTERIA ASSOCIATED WITH BROILER PERFORMANCE ACROSS VARIOUS AUSTRALIAN FEEDING TRIALS

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Summary

Linkages were established between performance and gut microbiota from three poultry trials investigating effect of various diets and/or litter on broiler performance. Bacterial profiling was done to investigate changes in gut microbiota and identify potential performance related bacteria. Across all three trials four operational taxonomic units (OTU) within the ileum (180, 492, 564-566 and 936-938) and five OTU within the caeca (140-142, 216-222, 280-286, 312 and 482) were consistently identified. Among these OTU 492, 140-142 and 482 were more closely associated with improved performance, while OTU 564-566 was mainly associated with decreased performance. Targeted cloning and sequencing of 7 of these OTU revealed a possible 19 different bacteria species, indicating many of these OTU contain several bacteria which may be contributing to the increase or decrease of a particular performance related OTU. Many of these bacteria were identifiable to the species level; however, the majority remain unclassified bacteria. Where bacteria were identifiable to the phyla level, they belonged predominantly to the Firmicutes and Bacteroidetes.

I. INTRODUCTION

A favourable gut microbiota is important for the optimal growth and performance of chickens and several gut bacteria isolated from chicken have been shown to have various important biochemical properties. Alternatively, an unfavourable microbiota may promote clinical and sub-clinical 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. For example, the toxic metabolites generated by the bacterial β-glucuronidase in poultry have been shown to hinder performance or reduce feed utilization (Jin *et al*., 2000), while ammonia, produced by proteolytic bacteria such as *Clostridium* spp., *Enterococcus* spp. and *Bacteroides* spp, have toxic effects on enterocytes (Rehman *et al*., 2007). Furthermore, some members of the genera *Clostridium*, *Lactobacillus*, *Fusobactrium*, *Bacteroides*, *Bifidobacterium*, *Peptostreptococus*, and *Streptococcus* produce bile salt hydrolase compounds which lower the detergent properties of bile acids in the emulsification of fat leading to growth depression in chickens (Knarreborg *et al*., 2002, Rehman *et al*., 2007).

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). The aim of this study was to determine if particular broiler gut bacteria could be consistently linked with improved or decreased performance across three performance trials.

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II. MATERIALS AND METHODS

Linkages were established with three poultry performance trials between 2007-2008. These trials evaluated influence of various dietary and/or litter materials on poultry performance as measured by feed conversion ratio (FCR). Within each trial diets were formulated to have similar energy and nutrient specification although composition of raw ingredients may have varied between dietary treatments. Trial 1 was done at the Queensland Poultry Research Centre, Alexandra Hills Qld in 2007. This trial (Evaluation of sorghum grains from Qld and NSW for broiler growth performance in a semi-commercial environment) was led by Dr R Perez-Maldonado and supported by RIRDC (project DAQ-326A). Trial 2 was done at Inghams Enterprises Pty Ltd., Leppington NSW in March 2008. Trial 2 (The effect of litter and dietary fibre on gut development, nutrient digestibility and gut microbiota) was led by Dr L Mikkelson and supported by the Australian Poultry CRC (project 06-18). Trial 3 was done at Inghams Enterprises Pty Ltd., Leppington NSW in November 2008. Trial 3 was led by Dr R McAlpine and K Balding and was evaluating commercial broiler feeds produced in various Inghams' feed mills across Australia.

Where significant differences were detected in FCR (mean $\pm$ SE) the poultry gut microbiota was investigated in an attempt to identify bacterial indicators potentially linked with broiler performance. A segment (3 cm) of tissue and associated digesta was collected from the mid-point of the ileum and one caeca (n=12/treatment on day 42 of age for trial 1 and n=24/treatment on days 35 and 42 for trials 2 and 3 respectively). All samples were stored at -20°C and freeze dried prior to nucleic acid extraction. Total nucleic acid was extracted using a modified SARDI proprietary method. Bacterial communities were analysed by terminal-restriction fragment length polymorphism (Torok *et al*. 2008). Multivariate statistical methods were used to identify significant differences in bacterial community composition between treatments within each experiment and identify potential operational taxonomic units (OTU) associated with performance differences. OTU can represent particular bacterial species or taxonomically related groups of bacteria. Identity of OTU of interest were determined using targeted cloning and sequencing (Widmer *et al*. 2006)

III. RESULTS

In trial 1, FCR (21-42 days) was significantly better for birds raised on either a commercial sorghum diet (1.85±0.01) or commercial sorghum diet supplemented with phytase (1.86±0.01), as compared with a control wheat based diet supplemented with xylanase (1.99±0.02). Microbial profiling of the caecal samples showed that there were no significant (P>0.05) differences between dietary treatments. However, within the ileum there were significant (P<0.05) differences in bacterial communities of birds fed the wheat plus xylanase diet when compared to birds fed either the sorghum commercial diet or sorghum commercial diet supplemented with phytase. OTU 76, 180, 468, 492, 564, 936 were identified as contributing to differences in ileal microbial community composition between birds raised on the wheat control diet and birds raised on either of the sorghum diets. OTU 76, 180, 468, 492 and 936 were more abundant in the better performing sorghum diets, while OTU 564 was more abundant in the poorer performing wheat control diet.

In trial 2, FCR (0-35 days) was significantly better for female broilers fed a low fibre diet and reared on hardwood litter (1.51±0.02) as compared to those fed either a low fibre diet and reared on paper litter (1.58±0.02) or a high fibre diet and reared on hardwood litter (1.56±0.02). Microbial profiling of the ileal samples showed there were no significant (P>0.05) differences between any of the dietary/litter treatments. However, for both male and female birds, caecal microbial communities were significantly (P<0.05) different between

birds fed a low fibre diet and reared on paper litter versus birds fed a low fibre diet and reared on wood litter. OTU 92-94, 142, 198, 206-208, 216-218, 222, 282, 284-286, 312, 482, 542 and 522 were identified as contributing to differences in caecal microbial community composition between female birds in these two groups. OTU 92-94, 142, 198, 206-208, 482, 542 and 522 were more abundant in the group with improved performance as measured by FCR, while OTU 222, 282, 284-286, and 312 were more abundant in the lower performing group.

In trial 3, FCR (corrected to 2.6kg live weight) was significantly better for broilers fed diets produced in mills in Western Australian (1.55 ± 0.01) and Tasmanian (1.50 ± 0.01) as compared with diets produced in mills in Queensland (1.58 ± 0.01) and New South Wales (1.60 ± 0.01). Microbial profiling of the ileal and caecal samples showed that there were significant ($P<0.05$) differences between microbial communities of birds on the two better performing diets versus birds raised on the two poorer performing diets. Within the ilea OTU 180, 188, 454, 492, 506, 566, 576, 872 and 938 were identified as contributing to differences in microbial composition between improved and poorer performing birds as measured by FCR. OTU 454, 492 and 506 were more abundant in the higher performing groups, while OTU 180, 188, 566 and 938 were more abundant in the lower performing groups. Within the caeca OTU 140-142, 212, 218-220 284-286, 312, 482, 488 and 536 were identified as contributing to differences in microbial composition between improved and poorer performing chickens. OTU 140-142, 218-220, 284-286, 312, 482, 488, and 536 were more abundant in the better performing groups, while OTU 212 was more abundant in the lower performing groups.

IV. DISCUSSION

Linkages were established with three poultry performance trials investigating effect of various feed and/or litter on broiler performance. Bacterial profiling was done to investigate changes in gut microbiota and identify potential bacterial indicators linked with performance. Across all three trials four OTU within the ilea (180, 492, 564-566 and 936-938) and five OTU within the caeca (140-142, 216-222, 280-286, 312 and 482) were consistently identified. Targeted cloning and sequencing of 7 of these OTU revealed a possibility for 19 different bacterial species, indicating many of these OTU contain several bacteria which may be contributing to the increase or decrease of a particular performance related OTU. Many of these bacteria were identifiable to the species level however, the majority remain unclassified bacteria. Where bacteria were identifiable to the phyla level they belong predominantly to the Firmicutes and Bacteroidetes. The relative abundance of the Bacteroidetes and Firmicutes have been shown to differ in genetically predisposed obese mice versus lean mice (Turnbaugh *et al.*, 2006) indicating particular bacterial groups have increased capacity for energy harvest. Although many of these potential performance related bacteria are unclassified they do show high sequence similarity (90-100%) with other unclassified bacteria in public genome sequence databases. Many of these identified similar sequences have been obtained from studies investigating the relationship between the gut microbiome and host metabolic phenotype, innate immunity and gut microbiota, gut microbiota in various host species, and several unpublished molecular poultry gut microbiota studies.

V. CONCLUSIONS

These results are promising in our quest to identify gut bacterial species linked to broiler performance, however further well-planned work is required to validate findings. Specific

tests for performance related bacteria would allow us to better evaluate dietary treatments in achieving an optimal gut microbiota for broiler performance and production.

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EFFECTS OF PHYTOGENICS AND ZINC BACITRACIN ON PERFORMANCE AND INTESTINAL HEALTH STATUS OF BROILERS

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Summary

A 35-day trial was carried out to determine effects of phytogenics or zinc-bacitracin on broiler performance and intestinal lesion scores. 2016 Cobb and Ross day-old broilers were assigned to four treatments: (1) negative control, (2) positive control: zinc-bacitracin, (3) NC + phytogenics and (4) Treatment 3 plus coccidiosis vaccination (Paracox-5). Birds were fed starter (day 0–7), grower (day 8–21) and finisher (day 22–35) diets based on corn and soybean meal. Performance parameters did not differ (P > 0.05) between Cobb and Ross broilers. After 35 days the lowest body weight recorded was for the control treatment (1435 g), whereas the highest body weights were recorded for birds in Treatments 3 and 4 (1508 and 1503 g, respectively), these being significantly (P < 0.05) heavier than birds in the negative and positive control. Total feed consumption over the five-week period did not differ (P > 0.05) between treatments. Feed conversion efficiency (FCE), however, was lowest in Treatment 1 (468 g/kg), and differed numerically (P > 0.05) from Treatment 3 (516 g/kg). In contrast, FCE was improved (P < 0.05) in comparison with the control in Treatments 2 and 4 (527 and 519 g/kg, respectively). Feed consumption prior to each feed change did not differ between treatments (P > 0.05), but on the day of the first change (day 7) Treatment 1 recorded the lowest intake (29.5 g), whilst birds on Treatment 4 consumed the most feed (32.2 g; P < 0.05). Feed intake in treatments 2 and 3 (31.1 and 31.5 g, respectively) were higher (P < 0.05) than in the negative control, but numerically lower than in Treatment 4 (P > 0.05). There were no differences in feed intake at the second feed change (day 21). Application of the coccidial vaccine in Treatment 4 did not affect performance parameters (P > 0.05). In spite of small differences in airsac and trachea lesions, the health status of the birds was excellent throughout the trial. In conclusion, phytogenics and zinc-bacitracin beneficially affected performance of broilers reared under good hygienic conditions with little intestinal challenge.

I. INTRODUCTION

Phytogenic compounds are regarded as potential modifiers of gut function and performance, hence representing potential alternatives to antimicrobial growth promoters such as zinc-bacitracin. Indeed, a growing number of scientific reports show that these compounds exert performance-enhancing effects in poultry (Mountzouris et al., 2009; Windisch et al., 2008). The objective of this study was to investigate two areas related to gut function and destabilization during periods of intestinal stress, and the potential of a phytogenic feed additive or and zinc bacitracin to alleviate this stress. The first deals with stress related to the change-over from one feed to the next, and the other, the effect of vaccinating for coccidiosis.

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II. MATERIALS AND METHODS

2016 as-hatched Cobb and Ross broilers, treated at day old with IB-H120 and ND-VH, were used in the trial. They were placed 63 to each of 48 floor pens in a tunnel ventilated broiler house at an initial stocking density of 14 birds/pen. Cross ventilation was used initially to provide fresh air, and gas brooders, centred over the junction between four pens, were used to provide warmth. Control over these conditions was automatic according to the settings applied. After 14 days, use was made of longitudinal ventilation when necessary.

A commercial feed (Meadow Feeds, Pietermaritzburg) without antibiotics, growth promoters or coccidiostats, was used as control. Three phases were implemented: Starter, Grower and Finisher and the trial was terminated at 35 d. The starter diet, in mash form, was fed for 7 d. Feed remaining in the troughs at that point was removed and the grower diet introduced from 8–21 d. Grower and Finisher diets were pelleted. The following treatments were implemented (1) Negative control (NC) diets, (2) Positive control + AGP (Zinc Bacitracin at 125 g/t), (3) NC + Phytogenics (Biomin$^®$ P.E.P. 125 poultry, 125 g/t) and (4) NC + Phytogenics + coccidiosis vaccination. In Treatment 4 Paracox^{TM}- 5 was used to vaccinate the broilers against coccidiosis. Access to nipple drinkers in pens receiving coccidiosis vaccine was barred. Water with vaccine was given in open troughs. None of the birds was treated with a coccidiostat during the trial, nor was it necessary to treat them prophylactically.

Body weight and food intake were recorded at 7, 21 and 35 d. Vent scoring to indicate presence of loose droppings was done on a daily basis. Daily measurements of feed intake were carried out for one day before and three days after the two periods of feed change by weighing the feeders on days 6–9 and 20–23. Lesion score of the intestines was conducted on ten birds per treatment at the end of the trial. The measurements were made using a scoring system that is used in practice for evaluating the efficacy of coccidiostats. Each bird was scored for lesions in the trachea, airsacs and gizzard (erosion), for the presence of *Eimeria* species, and for the status of the Bursa of Fabricius.

III. RESULTS

Performance parameters did not differ (P > 0.05) between Cobb and Ross broilers. After 35 days the lowest body weight recorded was for the control treatment (1435 g), whereas the highest body weights were recorded for birds in Treatments 3 and 4 (1508 and 1503 g, respectively), these being significantly (P < 0.05) heavier than birds in the negative and positive control (Figure 1). Total feed consumption was 3080, 2782, 2836 and 2812 g in Treatments 1, 2, 3 and 4, respectively, and did not differ (P > 0.05) between treatments. Feed conversion efficiency (FCE), however, was lowest in Treatment 1 (468 g/kg), and differed numerically (P > 0.05) from Treatment 3 (516 g/kg) (Figure 2). In contrast, FCE was improved (P < 0.05) in comparison with the control in Treatments 2 and 4 (527 and 519 g/kg, respectively).

Feed consumption prior to the first feed change did not differ between treatments (33.3–35.7 g; P > 0.05), but on the day of the first change (day 7) Treatment 1 recorded the lowest intake (29.5 g), while birds on Treatment 4 consumed the most feed (32.2 g; P < 0.05) (Figure 3). Feed intake in treatments 2 and 3 (31.1 and 31.5 g, respectively) were higher (P < 0.05) than in the negative control, but numerically lower than in Treatment 4 (P > 0.05). There were no differences in feed intake at the second feed change (day 21). Application of the coccidial vaccine in Treatment 4 did not affect performance parameters (P > 0.05).

The health status of broilers at the end of the trial was considered to be excellent taking into account the relative absence of lesions in the airsacs, trachea and gizzard, and the

presence of only *E. maxima* in very few of the birds sampled (Table 1). The size of the Bursa was consistent with healthy, uninfected birds in all cases. No statistical analysis was possible on these data.
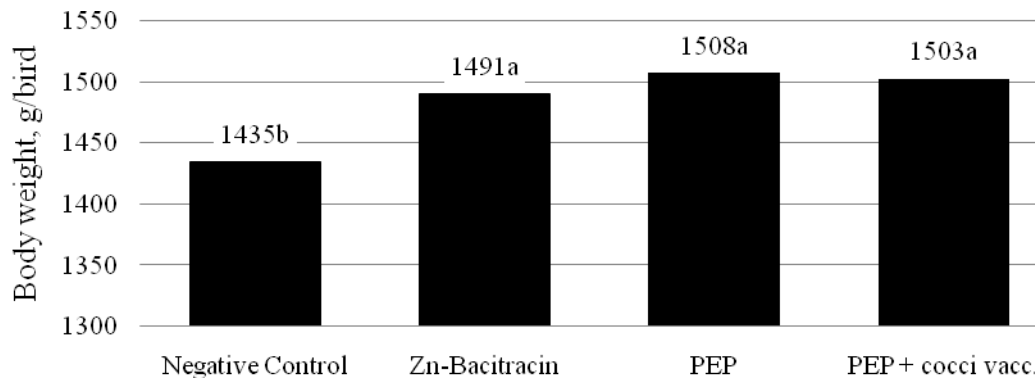

Figure 1.   Body weight as affected by antibiotics, phytogenics and phytogenics plus coccidiosis vaccination. [a,b]Means with a different superscript differ significantly (P < 0.05).

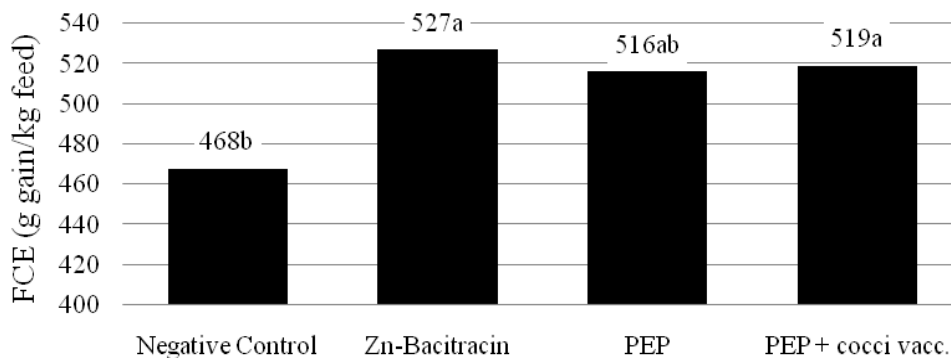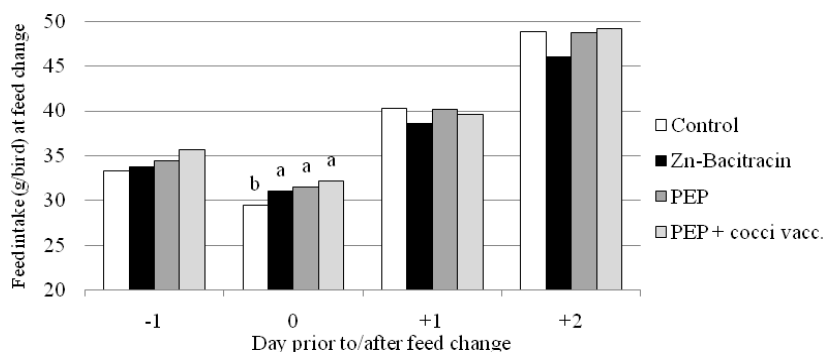Figure 2.   Feed Conversion Efficiency (FCE) as affected by antibiotics, phytogenics and phytogenics plus coccidiosis vaccination. [a,b]Means with a different superscript differ significantly (P < 0.05).

Figure 3.   Feed intake per bird prior to and following the first change in feed composition

Table 1.   Incidence of various lesions, and bursa size, of 10 birds from each treatment

Treatment	Airsac lesions[1]	Trachea lesions[1]	Gizzard erosion[1]	*E. maxima*	Bursa Size[2]
1	2	0	1	8	5
2	2	0	1	1	6
3	5	1	1	0	6
4	3	0	0	1	5

[1] Scoring: 0–5 (0 = no lesions × number of birds showing lesions)

[2] Scoring: 0–6 (6 being a healthy Bursa for that age)

IV. DISCUSSION

Changes in feed composition represent a potential stressor, which may result in a destabilization of the gut due to a change in intestinal microflora. This destabilization often presents as visual wet droppings for a few days and a lag in daily weight gain. This would be demonstrated by a reduction in feed intake resulting from the feed change, which confirms previous reports (Windisch et al., 2008). The addition of the phytogenic feed additive improved performance of broilers to 35 d in this trial. Although the total amount of feed consumed per bird did not differ between treatments, the improved growth rate at a similar feed intake resulted in improved feed conversion efficiencies when this feed additive was included. Effects were, in most cases, similar to those obtained with zinc-bacitracin. In conclusion, phytogenics and zinc-bacitracin beneficially affected performance of broilers reared under good hygienic conditions with little intestinal challenge. The additional feed consumption in these treatments at the first feed change might have been partly responsible for the heavier body weights of birds at the end of the growing period.

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THE ABILITY OF GREEN TEA TO POSITIVELY MODIFY THE GUT MICROFLORA IN BROILER CHICKENS

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Summary

The objective of the current study was to investigate the influence of two green teas [normal (N-GTE) and selenium-containing (Se-GTE) green teas] on changes in gut bacterial population of broiler chickens. The effects of green teas on faecal and caecal microflora, and faecal bacterial metabolic activities were studied in broilers fed a basal diet or basal diet supplemented with 1.0% green tea for 35 days. Gut microbial communities were analysed by the Florescent in Situ Hybridisation (FISH) technique at 7, 21, and 35 days of age. Dietary inclusion of green tea had a positive effect on both feed efficiency and on the gut microflora with an increased numbers of beneficial bacteria (*Lactobacillus spp.* and *Bifidobacterium spp.*) and a reduced number of pathogenic bacteria (*Clostridium spp.* and *Bacteroides spp.*). Although both teas showed beneficial effects on feed efficiency, Se-GTE was more beneficial than N-GTE in the positive modulation of gut microflora. Relative to the control group, the level of faecal bacterial ß-glucuronidase, an enzyme generated mainly by *Escherichia coli*, *Bacteroides spp.*, and *Clostrdium spp.*, was decreased significantly while the level of ß-glucosidase, an enzyme generated mainly by lactobacilli and bifidobacteria, was increased significantly in the faecal contents of the chickens treated with both teas. Moreover, the pH of the faecal contents collected from the birds fed diets with green teas was significantly lower than that of the control group. In conclusion, the results of this study are beneficial in developing and evaluating natural alternatives to in-feed antibiotics for sustainable poultry production and also provide evidence that gut microbial community composition is associated with growth performance.

I. INTRODUCTION

The role of commensal gut microflora in animal production is receiving much interest, particularly with the global trend of moving away from in-feed antibiotics. The gut contains a complex microbial community including potentially beneficial and pathogenic bacteria. An unfavourable microflora may promote enteric infections, leading to decreased growth rates and increased mortality. Consequently, a favourable gut microflora is necessary for the optimal growth and performance of chickens.

Green tea is derived from the leaves and fine stems of the leguminous shrub, *Camellia sinensis* with health promoting effects mainly attributed to its polyphenol content (Wolfram et al., 2006) and antioxidant activity (Molan et al., 2009). Green tea has been shown in rodent studies (Molan et al., 2007) to modify gut microflora, which may impact upon the digestibility of nutrients, and affect chick performance. In this study changes in growth and feed efficiency were measured and gut microbial communities analysed to assess the effect of green tea dietary supplementation.

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II. MATERIALS AND METHODS

Two experimental diets were prepared by the addition of one of two types of powdered green tea to a wheat based control diet at an inclusion rate of 1% giving a total of three diets. The green tea types used were either locally sourced Chinese green tea (N-GTE) or a high selenium green tea (Se-GTE) sourced from China. The diets were formulated to meet or exceed the NRC (1994) requirements for all nutrients for broilers and were pelleted at 70 ^{0}C. Each diet was fed to six cages (8 birds/ cage). The birds received fluorescent illumination for 20 hours per day, and were allowed free access to the diets and water. Body weights and feed intakes were measured weekly. In addition, a cohort study (n=192) was undertaken to provide sampling of digesta on days 7, 21 and 35.

Bacterial measurement was undertaken on days 7, 21 and 35 using fluorescent in situ hybridization (FISH) analysis of microflora. The probes used in the study were specific for *Lactobacillus spp.*, *Bifidobacterium spp.*, *Clostridium spp.,* and *Bacteroides spp.* These were commercially synthesised and labelled with the fluorescent dye Cy3 (GeneWorks, Australia). The procedure described by Dinoto et al. (2006) was followed with some modifications. The β-glucuronidase and β-glucosidase activities were determined aerobically according to the method of Goldin et al. (1980) and as described by Preter et al. (2008) with some modifications. Faecal pH was measured on days 7, 21 and 35. The pH was measured using a digital pH-meter at room temperature (20-22 ^{o}C).

Populations for each bacterial group were expressed as log number of bacterial cells/ gram caecal materials. Logarithmically-transformed data were analysed by one way analysis of variance using SAS (version 9.1) with the level of significance set at $P < 0.05$.

III. RESULTS AND DISCUSSION

Normal Chinese green tea (N-GTE) supplementation reduced ($P < 0.05$) weight gain during the 5-week trial period, and high selenium green tea (Se-GTE) supplementation reduced ($P < 0.05$) weight gain on days 7 and 35. Feed intake was reduced ($P < 0.05$) in both treatment groups over the whole trial period, but feed efficiency was improved ($P < 0.05$) as green tea supplementation reduced feed intake to a greater extent than weight gain.

Dietary inclusion of green tea had a positive effect on the gut microflora (Table 1) with an increased numbers of beneficial bacteria (*Lactobacillus spp.* and *Bifidobacterium spp.*) and a reduced number of pathogenic bacteria (*Clostridium spp* and *Bacteroides spp.*). Differences between the two green tea types were observed, with Se-GTE more beneficial than N-GTE in the positive modulation of gut microflora. A number of studies have shown that increasing the numbers of lactic acid bacteria (lactobacilli and bifidobacteria) in the colon reduces the formation of ammonia, skatole, harmful amines and other procarcinogens in the large intestine and the carcinogenic load on the intestine of humans (Burns and Rowland 2000; Yamamoto et al., 1997).

Table 1.   Influence of dietary treatments on the enumeration of *Bifidobacterium* species, *Lactobacillus* species, *Bacteroides* group and *Clostridium* species [Log_{10} cells/g of wet caecal contents] in caecal samples of broilers

	Day 7		Day 21		Day 35	
	$\underline{Log_{10}}$	$\underline{SE}$	$\underline{Log_{10}}$	$\underline{SE}$	$\underline{Log_{10}}$	$\underline{SE}$
Bifidobacteria						
Control	7.12	0.021	6.98	0.040	7.19	0.023
N-GTE	7.13	0.025	7.30***	0.044	7.27**	0.024
Se-GTE	7.20*	0.021	7.29***	0.029	7.32***	0.012
Lactobacilli						
Control	6.06	0.029	5.91	0.062	5.68	0.059
N-GTE	6.06	0.039	5.98	0.045	6.16***	0.022
Se-GTE	6.16*	0.032	6.16**	0.040	6.20***	0.024
Clostridia						
Control	7.14	0.032	7.00	0.027	7.08	0.020
N-GTE	6.96***	0.037	6.77***	0.036	6.98	0.058
SE-GTE	6.82***	0.039	6.77***	0.031	6.42***	0.043
Bacteriodes						
Control	7.20	0.033	7.06	0.031	7.34	0.027
N-GTE	7.10	0.065	7.03	0.047	6.53***	0.034
Se-GTE	6.82***	0.070	6.98	0.030	6.22***	0.045

* P < 0.05; ** P < 0.01 and *** P < 0.001 (significantly different from control).

Inclusion of both green teas in the diet resulted in a significant reduction (P < 0.01 to 0.0001) in the numbers of pathogenic bacteria. Physiological concentrations of green tea polyphenols and extracts have been shown in *in vitro* studies to delay or inhibit the growth of a wide range of pathogenic strains of enteric bacteria, including pathogenic strains of *Escherichia coli* (Yam et al., 1997; Ciraj et al., 2001; Ishihara et al., 2001).

Relative to the control group, the level of faecal bacterial ß-glucuronidase, an enzyme generated mainly by *Escherichia coli*, *Bacteroides spp*., and *Clostridium spp*., was decreased significantly (P < 0.05) in the faecal contents of the chickens fed diets with both green teas. Moreover, the level of ß-glucosidase, an enzyme generated mainly by lactobacilli and bifidobacteria, was increased significantly (P < 0.05) in the faecal contents of the chickens fed diets containing with both green teas when compared with the control group.

In addition, the pH of the faecal contents from birds fed diets with Se-GTE was significantly lower (P < 0.05) than that of the control group. Supplementation with N-GTE resulted in a significant decline (P < 0.05) in the pH at 21 and 35 days of age. The pH was significantly lower in the faecal samples collected from birds supplemented with Se-GTE than those supplemented with N-GTE after 7 days (P < 0.01) and 35 days (P < 0.05). The lower pH in the faecal samples of birds fed diets with green teas, compared to the controls, may be related to the growth of lactic acid bacteria.

IV. CONCLUSIONS

In conclusion, the present data suggest that green tea supplementation may be beneficial in maintaining a healthy gut microflora in the absence of in feed antibiotics and assist in efficient feed utilisation in broilers.

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EFFECTS OF NOVEL FEED ADDITIVES ON GUT HEALTH AND OVERALL PERFORMANCE IN BIRDS CHALLENGED WITH *CLOSTRIDIUM PERFRINGENS*

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Summary

The capacity for *Lactobacillus johnsonii* and an organic acid (OA) blend to prevent necrotic enteritis (NE) was studied. Additionally, we evaluated the influence of *Clostridium perfringens* challenge, zinc bacitracin (ZnB), *L. johnsonii* and OA on the intestinal microbiota. Cobb 500 birds were allocated into six groups; unchallenged (Control), challenged (Cp), zinc bacitracin (ZnB), organic acid (OA), *L. johnsonii*, and vehicle (n = 25 birds/pen, 8 pens/treatment). All birds were challenged with *C. perfringens* except for the Control group. Only birds fed ZnB were protected from NE as indicated by maintenance of body weight, low mortality and clostridia levels, and decreased intestinal macroscopic lesion score compared to Cp-challenged controls. *L. johnsonii*-fed birds had reduced lesion scores whilst OA-fed birds had reduced clostridia levels. Both *L. johnsonii* and OA-fed birds had improved feed conversion ratios; however, mortality and body weights were not improved by either treatment. Microbial profiling indicated that *C. perfringens* significantly altered the jejunal microbiota. The microbiota of ZnB-fed birds was different to all other treatments. Whilst OA and *L. johnsonii* altered some intestinal parameters, no protection against NE was observed. The search for alternatives to antibiotics is important for the poultry industry. Knowledge of the mechanisms involved in ZnB-mediated protection may lead to the identification of compounds (or combinations) that promote a similar intestinal environment.

I. INTRODUCTION

Necrotic enteritis (NE) caused by the bacterium *Clostridium perfringens*, is one of the world's most prominent and severe poultry diseases. The economic impact of NE on the world-wide poultry industry is estimated at over $2 billion per annum (Choct and Kocher, 2008). NE is typified by intestinal lesions, diarrhoea, impaired digestive function, reduced nutrient absorption, and decreased feed intake (Kocher et al., 2004). In its acute form, NE can cause significant flock mortality over a period of several days whilst sub-clinical NE can significantly impair bird performance.

Currently in Australia, NE is controlled by the in-feed supplementation of antibiotics such as zinc bacitracin (ZnB). The European Union have enforced a ban on the use of in-feed antibiotics, and consumer pressure in other regions may force similar restrictions on antibiotic use. Therefore, alternative strategies for the control of NE are needed which can limit the health and economic impacts of the disease. The aims of this study were to assess the capacity for an organic acid (OA) product and a candidate probiotic organism, *Lactobacillus johnsonii*, to reduce the severity of NE in broilers. In addition, we aimed to assess the effects of these compounds on the intestinal microbiota.

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II. MATERIALS AND METHODS

Twelve hundred male Cobb 500 birds were randomly allocated to 48 pens (n=25 birds/pen) and assigned to six treatment groups (8 pen replicates/treatment); unchallenged (Control), *Clostridium perfringens*-challenged (Cp), ZnB (45 ppm + 100 ppm monensin in the diet), OA (2 kg/ton in the diet), *L. johnsonii* (10^9 cfu/ml in PBS, administered orally on days 1, 3, 7, and 12), or vehicle control (PBS gavage on corresponding days). All groups were challenged with *C. perfringens* except for the Control group. The NE challenge procedure was carried out as described previously (Kocher et al., 2004; Mikkelsen et al., 2009). Birds were fed a starter diet from placement until day 7, and again between days 15-22, with finisher diet from day 23 until completion of the trial. Between days 7-15, birds were fed a high protein diet (50% fishmeal and 50% starter) to facilitate NE development. On day 9, all birds (except unchallenged controls) were given a suspension of 2,500 oocysts of *Eimeria acervulina*, *E. maxima* and *E. tenella* in 1 ml PBS. On day 15, birds in challenged groups were individually inoculated with 1 ml of *C. perfringens* at a concentration of 3.5 x 10^8 cfu/ml.

Live weight and feed consumption were recorded on days 0, 9, 14, 21 and 28 and feed conversion ratios were calculated. On days 15 and 18 two chickens per pen (n=16 birds/treatment) were selected for intestinal lesion scoring and clostridia enumeration. The small intestine from each bird was incised longitudinally and examined for gross necrotic lesions. Lesions were scored according to the previously described criteria (Prescott et al., 1978). Clostridia enumeration was performed on days 15 and 18 as described previously (Mikkelsen et al., 2009). On day 18, 2 birds in each pen (n=16 birds/treatment) were killed for tissue collection. A segment (3 cm) of tissue and associated digesta was collected from the mid-point of the jejunum and stored at 4°C until later frozen for microbial profiling by terminal-restriction fragment length polymorphism (T-RFLP) analysis and assessment of *Lactobacillus* species by denaturing gradient gel electrophoresis (Lac PCR-DGGE).

III. RESULTS

On day 9, prior to *C. perfringens* challenge, birds treated with *L. johnsonii* had a significantly greater body weight compared to birds fed basal diet (Unchallenged and Cp-challenged control groups; P<0.05; Table 1). On days 21 and 28, birds fed ZnB had the greatest body weights compared to all other Cp-challenged groups (P<0.05). The mean body weight of ZnB-fed birds was comparable to unchallenged birds (P>0.05). Birds fed ZnB had a lower FCR compared to other Cp-challenged groups in the periods between days 0-21 and 0-28 (P<0.05). Birds fed OA had a significantly lower FCR compared to Cp-challenged controls between days 0-21 and 0-28 (P<0.05). In the period between days 0-28, birds treated with *L. johnsonii* also had a lower FCR compared to Cp-challenged controls (P<0.05).

In the period following *C. perfringens* challenge (days 14-21), bird mortality was significantly elevated in Cp-challenged controls, vehicle controls, OA and *L. johnsonii*-treated groups compared to unchallenged birds and challenged birds fed ZnB (P<0.05; Table 1). On day 18, ZnB and OA treatment significantly reduced clostridia levels compared to Cp-challenged controls (P<0.05; Table 2). On day 18, duodenum macroscopic lesion scores were significantly greater in Cp-challenged controls, vehicle controls and OA-fed birds compared to unchallenged controls (P<0.05). ZnB and *L. johnsonii*-treated birds had significantly lower duodenum lesion scores compared to Cp-challenged controls, vehicle controls and OA-fed birds whilst having a lesion score comparable to unchallenged birds (P>0.05). In the jejunum, day 18 lesion scores were significantly greater in Cp-challenged controls, vehicle controls and OA-treated birds compared to unchallenged controls (P<0.05). ZnB and *L. johnsonii*-treated birds had a significantly lower score compared to Cp-challenged controls (P<0.05).

Table 1.   Body weight, feed conversion and mortality

	Control	Cp	ZnB	OA	Vehicle	*Lj*	P
Weight							
0	37 ± 0.2	38 ± 0.4	38 ± 0.3	37 ± 0.2	37 ± 0.1	38 ± 0.3	-
9	196 ± 1^{bc}	192 ± 5^{c}	201 ± 4^{abc}	205 ± 2^{ab}	201 ± 1^{abc}	208 ± 2^{a}	**
14	380 ± 4	368 ± 6	386 ± 5	380 ± 4	378 ± 5	383 ± 5	-
21	800 ± 10^{a}	552 ± 26^{b}	817 ± 12^{a}	567 ± 20^{b}	580 ± 14^{b}	574 ± 13^{b}	***
28	1374 ± 17^{a}	1008 ± 46^{b}	1420 ± 14^{a}	1048 ± 30^{b}	1082 ± 15^{b}	1065 ± 21^{b}	***
Mortality							
0-9	0.5	3.0	3.0	1.5	2.0	2.0	-
9-14	2.5	1.0	0.5	3.0	1.0	1.5	-
14-21	5.5^{a}	36.0^{b}	7.5^{a}	30.5^{b}	35.5^{b}	35.5^{b}	***
21-28	1.0	0.0	2.5	1.0	0.0	1.0	-
FCR							
0-21	1.29 ± 0.01^{c}	1.58 ± 0.06^{a}	1.25 ± 0.01^{c}	1.47 ± 0.02^{b}	1.56 ± 0.04^{ab}	1.53 ± 0.03^{ab}	***
0-28	1.41 ± 0.02^{cd}	1.66 ± 0.08^{a}	1.37 ± 0.01^{d}	1.51 ± 0.01^{bc}	1.59 ± 0.04^{ab}	1.51 ± 0.03^{bc}	***

Live weight data (g) and feed conversion ratio (FCR) are expressed as mean $\pm$ SEM (n=8 pens/treatment). Mortality data are expressed as percentage (%). Values within a row that do not share a common letter are significantly different (P<0.05). ** P<0.01; *** P<0.001. Cp, *Clostridium perfringens*; Lj, *Lactobacillus johnsonii*; OA, organic acid; ZnB, zinc bacitracin.

Table 2.   Macroscopic intestinal lesion scores and clostridia enumeration

	Control	Cp	ZnB	OA	Vehicle	*Lj*	P
Duodenum lesions							
Day 15	0.0 ± 0.0^{a}	0.84 ± 0.20^{bc}	0.0 ± 0.0^{a}	1.13 ± 0.17^{c}	1.13 ± 0.15^{c}	0.38 ± 0.17^{b}	***
Day 18	0.0 ± 0.0^{a}	0.56 ± 0.17^{b}	0.06 ± 0.04^{a}	0.28 ± 0.10^{b}	0.53 ± 0.17^{b}	0.09 ± 0.09^{a}	***
Jejunum lesions							
Day 15	0.0 ± 0.0^{a}	0.31 ± 0.14^{b}	0.0 ± 0.0^{a}	0.78 ± 0.27^{b}	0.59 ± 0.20^{b}	0.28 ± 0.13^{b}	**
Day 18	0.0 ± 0.0^{a}	0.75 ± 0.27^{c}	0.0 ± 0.0^{a}	0.50 ± 0.18^{bc}	0.38 ± 0.10^{bc}	0.19 ± 0.11^{ab}	***
Clostridia (log_{10} cfu/g)							
Day 15	3.50 ± 0.12	3.81 ± 0.28	3.60 ± 0.20	5.14 ± 0.69	3.71 ± 0.43	3.47 ± 0.24	-
Day 18	3.69 ± 0.28^{ab}	4.23 ± 0.35^{a}	3.45 ± 0.32^{b}	3.42 ± 0.16^{b}	4.16 ± 0.22^{a}	4.44 ± 0.35^{a}	*

Lesion scores and clostridia levels (log_{10} cfu/g digesta) are expressed as mean $\pm$ SEM. Values within a row that do not share a common letter are significantly different (P<0.05). * P<0.05; ** P<0.01; *** P<0.001.

Unchallenged control birds had a significantly different microbial profile in the jejunum compared to Cp-challenged controls (P<0.05; Table 3), indicating that the challenge procedure had a direct impact on the overall microbiota. Unchallenged birds also displayed a significantly different microbial profile compared to ZnB, OA and vehicle-treated birds (P<0.05), with a strong trend toward a difference compared to *L. johnsonii*-treated birds (P<0.1). The microbial communities of Cp-challenged controls were significantly different compared to ZnB and vehicle-treated birds (P<0.05); however, they were not significantly different compared to OA and *L. johnsonii*-treated birds (P>0.05). Birds fed ZnB had a significantly different microbial composition compared to all other treatments (P<0.001). Birds treated with OA, vehicle and *L. johnsonii* were not significantly different to one another; however there appeared to be a trend toward a difference between OA and vehicle-treated birds (P<0.1). *C. perfringens* challenge significantly influenced the *Lactobacillus* communities of broilers (P<0.05; data not shown). Birds fed ZnB had a significantly different *Lactobacillus* profile compared to all other treatments (P<0.05). Interestingly, *L. johnsonii* was only detected in half of the birds treated with *L. johnsonii* on days 1, 3, 7 and 12.

Table 3.   One-way analysis of similarities (ANOSIM) of microbial communities

	Control	Cp	ZnB	OA	Vehicle	*Lj*
Control	-	**0.134**	**0.404**	**0.132**	**0.127**	**0.066**
Cp	*0.012*	-	**0.299**	**0.004**	**0.103**	**-0.01**
ZnB	*<0.001*	*<0.001*	-	**0.457**	**0.546**	**0.374**
OA	*0.014*	*0.388*	*<0.001*	-	**0.060**	**-0.005**
Vehicle	*0.021*	*0.024*	*<0.001*	*0.084*	-	**-0.008**
Lj	*0.078*	*0.543*	*<0.001*	*0.483*	*0.513*	-

Data are expressed as R-statistic (bold), with significance level (italics). P<0.05 was considered significant. The global R-value was 0.178 at a significance level of 0.0001 which is considered significant.

IV. CONCLUSION

In-feed supplementation with *L. johnsonii* or OA did not successfully prevent the onset of NE in broilers, whilst conventional treatment with ZnB prevented the development of NE and maintained bird performance during the challenge. *C. perfringens* challenge appeared to significantly alter the microbiota, and ZnB shifted the intestinal microbial communities compared to all other treatments. The unique microbial profile produced by ZnB may be associated with improved health and performance. The potential role of probiotics and OAs in the prevention of NE remains unclear; however, the observations that OA decreased intestinal clostridial load and *L. johnsonii* decreased lesion scores whilst both partially improved FCR indicate that other compounds and organisms of this nature, and combinations of OAs and probiotics may indeed have the capacity to reduce the severity of NE.

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FEED ADDITIVES INFLUENCE GOBLET CELL DISTRIBUTION AND VILLUS-CRYPT ARCHITECTURE IN BROILERS AFTER NECROTIC ENTERITIS CHALLENGE

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Summary

The probiotic, *Lactobacillus johnsonii* and a commercial organic acid (OA) blend were histologically evaluated as potential non-antibiotic preventatives for necrotic enteritis (NE). A total of 1200 Cobb 500 broilers were randomly assigned to the following six treatment groups; unchallenged (Control), *Clostridium perfringens* challenged (Cp), zinc bacitracin (ZnB), organic acid (OA), vehicle and *L. johnsonii* (*Lj*) (n=25 birds/pen, 8 pens/treatment). All treatment groups with the exception of the Control group were challenged with *C. perfringens* (Cp). Histological examination revealed that OA and *Lj* were unsuccessful in preserving intestinal architecture, however ZnB maintained villus:crypt structure comparable to the Control group. Total goblet cell (GC) number/mm villus surface length was not significantly different amongst any of the dietary treatment groups. No interactions between dietary treatment and mucin type were observed. The number of neutral mucin containing GC was significantly greater in comparison to the number of total acidic mucin containing GC in all dietary treatment groups. Cp challenge did not appear to affect the number of neutral or acidic mucin GC however ZnB decreased the number of both neutral and total acidic mucin GC. No significant differences in acidic mucin subtypes (sulphated, sialylated and intermediate) were observed amongst dietary treatments. Sulphated mucin containing GC were the dominant subtype within all treatment groups. Understanding how Cp affects the intestinal wall will assist in the search for NE preventatives.

I. INTRODUCTION

Necrotic enteritis (NE), caused by *Clostridium perfringens* (Cp), is a serious disease threatening the poultry industry worldwide (McDevitt et al., 2006). It is characterised by mortality and intestinal lesions in the clinical form, whilst sub-clinical NE is associated with diarrhoea and lowered feed efficiency (Van Immerseel et al., 2004). For many years NE has primarily been controlled by the inclusion of in-feed sub-therapeutic antibiotics. The withdrawal of in-feed antibiotics in the European Union and voluntary reductions in other regions has increased the incidence of NE, hence there is a need to identify non-antibiotic alternatives for NE prevention. The probiotic, *L. johnsonii* (*Lj*) and a commercial organic acid (OA) blend have demonstrated potential as antibiotic alternatives (La Ragione et al; Zelenika et al., 2005). *Lj* has been shown to suppress all aspects of colonisation of Cp, possibly by immunomodulation or competitive exclusion (La Ragione et al., 2004), whilst OA has improved bird performance, perhaps by disrupting the proton gradient across the bacterial cell membrane (Zelenika et al., 2005). Probiotics and organic acids have also been shown to alter the mucin profile during bacterial challenge or in unchallenged birds respectively.

The mucus layer is the first line of defence encountered by gut bacteria. Mucins, which are the primary constituent of the mucus layer, are high molecular weight

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glycoproteins secreted by intestinal goblet cells (Smirnov et al., 2006). Goblet cells can be histologically separated into two types; those that produce neutral or acidic mucin on the basis of terminal sugars. The latter can be further divided into two subtypes; sulphated or sialylated. Diet and bacteria are known to influence mucin subtype distribution, however little is known in regard to changes in mucin profile during Cp challenge. It is well known that NE disrupts intestinal integrity and villus:crypt architecture, consequently lowering nutrient absorption and bird performance. In-feed antibiotics are known to reduce NE associated intestinal damage however there is minimal literature on the ability of organic acids or probiotics to prevent gut damage in NE challenged birds. This study investigated the effect of *Lj* and an OA blend on the intestinal morphology and mucin profile of Cp challenged broilers to aid evaluation of their potential as non-antibiotic preventatives for NE.

II. MATERIALS AND METHODS

This NE challenge experiment was carried out at the University of New England (Armidale, NSW, Australia) under the guidance of Dr. Lene Mikkelsen and colleagues, as part of CRC project 05-2 managed by SARDI researchers. A total of 1200 male Cobb 500 broilers were reared from day old in 48 pens (n=25 birds/pen). Pens were randomly assigned to the following six treatment groups (8 pens/group) unchallenged (Control), *C. perfringens* challenged (Cp), ZnB (50 ppm in the diet), OA (2kg/ton in the diet), *Lj* (10^9 cfu/ml *L. johnsonii* in PBS on days 1, 3, 7 and 12 administered by oral gavage) or vehicle (PBS gavage on corresponding days). All treatment groups with the exception of the Control were inoculated with a suspension of three *Eimeria* sp. on day 9 and Cp type A (3.5×10^8 cfu/ml) on day 15, according to the NE challenge procedure described previously (Kocher et al., 2004; Mikkelsen et al., 2009). Birds were fed a starter diet and corresponding dietary treatments from days 0-6 and 15-22 inclusive and a finisher diet until day 28. Between days 7-15 birds were fed a high protein diet (50% fishmeal and 50% starter) to aid NE development. On day 18, 1 cm tissue samples were collected from the midpoint of the duodenum, jejunum and ileum (n=12 birds/treatment) and later embedded in paraffin.

Standard haematoxylin and eosin staining was performed on 8 μm jejunal sections. Villus height (VH), crypt depth (CD) and villus width (VW) were measured from 15 randomly selected villi and associated crypts from each bird by microscopic examination. Apparent villus surface area (SA) was calculated using the formula SA=2π(VW/2)(VH) (Sakamoto et al., 2000).

Periodic acid-Schiff (PAS) staining methods were used to identify neutral mucin containing GC and high iron diamine-alcian blue (HID-AB) pH 2.5 staining was used to distinguish between the two acidic mucin subtypes; sialylated and sulphated, on 10 randomly selected villi for each bird. GC containing both sulphated and sialylated mucin concurrently were termed intermediate. Light microscopy and image analysis were used to determine the mean number of GC per unit of villus surface length (mm).

III. RESULTS

Birds from the Control and ZnB treatment groups had significantly greater villus heights and VH:CD and reduced crypts and villus width (P < 0.05; Table 1) in comparison to birds from the Cp, OA, Vehicle and *Lj* treatment groups. ZnB fed birds maintained an intestinal villus:crypt architecture comparable to Control birds (P > 0.05; Table 1). No significant differences in any of the intestinal histomorphological parameters were observed amongst the Cp, OA, Vehicle and *Lj* treatment groups with the exception of the OA group displaying a significantly greater mean villus width in comparison to the Cp group (P < 0.05; Table 1).

Table 1   Histomorphological parameters in the jejunum from 18 day old broilers, 3 days post *Clostridium perfringens* challenge[1]

	Villus height (μm)	Crypt depth (μm)	VH:CD	Villus width (μm)	Apparent villus SA[2] (x10^5 μm^2)
Control	880 ± 39.4 [x]	182 ± 12.3 [y]	5.3 ± 0.4 [x]	68 ± 4.5 [z]	1.9 ± 0.1
C. perfringens	656 ± 64.2 [y]	306 ± 14.7 [x]	2.3 ± 0.3 [y]	85 ± 2.5 [y]	1.8 ± 0.2
Zinc bacitracin	997 ± 42.4 [x]	221 ± 10.2 [y]	4.9 ± 0.3 [x]	73 ± 1.9 [z]	2.3 ± 0.1
Organic acid	550 ± 66.7 [y]	298 ± 17.0 [x]	2.1 ± 0.2 [y]	97 ± 2.9 [x]	1.7 ± 0.2
Vehicle	576 ± 61.7 [y]	308 ± 18.5 [x]	2.1 ± 0.3 [y]	92 ± 2.8 [xy]	1.7 ± 0.2
L. johnsonii	641 ± 60.4 [y]	307 ± 12.4 [x]	2.3 ± 0.2 [y]	93 ± 2.7 [xy]	1.8 ± 0.2

[1]Values are means ± SEM (n=12 birds/treatment). Means within the same column with different superscripts differ significantly (P < 0.05) [2] SA; surface area.

No significant difference in total GC number was observed amongst treatment groups (P > 0.05; Table 2). No interaction amongst dietary treatments and mucin type was evident. Cp challenge did not appear to affect the number of neutral or total acidic mucin GC; whilst ZnB decreased the number of neutral mucin GC in comparison to birds in the Cp group and the number of total acidic mucin GC in comparison to birds in the Control, Cp and *Lj* group. The number of neutral mucin containing GC was significantly greater than total acidic mucin containing GC in all dietary treatment groups (P < 0.05; Table 2).

Table 2   Goblet cell (GC) mucin composition and total GC numbers in the jejunum of 18 day old broilers, 3 days post *Clostridium perfringens* challenge[1]

	Neutral	Total acidic	Total GC
Control	75.0 ± 3.1 [xy]	65.0 ± 2.0 [x]	139.8 ± 4.4
C. perfringens	80.6 ± 3.1 [x]	63.6 ± 4.8 [xy]	144.2 ± 7.2
Zinc bacitracin	68.8 ± 2.2 [y]	56.8 ± 1.8 [z]	125.6 ± 3.7
Organic acid	74.4 ± 2.1 [xy]	59.3 ± 1.6 [yz]	133.7 ± 2.5
Vehicle	73.1 ± 2.7 [y]	58.7 ± 3.1 [yz]	131.9 ± 5.2
L. johnsonii	75.0 ± 2.3 [xy]	62.5 ± 2.6 [xy]	137.5 ± 4.0

[1]Values are means ± SEM of the number of goblet cells/mm of villus surface length (n=12 birds/treatment). Means within the same column with different superscripts differ significantly (P < 0.05).

There were no significant differences in acidic mucin subtype amongst dietary treatments (P > 0.05; Table 3). However the number of acidic mucin subtypes was significantly different within each dietary treatment (P < 0.05; Table 3). Sulphated mucin was the dominant mucin subtype within all dietary treatments; whilst numbers of intermediate mucin containing GC were the lowest (P < 0.05; Table 3).

Table 3   Acidic goblet cell mucin composition in the jejunum of 18 day old broilers, 3 days post *Clostridium perfringens* challenge[1]

	Sulphated	Sialylated	Intermediate
Control	32.6 ± 5.2[a]	22.3 ± 4.4[b]	11.0 ± 1.3[c]
C. perfringens	40.0 ± 6.2[a]	16.1 ± 3.3[b]	7.5 ± 1.6[c]
Zinc bacitracin	35.4 ± 4.6[a]	12.8 ± 3.4[b]	8.6 ± 1.5[c]
Organic acid	33.0 ± 5.0[a]	18.9 ± 3.6[b]	7.4 ± 1.3[c]
Vehicle	29.0 ± 4.4[a]	20.4 ± 4.0[b]	9.3 ± 1.3[c]
L. johnsonii	33.0 ± 5.4[a]	22.9 ± 4.9[b]	6.7 ± 1.1[c]

[1]Values are means ± SEM of the number of goblet cells/mm of villus surface length (n=12 birds/treatment). Means within the same row with different superscripts differ significantly ($P < 0.05$).

IV. CONCLUSION

The probiotic, *L. johnsonii* and an organic acid blend did not successfully ameliorate histological signs of intestinal damage or alter the intestinal mucin profile in broilers challenged with Cp. Conventional treatment with zinc bacitracin maintained intestinal villus:crypt architecture in Cp challenged birds. The mucin profile did not appear to be affected by Cp challenge, however zinc bacitracin decreased the number of neutral and total acidic mucin containing GC. Neutral mucin was the dominant mucin type contained in GC irrespective of treatment, whilst sulphated mucin was the primary acidic mucin GC subtype. Thus it is possible that Cp does not influence the mucin profile however further investigation is required, particularly into the function and structure of acidic mucin subtypes. Although *L. johnsonii* and the organic acid blend have not demonstrated potential as antibiotic alternatives in this study, similar compounds and organisms, which can prevent Cp colonisation and maintain gut integrity, may improve bird performance and prevent NE.

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