14TH ANNUAL AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

SYDNEY, NEW SOUTH WALES

11 – 13 FEBRUARY 2002

Organised by

THE POULTRY RESEARCH FOUNDATION
(University of Sydney)

and

THE WORLD'S POULTRY SCIENCE ASSOCIATION
(Australian Branch)

Papers presented at this Symposium have been refereed by external referees and by members of the Editorial Committee. However, the comments and views expressed in the papers are entirely the responsibility of the author or authors concerned and do not necessarily represent the views of the Poultry Research Foundation or the World's Poultry Science Association.

Enquiries regarding the Proceedings should be addressed to:

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

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

ISSN-1034-6260

AUSTRALIAN POULTRY SCIENCE SYMPOSIUM
2002

AUSTRALIAN POULTRY AWARD

Previous recipients of the award are:

1964	Mr A.O. Moll	1983	Dr R.K. Ryan
1965	Dr M.W. McDonald	1984	Mr C. Donnelley
1966	Professor R.B. Cumming	1985	Dr P. Gilchrist
1967	Mr F. Skaller	1986	Dr C.A.W. Jackson
1968	Professor G.L. McClymont	1987	Mr E. Rigby
1969	Dr S. Hunt	1988	Mr W. Shaw
1970	Dr L. Hart	1989	Dr H. Bray
1971	Mr N. Milne	1990	Dr M. Mackenzie
1972	Mr R. Morris	1991	Professor D.J. Farrell
1973	Mr J. & Mr R. Ingham	1992	Dr B.L. Sheldon
1974	Mr S.J. Wilkins	1993	Mr R. Macindoe
1975	Professor C.G. Payne	1994	Mr B. Bartlett
1976	Mr W. Stanhope	1995	Dr R.A.E. Pym
1977	Professor B. Sinkovic	1996	Dr E.E. Best
1978	Mr J. Douglas	1997	Mr M. Peacock
1979	Mr D. Blackett	1998	Professor D. Balnave
1980	Dr A.F. Webster	1999	Dr H. Westbury
1981	Mr R. Fuge	2000	Mr L. Brajkovich
1982	Dr J.G. Fairbrother		

SYD WILKINS MEMORIAL PRIZE

Previous recipients of the prize are:

1984	Jennifer York	1993	Zee Upton
1985	Ian Wallis	1994	No Award
1986	Tom Scott	1995	Sandra Sapats
1987	No Award	1996	Carmel Ruffolo/Chris Siatskas
1988	Darren Shafren	1997	No Award
1989	No Award	1998	Wendy Muir
1990	Mingan Choct	1999	No Award
1991	Kevin Sanderson	2000	No Award
1992	No Award		

SPONSORS of the 2002
AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

Speaker Sponsors

Bartter Enterprises Pty Ltd
BASF Australia Ltd
Inghams Enterprises Pty Ltd
Novus International Pty Ltd
Ridley AgriProducts Pty Ltd
RIRDC Chicken Meat Program
RIRDC Egg Program
Roche Vitamins Australia Pty Limited
Tegel Foods Ltd

Gold Sponsors

ADM Australia Pty Ltd
Alltech Biotechnology Pty Ltd
Degussa Australia Pty Ltd

Silver Sponsors

Aventis Animal Nutrition
Baiada Poultry Pty Ltd
Elanco Animal Health
Kemin (Aust) Pty Ltd
Phibro Animal Health
Weston Animal Nutrition

Conference Material Sponsors

Alpharma Animal Health
Finnfeeds International Pte Ltd

CONTENTS

INVITED PAPERS

CONTRIBUTED PAPERS

THE POULTRY REVOLUTION: ISSUES, OBSTACLES AND OPPORTUNITIES

D.J. FARRELL

Summary

The Livestock Revolution has been, and will continue to be, spearheaded by the Poultry Revolution. Developing rather than developed countries will experience the continuing expansion of the poultry industry. China and India (now largely non vegetarian) are the two major players. Bird performance is predicted to improve in all categories. Food and feed, particularly their safety, are big issues. The widespread acceptance of genetically modified grains in feedstuffs is uncertain. Trading of poultry products will rise, with the US, Brazil and Thailand as major exporters. Australia must expect importation of chicken meat to occur. There may also be opportunity to export Australian chicken meat into several countries. Japan and China are predicted to increase chicken meat imports by 6% per year to 2008. The outlook for the egg industry, particularly in Australia, is not buoyant, but there are opportunities to increase production.

I. INTRODUCTION

Affluence is the force driving livestock production. As developing countries increase their gross national production (GNP), their people demand more animal products. In the next 20 years we will witness the Poultry Revolution as a major contributor to the Livestock Revolution (Delgado *et al*., 1999). Currently 23 % of the world's population consume four times the amount of animal protein as those in the rest of the world. The anticipated Livestock Revolution will not occur in Europe or in Australia, but in the Asia-Pacific region, South America and sub Saharan Africa (Table 1). Despite the dramatic downturn in the economy of several countries in the Asian region starting in 1997 (Farrell, 2000a), economic growth is recovering in most countries. China continues to head the list; achieving in 1996 economic growth of almost 10 % and currently over 6 %.

It is against this background that we must first examine the important domestic livestock production and their role in meeting the demand for animal protein that is predicted to occur during the next 20 years. Poultry production will then be reviewed.

The two countries that are most likely to drive the Livestock Revolution are China and India with a combined population of 2.3 billion. Traditionally Indians have been largely vegetarians, partly because of religious belief but also because they did not have the funds to purchase livestock products. Almost 75% of all Indians are now non vegetarian and for 92% of those, chicken is the meat of choice. Current *per capita* uptake in India of eggs, chicken meat and pig meat is only 1.8 kg, 0.6 kg and 0.6 kg per year respectively, while *per capita* milk consumption is 73.1 kg and expanding rapidly (FAO, 2001). It is not only livestock in these countries that we must consider, but the impact of the one billion increase in world population that will occur by 2015 and almost exclusively in developing countries. Their current combined human population is almost 5 billion.

Pig meat is the world's most popular animal protein, in no small measure because of the *per capita* consumption in mainland China of 54.1 kg per year. This is forecast to increase to 56.5 kg by 2010 (Anonymous, 2001a), or an additional 300 million metric tonnes (mt) of pig meat.

School of Land and Food Sciences, The University of Queensland, Brisbane, Qld 4072.

The Livestock Revolution is not just about to start, it began well over a decade ago. The rate of increase was far more rapid in the developing than in the developed countries developed world. It should be made clear that most developing countries were starting from a very low base and many still are. Both China and India each has a middle class population equal to that of the US and which enjoys a relatively high income. It is this group that will continue to drive the Livestock Revolution, particularly the demand for poultry products.

A major concern in the Livestock Revolution is that increase in output has been achieved mainly by increasing animal numbers and not by increasing production per head in developing countries, thus often leading to environmental damage (Delgado *et al.*, 2000).

Table 1. Global production of meat, milk and eggs (millions of metric tonnes) in the developed and developing countries (FAO, 2001) and those predicted by Delgado *et al*. (1999) for the year 2020. NA = not available.

Commodity	Region	Year				
		1994	1996	1998	2000	2020
Beef & veal	World	53.2	54.6	55.1	57.2	82.0
	Developed[1]	31.8	31.0	30.2	30.5	38.0
	Developing[2]	21.3	23.6	24.5	26.6	44.0
Pig	World	77.5	78.4	86.7	90.9	122.0
	Developed	36.2	36.0	37.7	37.2	41.0
	Developing	41.4	42.4	49.9	53.7	81.0
Poultry	World	50.6 (43.5)[3]	56.0 (47.5)	61.7 (52.5)	66.5 (56.9)	83.0
	Developed	27.4 (22.9)	29.1 (24.2)	30.5 (25.5)	31.8 (26.7)	36.0
	Developing	23.2 (20.6)	26.9 (23.4)	31.2 (27.0)	34.7 (30.2)	47.0
Sheep & goat	World	10.6	10.3	10.9	11.3	
	Developed	3.8	3.5	3.4	3.3	NA
	Developing	6.8	6.8	7.5	8.0	NA
Milk	World	534.9	549.2	562.7	568.5	772.0
	Developed	350.0	342.0	343.0	344.0	371.0
	Developing	185.0	207.0	220.0	224.0	401.0
Eggs	World	45.0	50.0	51.7	54.7	NA
	Developed	17.9	17.6	18.0	18.4	NA
	Developing	27.0	32.4	33.7	36.4	NA

[1] Australia, Canada, Eastern Europe, European Union, other Western European countries, Israel, former Soviet Union, Japan, New Zealand, South Africa, United States

[2] All other countries in FAO Statistical Database

[3] Broiler meat

II. FOOD AND FEED

Food (for humans) and feed (for livestock) are both critical and contentious issues in the future of livestock production. About one third of all grain produced is fed to livestock, mainly pigs (12%) and poultry (9%) (Martin, 2001). Much less feed grain is used in developing than developed countries. Cultivated land has remained constant at 700 million ha and is now shrinking, with no more arable land available especially in Southeast Asia (FAO, 2001). Prediction to 2010 of additional food and feed grain needs made by Farrell

(2000b) and Delgado *et al.* (1999) differ markedly (increase by 22 million vs. 11 million mt each year) with some explanations. Farrell (2000b) predicted a further 15 million mt mainly of protein concentrate and 11 million mt for food each year, giving an additional total for food and feed of 525 million mt by 2010. Predictions are hampered by uncertainty of livestock numbers that are extensively managed. In China, backyard production accounts for almost 80% of the 450,000 pigs produced each year (Anon, 2000a). Backyard poultry contributes significantly to meat and egg production in developing countries but intensive production is increasing rapidly.

For the first time there was in 1997 a 24% decline in the global production of manufactured feed due mainly to the economic climate in Asia. The industry is now in an advanced stage of recovery. Poultry accounts for 35% of all manufactured feed (Farrell, 2000b).

Evans (2000) maintains that a combination of increased grain production on existing land and the use of marginal land is part of the solution. Farrell (2000b) argued that the environmental cost of such an expansion is too great and, over the long term, unsustainable. Oliver (1998) predicted that, 'if world consumption of animal protein rises to 75-80% of North American levels, based on yield and available land, we will need three or more Earths to produce it'. The recent increase in the price of crude oil and the low price of maize has posed a threat to the feed industry. It is estimated that 21% of the 240 million mt of maize grown in the US will be used to produce 20 billion litres of ethanol in 2005 (Lyons and Bannerman, 2001) and not be available as stock feed.

Despite future concerns of the feed supply and the very large increase in demand during the previous decade, the price (in $US) of cereal grains and most feedstuffs, adjusted for inflation, has declined dramatically (Delgado *et al*., 1999). In the past 5-6 years, the actual price (in $US) of grains has declined. Today maize and soybeans, the basis of intensive livestock diets, are at an almost all-time low. Price is not predicted to rise substantially in the next 10 years (Delgado *et al*., 1999), although this is debatable (Farrell, 2000b).

Changes are happening. Use of meat and bone (M&B) meal in animal feeds is now banned in the EU. Brookes (2001) calculated that 3.9 million mt of sunflower meal, or 3 million mt of soybean meal (SBM)/year will be required to replace the M&B meal. Ninety seven percent of the SBM presently used in the EU is imported and contributes 53% of total protein in manufactured feed used in the EU. There is also the cost of disposal of M&B meal. In the not too distant future, tallow, poultry offal meal, feather meal and fishmeal will come under scrutiny and likely suffer the same fate as M&B meal. There is also concern in the EU that some fishmeal may be contaminated with M&B meal.

In Australia, the pig and poultry industries rely heavily on M&B meal, not only to supply protein but also phosphorus (P). Poultry offal meal is also produced here and fish meal imported. Again the questions arise of how long these byproducts will be acceptable in diets of livestock. Already there is emphasis here on animals grown or producing on all vegetable diets and for product differentiation and other considerations are becoming important issues in consumer choice (Annison, 2000).

The intensive industry in Australia is fortunate in having an ample supply of different feed grains, oil seed meals and legume seeds, but there will be competition for these from a rapidly expanding livestock industry in Asia (see Farrell 1997 for review). Again the question of long term sustainability arises. John Williams, a soils scientist with CSIRO, recently described our Australian agricultural practice as 'the road to suicide' (Lowe, 1999). Rehabilitation of saline soils will cost $A46 billion (Lowe, 2001), although D. Avery (pers. comm., 2001) claims that biotechnology at the University of California (Davis) has resulted

in crops that will desalinate the soil, e.g. tomatoes, rape, and these will provide the solution to rehabilitation.

III. THE POULTRY INDUSTRY

(a) Poultry Meat

The Poultry Revolution is the spearhead of the Livestock Revolution and showing unrelenting growth, particularly in the last decade (Table 1). Between 1997 and 2020 poultry meat is expected to increase market share from 28% to 40% of all meat consumed (Delgardo *et al.*, 1999). The term poultry includes broiler chickens, spent laying hens and some minor domestic species used for meat. The global chicken meat industry was reviewed by Rothwell (2001) in a conference in New Zealand, and the future of poultry as a food source by Sheldon (1998) in Japan.

The US has led the way in broiler meat production technology in the industrialised process and laid the foundation for the advances in breeding, feeding, housing, disease control and management to make the industry the most efficient of all the livestock industries and the technology portable. The US is the leading producer of chicken meat with 15 million mt per year followed by China (12 million mt). The US exports 2.9 million mt, almost 20% of production. Tyson Foods is the major producer accounting for one quarter of the country's chicken output. Cheeke (1999) is highly critical of the USA broiler industry, almost all of which is in the Southern States. Workers in poultry processing plant are poorly paid and have few benefits. Allowing for inflation, real earnings have declined. Cheeke (1999) cites examples of political influence (gerry mandering) and politicians receiving gifts, resulting in hefty company fines.

The performance of broiler chickens continues to improve at what appears to be at a never-ending rate. Predictions (McKay *et al*., 2000) for the future are impressive (Table 2).

Table 2. Past and predicted performance to 2007 by Ross Breeders for male broilers (McKay *et al*., 2000)

	Year				
	1976	1987	1997	2001	2007
Live weight @42d (kg)	1050	1775	2425	2650	3000
Days to reach 2 kg (d)	63	45	37	35	33
FCR to 2 kg (kg/kg)	2.50	1.90	1.65	1.50	1.25
Feed to 2.5 kg Γ (kg)	5.0	3.8	3.3	3.0	2.5
Carcass yield (%)	66.7	67.8	69.5	70.3	71.5
Breast meat @ 2kg (%)	12.6	14.5	16.1	17.3	19.1
Feed (kg) per kg breast meat	20	13	10	8.7	6.5

Annual *per capita* consumption of broiler meat has continued to increase in the US and is currently at 35 kg. Whole chickens are sold largely fresh and the breast is the main component of chicken parts (Table 3). Consumption of turkey meat in the US is more or less stagnant at 8.2 kg per person per year. In contrast to the Australian broiler industry, very few chickens are now marketed whole; the vast majority are for further processed meat and parts (Table 3).

In the US, dark meat is less favoured than white meat; significant amounts mainly of dark meat are exported and marketed often at well below cost of local production. These exports have had a significant negative effect on the broiler industry in the Philippines and South Africa, for example. The counter argument is that imported, low-cost chicken meat is

more affordable to the poor who need animal protein. Tan (2000) reported that US exports of leg quarters to the Philippines increased from 2,753 mt in 1998 to 31,899 mt in 1999. She also states that 'there is rampant smuggling in the country'.

Table 3. Market share (%) of broiler meat in the US domestic market (Mellor, 2000)

Year	Whole	Cut-up/parts	Further processed
1997	12.0	47.5	40.5
1998	12.0	47.0	41.0
1999	10.0	46.5	43.5
2000 (estimated)	9.0	46.0	45.0
2000[1]	40.0	50.0	10.0

[1]Australian Chicken Meat Industry.

Despite forecasts of economic downturn and continual economic problems in some Asian countries, the poultry industry continues to expand at a rate of about 4% per annum (Rothwell, 2001). The industry has expanded at a faster rate in developing than in developed countries and will continue to do so. They now account for more than half of the global production (Table 1), driven largely by China. Of China's 20 million mt of poultry meat, duck meat and goose meat combined account for about 3 million mt/year, with both industries expanding rapidly in Asia.

(b) Egg Production

There has been a tendency to ignore eggs in a wide-ranging discussion of the poultry industry. In the developing world, egg production is twice that of the developed world, with more rapid growth. In the developed world egg consumption is almost static, particularly the EU. China dominates global egg production with over 18 million mt/year or almost 300 eggs/person. Ninety percent of all eggs produced are sold in shell. The US is next with 4.8 million mt (259/person) and increasing at 2% per year. Production of eggs in the US is dominated by three companies with over 50% of market share. Unlike in China, in the US almost 50% of all eggs produced are broken out and used in the food industry. The laying industry in China comprises much smaller farms than in US. The largest has less than 2 million layers. There is substantial backyard egg production (>70%) which lends some uncertainty to the statistics on production. Japan is a large consumer of eggs and next behind the US with 2.5 million mt/year.

IV. ISSUES AND OBSTACLES

(a) Globalisation

Several major issues confront the poultry industry. Perhaps the issue that raises several questions is globalisation. Globalisation has been defined by Annison (2000) as 'the removal of barriers to information, capital, services and goods flows (i.e. reduction or removal of barriers to trade).' This is being promoted heavily by the G8 countries who seem likely to benefit most from a perceived level playing field. One third of US farm income comes from export sales (Martin, 2001). The effects of trade liberalisation on the poultry industry in Asia was reviewed by Templeman and Kerkwijk (2001 a, b). Several countries will not have the capacity to compete for market share. Countries such as China, Brazil and

Thailand who can produce chicken meat cheaply will swamp countries such as the Philippines, Malaysia and Indonesia who rely heavily on the importation of breeding stock, feed and other consumables.

European livestock industries are starting to move off shore. Already a large piggery is about to be established in Queensland by the Danes, and the Dutch have moved into China. The poultry industry in several high-cost countries is relocating. A French producer has already relocated to Brazil, and a British producer to Hungary to allow expansion. The US export market for chicken meat may change considerably. Subsidised exports under the heading of 'foreign aid' have been one way in which the US has made market penetration into Russia for example (Templeman and Kerkwijk, 2000a). Japan is a major importer of poultry meat. China (42%) is currently the major supplier to Japan, followed by Thailand (22%), Brazil (20%) and the USA (16%). Australia has so far managed to resist importation of poultry products and in 1999 exported few eggs (564 mt) and some chicken meat (14,000 mt) mainly to the Pacific Islands. The recent outbreaks of Newcastle Disease (ND) have brought chicken meat exports to a halt. The ban should be lifted by the end of 2001. There is little doubt that with globalisation the Australia chicken meat industry will be under threat. Despite the highly devalued $A against the $US, several countries in Southeast Asia have not depreciated greatly against the $A, particularly the Thai baht. Thailand has been thwarted previously in its attempts to export chicken meat into Australia. Templeman and Kerkwijk (2000b) concluded, 'At the end of the day, governments cannot stop the entrance of foreign products into their local market.' This is also the view of at least one Australian broiler producer (McErlane, 2001a).

A major force in the broiler industry is Brazil, producing almost 5 million mt of chicken meat per year. Brazil is self sufficient in maize and soybean and has arguably the most sophisticated production system in the world. This, combined with the cheap labour, and a greatly devalued currency, has allowed Brazil to become the world's second largest broiler meat exporter (1.1 million mt/year) and forecast to increase by 16 % between 2000 and 2001. In the UK 40% of chicken meat consumed was imported mainly from Brazil and Thailand. Chicken breast is produced in Brazil about 70% cheaper than in the UK (Hambly, 2001). Poultry exports from Thailand are forecast to increase by 6 % and reach 360,000 mt in 2001. Over 40% will go to Japan as value added products, but the EU will import 36%. Thailand is almost self sufficient in maize but imports almost half of its soybeans and soybean meal and half of its fishmeal usage.

(b) Disease

Outbreaks of human disease and food poisoning spread through livestock and manufactured feed have made the consumer cautious and placed supermarkets on the alert (Ratcliff, 2000). A recent outbreak of foot and mouth disease (FMD) in cloven-hoofed livestock has highlighted just how difficult it is to contain such outbreaks under the present system of production and marketing. The cost in terms of money and the loss of valuable breeding stock has been enormous. Grains contaminated with FMD virus may threaten the global grain trade (Best, 2001).

The major issue now is food safety. David Byrne (cited by Lyons 2001), the European Commissioner for Food and Public Health stated, "*Safety is the most important ingredient in our food. Europe must have the capacity to ensure that we can deliver this to our customers*".

The Australian poultry industry has made giant strides in preventing outbreaks of several avian diseases through vaccination programs, excellent hygiene and management. However disease outbreak is always high risk given the intensification of stock. Several

exotic diseases endemic in adjacent countries pose a continual threat to all animal industries. Several serious outbreaks of avian influenza and more recently ND in Australia have been successfully contained. Biosecurity measures are in place and are being continually reviewed in order to meet emergency situations (Fairbrother, 1999).

With the importation of brown egg layers into Australia, the incidence of Marek's disease (MD) increased markedly with high mortality before and during egg production. Mortality was reduced with more effective management, vaccines, and the introduction of defined handling and administration procedures for the vaccine (Jackson, 2000). Recently sound management practices have been relaxed and MD may be on the increase (R. Jenner, pers com., 2001).

(c) Animal Welfare

This is an emotive and contentious issue. It is almost continuously in the public eye. In 2012, the EU will ban the use of battery cages in the layer industry. In the meantime, birds will be given greater floor space. Alternative systems will increase the cost of production by 12% for enriched cages, 17% for aviary cages, and by 38% for free range (van Horne, 2001).

In the EU there is a gradual move away from cage egg production. Eggs from alternative systems (mainly deep litter) have 40% of the Dutch supermarket sales and trade at a premium price of 20-25% (van Horne, 2001). Because of the enormous capital cost that will be needed to purchase furnished cages by 2012, the threat of importation of cheap eggs from non EU countries, the future of the egg industry in Europe is in doubt (Windhorst, 2001). In the US the fast food chain McDonalds has advised all egg suppliers of minimum cage size and has banned forced moulting (Boersma, 2001). Other sensitive issues relate to beak trimming and toe clipping of layers (Broom, 2001). Welfare is more than just cage management. Stewart (2001) outlined other issues such as stockmanship and husbandry training.

Two metabolic diseases of the broiler industry are ascites and leg disorders. Both respond to management practices although genetic selection has helped to reduce the incidence of both (McKay *et al*., 2000). They are still important welfare issues. Broom (2001) cited data in which 90% of broiler chickens surveyed had walking impairment in the week before slaughter and 26% had severe impairment. Reduced growth rate has been suggested as one solution.

(d) Environmental Concerns

The fragile nature of the environment is of increasing concern. Australia does not have a good record in environmental management. There is increasing pressure on the poultry industry to improve waste management, to reduce pollution and to control noise level and odour. A new Victorian Code of Practice may force new poultry producers to relocate onto large acreages at great cost (Anonymous, 2001b). Poultry contribute to the green house gases, particularly the production of nitrous oxide. Disposal of poultry manure adds an additional burden on soil N and P which then find their way into water run off. Strategies are in place to decrease the crude protein and P contents of poultry diets, e.g. phytase addition. Environmental issues are not only of concern to the poultry industry but are being increasingly driven by public pressure groups, e.g. Greenpeace, RSPCA.

There are 10,000 mt of antibiotics used in the EU. Up to half of these end up in animals and up to 50% is passed out unchanged (Pearce, 2000). The removal of most antibiotic growth promoters (AGP) from broiler feeds in the EU has placed the industry under considerable pressure. Not only did they improve bird performance but they reduced the

incidence of necrotic enteritis. Alternative measures are now being examined (Ratcliff, 2001a) including the inclusion of capsicum to reduce *salmonella enteriditis* in broilers (Anonymous, 2001), and xylanase to reduce *Campylobacter jejuni* in the gut of chickens (Fernandez *et al.*, 2000).

Several supermarket chains in the UK have excluded the use of all AGP in chicken meat production. These same supermarkets are expanding rapidly into the Asia-Pacific region and will demand the same high standards of poultry production as markets in the EU. Countries in this region experience difficulties in obtaining a supply of clean water, mycotoxin-free feed and in maintaining the high hygienic standards needed to meet export requirements.

A committee was established in Australia to examine the use of antibiotics in food-producing animals. Grimes (2000) reported findings; the important one was that 'The Australian poultry industry will rely less on antibiotics for control of bacterial disease'. Australia will soon follow the EU and ban completely all AGP, accelerated by the vanomycin-resistant enterococci concern here.

V. OPPORTUNITIES

The global wastage of cereal grains from pests, disease and weed problems is estimated to be 42% (Evans, 2000). The solution to this wastage may lie in biotechnology. Oliver (1998) maintains that agricultural biotechnology 'is limited only by the imagination of its practising scientists'. In 1995 there were no genetically modified (GM) soybeans. In 2000 over half the soybeans grown in the US and 90% in Argentina were genetically modified (Martin, 2001). An improvement in nutrient content and a reduction in current grain wastage, mainly through use of GM grains, will increase production and could add over 800 million mt of food and feed grains to the world supply (Evans, 2000). Williams (2001) gave an excellent review of the economic aspects of GM crops at this symposium. This is a contentious issue; in the EU, Korea, the Middle East, and Japan, GM grains have met stiff consumer resistance but this is softening. McDonalds, the fast-food chain, has instructed that all products used in the UK, Sweden, Germany and Denmark be from livestock fed on non-GM sources (Ratcliff, 2001b). The central argument is risk versus benefit (Williams, 2001). Attitudes may change if non GM foods become more expensive than those that are GM. The use of GM feed additives could extend to amino acids, some vitamins and enzymes, and these may also have to be removed from poultry diets.

There is increasing opportunity for special purposes for eggs other than as a food, particularly in the rapidly growing field of biotechnology (Sim *et al.*, 2000). Eggs can be designed to deliver antibodies to prevent disease, to deliver cancer drugs (Anonymous, 2000b), or to enhance growth. This can also be achieved by adding conjugated linoleic acid to the feed (Walker, 2000). The egg as a functional food can be enriched with several minerals (iodine, selenium and iron), vitamins (B_{12}, folate) and special fats (e.g. omega-3). These speciality products are gaining increased market share.

VI. THE AUSTRALIAN POULTRY INDUSTRY

The Poultry Revolution has been no less successful in Australia than elsewhere with an annual growth of 4-5%. Chicken meat production, now 33 kg per person/year, has increased consistently and is forecast to rise to 34 kg in 2002 and 35.5 kg by 2006. Annual production exceeds $2.5 billion. Other poultry meats (duck, turkey) have remained low and relatively constant at about 340 g/head over the past three years, although duck production may be increasing slowly. Two companies supply about 70% of the Australian market, both

are now family owned. They differ in that one uses contract growers, the other grows its own chickens. There are nine other important chicken meat processors. So far there has been little interest in Australia in free-range chicken meat, although 'corn fed' chickens with yellow skin attract a premium price. In France, Label Rouge accounts for about 20% of the chicken meat market (Ratcliff, 2001a).

The egg industry has not fared well. *Per capita* consumption has decreased over the past 20 years to about 140 eggs/year worth $340 million, although there are encouraging signs of a turn around. Of all eggs sold, 5% are free-range and 2.5% are barn eggs (RIRDC, 2001). In the UK only 70% of eggs produced are from caged birds and McDonalds use only free-range eggs in their food products (McErlane, 2001b).

The deregulation of the industry has resulted in a significant 'shake out', often with substantial oversupply driving down egg prices and then rebounding as producers cut back on bird numbers. There are a few producers who are expanding, some with state-of-the-art technologies such as fully enclosed, environmentally-controlled houses. A new $A14 million layer complex has just opened in WA. There is little doubt that this trend towards fewer egg producers will continue.

Recent importation of brown-egg laying strains has been questioned. With these came management and disease problems, and sometimes too large an egg. Their feed consumption and nutrient needs are substantial compared to the White Leghorn strain for example. Although egg production and persistence is slightly better than the Leghorn (Robinson *et al.*, 2001), the introduction of brown-egg layer strains may not have been justified.

Export opportunities in the Australian egg industry are few until the country is officially free from ND, expected before the end of 2001. Countries in the region such as Singapore, Samoa and Fiji grow few crops for livestock feed and have small or no intensive animal industries. There are opportunities to export both chicken meat and eggs to these countries.

There has not been sufficient emphasis given to promoting the excellent nutritional value of the egg, particularly for the vulnerable groups in society. Not only is the egg a meal in itself, but it delivers the highest quality protein available, with all of the vitamins (except C) and minerals; it contains only 2 g of saturated fats with an excellent ratio of polyunsaturated fats to saturated fats. Packaging of small eggs in 'personal' cartons specifically for young children appears to offer opportunity. Speciality eggs produced under different management systems (aviaries, barn, free range, organic), on all vegetable diets, and as functional foods, are making market penetration. However, a major issue is the marketing itself. Seventy percent of eggs are sold through the big supermarket chains who essentially control the market and the price. There appears to be an unreasonable 'mark up' between the price received by the producer and that paid by the customer. In addition, there may be a hefty sum demanded from packers and distributors by the supermarket to obtain shelf space.

The issue of caged layers will not go away. There is increasing public resistance to keeping hens in cages. The reaction of the Australian public to alternative methods of housing layers has yet to be assessed and may be constraining replacement and upgrading of existing layer facilities.

Consumer confidence in poultry products is the major issue. It is still not uncommon to see in butcher shops in Australia a sign assuring their customers that their chickens are produced on 'antibiotic and hormone free feeds'. It is extremely difficult to erase these negative aspects of poultry meat and cholesterol in eggs from people's minds. Dietary cholesterol is still being promoted by those with vested interests (Smith and Pinckey, 1991) even though it is essentially a non issue. McNamara (2000) reported a significant inverse relationship ($P = 0.0053$) between egg consumption and the incidence of cardio-vascular

disease in 24 countries examined. It is up to the poultry industry and the stock feed industry to erase these impediments and to emphasise food safety and quality as their major concern.

The chicken meat industry is starting to benefit from the recent increase in the price of beef and pork. The outbreak of the Nipah virus in Malaysia killing one hundred humans and the slaughtering of over one million pigs opened the door to the Australian pig producers to export chilled pig meat to Singapore, currently almost 45000 mt/year.

The major threat to the chicken meat industry is importation of cooked/frozen chicken meat. Australia has managed to prevent this to date, but how long will it last? The aggressive marketing (or dumping) of poultry parts by the USA, the competitiveness of Brazil in the export market and the proximity of Thailand is exerting pressure on the Australian chicken meat industry. Interestingly Ratcliff (2001b) predicted a decrease of 1.6% in Thailand's export by 2008, and China's imports of poultry meat increasing by 52% and Japan 58% by 2008. One of the most exciting technologies on the horizon is the ability of biotechnologists to determine the sex of the chick. This would provide great economic savings for the egg industry where currently male chicks are discarded. The broiler industry will benefit from selection of the faster growing, more feed efficient male birds. It would also lead to greater uniformity of chickens at slaughter, an important industry objective.

VII. CONCLUSIONS

There is little doubt that the Poultry Revolution will continue and the main beneficiaries will be those in developing countries, many of which are rapidly becoming affluent with some embracing globalisation. Demand will be for wholesome, safe food. There is opportunity for the Australian poultry industry to help meet this demand. To date the industry has been complacent and inward looking. With the very low Australian dollar, the poultry industry is well positioned to seek out markets in the Asia-Pacific region. Already the pig industry here is successfully meeting the stringent market requirements of Japan and Singapore. These poultry markets may well be niche markets with special needs. Japan is forecast to reduce its chicken meat production by 13 % and to increase imports by 49 % during the next eight years (Ratcliff, 2001b). Australia is in a strong position with an adequate feed supply. Many countries in the region are net importers of feedstuffs. On the other hand, there is the ever looming shadow of poultry imports, and the industry must brace itself for this eventually occurring and should now take the necessary steps to minimise the effects.

Egg consumption is unlikely to increase significantly but there are niche markets to be exploited. Surprisingly there is small importation of liquid egg into Australia (641 mt/year). In the long term there are big issues to be addressed: the rapidly increasing world population, the supply of food and feed, the use of GM feeds and additives in livestock production, animal welfare, the effects of agriculture and livestock on the environment and the degradation of our soils, to name a few. These issues will impact directly on the poultry industry; in the meantime the revolution will continue.

REFERENCES

Annison, G. (2000). *Asian-Australasian Journal of Animal Science*, **13**: Supplement July 2000c: 405-410.

Anonymous (2001b). *Poultry News*, **1**: (4) 15.

Anonymous (2000a). *Pig International*, **30**: (1) 21-24.

Anonymous (2000b). *New Scientist*, **168**: (2268) 5.

Anonymous (2001b). *New Scientist*, **171**: (2305) 5.

Best, P. (2001). *Feed International*, **22**: (8) 6-10.
Boersma, S. (2001). *World Poultry*, **16**: (12) 26-27
Brookes, G. (2201). *Feed International*, **22**: 6-8.
Broom, D.M. (2001). *Proceedings Australian Poultry Science Symposium*, **13**: 79-82.
Cheeke, P.R. (1999). *Journal of Animal Science*, 77: 2031-2035.
Crimes, T.M. (2000). *Proceedings Australian Poultry Science Symposium*, **12**: 10-15.
Delgado, C., Rosegrant, M., Steinfleld, H., Ehui, S. and Courbois, C. (1999). Livestock to 2020: The Next Food Revolution. International Food, Policy Research Institute, Washington, D.C.
Evans, L.T. (2000). The Food and Environment Tightrope, pp. 149-154. ACIAR Monograph No. 23. Canberra.
FAO (2001). Statistical Databases and Agriculture, http:faostat.fao.org/default.htm.
Fairbrother, J. (1999). *Proceedings Australian Poultry Science Symposium*, **11**: 23-38.
Farrell, D.J. (1997). In: *Recent Advances in Animal Nutrition in Australia 1997.* Eds. J.L. Corbett, M. Choct, J.V. Nolan and J.B. Rowe, University of New England, Armidale, pp. 136-145.
Farrell, D.J. (2000a). *Proceedings Australian Poultry Science Symposium*, **12**: 67-73.
Farrell, D.J. (2000b). The Food and Environment Tightrope, pp. 97-121. ACIAR Monograph No. 23. Canberra.
Fernandez, F., Sharmu, R., Hinton, M. and Bedford, M.R. (2000). *Cellular and Molecular Life Sciences*, **57**: 1793-1801.
Grimes, T.M. (2000). *Proceedings Australian Poultry Science Symposium*, **12**: 10-16.
Hambly, P. (2001). *Poultry News*, **1**: 22-23.
Jackson, C.A.W. (2000). *Proceedings 2000 Poultry Information Exchange 2001*, ANA Gold Coast, pp. 45-51.
Lowe, I. (1999). *New Scientist*, **165**: (2212) 57.
Lowe, I. (2001). *New Scientist*, **170**: (2286) 51.
Lyons, T.P. (2001). *Proceedings Alltech's 17th Annual Symposium.* Eds. T.P. Lyons and K.A. Jacques, Nottingham University Press, UK, pp. 1-23.
Lyons, T.P. and Bannerman, J. (2001). *Proceedings Alltech's 15th Annual Asia Pacific Lecture Tour*, 115-129.
Martin, M.A. (2001). *Outlook on Agriculture*, **30**: 11-19.
McErlane, B. (2001a). *Poultry News*, **1**: (4) 4.
McErlane, B. (2001b). *Poultry News*, **1**: (5) 12-15.
McKay, J., Barton, N.F., Kverhuis, A.N.M. and McAdam, J. (2001). *Proceedings XXI World's Poultry Congress*, S2.601, Montreal, August-20-24, 2000.
McNamara, D.J. (2000). *World Poultry*, **16**: (8) 24-25.
Mellor, S. (2000). *World Poultry*, **16**: 12-14.
Oliver, J. (1998). *The Agricultural Biotechnology Bulletin*, July 1998, 10-11.
Pearce, F. (2000). *New Scientist*, **165**: (2226) 20.
Ratcliff, J. (2000). *Proceedings Australian Poultry Science Symposium*, **12**: 25-31.
Ratcliff, J. (2000b). *Proceedings 2nd International Broiler Nutritionists Conference*, Rotarura, NZ, pp. 7-19.
Ratcliff, J. (2001a). *Proceedings of Alltech's 15th Annual Asia Pacific Lecture Tour*, 55-77.
RIRDC (2001). www.rirdc.gov.au
Rothwell, G. (2001). *Proceedings 2nd International Broiler Nutritionists Conference*, Rotarura, NZ, pp. 1-16.
Sheldon, B.L. (1998). *Proceedings 6th Asian Pacific Poultry Congress*, Nagoya, Japan. June 4-7. 1998, pp 1-8. Japan Poultry Science Association.

Sim, J., Nakai, S. and Gunter, W. (2000). Egg Nutrition and Biotechnology. 495 pp. CABI Publishing, UK.

Smith, R.L. and Pinckey, E.R. (1991). The Cholesterol Conspiracy. Warren H. Green, inc. USA

Tan, I.Q. (2000). *Asian Poultry*, May/June 17-18.

Templeman, K-A. and Kerkwijk, E. (2001a). *Asian Poultry Magazine*, May/June, 16-18.

Templeman, K-A. and Kerkwijk, E. (2001b). *Asian Poultry Magazine*, July/August, 28-31.

Van Horne, P. (2001). *Poultry International*, **40**: (4) 28-34

Walker, M. (2000). *New Scientist*, **165**: (2221) 9.

Williams, P.V. (2001). *Proceedings Australian Poultry Science Symposium*, **13**: 1-11.

Windhorst, H. (2001). *Poultry International* **40**: (7), 26-32.

DEVELOPMENTS IN THE US POULTRY INDUSTRY: HOW RESEARCH CENTERS CAN MEET INDUSTRY NEEDS

J.H. DENTON

Summary

The Center of Excellence for Poultry Science at the University of Arkansas is the first new program to be established with Poultry Science as the focus in over 40 years. Speaking strictly from a personal perspective, it has been the most challenging, exciting, and rewarding experience of my professional career. An accurate description of the Poultry Center is that we are a "work still in progress". We are working very hard to develop an outstanding faculty program consistent with the outstanding facilities that have been provided for us. We understand our mission of service is to provide the research and continuing education needs of our personnel in the poultry industry as well as to assist our young people with their education needs in preparation for their careers. If we are successful in maintaining our strong industry relationships and meeting their needs and expectations, with time, we will succeed in becoming recognized as the Center of Excellence for Poultry Science, a designation that cannot be claimed but must be earned.

I. INTRODUCTION

The concept for the development of the Center of Excellence in Poultry Science at the University of Arkansas originated January through April, 1990. This development was accomplished by the Strategic Planning Council chaired by R. H. Forsythe, which comprised prominent members of the poultry industry, allied industries, government officials, and University of Arkansas Division of Agriculture. One of the guiding principles that was integral to this development was the recognition by the administration of the University of Arkansas that prioritization of program resources is essential to achieving program excellence. It is no longer possible to be everything to all parties. We must determine where program need and program support can be complementary. The Arkansas poultry industry (broilers, turkeys, eggs) accounts for almost one-half (45%) of total agricultural income and provides employment for 1 of every 12 people in the state.

Clearly, the opportunity for developing a program of excellence at the University of Arkansas with the greatest potential for success was in Poultry Science. The vision for the development of the Center encompassed the needs for providing research in poultry science, education in poultry science, technology transfer in poultry science, and continuing education for industry professionals through extension education. These needs were to be addressed in a comprehensive program that provides these services for all components of the industry from primary breeder companies through integrator companies involved in production, processing, and marketing, including the allied industries, and ultimately the consumers of poultry products. In order to achieve the desired recognition as a regional center it was essential to recognize that our mission is to provide the research and education needs required to support today's poultry industry, which is in reality a food industry.

Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701, USA.

II. THE PARTNERSHIP

In order to achieve the targeted goal required for the construction of the physical facilities it was necessary to develop a partnership that consisted of federal, state, and industry contributors. The goal of $20 million was met through a USDA Special Grant of $10 million, a $5 million commitment from the Arkansas College Savings Bond program, and a $5 million contribution from the Arkansas poultry industry and regulated utilities. These commitments were achieved during the 3-yr period from 1992 to 1995. The allied poultry industries were the primary contributors of the $5 million goal identified in the Capital Equipment Campaign, approximately one-third of which has been realized.

III. THE FACILITIES

The research facilities for The Poultry Center include seven specialized components designed to address the comprehensive needs of the research and teaching faculty associated with the poultry program. The showcase for the Poultry Center is the 112,000 ft^2 John W. Tyson Building. The facility has approximately 70% of the space dedicated to research laboratories, teaching laboratories, and classrooms; the other 30% is allocated for faculty, graduate students, support staff, and administrative offices as well as conference rooms, faculty, and student lounges. The building boasts several outstanding features including the atrium that includes the Pioneers Room, which is dedicated to the 32 founders of the Arkansas Poultry Industry. Highlights of the research and teaching components include the 172-seat Leland Tollett Auditorium, which is equipped with state-of-the-art audiovisual capabilities, including both satellite downlink and uplink. The Tyson Building also houses the Central Analytical Laboratory, which provides the core analytical services for our faculty and graduate students, the Cell Isolation and Characterization Laboratory, which contains the flow cytometer utilized for cell isolation and characterization, the Core Biotechnology Laboratory with DNA sequencers, microarray and synthesizer, and the student computer teaching laboratory, which consists of 24 work stations plus the master work station for the instructor, which are all networked.

Our new research facilities also include a pilot feed mill, which began operations in 1993. The mill consists of a 1-ton Weightronix mixer with a California pellet mill in order to maintain feeding programs consistent with the commercial poultry industry. In addition, we have a 10,000 ft^2 Poultry Health Lab with eight suites for performing separately monitored trails. Four of these suites have the capability of achieving biosafety level 3 (P3) biosecurity. The research facilities at the University of Arkansas also include a new 12,000 ft^2 pilot processing plant that includes slaughter, evisceration, and further processing areas. The automated processing system, which is geared to research line speeds, was installed to simulate commercial processing systems for most parameters except line speed. This facility began operations in the fall of 1996. The University of Arkansas also has a four house, 40 x 400 ft. commercial broiler research unit that is designed to evaluate the energy utilization efficiency of house type and ventilation systems for the commercial broiler industry. The commercial broiler unit is also utilized to conduct alternative heat source, alternative bedding source, heat recovery, ammonia-CO_2, and pharmaceutical field test research projects. The flocks produced in these facilities are contracted with a broiler integrator and treated as any other contract producer to the extent possible.

A floor pen broiler breeder research unit is being constructed for evaluation of genetic, health and nutrition projects related to the primary breeder industry. The houses have each been subdivided to create multiple independent units per house with completely

separate feeding systems, watering systems, utilizing cool cell ventilation systems. The purpose of this unit is to provide the system for evaluating in small scale trials research projects which may be applied to commercial situations and to allow poultry science students the opportunity to gain hands-on management experience involving broiler breeders. The facilities at the University of Arkansas also include a 17-house poultry research farm with floor pens, battery cage, and environmental chamber facilities. In addition, the veterinary research farm has houses equipped for battery cage research and diagnostic facilities.

IV. FINANCIAL SUPPORT

The total investment in physical facilities for the Poultry Center is nearing $28 million. However, as we all know, the money invested in bricks and mortar only provides a suitable work location. The support for the scientific faculty, and therefore the real work, holds the key to success for the entire program. To the complete credit of the poultry industry and the legislature of the state of Arkansas, each had the vision to make sure that both sides of the equation were balanced. The base program support for the Poultry Center was phased in during three biennial budget cycles. The base program for the Poultry Center stands at $6.2 million annually.

In addition to the base program budget, we also have relied upon the capital equipment campaign to provide the needed resources to achieve the extra margin in scientific capability that will allow us to reach our goal of becoming a center of excellence. We have been able to recruit the faculty which allows us to effectively compete in the more traditional competitive grants arena (USDA, National Institutes of Health, National Science Foundation, Environmental Protection Agency, Food and Drug Administration, etc.). We have also become the type of mission-oriented program that has been successful in competing for industry-sponsored research. The total external support for the program is approximately $1.4 million annually.

V. THE RESEARCH PROGRAM

The research program for the Poultry Center, in order to be successful, must meet the poultry industry's expectations in the broadest sense of the total program. By the term "total program" we mean that our research must be relevant and current with regard to all phases of the industry. Our program is designed to be effective in meeting the needs of primary breeder personnel, broiler production, broiler breeder, hatchery, feed mill, processing plant, research and development, quality assurance, and management personnel in all phases of the allied poultry industries, including pharmaceutical, vaccine, equipment, and technical service companies.

In order to achieve these goals, we must achieve the appropriate balance of basic and applied research, even though I think that these terms may be overemphasized. For a multitude of reasons, the basic research commitment to poultry has been shrinking for a number of years. Quite simply stated, we have reached the time in which any basic research of significance to the poultry industry will most likely come from programs in which only the primary emphasis is poultry based. Therefore, the terms basic/applied may not be appropriate to describe our program. The more appropriate term for our overall research program would be "mission-oriented". In this context, we feel that regardless of the level of science involved, such as immunology, molecular biology, molecular genetics, molecular virology, biochemical nutrition, or reproductive physiology, we are going to utilize the necessary tools to achieve the desired objective. To that end we must also remember that our

mission is to serve the scientific needs of the food industry that we call poultry.

The current research faculty in our program include 26 poultry science faculty, 6 USDA Poultry Production and Product Safety Research Unit scientists, and several associated faculty in Biological and Agricultural Engineering, Biological Sciences, Animal Science, Entomology, Agronomy and Food Science. To that end, the program is strongly multidisciplinary and will become even more so as the program evolves and matures.

VI. THE ACADEMIC PROGRAM

The lifeblood of any poultry science program, especially an emerging program such as that at the University of Arkansas, is the undergraduate student enrolment. In order to meet the personnel needs of our industry, we have begun the process of strengthening our academic program. The two primary initiatives that we have developed are 1) an intensified and coordinated student recruiting program and 2) and extensive revision of the undergraduate and graduate curriculum. The recruiting effort is a multifaceted effort; two of the major components are directed to work capitalizing on the youth poultry program for 4-H and Vocational Agriculture students, and a concerted effort to provide workshops for high school science, chemistry, and math teachers to familiarize them with the career options available to Poultry Science majors. Our enrolment has almost tripled during the past 8 years. With the assistance of industry personnel during a 2-day faculty retreat, our undergraduate curriculum has been significantly revised to incorporate industry's needs.

A strong supporting component for our students include a very generous, industry-supported scholarship program. We currently have 52 students receiving scholarships totaling approximately $73,000. In addition to their scholarship support, our industry also provides summer internship opportunities for our students to obtain real world working experience.

We have also worked at developing regional collaborations with other colleges and universities. We currently have articulation agreements with schools in Oklahoma and Missouri that allow students to transfer to the Poultry Science Department at the University of Arkansas after they have completed a 2-year curriculum that is consistent with our program.

VII. THE EXTENSION PROGRAM

The faculty staffing plan for the Poultry Center includes positions that have extension components designed to serve the poultry and related industries. When our program was first established in 1992 there were two positions with extension responsibility. We now have seven faculty with extension responsibility. The expertise of our faculty covers production/nutrition, processing/product technology, food safety in processing/food service, poultry health, economics, and hatchery/breeder management issues. The extension faculty are also an integral component of the youth development/recruiting initiative. We also collaborate with our extension colleagues in Oklahoma, Kansas, and Missouri. This is an important partnership, and is one that we expect to become more effective as we mature as a program.

VIII. INDUSTRY RELATIONSHIPS

The key to the success of the development of the Poultry Center program is maintaining strong and effective industry relationships. This success is accomplished through the Poultry Center Advisory Committee concerning the center development process,

program priorities, and current program thrusts. In addition, the Poultry Federation Research Advisory Committee (Arkansas, Oklahoma, Missouri), which is composed of a broad representation of industry personnel, meets quarterly to review active projects, receive program updates, and address priority concerns. There are also periodic briefings relative to ongoing projects.

The Center faculty and administration also work actively with all of the Poultry Federation affiliate organizations in program planning, speaker scheduling, and other related activities. These activities include the Poultry Federation Feed Manufacturer's sponsored Nutrition Conference, the Poultry Federation Poultry Processor's Workshop, the Poultry Federation Turkey Committee Meeting, the Poultry Symposium, and the Egg Council. In addition to the industry education programs, the Center faculty and students also participate in the fund raising activities of the Allied Industries Committee during the Arkansas State Fair in the Chicken Kitchen.

Some of the material presented in this paper appeared in *Poultry Science* **77**: 214-216.

MODULATION OF NUTRITIONAL STATUS BY THE IMMUNE RESPONSE

E.A. KOUTSOS and K.C. KLASING

Summary

An immune response to an infectious challenge changes the partitioning of nutrients away from productive processes such as growth toward the immune response and especially the production of acute phase proteins. We estimate that the immune system utilizes only about 1.2% of the lysine intake in a healthy growing broiler chick. During an infectious challenge, additional nutrients are needed to support the clonal proliferation of lymphocytes, formation of germinal centers, recruitment of new leukocytes from bone marrow, and the synthesis of acute phase proteins by the liver. These processes increase lysine needs for immunocompetence to 6.7% of intake. For some nutrients, like methionine and lysine, decreased growth completely compensates for increased needs for immunocompetence. The requirement for others, such as antioxidant nutrients, may be increased.

I. NUTRITION AND IMMUNOLOGY INTERACTIONS: AN OVERVIEW

The immune system is made up of a large number of cells and organs that interact to defend the body against non-self antigens. To be effective, the immune system must survey all possible sites of antigen entry (primarily mucosal tissue including the gut, nose, eyes, skin and lungs), be able to recognize and respond to an acute or chronic antigen exposure, and maintain memory of previous encounters.

The innate immune response is the first line of defense against an antigen and generally consists of antigen recognition, cell recruitment, phagocytosis and/or destruction of the antigen, and then presentation of antigen to other immune cells. In the course of the innate immune response, a cascade of cytokines (termed the pro-inflammatory cytokines) including interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α (TNF-α) provide a communication network within the immune system as well as between immune cells and other cells of the body. These cytokines act locally and systemically to regulate immune responses, intermediary metabolism, and the endocrine system. The production, activity, and degradation of the pro-inflammatory cytokines are regulated by various factors including glucocorticoids, other cytokines, acute phase proteins, receptor antagonists and soluble receptors, as well as autocrine effects of the cytokines themselves. In addition, nutrition may affect the production of the pro-inflammatory cytokines as well as their regulators.

The acquired immune response consists of B and T lymphocytes that interact to defend the body against intracellular pathogens (primarily a cell mediated, cytotoxic T lymphocyte (CTL) mediated response), extracellular pathogens (primarily a humoral, B lymphocyte mediated response), and against tumor cells (accomplished by cell mediated and/or humoral responses, as well as by natural killer cells). These components of the immune system must respond to antigen that is presented by macrophages and other innate immune system cells, must be specific enough to respond to foreign antigen, but must not be auto-reactive. In addition, lymphocytes are responsible for maintaining memory of previous antigen exposure, and thus enabling a quicker, more effective response to a pathogen upon secondary exposure.

Department of Animal Science, University of California, Davis, CA, USA

II. IMPACT OF IMMUNE RESPONSES ON GROWTH AND NUTRITION: THE PROCESSES

Interestingly, reductions in production parameters associated with disease challenges are often a consequence of mounting an immune response rather than a specific effect of an invading pathogen on host tissue. For this reason, preventing exposure to pathogens through practices such as biosecurity programs, vaccination schedules, and feeding of antibiotics, probiotics or other compounds that can modulate gut microflora populations can prevent the onset of the immune response in the first place.

The first mechanism by which the immune system impacts nutrition is based upon the requirement for nutrients to maintain immune cell populations and to support proliferation and production of cytokines, antibodies, acute phase proteins etc. when a challenge occurs. The actual nutrient requirements to maintain and run the immune system have not been fully quantified, due to the diversity of cell types involved in various immune responses, the broad spectrum of possible immune responses, and the fact that immune cells are spread around the entire body, rather than being localized within a single organ. However, it has been demonstrated that the innate immune response is more nutritionally demanding than the acquired immune response. In fact, it is the production of acute phase proteins by the liver that comprises the majority of nutrient input into an immune response, while other immune processes such as antibody production are less costly (Klasing and Calvert, 2000).

The pro-inflammatory cytokine cascade directly modulates animal behavior. Lethargy, reduced social interactions, and anorexia result from the actions of IL-1 and TNF-α on the brain. These behavioral changes result in reduced feed intake and subsequent weight loss. Lethargy partially reduces the energy requirements for voluntary activity, thereby reducing the effects of anorexia on body catabolism.

An immune response can also alter intermediary metabolism. For example, the acute phase response to inflammation (APR) enhances skeletal muscle catabolism and hepatic protein synthesis, which is in direct contrast to the use of amino acids for skeletal muscle deposition during anabolic growth phases [see review by (Bistrian *et al.*, 1992; Klasing, 1998a)]. This change in protein turnover within tissues reflects a need for substrates for production of the acute phase proteins, and presumably provides enhanced surveillance of cellular contents (including possible pathogens) by the immune system. In addition, the APR is associated with increased rates of glucose oxidation and gluconeogenesis, as well as increased hepatic lipid synthesis from fatty acids release by adipocytes (Beisel, 1977). Finally, antioxidants (particularly vitamin E) may be used to a greater extent during an immune response, to protect non-infected cells from the damaging effects of the reactive oxygen species by immune cells. Leukocytes release bursts of reactive oxygen species (ROS) when they encounter pathogens. ROS kill pathogens and cells infected by them; however, they also cause lipid peroxidation and DNA damage of non-infected cells that lack adequate antioxidant protection. For this reason, the requirement for antioxidant nutrients may be increased during an inflammatory response (Webel *et al.*, 1998; Yoshida *et al.*, 1999).

Nutrient partitioning, or the localization of specific nutrients within tissues, may be altered during an immune response. As previously stated, amino acids are re-partitioned to the liver, where production of acute phase proteins occurs. Some acute phase proteins bind minerals, resulting in reduced plasma iron and zinc. Reductions in plasma mineral concentrations effectively "starves" pathogens of essential nutrients. While synthesis of metal-binding proteins is increased, synthesis of other proteins is reduced (negative acute phase proteins), also dramatically affecting plasma nutrient levels. For example, the production of retinol-binding protein is reduced during an immune response, resulting in

lowered plasma vitamin A during disease (Rosales *et al.*, 1996). Concomitantly, triacylglycerol uptake by tissues other than the liver is reduced in response to APR [see review by (Grunfeld and Feingold, 1992)], resulting in increased plasma lipid levels. Additionally, hepatic fatty acid uptake is increased in response to the pro-inflammatory cytokines.

The immune response can directly impact nutritional status by modulation of nutrient absorption. In general, due to a reduction in food intake, nutrient absorption will be reduced overall. In addition, the absorption of specific nutrients is purposefully impaired. For example, dietary iron absorption is reduced, presumably to prevent pathogens from obtaining this limiting nutrient (Weinberg, 1974; Weinberg, 1999). Water absorption is significantly reduced by sepsis (Kanno *et al.*, 1996), as is sodium, chloride and glucose absorption, and flux of these nutrients is often directed out of the body, as is the case with diarrhea.

An immune response has profound effects on the hormonal milieu. Pro-inflammatory cytokines decrease anabolic hormones such as growth hormone (GH) (Elsasser *et al.*, 1997), insulin-like growth factor 1 (IGF-1) (Elsasser *et al.*, 1995) and increase the release of catabolic hormones such as glucocorticoids [see review by (Elsasser, 2000)]. At the same time, a reduction in food intake also leads to reduced IGF-1 levels, thus promoting the catabolism of skeletal muscle. The APR is also associated with insulin resistance, as well as an increase in insulin and glucagon levels and subsequently, increased glucose oxidation (Wan *et al.*, 1989). Hormonal modulation by the immune system provides an effective mechanism for changes in substrate partitioning.

If pathology occurs to a particular tissue during an immune response, metabolic and nutritional consequences may be severe. For example, intestinal pathogens may cause significant damage to the gut mucosa, thereby reducing nutrient absorption and enhancing blood loss (also directing nutrients away from the body). These pathogen-induced metabolic derangements must be added onto the purposeful alterations initiated by the immune system.

Once a bird has mounted an effective immune response and cleared a pathogen, levels of pro-inflammatory cytokines decline and food intake returns to normal. In fact, a period of compensatory growth typically ensues. The amino acid requirements to support compensatory growth are higher than normal. Presumably the requirements for other nutrients are increased as well. If higher levels of amino acids are not provided, the compensatory growth does not occur and the bird lags behind. Consequently, higher nutrient levels following the immune response are probably more important than during the immune response.

III. IMPACT OF IMMUNE RESPONSES ON GROWTH AND NUTRITION: THE RATES

We have recently attempted to estimate the quantitative needs of immune defenses in growing broiler chicks (Klasing, 1998b; Klasing and Calvert, 2000; Klasing and Leshchinsky, 1999). Lysine was used as the currency in this exercise - not because of any paramount importance of lysine, but because this amino acid is commonly used as a reference nutrient (e.g. ideal protein systems). In this exercise, we estimated the amount of lysine used to maintain an affective immune system in the absence of major infectious challenges and then added on the extra needs to support a robust innate and adaptive immune response triggered by a pathogen challenge.

The overall maintenance costs of owning an immune system can be put in perspective by examining the weight of immune tissues relative to body weight (Table 1). Nutritional costs of the immune system must also consider turnover rates of cells and macromolecules (Table 2). Nearly half of the lysine used for maintaining the immune system is for IgA secretion, especially along the mucosa. We estimate that the immune system utilizes only

about 1.2% of the lysine intake in a growing broiler chick and much of the rest is used for tissue accretion. However, maintaining the immune system accounts for about 10% of the maintenance component of the lysine requirement the chick.

When the immune system responds to an infectious challenge, additional costs include the clonal proliferation of lymphocytes, formation of germinal centers in lymphoid tissues for affinity maturation of immunoglobulin, the recruitment of new monocytes and heterophils from bone marrow, and the synthesis of effector molecules (e.g. immunoglobulin). Although the rate of synthesis of immunoglobulins (Ig) specific for a pathogen increase tremendously (Cohn and Langman, 1990), the total amount of Ig produced during most infections is not increased remarkably because most Ig are not directed toward the pathogen and the rate of their synthesis does not change. However, the production of acute phase proteins by the liver requires nutritionally relevant amounts of lysine. The high cost of an acute phase response likely explains why an infectious challenge causes changes in body condition and energy metabolism that are much greater than can be reconciled by summation of the substrates needed for leukocytes themselves. In the case of lysine, 6.7% of the intake is used to support the defensive responses, most of which is used for synthesis of acute phase proteins (Table 2). In total about 70% of the reduced performance that occurs during an infectious challenge can be attributed to decreased intake and the remaining 30% is due to inefficiencies (Klasing *et al.*, 1987), including diversions to support the immune response.

Several experiments have examined whether higher concentrations of dietary nutrients ameliorate the impaired growth caused by an immune response. In the case of lysine, methionine, copper and zinc, high levels have little effect on growth rates (Klasing and Barnes, 1988; Klasing and Calvert, 2000; Koh *et al.*, 1996). Increasing the energy density of the diet improves gain of broilers undergoing an acute phase response (Benson *et al.*, 1993) but not turkeys (Piquer *et al.*, 1995).

Table 1. Size of leukocyte pools[1].

Cell type	Concentration (10^9/kg BW)	Mass (g/kg BW)	Lysine content[2] (µmol/kg BW)
Lymphocytes	15.2	2.43	170
Granulocytes	6.93	1.39	97
NK cells	0.29	0.06	4
Macrophages/monocytes	1.1	0.28	19
Total of leukocytes	23.52	4.15	291
Total Ig[3]		1.28	736
Whole body	-	1000	70000

[1]From Klasing and Calvert, 2000 .
[2]Assumes 70 µmol lysine per gram cells and 575 µmoles lysine/g Ig.
[3]Assuming that IgY and IgA concentrations in interstitial fluids are similar to that in blood plasma (BW = body weight; Ig = immunoglobulin).

Table 2. Daily rate of leukopoiesis, Ig synthesis, and growth in young chicks.[1]

	Normal		LPS challenged	
Process	Production (mg/kg)	Cost (µmolys/kg)[2]	Production (mg/kg)	Cost (µmol lys/kg)
Leukopoiesis in all tissues	650	45.5	1300	90.9
Ig synthesis[3]	114	65.6	121	69.6
Acute-phase protein synthesis[4]	~0	~0	710	386
Total for immunocompetence	764	111.1	2131	546.5
Body weight gain[4]	85000	5950	72446	5212
Lysine intake	-	9520	-	8311
% of intake used for immune processes		1.17		6.71
% of intake used for growth		62.50		62.70

[1]From Klasing and Calvert, 2000.
[2]Data are on a kg body weight per day basis.
[3]Assuming the 25% increase in Ig requires 4 days to accomplish (as in a secondary exposure to an antigen).
[4]Data taken from 14 d broiler chicks.

IV. CONCLUSION

The first priority for animal production systems must be to reduce the incidence of disease challenges. Secondly, changes in diet formulation should be made during disease challenges to account for reduced food intake. Finally, after an immune response, dietary strategies must account for compensatory growth and for regeneration and healing of damaged tissue.

Nutrient requirements to maintain a capable immune system must account for maintenance of immune cell populations, their normal cellular functions and the memory of previous exposures. In addition, mounting an immune response may modify the use and/or supply of nutrients. These nutrients may be derived from dietary sources, but often are re-partitioned from productive processes such as growth and reproduction to the immune response. At the same time, voluntary food intake and activity are reduced in diseased animals, resulting in reduced nutrient uptake and subsequent weight loss.

When formulating diets for animals facing disease challenges, it is important to remember that food intake may be depressed. Therefore, if some nutrients are required at increased levels during disease (eg. antioxidant nutrients), the actual level of incorporation of these nutrients into the diet must be greater to overcome the reduction in dietary intake. For this reason, it may be necessary to formulate diets for pre-exposure, disease states, and recovery periods.

REFERENCES

Beisel, W.R. (1977.). Metabolic and nutritional consequences of infection. In *Advances in Nutritional Research*, ed. H. H. Draper. pp. 125-133. Vol. **1**. New York: Plenum.

Benson, B.N., Calvert, C.C., Roura, E. and Klasing, K.C. (1993). *Journal of Nutrition,* **123**:1714-1723.

Bistrian, B.R., Schwartz, J. and Istfan, N.W. (1992). *Proceedings of the Society for Experimental Biology and Medicine,* **200**:220-3.

Cohn, M. and Langman, R.E. (1990). *Immunological Reviews,* **115**:11-147.

Elsasser, T.H., Caperna, T.J. and Rumsey, T.S. (1995). *Journal of Endocrinology,* **144**:109-117.

Elsasser, T.H., Kahl, S., Steele, N.C. and Rumsey, T.S. (1997). *Comparative Biochemistry and Physiology,* **116A**:209-221.

Elsasser, T.H., Klasing, K.C., Filipov, N. and Thompson, F.,. (2000). The metabolic consequences of stress: Targets for stress and priorities for nutrient use. In *The Biology of Animal Stress*, eds. G.P. Mobergand and J.A. Mench. pp. 77-110. New York: CAB International Press.

Grunfeld, C. and Feingold, K.R. (1992). *Proceedings of the Society of Experimental Biology and Medicine,* **200**:224-227.

Kanno, S., Emil, S., Kosi, M., Monforte-Munoz, H. and Atkinson, J. (1996). *American Surgeon,* **62**:793-9.

Klasing, K.C. (1998a). *Poultry Science,* **77**:983-989.

Klasing, K.C. (1998b). *Poultry Science,* **77**:1119-1125.

Klasing, K.C. and Barnes, D.M. (1988). *Journal of Nutrition,* **118**:1158-64.

Klasing, K.C. and Calvert, C.C. (2000). The care and feeding of an immune system: an analysis of lysine needs. In: *Proceedings of the VIIIth International Symposium on Protein Metabolism and Nutrition,* eds. G.E. Lobley, A. White and J.C. MacRae. Wageningen: Wageningen Press, 2000, pp. 253-264.

Klasing, K.C., Laurin, D.E., Peng, R.K. and Fry, D.M. (1987). *J. Nutr.* **117**:1629-1637.

Klasing, K.C. and Leshchinsky, T.V. (1999). Functions, costs, and benefits of the immune system during development and growth. In *International Ornithology Congress, Proceedings*, eds. N. J. Adams and R. H. Slotow, pp. 2817-2832.

Koh, T.S., Peng, R.K. and Klasing, K.C. (1996). *Poultry Science* ,**75**:867-872.

Piquer, F.J., Sell, J.L., Sotosalanova, M.F., Vilaseca, L., Palo, P.E. and Turner, K. (1995). *Poultry Science* **74**:983-997.

Rosales, F.J., Ritter, S.J., Zolfaghari, R., Smith, J.E. and Ross, A.C. (1996). *Journal of Lipid Research,* **37**:962-71.

Wan, J.M., Haw, M.P. and Blackburn, G.L. (1989). *Proceedings of the Nutrition Society,* **48**:315-35.

Webel, D.M., Mahan, D.C., Johnson, R.W. and Baker, D.H. (1998). *Journal of Nutrition,* **128**:1657-60.

Weinberg, E.D. (1974). *Science,* **184**:952-6.

Weinberg, E.D. (1999). *Emerging Infectious Diseases,* **5**:346-352.

Yoshida, N., Yoshikawa, T., Manabe, H., Terasawa, Y., Kondo, M., Noguchi, N. and Niki, E. (1999). *Journal of Leukocyte Biology*, **65**:757-63.

THE INTERFACE BETWEEN MANAGEMENT AND THE CHICKEN: THE ROLE OF THE IMMUNE SYSTEM

M.E. COOK

Summary

New strategies on the control of the microbial/immune and immune/nonlymphoidal interfaces are critical in discovering alternate strategies to antibiotics as growth promotants. These strategies must not result in resistant microbes and must enhance or maintain immune function.

I. INTRODUCTION

Although plants and animals began to be acquired as domesticants nearly 10,000 years, it has only been the last 50 years in which animals have been intensively raised for food. In 1892 Wehman wrote “Poultry, to be successful on a large scale must be kept in small colonies of about 50 birds, for many more than that number in a single house is apt to cause sickness or disease ere long among”. Given the scale and concentration of the modern poultry industry, one must ponder whence we have come and question how it was accomplished.

Two developments, namely vaccination and antibiotics, allowed the microbial villain of the piece to be overcome sufficiently for the movement from small animal husbandry schemes to the large scale consolidated units today. Before we focus on these developments and the villain, let us consider the most obvious needs of consolidation.

One of the most obvious phenotypes that needed to be modified in the process of animal domestication and consolidation was behavior. The pheasant, one of the more recent wild animals in the US to be placed into domestic conditions in large scale will serve to illustrate domesticated selection. To produce a released bird, ready for hunting season and capable of building a stable flock (the long term goal) these fowl had to be brought into captivity. However, placing rearing pheasants had unintended consequences in that captivity adjusted behavior as successive generations over time were bred in confined space. When wild pheasants were first placed into confinement, they were put into outdoor flight pens often 200-300 feet long and 50 feet wide. They were rectangular in configuration with posts holding containment wire or nylon netting. However, the posts supporting the wire or nylon netting needed interior support to prevent post collapse, especially during heavy snow and ice. Hence, the original flight pens had an obstacle to flight (the support post) that would accidentally kill the flying pheasant. The consequences of breeding 30 generations of pheasants in this consolidated environment were that birds surviving to breeding age were the least likely to fly. When confronted by the caretaker, the most likely survivor fled on foot. Hence, over the course of many generations, game farm pheasants became runners, not fliers. Leading game breeders recognized the problem, namely that management practices had selected for a tame species. Recognition of this problem led game producers to import wild individuals from China. These were used to breed back traits lost in the more domesticated birds. However, current flight pen construction had to change.

Confinement and rearing of a wild species, pheasant, serves to illustrate the consequences of consolidation and the rapid change of a species based upon a simple decision on whether the support beam is on the inside of a flight pen or on the outside. Our subtle decisions made in the short period of animal domestication and consolidation have had dramatic consequences on the nature of living organisms. Hundreds of management factors

---

University of Wisconsin, Madison, Wisconsin, USA

have influenced our process of changing the nature of our domesticated animals. Contemplation of the role of management decisions on animal change is critical in determining the future and sustainability of those decisions.

In the following paper, discussion will be narrowed to one well-defined system I will call 'the ecosystem of the chicken house'. Since this ecosystem is far more complex than can be dealt with here, the discussion will be limited to several ecological interfaces: 1) the interface of select management decisions and the chicken, 2) the interface between the chicken and its microbes (villains), and 3) the interface between time and the chicken which reflects management-induced genetic change. It is in this context that we begin to understand how our rearing practices, including antibiotic use, have altered the nature and physiology of the chicken. It is also in this context that we must view the value and the cost of using antimicrobials and seek new directions to maximize growth of intensively-reared birds.

II. THE INTERFACE BETWEEN MANAGEMENT AND THE CHICKEN: CONSOLIDATION, VACCINATION AND ANTIBIOTIC USE

Wehman (1892) clearly described management strategies from the beginning of chicken domestication to the beginning of the 20th century. Where earlier strategies might be described as 'divide and conquer' or 'don't put all your eggs in one basket'; the modern poultry industry has adopted the riskier strategy of putting all of its eggs in one basket, ie intensive rearing. Such risk taking is largely based on a central idea or ideology. In the case of the chicken, there were two management strategies that allowed the industry to move from 50 bird flocks to the 1 million plus flocks (layers) or 1 million processed broilers per week in a 30 mile radius of an integrated broiler unit. These two strategies were adapted by early leaders in the poultry sector in order to consolidate animal units and to maintain a competitive edge in animal agriculture. This assured, in the case of chickens, that a valuable food (eggs and meat) would be available to the consumer at fractions of its cost in 1900. The success of these two management strategies remain a marvel, and assured the modern world that food would not be a deterrent to human pursuits. The companies involved should be applauded for the risk taking that gave rise to our surplus food supply.

Antibiotics and vaccination are the two management strategies that moved us from 50 bird flocks to multimillion bird unit production facilities. The entire animal food industry hinges on these two technologies and discoveries. Vaccination was not only one of the most brilliant discoveries of mankind, one only need examine the history of poultry science to learn the valuable lesson achieved in vaccination strategies to prevent disease. Once scientists realized that the microscopic world had its own weaponry, antibiotics, we began using antibiotics against microbes. This brilliant strategy in disease prevention was quickly moved to the chicken house. An added benefit appeared in that when using antibiotics in animal agriculture to fight disease, animals grew faster and used less food per unit body weight gain. This remained a mysterious marvel for generations until the explanation was provided by Kirk Klasing of U.C. Davis (discussed later).

Vaccination and antibiotics became crucial tools in the consolidation of the poultry industry. The interface between the chicken and the microbial world was subdued though vaccination and antimicrobial use. The revelation that continual feeding of antibiotics promoted growth and feed efficiency resulted in the concept of 'subtherapeutic' use of antibiotics on a continual basis in the chicken ecosystem. The ramifications of continual antimicrobial use for performance effects in the chicken house were far-reaching. Poultry products became affordable to all households. Soon antibiotic use in animal feeds represented 50% of antibiotics made in the US. This meant that pharmaceutical companies could finance the discovery of new human cures, partly on the back of profits realized by antibiotic use in

animal agriculture. Had it not been for this link between chicken growth and the cures of human diseases, we may have never enticed pharmaceutical companies to risk investments required to generate some of these products. The cost of launching a new antibiotic for use in human medicine, from concept to product, is a staggering $350 million investment. In view of such an investment, one can begin to realize the importance of the chicken's growth response to antibiotics in curing our children's ear infections. More than 7.5 billion broilers are raised a year in the US. Antibiotics have historically improved growth 5-10%. With these numbers, the importance of the chicken in medicinal development is evident.

However, all is not well in the ecosystem of the chickenhouse. We are seeing vaccine failure; and there is evidence of emerging pathogens and resistance to the antibiotic arsenal used against them. There is a revolution in the making and it extends beyond the doors of the chicken house. In fact, it extends to the business plans of leaders in the pharmaceutical houses that oblige and are accountable to stockholders and boards of directors. If the chicken is losing its battle against its pathogens, and if the chicken is not responding to the arsenal, why continue the $350 million investment to keep you and the chicken protected from infectious disease?

The chicken population was consolidated using the management practices of vaccination and antibiotics. Initial use of antibiotics was not for the purpose of promoting improved growth and feed efficiency, but its value was recognized and so the practice grew. The chicken ecosystem had three management pressures driving change: the force of consolidation, the use of vaccination and the continuous use and therapeutic use of antimicrobials.

Consolidation was, in its own right, a pressure for change in disease patterns that all of us recognize. Once winter sets in, and people spend more time indoors, the disease of one become the disease of all in the household, classroom, office or movie theatre. When we brought chickens together in densities less than one square foot per bird, we created an environment in which disease could quickly spread among large numbers. In the chicken house we have seen two events that were catastrophic to the poultry industry in recent history: the Newcastle disease outbreak of the early 1970s in California and the influenza outbreak in the 1980s in Lancaster, Pennsylvania. Both outbreaks, while very contained to their respective regions, cost the consumer hundreds of millions of dollars in increased food costs. Few realize that our animal food production does not far exceed human demand. The fact is that our production, particularly for inelastic markets such as eggs, barely exceeds human needs, hence the low price. During the two historical outbreaks, prices for poultry products reached an all time high even though only a small part of the nation's production was affected.

Consolidation of an animal species increases the likelihood of the transmission of a pathogen among individuals. If we consider the strategy of the villain, the infectious organism, consolidation is the prefect media to achieve its goals. Keeping a flock of no more than 50 birds was a perfect strategy for prevention in the 1890s, but is not sustainable if we are to maintain a low cost food supply. Hence, the greater the consolidation, the lower the food cost, but the greater the chance of the spread of an introduced infectious pathogen. Ultimately, consolidation and low food prices have driven the need for vaccination and antibiotics.

The tool of vaccination (not to be discussed at length here) was the most important vehicle for consolidation of humans and their animals. Controlled exposure to potential insults could assure that an army of educated defenders (antibodies) were in place if the attack came. Most interesting, the agricultural community largely ignored the cost of maintaining a specifically trained militia designed for only one purpose: to destroy only one enemy and often at only one frontal attack. This cost will be explored in more detail later.

Of all management strategies for consolidation, none have come under more scrutiny than the use of antimicrobials. It is interesting that we group a wide range of biologically active compounds under a single term; but however diverse their activity in controlling infectious diseases, they, as do vaccines and consolidation, result in change of the chicken ecosystem. Hence, these three management pressures (consolidation, vaccination, and antibiotic use) have placed a powerful force on the chicken ecosystem. For many generations, forced change has occurred rapidly.

III. BACTERIA, ANTIMICROBIALS AND IMMUNE RESPONSE: WHY THE CHICKEN RESPONDS TO ANTIBIOTICS

Despite the modern misconception that if it was not published yesterday, it is not relevant to the problems of today, many of the answers to today's questions can only be found in works dating back decades. Lev and Forbes (1959) published a paper that is pivotal in the understanding of the ecosystem of the chicken house. They showed that chickens raised in germ free environments grew faster than those exposed to conventional bacterial flora. More importantly, they showed that the feeding of penicillin (an antibiotic known to be a growth stimulant in poultry) had no growth promoting effects in germ free environments in contrast to potent growth stimulation in bacterially contaminated environments. The improvement in growth in germ free conditions was greater than 10%. Even more importantly, antibiotics only partially alleviated the growth suppression associated with the exposure to naturally occurring microbes. Hence, their work showed that there was room for expression. It was this study and similar ones in the same era that turned a colleague, Kirk Klasing, and I independently toward the investigation of the meaning in Lev and Forbes' findings.

Kirk Klasing showed that injecting the cell wall of *Escherichia coli* (endotoxin) into chickens caused them to grow slower or lose weight. Why? Because the exposure of an animal to a normal flora antigen had such a negative impact on growth. The reduction in body weight gain following endotoxin exposure was 30%. In my calculations of feed conversion from his data, those not injected with endotoxin had a feed conversion of 2.63 versus 3.22 for the endotoxin-injected birds (Klasing et al., 1987). This difference in feed conversion was 59 points with each point valued at over $15 million (7.5 billion broilers in the US at average weight of 4 lbs). His work also showed that the type of immune stimulant was not responsible for the reduction in gain. Chicks injected with sheep red blood cells also grew 17% slower than the control birds.

While never published in a full length manuscript, a group at Mississippi State University reported that "vaccination of broilers resulted in lower final body weights, poorer feed conversion and higher 8 day and 42 day mortality. Vaccination reduced overall performance in the absence of overt disease" (Chamberlee et al., 1992). We also observed a similar effect in ducks. In a study with a commercial line of ducks, we injected either a saline control or a standard killed bacterin of *Pasteurella antipestifer*. The injections were given at day 12 and again 10 days later. The ducks were then raised to market age. The final carcass weight of the ducks injected with the killed bacterin was reduced 9% and the amount of breast meat was reduced 5.4%. These data clearly showed that there was a significant cost associated with vaccination. In addition, the decreased growth and feed efficiency observed with a diverse range of antigens suggested that the reduced performance was not antigen mediated, but perhaps related to the immune response.

During the response of the immune system to a stimulus, immune cells such as the macrophage destroy and process (degrade) the stimulant and present specific parts of the stimulant to white blood cells known as lymphocytes. There are two primary classes of

lymphocytes known as T and B cells. These cells proliferate to form clones of cells specifically targeted to the antigen presented. The cloned cells have increased capacity to respond in a rapid defense if exposed to the antigen in the future. The macrophage is also responsible for producing cell signals, known as cytokines, which up-regulate the immune cells during their cloning. The cytokines, interleukin-1 (IL-1) and tumor necrosis factor (TNF), are two major cytokines released from the macrophage during the immune response to an antigen.

Klasing *et al*. (1987) went on to show that the growth depression associated with endotoxin (*E. coli* cell wall) injection could be produced by a direct injection of IL-1. He also showed that IL-1, when placed on cultured muscle strips, increased muscle degradation and decreased protein synthesis. Hence, the growth depression associated with immune stimulation was the result of the release of the immune cytokines and not the direct effect of the immune stimulant. Everyone can relate to the consequence of immune stimulation. When we develop an infectious disease, we lose our appetite and we lose weight. The pathogen is not responsible for these physiological changes, it is the result of immune cytokines. The use of recombinant cytokines as immunotherapy was of great interest when first discovered. However the side effects were so severe that routine use of IL-1 and TNF was never realized in human medicine. Most of you who are reading this paper are involved in animal agriculture. Those of you who are actively involved in poultry production can relate to the effects of immune stimulation. In our research involving growing broilers or turkeys, we often go into the growing facility to weigh birds. During the process of data collection, litter is stirred causing the air to be filled with dust. In this dust is fecal matter rich in killed bacterial cells and hence endotoxin. After breathing this dust for several hours, we all experience similar signs: loss of appetite, low grade fever and fatigue. These effects are immune related. Within the next 24 hrs, the immune system slows and the adverse effects of immune stimulation are resolved. Since it is the immune products that suppress growth, it becomes evident that immune suppression or removal of the immune stimulant should enhance growth in the absence of disease.

Both immune suppression and reduction of immune stimulants (such as through the use of antibiotics) represent two major management strategies used to consolidate poultry. While both management strategies have moved animal agriculture toward more efficient production of food, there is a long term cost associated with these strategies.

IV. THE COST OF ANTIMICROBIAL USE

If animals could be reared in the absence of immune stimulation, the added performance would be valued in the hundreds of millions of dollars in the US. However, it is unlikely that such a process could be economic. Improved sanitation has been shown to minimize decreases in growth associated with the immune response (Roura and Klasing, 1993). However, in certain species one strategy used to reduce immune stimulation is ‘all in/all out’ management. In this scenario, animals are placed in a growing setting only with others of the same age. By doing so, older animals, which often become carriers of infectious pathogens, do not expose younger animals to disease agents. The swine industry experienced major improvements in growth rates when they moved from facilities containing multi-age animals to segregated early weaning strategies where piglets were removed from the sow at an early age and reared in isolation.

Since the 1950s, another management practice used to reduce immune-induced growth suppression involved the use of antibiotics. It was observed that feeding low levels of dietary antibiotics on a continuous basis improved growth and feed efficiency. The reason for the improved performance was that antibiotics reduced the bacterial load (immune

stimulants) in the gut, decreased the level of immune stimulation, and hence prevented the catabolic nature of the immune response. By the 1970s, over 50% of all antibiotics produced in the US went into animal feeds (Von Houwelling, 1978). In some countries, antibiotic use in animal feed was more than 1000 times the use in human medicinals (Witte, 1998).

Witte (1998) reported the consequence of antimicrobial use in animal feeds. The study he reported was perhaps the best longitudinal study illustrating that antimicrobials in animal feed confers resistance to organisms in humans. In 1983 in East Germany, pigs were tested for resistant microbes to the antibiotic nourseothricin prior to its use in swine diets. No resistance was observed. Beginning in 1983, nourseothricin was used as a growth promotant in swine diets. By 1985, microbes with resistance to the antibiotic were observed in the intestinal tract of pigs and on the processed meats. By 1990, resistant *E. coli* was found in the farmers and individuals in the community. In 1987, Shigella (a human pathogen and an organism not associated with pigs) was expressing resistance to nourseothricin. It is now well recognized that bacteria can transfer antibiotics resistance across species of bacteria. This resistance can be transferred both by plasmids as well as genomically. Hence, targeting these immune stimulants as a strategy for enhancing growth rate ultimately confers resistance.

What alternatives are available to assure improved growth and feed efficiency without directing the therapy to the microbial flora, or without suppressing the inflammatory response? Dafwang *et al*. (1987) showed that when broilers were provided with more floor space, the depressed performance associated with consolidation was reduced. In fact, these studies showed that at only the highest densities were antibiotics effective at enhancing growth rate. While these results looked promising, the cost associated with doubling the floor space for 7.5 billion broilers would be prohibitive. Our data also showed that increasing the density of broilers resulted in a reduced size of select lymphoid organs (Bursa of Fabricius and thymus). While antibiotics enhanced the growth rate of broiler chicks raised at high densities, the use of antibiotics was ineffective at restoring the size of the bursa and thymus associated with dense populations of broilers.

A number of antibiotics have been banned in Europe, in part because it is feared that the generations of antibiotic resistance will increase human disease with no effective therapeutic for treatment. Logic would have it that similar bans should be proposed in the United States. A removal of antibiotics as growth promotants could cost poultry and swine producers as much as a billion dollars. One must also consider that the poorer feed efficiency could significantly increase the demand for corn and soybean meal. In addition, animals not fed antibiotics would grow slower and hence would not reach their market weight until days later than those fed antibiotics. This would decrease the number of animals moving through the existing infrastructure. Unless new animal units were constructed, total animals produced would decline. Even more important is the potential negative affect antibiotic removal could have on human health. The continuous use of antibiotics reduces the bacterial load on an animal and hence the final meat products. Would animal products from animals not fed antibiotics represent an even more serious food safety risk? Another indirect means by which a ban on antibiotics could affect human health involves the pharmaceutical manufacturers. Since animal agriculture represents a source of income for antibiotic manufacturers, what will be the likely outcome if this source of income is lost? Will a company be eager to spend the $350 million needed to create a new antibiotic if it will have lost a major market which helps defray these costs? If so, then the future generation of new antimicrobials for human health could be (or is) at great risk. As one thinks about these issues, it would appear that we have created a trap that may be difficult to avoid without a major restructuring of the animal industry and their allied industries. It is clear that research is needed to clearly define the costs associated with both the use and avoidance of antibiotics in animal feeds.

V. GENETICS

Our discussion to this point clearly shows that the interface between management strategies and the microbial world is greatly linked to the immune response of an animal, with critical points of growth and feed efficiency being the driving variables needing optimization. Most of our management strategies in consolidated animal units attempt to minimize the inflammatory process, whether intentionally or by chance. The unexpected consequence, particularly with regard to the microbial world, of antibiotics resulted in increased resistance and hence a potential human hazard. The microbial immune interface has another dimension worthy of discussion: the effects on genetic selection. While improved growth was achieved by reducing immune stimulation (Lev and Forbes, 1957 only showed a partial restoration of growth through the use of antibiotics), genetic selection for growth rate and feed efficiency was not without its effect. As previously discussed, symptoms associated with an immune reaction include decreased body weight (or rates of weight gain) and poorer feed efficiency (or anorexia). In addition, immune stimulation can actually enhance mortality. These are the very endpoints we wished to improve and the reason for our desire to reduce the level of immune stimulation using antibiotics. However, if you were an animal geneticist selecting commercial breeding stock and looking for the birds which grow the fastest and convert feed the most efficiently, which bird would you select? Would the bird with the greater or lesser inflammatory response perform best? The birds that are selected as the superior performers in theory should have the poorest immune response. In fact, from the geneticist's point of view, the less growth depression due to immune stimulation the better. Generation after generation of selecting animals that perform in the top 20% in the typical immune stimulating environment loaded with airborne endotoxin has resulted in an animal that is less likely to mount an inflammatory response associated with the cytokines IL-1 and TNF (M.E. Cook, unpublished data).

We became very interested in the effects of genetic selection on the immune response of an animal. Access to such genetic lines however proved difficult. Fortunately, a duck company, Maple Leaf Farms, was interested in this question as well. This highly vertically-integrated company had its own breeding program where performance traits were selected. While the studies conducted were not pure and ideal, we were able to gain limited insight into the influence of selection for performance on immune responses. In our first study, we compared a T cell dependent immune response to phytohemagglutinin-P. Fortunately, the company had a line of ducks that did not have heavy selection pressures (we called this line the control). The other lines were selected for rate of gain, breast meat yield, or feed efficiency. All lines selected for performance traits had reduced immunoreactivity of 28% or more when compared to the line with less (or no) selection pressure. We expanded our test to include antibody synthesis in response to an antigenic stimulus. The antibody response was 29 to 79% less in lines selected for improved performance when compared to our control. Dr. Venelin Kounev soon joined our group to try to improve our understanding of growth and immune function. Working within a given elite duck line (the great grandparent lines) he was able to show a direct inverse correlation between body weight and cell-mediated immunity ($r = -.38$). This means that in this line of ducks, if the top performers were selected as grandparents for the next generation, those selected would have the poorest ability to generate an immune response to a stimulus.

Others were making similar observations. In a study by Sharaf *et al*. (1988), turkeys selected for enhanced egg production had decreased antibody titers in response to Newcastle Disease virus vaccination. Hence, these data suggested that genetic selection for enhanced performance (whether for growth, feed efficiency, or egg production) is associated with suppressed immunological function. Work has shown that the genetic overexpression of

genes for tumor necrosis factor (TNF) greatly retards growth and thriftiness of animals. Hence, the obvious effect of selection for growth in immune stimulating environments is the selection against catabolic cytokines. However, no data are available to directly support this hypothesis. We are actively engaged in such research but have failed to convince the scientific community of its merits

It appears that not only are management practices changing to enhance animal performance, but also that the immune system of our agricultural species is being modified to improve animal growth and feed efficiency. As there are consequences associated with an attempt to modify immune stimulation, there are likewise consequences in modification of the inflammatory process and other immune reactions. One consequence involves physiological processes that cells of the immune response are involved in that have little relationship to defense. Select cells of lymphoidal origin are responsible for the maintenance of tissue structure and function. The macrophage is essential in the repair and remodeling of tissues associated with growth, development and injury. Hence, one can predict that selection against inflammatory or immune processes may lead to physiological aberrations.

VI. OUT OF THE TRAP?

Clearly, new strategies are needed to assure animal growth, to maintain an immunological expressive animal and to reduce pressure on the microbial flora ecosystem. Such approaches should target neither the immune system nor the microbial flora. While these targets have rewarded us with improved animal growth and perhaps even wellbeing, these targets have limitations that can be pushed only so far. We have signs that suggest that it is time to remove pressures on the microbial flora and the animal's immune system. What are those new targets going to look like? If we need to find alternatives for maintaining the existing level of growth in animals, how will we structure these alternatives? It is now time to explore these ideas. We are landed in a kind of trap in that our management strategies of antibiotic use may lead to resistant disease organisms and our genetic selection practices result in 'immune suppressed' animals. Where is the door of opportunity? We have created a system of unintended consequences that demands creative thinkers.

VII. ALTERNATIVES: WORK WITH CONJUGATED LINOLEIC ACID

Initial work with nutrition and immunity examined pharmacological levels of certain nutrients in an effort to enhance immune function and was not promising. Working with two integrated turkey companies in the early 1990s, we reformulated the diets based on experience and literature reviewed (Cook, 1991). Both companies said the results were disastrous. It was then that I realized that there was a cost to immunological function, much as Kirk Klasing was demonstrating in his work. Other works in the literature, although sketchy, were showing similar effects. Nockels (1979) had shown that immune enhancement of guinea pigs using vitamin E had a deleterious effect on growth rate when the pigs were infected with equine encephalomyelitis virus. Gross (1992) also showed that while high dietary levels of ascorbic acid reduced lesions associated with Mycoplasma and *E. coli*, chickens fed the high ascorbic acid had much poorer feed conversion. It appeared that implementation of nutritional regimes proven to reduce pathogenesis of select infectious disease were costly. Enhanced immunoreactivity suppressed performance and hence an alternative appeared necessary in the management of performance in immunoreactive animals.

To understand alternative methods to prevent the immune-induced growth suppression and to find ways to improve animal performance without the aid of antibiotics, a more basic understanding of immune-induced growth suppression was needed. The question

that had to be answered was how do immune cytokines suppress growth? Rodemann and Goldberg (1982) had shown that muscle degradation associated with IL-1 was associated with increased production of prostaglandin E_2 (PGE_2). They went on to show (Goldberg *et al*., 1984) that when PGE_2 was directly applied to muscle strips, the degradation of muscle was increased. Based on these works, we began a series of studies to identify dietary factors that would prevent the wasting of body weight during the immune response. A number of compounds were identified, however, they all appeared to be immunosuppressive. Our goal was to prevent the loss of body weight in the immune challenged animal without having a negative effect on immune function.

We began work with conjugated linoleic acid (CLA) when Mike Pariza, a collegue in Food Microbiology and Toxicology, proposed feeding some laying hens CLA as he had found that it had potent anticarcinogenic activity. At that time, he believed that the anticancer activity of the compound might be related to antioxidant capacity. His goal was to feed laying hens CLA so he could harvest the eggs, make mayonnaise and determine if shelf life was extended. Since one mechanism in the reduction of tumor formation involved the immune system, we began collaborative studies on CLA and immunological function.

What was most appealing about CLA with regard to the Klasing model of immune-induced growth suppression was that CLA is a fatty acid markedly similar to linoleic acid (18:2, cis 9, cis 12), which was the precursor for PGE_2, the lipid mediator that caused muscle wasting. The double bond configuration of CLA (18:2) prepared in his laboratory was predominately cis 9, trans 11, or trans 10, cis 12. Even more perfect was that these fatty acids were naturally occurring (see www.wisc.edu/cook for more detail). As predicted, CLA prevented growth suppression resulting from immune stimulation (Cook *et al*., 1993; Miller *et al*., 1994). Later we found that it even protected against growth suppression associated with the direct injection of TNF and end wasting autoimmune disease. More exciting, CLA did not prevent immune-induced growth suppression by suppressing the immune system. In fact, it enhanced the immune response. This became our first alternative to the growth suppression caused by immune stimulation. Instead of targeting the microbial world (which would only develop resistance) or the immune system (a potentially bad idea) we target how nonlymphoidal tissue responded to the immune system (see US Patents: 5,430,066; 5,428,072; 5,827,885; 5,674,901 and 5,725,873).

An analogy may aid in explaining these results: If one thinks of the immune system as a military force, the immune reaction as a battle, and the animal's nonlymphoidal tissue (such as muscle) as the nonmilitary citizen where the battle is taking place, during conflict there is always collateral damage to nontargeted sites. Our hope is to minimize this collateral damage by erecting barriers. CLA was found to be a biological barrier to the collateral damage associated with the immune response.

Another area we thought would be a beneficial control point in protecting against the collateral damage of the immune response is the intestine. Of all places in the animal's body, the intestine hosts the greatest quantity of immune stimulants. As was previously mentioned, one of the consequences of the immune response is a reduction in feed intake. Work on our campus by Donna McCarthy (Daun and McCarthy, 1993) had shown that IL-1 induces anorexia in part by causing the release of the gut peptide, cholecystokinin (CCK). CCK in turn induces a satiety effect and alters gut motility. We reasoned that if we could interfere with the actions of CCK, then we could prevent reduced feed intake associated with the immune response. Literature suggested that CCK was released into the lumen of the intestine. This source of CCK was targeted using antibody generated against CCK. We selected the laying hen as our source of anti-CCK production since hens can be stimulated to produce high quantities of antibodies in the egg yolk. When egg powder containing antibodies to CCK

was fed to broiler chickens, growth rate and feed efficiency improved. Antibodies to other gut peptides also showed similar benefits (see US patents 5,827,517; 5,725,873 and 5,989,584). While these anti-CCK antibodies were not found to stimulate food intake, they proved very effective as growth promotants. We have continued our efforts to make antibodies to other physiological processes involved in the immune response with remarkable success.

Another area in regulating immune-induced growth suppression involved the development of a method to continuously monitor when an animal is immunologically challenged. We reasoned that if we could know rapidly and noninvasively when an animal was undergoing an immunological reaction, that animal could be treated accordingly. For example, is there a means to continuously monitor layers in a large complex (a million or more) to know if a disease is in the early stages of development? If a rapid method of detection could occur, then these diseased animals could be removed from the flock or specifically treated. To accomplish this goal we used the natural fractionation of isotopes of carbon (US patent 5,912,178). During enzymatic processes, enzymes discriminate against substrates containing ^{13}Carbon and preferentially use substrate with ^{12}C. We reasoned that during the immune response, as skeletal muscle is degraded and amino acids are released, these amino acids have two pathways of metabolism. They can be reused for acute phase protein synthesis or burned to CO_2 and expired. Since the complete metabolism to CO_2 has many enzymatic steps, we predicted that the amount of ^{13}C in breath would decrease during the catabolic response and indeed it does. Hence, one could envision the continuous sampling of CO_2 from ventilation exhaust for monitoring ^{12}C:^{13}C ratios.

REFERENCES

Chamberlee, T.N., J.R. Thompson and J.P. Thaxton. (1992). Effects of day old vaccination on broiler performance. *Poult. Sci.* **71**(Suppl. 1):144 (Abstr.).

Cook, M.E., C.C. Miller, Y. Park and M. Pariza. (1993). Immune modulating by altered nutrient metabolism: Nutritional control of immune-induced growth depression. *Poult. Sci.* **72**:1301-1305.

Dafwang, I.I., M.E. Cook and M.L. Sunde. (1987). Interaction of dietary antibiotic supplementation and stocking density on broiler chick performance and immune response. *Br. Poult. Sci.* **28**:47-55.

Daun, J.M. and D.O. McCarthy. (1993). The role of cholecystokinin in interleukin-1 induced anorexia. *Physiology and Behavior*, **54**:237-241.

Goldberg, A.L., V. Baracos, Rodemann, L. Waxman and C. Dinarello. (1984). Control of protein degradation in muscles by prostaglandins, Ca++ and leukocyte pyrogen (interleukin-1). *Federation Proc*., **43**:1301-1306.

Klasing, K.C., D.E. Laurin, P.K. Peng and D.M. Fry. (1987). Immunologically mediated growth depression in chicks: Influence of feed intake, corticosterone and interleukin-1. *J. Nutr.* **117**:1629-1637.

Lev, M. and M. Forbes. (1959). Growth response to dietary penicillin of germ-free chicks with a defined intestinal flora. *Br. J. Nutr.* **13**:78-84.

Miller, C.C., Y. Park, M.W. Pariza and M.E. Cook. (1994). Feeding conjugated linoleic acid to animals partially overcomes catabolic response due to endotoxin injection. Biochem. *Biophys. Res. Comm*, **198**:1107-1112.

Nockels, C.F. (1979). Protective effects of supplemental vitamin E against infection. *Federation Proc*., **38**:2134-2138.

Rodemann, H.P. and A.L. Goldberg. (1982). Arachadonic acid, prostaglandin E2 and F2 influence rates of protein turnover in skeletal and cardiac muscle. *J. Biol. Chem.* **257**:1632-1638.

Roura, E. and K.C. Klasing. (1993). Dietary antibiotics reduce immunological stress elicited by poor sanitation or consumption of excreta in broiler chicks. *J. Nutr*. **122**:2383-2390.

Sharaf, M.M., K.E. Nestor, Y.M. Saif, R.E. Sacco and G.B. Havenstein. (1988). Antibody response to Newcastle Disease virus and *Pasteurella multocida* of two strains of turkeys. *Poult. Sci*. **67**:1372-1377.

Von Houwelling, C.D. (1978). Draft environmental impact statement. Subtherapeutic agents in animal feeds. Food and Drug Admin. Washington, D.C.

Wehman, H.J. (1982). Wehman's Practical Poultry Book. Wehman Bros., New York, pp. 110. Quoted in: Wilson, W.O. 1974. Housing In: American Poultry History 1823-1973. American Printing and Publishing, Inc., pp. 221.

Witte, W. (1998). Medical consequences of antibiotic use in agriculture. *Sci*. **279**:996-997.

DIETARY MANAGEMENT OF THE INTESTINAL MICROBIOTA USING PROBIOTICS AND PREBIOTICS IN HUMANS AND ANIMALS

A.R. BIRD[1] ,W.J. CROOM[2],Jr. and B.W. McBRIDE[3]

Summary

The gastrointestinal tract is colonized by complex microflora components which have positive and negative influences on host health. This balance can be altered by introducing desirable microorganisms (probiotics) or stimulating beneficial endogenous bacteria by using nondigestible food constituents (prebiotics). Although both strategies have a sound scientific basis, direct evidence for many of the stated claims is lacking. Results from livestock research are relatively sparse and often inconsistent, although recent poultry production trials demonstrate modest but nonetheless economically meaningful effects of probiotics on growth, mortality and pathogen contamination of carcasses. Scientific substantiation of probiotic and prebiotic efficacy is clearly a prerequisite for continued development and adoption by the poultry industry.

I. INTRODUCTION

Dietary manipulation of the composition and activity of the flora of the gastrointestinal tract has long been considered a means for promoting human health and well-being. In the early 1900s, Metchnikoff (1908) was advocating consumption of fermented dairy foods containing live bacterial organisms to supplant potentially harmful putrefactive microbes (‘autointoxication’) from the human gut. This and other early strategies for improving the gut microflora were supported by anecdotal information and based on the premise that the enteric flora was harmful to the host. This negative impression was reinforced by clinical and experimental research in the 1950s demonstrating improvements in human and animal health, and animal productivity, as a result of using oral antibiotics. The superior growth rates of germ-free animals, whose gut is sterile, relative to conventional counterparts provided further support for the notion that the intestinal microflora was inherently harmful to the host animal. However, there was also growing concern that antibiotic therapies, particularly those involving long-term regimens, enhance susceptibility to infection. Experimental studies were also showing that digesta and faecal extracts taken from healthy adults and administered to very young animals afforded protection against various pathogens, including Salmonella (Nurmi and Rantala, 1973). This practice became known as ‘competitive exclusion’ (Mulder *et al*., 1997).

The gut microflora is now considered to play a critical role in preventing colonization of enteropathogens. The developing body of knowledge about the innate defensive capabilities of the gastrointestinal ecosystem underpins contemporary strategies to improve livestock productivity and human health status through manipulation of the gut microflora. Commercial exploitation of the concept in the last decade has resulted in a dramatic rise in the number of food products and supplements marketed to consumers on the basis of gut

[1]CSIRO Health Sciences and Nutrition, Kintore Avenue, Adelaide, SA 5000.
[2]Department of Poultry Science, North Carolina State University, Raleigh NC, USA.
[3]Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario N1H2W5, Canada.

functionality. Products containing live microbial supplements (probiotics) and/or specific nondigestible food ingredients (prebiotics) that improve the gut microflora and purportedly deliver health benefits, ranging from improved digestive function to prevention of cancer, are widely available and heavily promoted in markets worldwide, including Australia. Development of similar commercial products for animal agriculture has also occurred, but on a much smaller scale.

The purpose of this review is to provide an overview of the gut microflora, its relevance to host health and resistance to disease, and to examine the potential of the aforementioned 'biotic' strategies for improving the properties of the microflora. Although most of the public-domain information on these particular topics relates to biomedical research on human health and nutrition, much of it is still pertinent to discussion of the impact of the microbial flora on health and performances of other species, including poultry.

II. PROPERTIES OF THE GUT MICROFLORA

The gastrointestinal tract of humans and other vertebrates is colonized by a complex assemblage of microorganisms, principally bacteria, of which the greatest density and species diversity occur in the hindgut. The healthy human colon, for instance, harbours in excess of 100 g of bacteria, numbering about 10^{14} organisms, and comprising >400 species representing about 40 genera (Bird *et al*., 2000). In birds, the specialised anatomical compartments of the avian gut, such as the crop and caeca, are also sites of dense microbial colonization (Barrow, 1992).

The various microbial communities that comprise the normal gut microflora have positive and negative influences on the host. Predominant groups such as the lactic acid bacteria, namely lactobacilli and bifidobacteria, are deemed beneficial because they supposedly generate a luminal environment unfavourable to the growth and survival of foreign or opportunistic microbes. In addition, they may indirectly influence resistance to infections by augmenting the host's cell-mediated and humoral immunity. Accordingly, maintaining a microflora in which high numbers of lactic acid bacteria prevail is considered important to the health of the gut. However, composition and metabolic activity of the microflora are influenced by a large variety of external factors. Environmental stressors, including nutrient deprivation, by augmenting numbers of pathogenic and putrefactive organisms, adversely affect the gut’s microbial balance, and may increase risk of certain acute and chronic diseases for the host.

The composition of the enteric microflora also influences the biochemical environment to which the bowel epithelial mucosa is exposed. Certain end-products of bacterial activity are vital for mucosal health (Bird *et al*., 2000). Conversely, particular species of enteric bacteria have been shown to convert innocuous chemicals found in the bowel lumen into highly toxic end-products. Potentially, these metabolites may damage the epithelial lining of the bowel wall, thereby weakening the defensive barrier and increasing risk of infection. Increased cellular turnover along with other repair mechanisms may result in higher tissue metabolic costs. Various enteric bacteria have been implicated in the pathogenesis of a range of non-infectious bowel disorders in humans, including cancer and mucosal inflammation. Extraintestinal tissues may also be adversely affected because certain lumenal toxins are absorbed by the large bowel.

III. PROBIOTICS AND PREBIOTICS - DEFINITIONS AND APPLICATIONS

(a) Probiotics

Probiotics are dietary preparations of live, nonpathogenic microbes which benefit the host by improving the 'balance' of gut bacteria perceived as either beneficial or harmful (Fuller 1989). Since its inception (Lilly and Stillwell, 1965), the definition of a probiotic has broadened to accommodate functionality independent of changes to the enteric microflora and effects mediated by components of microbial cells (Salminen *et al* 1999; Naidu *et al*., 1999). The term biotherapeutic agent (Elmer *et al*., 1996) was coined to describe probiotics with specific clinical applications. In humans, ingestion is the main mode of delivery and the large bowel the principal target for probiotic action. Also, the concept applies equally well to other epithelial surfaces, such as the skin and genitourinary tract.

For humans, the most common probiotic agents have been bacteria that produce lactic acid, particularly strains of lactobacilli and bifidobacteria of human origin, although numerous other bacterial species and non-bacterial microorganisms such as yeasts have been used. In comparison, animal probiotics span a broader taxonomic range of microorganisms, and include certain species of fungi. Human probiotics are mostly consumed as foods, fermented dairy products being a popular probiotic vehicle, or as nutraceuticals in the form of powders, tablets and capsules. Probiotic foods for humans occasionally include more than one probiotic strain or species, whereas animal probiotic formulations usually contain a multitude of different and often taxonomically diverse species. As a generalization, ingestion of sufficient numbers of viable probiotic cells results in a significant increase in faecal populations of the relevant probiotic organism within days of the treatment commencing. The response is dose-dependent and usually in the order of 1-2 log units relative to pre-ingestion counts. Animal studies have demonstrated that probiotic numbers are amplified along the length of the gut. For the response to be sustained, regular probiotic consumption is required. Once supplementation is discontinued, faecal probiotic counts return to baseline levels within about 5-10 days.

The issue of strain identification is an important one for successful future development and application of probiotics. Strains differ in their functional properties. A successful probiotic must fulfill important technological and functional criteria. For instance, it must retain its physiological properties during manufacture and incorporation into carrier food (eg. yogurt) or vehicle, and during storage. It must also retain its functionality in the gut ecosystem and so must be resistant to the effect of acid, bile and other chemical insults. Other intrinsic characteristics considered important for probiotic efficacy are the ability to remain viable within the gut. Recent studies, however, suggest that dead probiotic bacterial cells or parts can bind luminal toxins and also potentiate the gut immune system.

(b) Prebiotics

Practical limitations with the probiotic approach resulted in the development of the alternative strategy of prebiosis for managing gut microflora. Prebiotics, as defined originally, are "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995). It has been demonstrated conclusively that fructoligosaccharide (FOS) ingestion dramatically and selectively increases numbers of faecal bifidobacteria to the extent that these microbes predominate the large bowel flora (Gibson and Roberfoid, 1995). Relatively small doses of FOS (<15 g/d) have potent bifidogenic effects in humans and other animals (Bird, 1999). Increased bifidobacterial counts are detected within days of commencing FOS administration, and the extent of the increase tends to be dependent on prebiotic dose and on

the size of the baseline bifidobacterial population. The practice of using nondigestible carbohydrates to modulate the gut microflora was established well before the prebiotic concept was pioneered in 1995. Nonabsorbable sugars such as lactose, as well as small doses of FOS, were shown to reduce intestinal colonization to Salmonella as well as enhance resistance against Salmonella in chickens (Oyofo *et al*., 1989; Bailey *et al*., 1991). The range of dietary constituents with prebiotic properties continues to expand and now includes various nondigestible oligosaccharides other than FOS, as well as certain types of resistant starch (Bird, 1999; Bird *et al*., 2000). Preparations containing combinations of both probiotics and prebiotics, known as synbiotics or probiotic enhancers (Casas *et al*., 1998), may prove more effective than the individual component agents in selectively altering the intestinal microflora. For instance, faecal bifidobacterial densities were increased significantly in pigs by feeding either galactooligosaccaride or the probiotic *Bifidobacterium longum* 1941, however co-administration of both agents resulted in a further increase in the faecal bifidobacterial population (Topping *et al*., 1997). Because the response to the synbiotic was additive, it suggests differential modes of action for the prebiotic and probiotic. Mixtures of probiotic strains, assuming they each exert benefits by different mechanisms, may be more efficacious than preparations containing single cultures.

IV. RATIONALE FOR HEALTH PROMOTION

The concept of prebiosis and probiosis is predicated on the purported health-promoting benefits of a select group of enteric bacteria, notably bifidobacteria and lactobacilli. Health benefits may be acquired through various possible mechanisms, for instance, displacement of microbial populations that are potentially pathogenic or have the metabolic capacity to release compounds likely to cause acute or chronic disease in the host. Circumstantial evidence and observational studies provide support for augmenting Bifidobacterium and Lactobacillus populations in the gut. Evidence that an absolute or relative increase in specific probiotic bacterial populations in the colonic microflora actually confers a health benefit for the host is equivocal. However, a number of plausible biological mechanisms have been proposed to explain the purported health-promoting actions of probiotic bacteria, the main ones being: (1) suppression or elimination of enteropathogens directly through chemical antagonism (production of antimicrobial compounds), or indirectly through competition for essential resources (eg nutrients or epithelial binding sites), (2) suppression of bacterial toxins through lowered production or activity, and (3) potentiation of the host's immune defenses. Recent experimental evidence indicates that competition for adhesion receptors may be a particularly important defense against invading bacteria (Fuller, 1999), although there is evidence that other modes of action also operate (Casas *et al*., 1998). Similar mechanisms are believed to also account for the actions of prebiotics, although there has been less extensive study of these agents compared to probiotics.

V. EFFICACY OF PROBIOTIC AND PREBIOTIC APPLICATIONS

Administration of probiotics and prebiotics to humans and experimental animals has demonstrated conclusively that the gut microflora is amenable to dietary manipulation. Regular ingestion of large numbers of viable probiotic microorganisms or prebiotic agents elicits substantial qualitative and quantitative changes in the faecal flora of animals and humans. However, whether these microbial changes translate into biologically meaningful effects has yet to be resolved. Epidemiological data are limited, and provide no proof of a positive relationship between intake of probiotic foods and risk of chronic disease in humans

(Sanders, 2000). Results of numerous clinical trials evaluating efficacy of probiotics in the prevention or treatment of various common human disorders are also equivocal. Evidence supporting a role of probiotics in protection, or reduced severity or duration of diarrhoea in humans is weak, except for specific applications in the clinical management of acute rotaviral diarrhoeal disease in children and infants (Saavedra, 2001). Studies in domestic livestock are similar in that their outcomes are inconsistent, whereas research involving laboratory animals has demonstrated consistently that oral administration of specific, probiotic strains under well-controlled experimental conditions augments local and systemic immune responses, reduces intestinal and faecal densities of potential pathogens and improves indices of cancer initiation and progression (Hirayama and Rafter, 2000). Recent poultry production trials have also demonstrated that certain probiotics effectively reduce pathogen contamination of carcasses, improve survival, and increase growth and feed conversion in flocks managed under hygienic commercial conditions (Casas *et al*., 1998).

Possible reasons for the differential response to probiotics are numerous. Most human studies are carried out using healthy populations of subjects. It is possible that the response to probiotic agents is minimal in these circumstances, whereas they may be more substantial in situations when barrier function of the gut has been disrupted. Animal and human studies clearly demonstrate that probiotic functionality is entirely strain dependent. Furthermore, probiotics may differ in the degree of protection provided against a particular range of pathogenic organisms. Although numerous studies in humans and animals have established that probiotics modulate immune system function, as assessed by the response in a range of experimental endpoints (Arunachalam *et al*., 2000), it has yet to be shown that the various changes that have been observed correlate with enhanced resistance to infectious disease.

VI. ENERGETIC CONSIDERATIONS

Although the reasons for the use of probiotics in humans and poultry may have, on the surface, many similarities, the ultimate endpoint considerations in their use are different. The major consideration for the use of probiotics in humans is health maintenance or enhancement. In poultry, the primary consideration is economic return on a production system via enhanced growth and efficiency of feed utilization. Gaskins (2001, page 586) eloquently makes this distinction in his consideration of probiotic use in another domestic livestock species, the pig. He states, "Animal growth efficiency is, however, a concept introduced only upon domestication of the pig as a food animal. These issues provoke consideration of an optimal gut microflora for intestinal health vs. its effects on the efficiency of gastrointestinal and whole body growth throughout the productive life cycle of a pig". The same can be said for poultry.

In making this differentiation, one has to consider the implications of increased maintenance costs imposed on the intestinal tract by the presence of either autocthonous or allocthonous bacteria. (For a detailed consideration of this issue see review by Gaskins, 2001). Simply stated, does the presence of bacteria, beneficial or pathogenic, stimulate physiological responses by the host animal that increase its energetic needs? One can intuitively sense that an enteric pathogenic infection is going to place huge energetic costs on the host animal by requiring an extensive immune response, as well as tissue repair, as the result of epithelial denudation during the infection. However, the picture is not as clear in regards to probiotic organisms that are designed to enhance or establish "beneficial" autocthonous intestinal and colonic bacterial ecosystems. Do autocthonous or allocthonous bacteria stimulate the immune system to a heightened level of vigilance? Do probiotic bacteria compete with the host organism for nutrients otherwise readily digested, absorbed

and assimilated? Does the presence of autocthonous or probiotic organisms stimulate metabolically costly production of anutrients? Gaskins (2001) states, "Indeed, the growth-promoting effects of antibiotics are consistent with the possibility that the normal microflora negatively impacts the energetics of animal growth" (Gaskins 2001, page 602). It should be clear that the present authors are in complete agreement with Gaskins (2001) that autocthonous and allocthonous bacteria have very specific and measurable effects on gut function. It is our contention, however, that in healthy animals with normal intestinal microflora, these effects are highly specific, and protective or regulatory in nature and, in themselves, evoke no measurable increase in the amount of energy required by either the gastrointestinal tract or the whole animal. There are several reasons for our position. First, one argument used to emphasize the potential impact of autocthonous organisms on whole-animal maintenance requirements is the fact that the numbers of resident microorganisms in the digestive tract exceed the number of host-animal cells in the gut by an order of magnitude (Gaskins, 2001). This observation does not take into account the larger size and mass of the host cells, or their metabolic activity. The single layer of enterocytes accounts for 60-70% of the total oxygen consumption of the intestinal tissue and each enterocyte is many fold larger than resident bacteria. Another factor considered important by proponents of this theory is the ability of both the autocthonous and allocthonous bacteria to stimulate mucous production by the host goblet cells, as well as local and systemic immune response (Gaskins, 1997, 2001). Changes in diet composition, such as fiber level, can also elicit similar responses which are not considered detrimental.

It is assumed that this reaction to resident bacterial populations requires enough energy to significantly alter whole-animal energy balance. We have found indirect evidence that autocthonous populations of bacteria or protozoa have little influence on whole animal or intestinal tissue energetics in mice (Fan and Croom, unpublished observations). Mice fed sodium monensin, laidlomycin or laidlomycin propionate for two weeks exhibited virtually no changes in whole body oxygen consumption (2.37 and 2.45 μmol O_2 /min/ g mouse; $p<0.216$, control vs. monensin, respectively) and total jejunum oxygen consumption (2.11 and 2.26 μmol O_2/min/g jejunum; $p<0.256$, control vs. monensin, respectively). Monensin has been demonstrated to reduce the numbers of Clostridium perfringens in the colon (Elwinger *et al*., 1998), decrease the numbers of lactobacilli, and increase the numbers of coliforms and enterococci in the crop of broilers (Rada and Marounek, 1996). Furthermore, it has been shown to have no effect on Na^+- dependent glucose transport in the jejunum of chicks (Riley *et al*. 1986). The presence of autocthonous or allocthonous intestinal microorganisms should stimulate energy requiring processes, such as mucin secretion by goblet cells and local immune responses (Gaskins, 1997). Hence, monensin mediated reductions in commensal nonpathogenic bacteria in the intestinal tract should have a measurable decrease on intestinal oxygen consumption due to alterations in intestinal function and immune response. We have observed none. An important caveat of this observation, however, is the potential limited microbial action of monensin. Similar studies need to be conducted using compounds with a wide and better-described spectrum of antibiotic activity.

It is interesting to note that Madsen *et al*. (2001) have recently reported that treatment of control and IL-10 deficient mice (murine Crohn's disease model) supplemented with the probiotic consortium, VSL 33 (Bifidobacterium longum, B. infantis, B. breve; $9X10^{10}$, Lactobacillus acidophilus, L. casei, L. delbrueckii subsp. L. bulgaricus and L. planetarium; $8X10^{10}$; Streptococcus salivarius subsp. thermophilus; $20X10^{10}$), resulted in decreases in the secretion of the inflammatory cytokines, IFN-γ and TNF-α, and increases in the total load of bacteria in the colon of both groups. Madsen *et al*. (2001) suggest that intestinal epithelial cells are able to differentiate between commensal nonpathogenic bacteria and deliver distinct

cytokine responses to underlying immune cells. Gaskins (1997) has proposed similar differential immune responses to intestinal pathogenic and non-pathogenic bacteria. Lee (1984) has stated that the normal ambient immunological response of commensal nonpathogenic intestinal bacteria are low and only a limited number of autocthonous microorganisms are able to evoke immunological responses in the intestinal tract and that this property is "only of minor consequence in the microbial ecology of the gut" (Lee 1984, pages140-141).

Perhaps the most compelling data that suggests that intestinal non-pathogenic autocthonous or allocthonous organisms do not unduly influence the energetic balance of animals is the growing body of evidence demonstrating that supplementation with specific formulations of allocthonous organisms results in increased growth and feed efficiency (see review by Casas *et al*., 1998). As noted by Croom *et al*. (1993), any manipulations in the digestive function of animals that result in improvements in performance must be such that any increases in the energetic efficiency of the animal resulting from this manipulation must be larger than increases in maintenance costs incurred. Although many feel that the true value of the use of probiotics is during microbial challenges associated with less-than-ideal sanitation and environmental conditions, many studies conducted under very prudent management systems demonstrate significant improvements in growth and performance. Hence, these data, taken as a whole, are de facto proof that the argument that probiotic or allocthonous supplementation of healthy animals imposes a detrimental effect on the energetic requirements of supplemented animals is flawed.

VII. CONCLUSION

Dietary modulation of the properties of gut microflora to improve human health and well-being and improve animal productivity is an attractive concept built on solid science, but scepticism surrounds the ability of practical strategies such as probiosis and prebiosis to deliver meaningful benefits. Laboratory animal studies demonstrate that probiotic and prebiotic preparations are effective tools for altering the properties of the gut microflora and that these interventions result in measurable improvements in experimental endpoints, such as greater disease resistance. Although augmenting populations of select microbes in the bowel conceivably may incur an energetic cost, on balance any increase in energy expenditure is overshadowed by the various advantages of this intervention. Although the large body of evidence from experimental animal studies provides strong support for the prebiotic/probiotic concept, human studies have been disappointing in this regard. Earlier livestock production trials produced conflicting results, however more recent well-designed studies conducted under commercial conditions suggest that certain probiotic preparations are efficacious in improving health and other performance parameters in poultry. The key to the successful development of probiotic and prebiotic strategies appropriate to animal agriculture is the development of paradigms for the design of probiotic consortia based on the known beneficial actions of specific microorganisms on specific host-animal physiological processes (Fuller, 1989). Because of the paucity of this type of information, the rationale for use of probiotics can be described as being in a state of "scientific infancy".

REFERENCES

Arunachalam, K., Gill, H.S. and Chandra, R.K. (2000). *European Journal of Clinical Nutrition,* **54**: 263-267.

Bailey, J.S., Blankenship, L.C. and Cox, N.A. (1991). *Poultry Science*, **70**:2433-2438.

Barrow, P.A. (1992). Probiotics. The Scientfic Basis. R.Fuller (ed). Chapman & Hall, London.

Bird, A.R. (1999). *Asia Pacific Journal of Clinical Nutrition*, **8**(Suppl.):S32-S36.

Bird, A.R., Brown, I.L. and Topping, D.L. (2000). *Current Issues in Intestinal Microbiology*, **1**:25-37.

Casas, I.A., Edens, F.W. and Dobrogosz, W.J. (1998). Lactic Acid Bacteria: Micorbiology and Functional Aspects, S. Salminen and A. von Wright (eds) 2nd ed., Marcel Dekker, New York, New York.

Croom, W.J., Jr., Bird, A.R., Black, B.L. and McBride, B.W. (1993). *Journal of Dairy Science*, **72**:2112-2124.

Elwinger K., Berndson, E., Engstrom, B., Fossum, O. and Waldenstedt, L. (1998). *Acta. Veterinaria Scandinavica*, **39**: 433-441.

Fuller, R. (1989). *Journal of Applied Bacteriology*, **66**:365-378.

Fuller, R. (1999). Probiotics: A Critical Review. G.W. Tannock (ed). Horizon Sceintific Press, Wymondham, UK.

Gaskins, H.R. (1997). Gastrointestinal Microbiology: Volume 2, Gastrointestinal Microbes and Host Interactions, Mackie,R.L., White, B.A and Isaacson, R.E. (eds). Chapman & Hall, New York, New York.

Gaskins, H.R. (2001). Swine Nutrition, 2nd edition, A.J. Lewis, L. Lee Southern (eds). CRC Press, Boca Raton, FL, USA.

Gibson, G.R. and Roberfoid, M.B. (1995). *Journal of Nutrition*, **125**:1401-1412.

Hirayama, K. and Rafter, J. (2000). *Microbes and Infection*, **2**: 681-686.

Lee, A. 1984. Neglected Niches: The Microbial Ecology of the Gastrointestinal Tract in Chapter 3, Advances in Microbial Ecology. 8:115-162.

Lily, DM and Stillwell, R.H. (1965). *Science*, **147**:747-748.

Madsen, K., Cornish, A., Soper, P., McKaigney, C., Jijon, H., Yachimec, C., Doyle, J., Jewell, L. and De Simone, C. (2001). *Gastroenterology*, **121**:580-591.

Elmer, G.W., Surawicz, C.M. and McFarland, L.V. (1996). *Journal of American Medical Association*, **275**:870-876.

Metchnikoff, E. (1907). The Prolongation of Life: Optimistic Studies. Chalmers Mitchell, P. (ed). Heinemann, London.

Mulder, R.W.A.W., Havenaar, R. and Huis in 't Veld, J. (1997). Probiotics 2: Applications and Practical Aspects. R. Fuller (ed). Chapman & Hall, London.

Naidu A.S., Bidlack W.R. and Clemens, R.A. (1999). *Critical Reviews in Food Science and Nutrition*, **39**: 13-126.

Nurmi, I.E. and Rantala, M. (1973). *Nature*, **241**:210-211.

Oyofo, B.H., DeLoach, J.R., Corrier, D.E., Norman, J.O., Ziprin, R.L. and Mollenhauer, H.H. (1989). *Avian Diseases*, **33**:531-534.

Rada, V. and Marounek, M. (1996). *Annales de Zootechnie*, **45**: 283-288.

Riley, W.W., Jr., Esteve-Garcia, E. and Austic, R.E. (1986). *Poultry Science*, **66**: 2292-2298.

Saavedra, J.M. (2001). *American Journal of Clinical Nutrition*, **73**: 1147S-1151S.

Salminen, S., Ouwehand, A., Benno, Y. and Lee, Y.K. (1999). *Trends in Food Science and Technology*, **10**:107-110.

Sanders, M.E. (2000). *Journal of Nutrition*, **130**:384S-390S.

Topping D.L., Warhurst, M., Illman, R.J., Brown, I.L., Playne, M.J. and Bird, A.R. (1997). *Proceedings of Nutrition Society (Australia)*, **21**: 134.

NUTRITIONAL PHYSIOLOGY OF THE YOUNG TURKEY POULT

M.S. LILBURN

Summary

There are numerous factors which influence the ability of the newly hatched turkey poult to make the transition from *in ovo* development to the immediate post-hatch nutritional environment. These factors include the age of the hen, the length of the holding period post-hatch prior to placement, and nature of the starter diet in terms of ingredient and nutrient composition. For the first 5 to 7 days post-hatch, the relative growth of the intestine is far greater than overall changes in body weight and it is also during this period that functional digestive processes are developing. In addition to the challenges of acquiring functional maturity, the first days post-hatch also represent the period during which lipid based metabolic processes are rapidly switched over to carbohydrate based energy metabolism.

I. INTRODUCTION

One of the major factors contributing to the financial success of the turkey industry has been the continual genetic improvement in body weight and carcass conformation in commercial lines. In a long-term genetic experiment (>30 generations) originating with commercial lines available in the mid-1960's, Nestor *et al.*, (2000) demonstrated that selection for body weight alone at 16 weeks of age resulted in a near doubling of body weight, albeit with some decline in reproductive efficiency. These changes in absolute body weight at defined ages were also accompanied by differences in proportional or relative growth (Maruyama *et al*., 1998). While the output side of selection (body weight) has been well documented, it has been accomplished without any major changes in the input side of the selection equation (i.e. the definition of changes in nutritional requirements). If one were to look at the nutritional requirements published by the U.S. National Research Council (NRC) from the mid 1970's through 1994 (NRC, 1994), there were few if any changes in the published requirements for growing turkeys, particularly with respect to protein and amino acids. This does not imply that changes in nutritional requirements have not occurred, but suggests that this is an area of nutritional research that has not received much attention.

II. INTESTINAL DEVELOPMENT

The intestine of the developing turkey embryo doubles in size the last week of incubation from 450 mg on Day 22 to 1.2 g at hatch and then continues to increase in size exponentially to approximately 4 g and 7 g at 3 and 6 days post-hatch, respectively (Ding and Lilburn, 2000). The pattern of embryonic development reflects the primary importance of the yolk sac membrane with respect to nutrient packaging and transfer during incubation. The importance of the first four to six days post-hatch with respect to intestinal growth has also been previously observed by Sell *et al*. (1991) and Pinchasov and Noy (1994). With respect to the growth of distinct parts of the small intestine, increases in the relative weight and length of the jejunum and ileum occur at a faster rate than observed for the duodenum up to five days of age (Applegate and Lilburn, 1999). While it is well accepted that hen age and egg weight can positively influence the "*quality*" of poults at hatch, data from the above experiment showed that this was not due to any significant differences in gross measurements

Department of Animal Sciences, OARDC, Wooster, OH 44691.

within the small intestine.

Within the intestine itself, enterocyte proliferation and differentiation/maturation are not confined to the crypt and villus regions, respectively. Using non-isotopic measures of cellular replication, bromo deoxyuridine (BrDU) and proliferation cell nuclear antigen (PCNA), Applegate *et al*. (1999a) reported that enterocytes within the villus are actively proliferating prior to hatch (Day 27) through five to seven days post-hatch. This contributes to a near two-fold increase in villus height whereas crypt cell proliferation remains relatively constant. An interesting observation was that villus height in poults at hatch from older breeder hens (44 weeks) was 22.8 *u*m longer than in young breeder hens (34 weeks). These differences, however, did not influence subsequent enterocyte migration along the crypt-villus axis post-hatch. While some authors have shown positive correlations between intestinal maturation and nutrient intake (Uni *et al*., 1995), others have suggested that the intestine is a dynamic organ that can make adjustments depending upon nutrient availability and needs of the animal (Obst and Diamond, 1992; Starck, 1996).

The more important changes in intestinal development during the first week are with respect to maturity or increased functionality of the digestive and absorptive processes. Sell *et al*. (1991) reported little or no intestinal enzyme activity (amylase, maltase, lipase, trypsin) prior to hatch followed by a two to three fold increase through the first four days post-hatch. There was a continued increase through eight days post-hatch though not always in a simple linear, age dependent fashion. As the authors point out, however, enzyme specific activity (activity per unit of tissue or body weight) needs to be considered along with changes in total enzyme activity which is a function of the total amount of secretory tissue. For example, in the study by Sell *et al*. (1991), pancreas weight increased from approximately 0 .1 g/100 g body weight at hatch to 0 .35 g/100 g body weight at four days of age, similar to what was reported by Phelps *et al*. (1987). During this same period, there was also a three-fold increase in trypsin and lipase specific activity, so the combination of increases in tissue size and enzyme synthesis result in more than sufficient enzyme activity for adequate digestive processing.

III. THE NUTRITIONAL STATUS OF NEWLY HATCHED POULTS

In newly hatched poults, the residual yolk sac contains approximately 50% dry matter of which 33 to 38% is lipid (Applegate and Lilburn, 1996). Triglyceride accounts for roughly 57% of total yolk sac lipid at hatch which is approximately one gram. The remaining yolk lipids are split between primarily phospholipids (21%) and cholesterol esters (17%; Ding *et al.,* 1995). Lilburn (1998) calculated that the maintenance energy requirement of a 60 g poult at hatch would be approximately 34 kcal based on the equation 2.2 kcal/gram $BW^{2/3}$ (Hurwitz *et al*., 1980). If one g of triglyceride provides 9 kcal (9000 kcal ME/kg), then the residual yolk sac itself, does not appear to be the major source of energy it is often claimed to be. This observation places even greater importance on the ingredient makeup and nutrient digestibility of the initial starter diets.

The nature of metabolism during incubation places a tremendous emphasis on gluconeogenesis for the derivation of embryonic glucose, some of which is stored as glycogen in various tissues. John *et al.* (1987, 1988) reported that in poults, there was a 50% decline in tissue glycogen (i.e. pectoral muscle, liver) between 26 days of incubation and hatch. Typical industry practices such as 24 to 48 hour holding times together with handling stressors such as desnooding and beak trimming can result in poults that are essentially devoid of glycogen reserves by the time they reach the growing farm (Rosebrough *et al*., 1978; Donaldson *et al.,* 1991; Turner *et al.,* 1999b). The potential combination of events occurring prior to hatch can compromise poults with respect to glucose metabolism by the

time they have access to their first source of exogenous nutrients. This often contributes to "poor poult starts", a generic term often associated with listless poults and elevated early flock mortality (Phelps *et al*., 1987). This situation can be exacerbated in poults coming from young breeder flocks (Applegate and Lilburn, 1999) and has also been observed in other commercial poultry species (Applegate *et al*., 1999b).

The question then arises, to what extent can we influence the metabolic condition of poults with early nutritional intervention. Starter diets for turkeys are high in protein (28%; NRC, 1994) and depending upon the availability and choice of ingredients, this continues to place a heavy reliance on gluconeogenesis for generation of glucose. In an initial series of experiments, Turner *et al.* (1999a) fed diets that contained either a high level of corn (60%; CHO diet) with fishmeal (22%) as the primary protein source or a lower level of corn (26%) with 44% soybean meal as the primary protein source (54%). The latter diet also contained a high level of supplemental animal-vegetable fat (10.8%; FAT diet) to make it isocaloric with the CHO diet. Feed intake was determined and total excreta was collected from three to five, five to seven, and seven to nine days of age. Over all three collection periods, dry matter digestibility of the non-lipid portion of the diet was over 70% for the CHO diet compared with an average 52% for the FAT diet. When the utilization of individual fatty acids was determined, the apparent digestibility of palmitic (16:0) and oleic (18:0) acids was low, particularly in the FAT diet (16:0 – 52%; 18:0 – 27%) and these two fatty acids accounted for 40% of the total fatty acids within the commercial blend. The digestibility of the mono- (18:1) and polyunsaturated fatty acids (18:2, 18:3), however, was > 80% in the FAT treatment. This supports earlier reports with chicks (Carew *et al*., 1972) and poults (Salmon, 1977) and suggests that vegetable oils, with their high percentages of unsaturated fatty acids, might be preferential energy sources in specially developed pre-starter diets for poults. In a third set of diets, Turner *et al.* (1999a) incorporated a commercial blend of medium chain triglycerides (MCT; 76% C 8:0) into the diet in place of the commercial animal vegetable blend. Whilst non-lipid dry matter digestibility was no greater with this blend of lipids than with the FAT diet, the C 8:0 was over 95% digested.

A second set of measurements in the above experiments looked at the effects of nutrient sources on selected aspects of early poult performance. Two management treatments were imposed within the different dietary regimes, viz. immediate (FED) or delayed (48 hours; WH) access to feed and water. The latter management treatments were an attempt to mimic the breadth of placement times often encountered in commercial practice. Interestingly enough, immediate or delayed access to feed had no significant effects on liver size, liver glycogen, or plasma glucose on Day 0 (the first day with access to feed or water). Chronologically, this would actually correspond to Day 3 for the delayed access treatment. Within the FAT treatment, there were no differences between the FED and WH poults in liver glycogen or plasma glucose on Day 2 post-feeding. In the CHO treatment, however, WH poults had significant increases in both liver glycogen and plasma glucose compared with FED poults. This suggests that under an imposed stress such as delayed access to feed or water, the source of nutrients may be an important factor regulating metabolic homeostasis. Plasma glucose remained high in the WH-CHO poults five days post-feeding so the initial metabolic effects were not short-lived. These initial effects were similar in poults fed either the FAT or MCT diets. In response to a glucose challenge at either four days post-placement, WH poults had significantly higher plasma glucose levels 30 and 60 minutes post-challenge compared with FED poults. Within dietary treatments, glucose clearance in the poults fed the CHO diets was significantly improved at 60 minutes post-challenge and this was true at both four and seven days post-feeding.

The sum total of these experiments is a body of data which addresses several aspects of early poult nutrition. Prolonged holding of poults due to either optimization of hatch

numbers or extensive transportation distances can be partially accommodated with commercially available hatchery supplements. The choice of ingredients for pre-starter diets is also worth addressing from the perspective of formulating to maximize availability of nutrients, particularly carbohydrates, as opposed to formulating to a published set of protein, amino acid, or energy levels. This approach would allow the poult to more easily make the transition from yolk-lipid based metabolic processes *in ovo* to carbohydrate as the primary energy source post-hatch.

REFERENCES

Applegate, T.J. and Lilburn, M.S. (1996). *Poult. Sci.* **75:**1210-1216.

Applegate, T.J. and Lilburn, M.S. (1999). *Comp. Biochem. Physiol. Pt.B*: **124:**371-380.

Applegate, T.J., Dibner, J.J., Kitchell, M.L. Uni, Z. and Lilburn, M.S. (1999a). *Comp. Biochem. Physiol*. Pt.B. **124**:381-389.

Applegate, T.J., Ladwig, E., Weissert, L. and Lilburn, M.S. (1999b). *Poult. Sci*. **78**: 1485-1492.

Carew, L.B., Machemer, R.H. Jr., Sharp, R.W. and Foss, D.C. (1972). *Poult. Sci.* **51:** 738-742.

Ding, S.T., Nestor, K.E. and Lilburn, M.S. (1995). *Poult. Sci.* **74:**374-382.

Ding, S.T. and Lilburn, M.S. (2000). *Poult. Sci.* **79**:1460-1464.

Donaldson, W.E., Christensen, V.L. and Krueger, K.K. (1991). *Comp. Biochem. Physiol*. **100A**:945-947.

Hurwitz, S., Weiselberg, M., Eisner, U., Bartov, I., Riesenfeld, G., Sharvit, M., Niv, A. and Bornstein, S. (1980). *Poultry Sci.* **59:**2290-2299.

John, T.M., George, J.C. and Moran, E.T. Jr., (1987). *Cytobios* **49:**197-210.

John, T.M., George, J.C. and Moran, E.T. Jr., (1988). *Poult. Sci.* **67:**463-469.

Lilburn, M.S., (1998). *J. Appl. Poult. Res.* **7:**420-424.

Maruyama, K., Potts, W.J.E., Bacon, W.L. and Nestor, K.E. (1998). *Growth Dev. Aging* **62**:123-139.

National Research Council, (1994). Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Nestor, K.E., Anderson, J.W. and Patterson, R.A. (2000). *Poult. Sci.* **79**:445-452.

Obst, B.S. and Diamond, J. (1992). *Auk* **109:** 451-464.

Phelps, P.V., Edens, F.W. and F.W. Christensen, F.W. (1987). *Comp. Biochem. Physiol.* **86A:7**39-743.

Pinchasov, Y. and Noy, Y. (1994). *Comp. Biochem. Physiol.* **107A:** 221-226.

Rosebrough, R.W., Geis, E., Henderson, K. and Frobish, L.T. (1978). *Poult. Sci.* **57:**747-751.

Salmon, R.E. (1977). *Can. J. Anim. Sci.* **57:**427-431.

Sell, J.L., Angel, C.R., Piquer, F.J., Mallarino, E.G. and Al-Batshan, H.A. (1991). *Poult. Sci.* **70:** 1200-1205.

Starck, J.M. (1996). *Acta Anatom.* **156:** 289-306.

Turner, K.A., Applegate, T.J. and Lilburn, M.S. (1999a). *Poult. Sci.* **78:**1573-1580.

Turner, K.A., Applegate, T.J. and Lilburn, M.S. (1999b). *Poult. Sci.* **78:**1581-1587.

Uni, Z., Noy, Y. and Sklan, D. (1995). *Poultry Sci.* **74:** 1622-1629.

GENETIC, NUTRITIONAL AND MANAGEMENT ASPECTS OF TURKEY PRODUCTION

C. NIXEY

Summary

Genetic progress in improving growth rate of turkeys over recent years is reviewed. As this has been accompanied by an almost equivalent increase in daily food intake, factors which influence food intake have become more important and are discussed. The influence of early growth performance is shown to be of importance. Maximum house temperatures need to be reduced to allow full growth expression. There is also an interaction between temperature and stocking density. The lighting programme during early life will influence early livability and, in later life, influence the incidence of leg problems.

I. INTRODUCTION

This paper largely uses turkeys as the example but most of the comments apply equally to broiler chickens and to other farm animals. The application of scientific breeding programmes has resulted in dramatic changes in production parameters of several types of farm animals, none more so than in turkeys. Table 1 shows the increases obtained in growth rate in a large British United Turkeys (BUT) commercial cross, based on published performance data.

Table 1. Growth rate progress in the BUT Big 6 turkeys between 1984 and 2002

Year	21 week old males		16 week old females	
	Weight (kg)	Annual increase (%)	Weight (kg)	Annual Increase (%)
1984	15.91			
1986	16.84	2.92	8.76	1.08
1993	18.44	1.36	9.56	1.09
1996	19.05	1.10	9.70	1.01
1999	19.63	1.01	9.88	1.02
2002	20.58	1.61	10.25	1.25

(In 1966 BUT Large White turkey males weighed 10.7 kg and females weighed 6 kg at the above ages)

These changes have been achieved using traditional mass selection methods applied to large populations. Of more immediate interest is the progress in recent years, which indicates no slowing down of annual improvement. Even without new technologies, the turkeys available in five years time will be faster growing than those of today.

The changes in Europe and the USA have been gradual whereas, because of recent importations, the changes in the Australian turkey will have been more pronounced in the first year following the introduction of the imported material.

British United Turkeys Ltd., Hockenhull Hall, Tarvin ,Cheshire, CH3 8LE, UK.

Similar progress has been achieved for broilers. Owen (1999) quotes a body weight of 1.8 kg. for a broiler chicken being achieved in 35 days in 1999 compared to 56 days in 1974. This represents a reduction of just under one day per annum. In 1974, 4.05kg of food was required to produce a 1.8 kg broiler chicken compared to only 2.9 kg in 1999. Such a reduction in food intake, accompanied by an improvement in feed efficiency, would be expected to place increased demands on achieving the correct diet formulation, particularly with regard to vitamins and trace minerals. If this growth is being achieved, it must be presumed that the required amounts of amino acids and metabolizable energy are being consumed. The adequacy of available nutrients which do not have a direct effect on weight gain but which can influence skeletal growth and normal physiological development, however, must be questioned.

II. BODY COMPOSITION

Another factor which may influence nutrient requirements is a change in body composition over time. The meat yield, especially of breast meat, has increased considerably. This is particularly so for the broiler chicken, which started from a lower base level than turkeys, with a move, in recent years, towards strains with high breast meat yield. Table 2 shows a comparison at two ages between a standard strain and a strain selected for high breast meat yield.

Table 2. Comparison of breastmeat yield of two strains of broiler chicken (Hubbard-ISA published 1999 goals)

	Standard (Hubbard Classic)			High yield (Hubbard Hi-Y)		
Age (days)	Body wt. (g)	Evicerated. yield (g/kg)	Breast yield (g/kg)	Body wt. (g)	Evicerated. yield (g/kg)	Breast yield (g/kg)
42	2300	679	160	2240	685	172
49	2770	688	165	2710	694	177

The difference in breast meat yield between the two types would indicate that the high yielding strains being developed will have a higher requirement for dietary amino acids and hence a reduced ratio of amino acid to AME. At the same AME content, the increased requirement for dietary protein could be in excess of 1% for optimum breast meat yield.

III. FOOD FORM

The increased growth rates require an increase in daily food consumption to achieve the required nutrient intakes. Any factor which influences food intake will therefore be of increased importance. In some instances, particularly early in life, food intake can have a greater influence on performance than the feed formulation itself. This is illustrated by an experiment conducted by Nixey (1989) which compared the growth (0 – 4 weeks) for turkeys fed the same diet formulation either as in mash or as crumbles (Table 3). Large differences were seen between the body weights of the turkeys. At 28 days the mash fed birds were 23.3% lighter than those fed crumbles. Even at seven days, a difference of 16% was observed. Those given crumbles ate more food than those given mash.

The presentation of the food is also of importance. When poults were presented with food on floor trays for five days in addition to tubular feeders, they grew 8% faster to 14 days than those without food on floor trays (Nixey, 1989).

Table 3. A comparison of growth (g) to 28 days of age in turkeys fed the same diet as either mash or crumbles. (Nixey, 1989)

Age (days)	Crumbles (g)	Mash (g)	Difference crumbles vs mash (%)
1	55.8	55.7	+0.3
7	139.7	117.4	+16.0
14	318.0	246.6	+22.5
21	596.7	451.7	+24.3
28	1017.4	780.6	+23.3

In turkeys, early growth rate influences subsequent growth rate prior to processing. In the crumbles and mash experiment, where large differences were seen at four weeks of age, all the birds were given pelleted high or low protein diets from four weeks of age. Table 4 shows that even at 20 weeks, large differences in body weight were still apparent between the two groups. The only difference between the groups was that one group had been fed crumbles and the other mash to four weeks of age.

Table 4. Influence of feed form to 4 weeks and of dietary protein from 4 to 24 weeks on growth performance (kg) of turkeys to 24 weeks of age.

Age	4	8	12	16	20	24
0-28d Treatment						
		High protein programme (g/kg)				
	300	270	230	180	180	180
Crumbles	1.02	4.16	8.76	12.73	16.54	20.00
Mash	0.78	3.67	8.21	11.88	15.63	18.79
Difference	0.24	0.49	0.55	0.88	0.91	1.21
		Low protein programme (g/kg)				
	300	230	180	180	140	140
Crumbles	1.02	3.96	8.12	10.95	12.62	16.74
Mash	0.78	3.42	7.34	10.06	11.48	14.79
Difference	0.24	0.54	0.78	0.89	1.14	1.77

The above data support field experience where, often when flocks are under weight at 20 weeks of age, the problem can be traced back to low weights early in life. Compensatory or “catch up” growth may take place under ideal conditions but field experience indicates that it does not under commercial growing conditions unless the later feed programme has a higher amino acid content than normal. The above study (Table 4) shows some evidence of greater compensation on the high dietary protein regime, although differences still persisted.

IV. TEMPERATURE

Changes in the growth potential will also change the optimum growing temperature to achieve that potential. The process of digesting food creates heat as does muscle and tissue growth and activity of the bird. In order for the bird to maintain its core body temperature constant, the heat produced must be dissipated. If it cannot, food intake and hence growth rate are reduced.

The rate of heat loss is dependent on the ambient temperature. Emmans (1989) has produced a computer model which can be used to calculate the comfort zone of male turkeys at different ages (Figure 1).

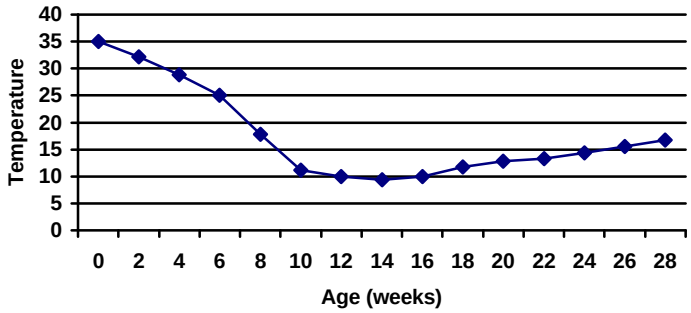


Figure 1: Comfort temperatures at different ages of large male turkeys (Emmans, 1989).

If turkeys are kept at temperatures above those shown in Fig 1, there will be a reduction in growth rate. The reason that the curve is at its lowest point at around 12 weeks of age is because weight gain is highest at this stage relative to the surface area of the bird. The latter is a major factor influencing rate of heat loss.

An indication of too high a brooding temperature can be obtained by observing turkey appearance at older ages. For example, a condition known by stockpeople as "split wings" is observed when the end primary feathers do not sit tightly against the body but jut out at an angle. This first appears very early in life. It can be caused by the poults staying in the hatcher too long but it can also be caused by excessive house temperatures early in life. This is illustrated in Table 5 from C. Nixey (unpublished results).

Table 5. Brooding temperature and incidence of split wings.

House temperature regime (0 – 14 days)	Split wings (%)
23 – 16^{o}C	19.5
30 – 26^{o}C	79.5

Field experience indicates that a growth response may be expected from a reduction in most house temperatures. For extended periods of the year, Australian turkey house temperatures are usually higher than optimal (Fig 1). However, in the cooler period of the year it should be possible to run the houses at a cooler temperature by increasing the ventilation; both the means and the end being beneficial to growth rate. The effect of high temperatures post brooding is illustrated in Table 6.

Table 6. The influence of two temperature regimes on body weight gain (kg) and food conversion ratio (FCR) in turkeys between 41 and 134 days. (from Veldkamp *et al.* 2000)

Temperature	15ºC	25ºC
Bodyweight gain (kg)	16.10	14.58
F.C.R. (g. food/ g. gain)	2.975	2.982

The greater the turkeys' growth potential, the lower is the optimum temperature. It is for this reason that females are more tolerant of higher temperatures than males.

There are management and nutritional strategies which can alleviate the effect of high temperatures. When formulating diets, ingredients can be included which produce less heat during digestion and metabolism. For example, fats can be increased at the expense of carbohydrates as an energy source. The fibre content of the diet can be reduced. Management factors include increasing the airflow over the bird to remove heat; cooling the drinking water or preventing it from getting too warm.

Both stocking density and ambient temperature will also have a negative or positive effect on growth rate due to the influence of radiant heat loss from the body. The closer the birds are housed, the slower will be radiant heat loss due to absorption of radiant heat from other birds. There is no published data on the influence of stocking density under commercial conditions. However, the Farm Animal Welfare Council (FAWC) of the U.K. has published a recommended formula for maximum stocking density per square metre based on the liveweight of the individual bird (Table 7).

Table 7. Indicated stocking densities for heavy medium strain turkeys based on the FAWC formula: m^2/ bird = 0.0459 x W(kg)$^{2/3}$

Age (weeks)	Males		Females	
	Bodyweight (kg)	Birds/m^2	Bodyweight (kg)	Birds/m^2
6	2.27	12.6	1.89	14.2
10	5.89	6.7	4.49	8.0
16	12.04	4.1	8.19	5.4
18	13.99	3.7	9.07	5.0
20	15.89	3.4	9.68	4.8
22	17.73	3.2		

As the growth potential of the bird increases, the stocking density (kg/m^2) increases if the same number of birds are put into the house. This is often overlooked by farmers. The effect is illustrated in Table 8 and is based on published growth goals in those years.

Table 8. The effect of maintaining constant bird numbers in a house as genetic growth potential increases.

a) Expressed as "1981 bird equivalents"

	Male	Female
1981	10000	10000
1999	12850	12770
	b) Expressed as "1986 bird equivalents"	
1986	10000	10000
1999	11660	11350

V. LIGHTING

A management factor not dependant on genetic change is the lighting programme the birds are grown under. Field experience indicates that if turkeys are kept on long light programmes such as 23 or 24 hours light/day, a moderate proportion of the flock will exhibit leg problems, not seen if the birds are given an eight hour dark period.

The improvement in growth rates detailed earlier have not resulted in increased incidences of leg problems. In part this is due to the elimination of mycoplasmas from the breeding stock and improved nutritional knowledge of mineral requirements for bone formation. Primary breeding companies also place great emphasis on bird mobility and the ability to carry the increased bodyweight.

For the first few days of life, an intermittent lighting programme such as three hours light, three hours dark is beneficial in improving livability. The extent of the improvement will depend upon the existing level of management. Fewer birds lose co-ordination and balance and the number of poults that do not learn to eat is also reduced. The programme tends to imitate nature where the poults retire periodically under their mother's wings.

Genetic improvements therefore mean that nutritional and management strategies must be regularly reviewed.

REFERENCES

British United Turkeys Big 6 Goals, British United Turkeys Limited, Warren Hall, Broughton, Chester CH4 0EW, England.

Emmans, G.C. (1989) *Recent Advances in Turkey Science*, Chapter 8. Eds. C. Nixey and T.C. Grey. Butterworth & Co. (Publishers) Ltd. London.

FAWC (1995) Report on the Welfare of Turkeys by Farm Animal Welfare Council, U.K.

Hubbard-ISA 1996 Goals, Hubbard-ISA, P.O. Box 415, Turnpike Street, Walpole, NH 03608 USA.

Nixey, C. (1989) *Recent Advances in Turkey Science*, Chapter 10. Eds. C. Nixey and T.C. Grey. Butterworth & Co. (Publishers) Ltd. London.

Owen, H. (1999) Broilers – 25 Years of Progress. Proceedings of ADAS/NFU Poultry Conference 1999. Cambridge, U.K.

Veldkamp, T. Ferket, P.R., Kwakkel, R.P., Nixey, C. and Noordhuizen, J.P.T. (2000). *Poultry Science*, **79:** 1472-1477.

PRODUCTION OF INTER- AND INTRA-SPECIFIC GERMLINE CHIMERAS IN POULTRY

S.L. PARDUE, S. D'COSTA, Y. SONG and J.N. PETITTE

Summary

In vertebrates, primordial germ cells (PGC) are extragonadal in origin and experience a complex migration to the gonadal anlage. Donor PGC have been obtained from avian embryos at various stages of development, e.g. the early blastoderm, germinal crescent, embryonic blood, and early primitive gonad. Primordial germ cells from donor chick and turkey embryos are capable of remigration to the gonads of recipient embryos resulting in the production of germline chimeras. It appears that the chemo-attractant produced by the avian gonad is not species-specific, given that turkey PGC were capable of migrating in chick embryos. Reports also confirm that gonadal PGC retain their ability to migrate even after they have initially colonized the gonad. The transfer of male PGC into female avian embryos could enhance the possibility of producing a greater frequency of male offspring. Similarly, the potential exists for the transfer of turkey PGC into chick recipients for the production of inter-specific gametes.

I. PRODUCTION OF AVIAN GERMLINE CHIMERAS

Unlike that observed in mammals, the avian male represents the homogametic sex, possessing only a ZZ sex chromosome genotype. Female birds are heterogametic, possessing both a Z and W sex chromosome. Therefore, it is the female that determines the gender of the progeny in birds. The development of specific methods of transferring primordial germ cells (PGC) to host embryos has the potential to alter gamete production. Such procedures could yield a number of novel characteristics or advantages that include: (1) the ability to potentially produce a disproportionately greater number of male offspring, (2) a dramatic reduction in the time required to produce viable turkey spermatozoa, (3) a significant savings in the production costs associated with the rearing of breeder flocks, and (4) enhancing the survival of endangered species.

Primordial germ cells are embryonic precursors of gametes observed in adults. These totipotent cells are unique in that they represent the genomic link from generation to generation. In birds, PGC arise from the epiblast of stage X embryos (Eyal-Giladi *et al*., 1981). These cells appear to be concentrated in the central region of the area pellucida of the stage X blastoderm (Eyal-Giladi and Kochav, 1976; Ginsburg and Eyal-Giladi, 1987; Kagami *et al*., 1997). Prior to stage X, no PGC [Stage Specific Embryonic Antigen (SSEA-1) positive cells] have been observed (Karagenc *et al*., 1996). By stage XII, the PGC have migrated to the region of the hypoblast (Sutasurya *et al*., 1983). During the process of gastrulation, the PGC move into the area commonly referred to as the germinal crescent (Swift, 1914). Following development of the extra-embryonic vasculature, the PGC enter the blood stream and migrate to the gonadal ridge (Swartz and Domm, 1972). It is here, in the primitive gonad, that the PGC mature and undergo gametogenesis (for a review, see D'Costa *et al*., 2001).

Department of Poultry Science, North Carolina State University, Raleigh, NC, 27695, USA

Historically, the production of germline chimeras has been viewed as a possible means to develop transgenic poultry. Methodologies for the production of germline chimeras have differed in many aspects, with the source of donor cells representing a principal difference.

Production of germline chimeras has been attempted by transfer of blastodermal cells (Petitte *et al*., 1990, 1993; Brazolot *et al*., 1991; Naito *et al*., 1991; Kagami *et al*, 1995; Kino, *et al*., 1997), germinal crescent cells (Reynaud, 1976; Wentworth *et al*., 1989; Vick *et al*., 1993), blood PGC (Yasuda *et al*., 1992; Tajima *et al*., 1993; Naito *et al*., 1994a, 1994b, 1998, 1999), cultured PGC (Chang *et al*., 1997), and gonadal PGC (Tajima *et al*., 1998; D'Costa, 1999).

Petitte and co-workers (1990) utilized blastodermal cells, isolated from stage X Barred Plymouth Rock (BPR) embryos, as donor cells in their experiments. Of the 53 embryos injected, only four (7.5%) survived to hatch. The decline in hatchability was associated with the removal of a portion of the shell to form a 0.5 cm window in the egg. Windowing alone resulted in a survival rate of only 9.1%. A total of six (11.35%) embryos and chicks exhibited somatic chimerism. The rate of germline chimerism was low, approximately two percent (1/53), suggesting that the incorporation of donor PGC into the host gonad occurred at a very low incidence. Of 717 offspring evaluated, only two (0.28%) were of the donor phenotype.

Yasuda, *et al*. (1992) injected (IV) approximately 100 chicken PGC, isolated from the blood of 2-day-old (stage 13 & 14) embryos, into stage 15 quail embryos. Subsequent histological analysis utilizing differential staining (Meyer, 1964; Swartz and Domm, 1972) of the quail gonad revealed that chicken PGC represented approximately 11% of the total PGC present.

Use of blood PGC is limited by their relatively low concentration. In embryonic blood, PGC represented less than 0.05% of the total cells (Yasuda, *et al*., 1992). Typically, the authors were able to collect 500-600 PGC from the blood of 20 embryos (1-5 µl per embryo). In a subsequent study, Tajima *et al*. (1993) also utilized blood PGC and produced 17 putative chimeras. After progeny testing, 76.5% of the birds were determined to be germline chimeras. However, relative production of gametes from the donor PGC was low, approximately 4.2-5.2%. Far greater donor PGC incorporation was reported by Naito *et al*. (1994a) when approximately 200 blood-derived PGC were injected into recipient embryos that had been bled prior to injection in an attempt to reduce the endogenous PGC population. Using a surrogate eggshell system, the authors noted a hatchability rate of 17.9 to 31.8%. Progeny derived from donor PGC ranged widely from 17.9 to 81.4%. In a companion study, experimental conditions were nearly identical with the major exception being that blood derived PGC were cryo-preserved in liquid nitrogen, thawed, and subsequently injected into recipient embryos (Naito *et al*, 1994b). Hatchability of manipulated embryos ranged from 15.6 – 33.3%. A 90% (9/10) rate of germline chimerism was reported. Donor derived offspring was lower than the previous study with a range of 6.9-17.1%. Later, Tajima and co-workers (1998) demonstrated that gonadal PGC could be frozen prior to injection into host embryos. Approximately 100 gonadal PGC were injected into stage 15-16 embryos following the removal of 4-8 µl of blood. Of the four adults screened, three were shown to be germline chimeras. An average of 9.7% of the offspring from these chimeras were donor-derived.

Chang *et al*. (1997) cultured gonadal PGC for five days prior to injection into recipient embryos. Recipient eggs were windowed at the inferior or small end and injected with 200-cultured gonadal PGC. In this system a hatchability rate of 58.8% was achieved. Ten percent of the recipients developed into germline chimeras. The frequency of donor-derived offspring was low (1.3-3.1%). Finally, frozen blastodermal cells have been utilized to produce germline chimeras (Kino *et al*., 1997). Approximately 500 blastodermal cells were injected into gamma irradiated (600 rads) hosts. Surrogate eggshell culture was used and hatchability ranged from

25.8–40.7%. However, injection of fresh PGC produced 53.2% germline chimeras, compared to 7.7 or 11.5% from frozen cells. These differences may be due in part to the rapid decline in PGC viability following thawing. Immediately following thawing, PGC viability was estimated to be approximately 60%. Within three hours of thawing, viability had fallen to 35%. This is in contrast to Naito *et al*. (1994b) who reported a viability rate of 94.2%.

In order to efficiently generate germline chimeras, endogenous PGC numbers must be reduced. A number of approaches have been utilized with varying degrees of success. Continuous exposure (20 days) to gamma irradiation (0.3-3.4R/hr, ^{60}Co) resulted in the complete destruction of oocytes at a dosage level of 3.4 and 1.8 R/hr (Mraz and Woody, 1973). However, hatchability was reduced at levels of 0.9 R/hr or higher. The application of continuous low-level gamma irradiation to reduce endogenous PGC is limited due to the relatively small numbers of eggs that can be exposed at any one time. Short-term exposure to a gamma source has also been attempted (Carsience *et al*., 1993; Thoraval *et al*., 1994; Maeda *et al*., 1998). In these studies, unincubated eggs were exposed to 500-700 rads just prior to the injection of stage X blastodermal or area pellucida cells. The incidence of germline chimerism following short-term gamma irradiation was highly variable. The basis for the inconsistent results where ascribed to "donor cells being injected into an inappropriate location…" (Carsience *et al*., 1993).

Attempts to sterilize recipient embryos using ultraviolet light have been described (Reynaud, 1969; 1976; Aige-Gil and Simkiss, 1991). Aige-Gil and Simkiss concluded "it is not possible to irradiate the germinal crescent, particularly at stage 4 of incubation, without inducing major abnormalities". The level of sterility appeared to be positively correlated with developmental abnormalities, thus limiting the practical use of UV-light as a means to reduce endogenous PGC.

The compound busulfan (1,4-butanediol dimethane sulfonate, BU) has been used as a chemotherapeutic agent in the treatment of leukemia (Bhagwatwar *et al*., 1996). In 1963, Hemsworth and Jackson demonstrated that the administration of BU in rats could markedly impair the development of PGC. Injection of BU into the yolk sac of chick embryos resulted in multiple malformations (Swartz, 1980). Hallett and Wentworth, (1991) also report significant declines in hatchability following injection of an albumen suspension of BU into quail eggs. In some BU treated quail, there appeared to be an absence of germ cells in the gonads, while other similarly treated birds appeared normal. The authors suggested that "inconsistencies in the delivery of BU to the embryo" might explain the observed variation. They concluded that discovering a non-toxic solvent system would be necessary to eliminate the inconsistent results associated with use of a suspension. Aige-Gil and Simkiss (1991) used saline or sesame oil suspensions of BU, or solublized BU in dimethyl sulphoxide (DMSO) in chick embryos. Administration of DMSO alone produced embryonic mortality, developmental delays, and malformations that exceeded those observed with saline. The teratogenic effects were greatly minimized when BU was suspended in sesame oil and injected into yolk. Injection of 100 µg BU in sesame oil resulted in a sterility index of 95+%. In a subsequent experiment, Vick and co-workers (1993) reported that the injection of 25, 50 and 250 µg BU significantly reduced gonadal germs cells in chick embryos. They estimated that BU treatment increased the rate of germline chimerism 3.5-fold when compared to non-BU treated embryos. Bresler *et al*., (1994) demonstrated that treatment with BU and subsequent injection of PGC could result in a significant repopulation of the gonad. Injection of 50 µg BU, suspended in sesame oil reduced PGC in the left and right gonad of 6 day-old chick embryos by 75 and 78%, respectively. Following the injection of a suspension of germinal crescent cells into BU-treated embryos, PGC numbers increased to 72 and 115% of controls for the left and right gonad, respectively.

Finally, dramatic reductions in endogenous PGC have been reported by the removal of 4-8µl of blood from recipient embryos at stage 14-15 (Naito *et al*., 1994b). This time coincides with the peak in PGC in circulation (Singh and Meyer, 1967). Singh and Meyer reported that peak blood PGC levels occurred at stage 15 with approximately 13 PGC per 10 µl. Using these data, one could predict that Naito and co-workers would have only removed 5 to 11 endogenous PGC by bleeding recipient embryos. However, Yasuda *et al*. (1992) reported higher levels of blood PGC being collected from stage 14 embryos. Approximately, 500-600 PGC were collected from 20 chick embryos when 1-5µl of blood was drawn. Assuming a mean blood collection volume of 3µl, the maximum concentration of PGC would be 10 per µl or roughly 7.5 fold higher than that reported by Singh and Meyer.

Reynaud (1969, 1976) was the first individual to report the production of turkey-chicken germline chimeras. In these studies, germinal crescents of chick embryos were exposed to UV-light in an attempt to reduce the endogenous population of PGC prior to the intra-vascular injection of turkey PGC obtained from turkey embryos. In the initial (1969) study, none of the injected embryos survived to hatching. Reynaud reported that turkey PGC had taken up residence in the embryonic chick gonad. His identification of turkey PGC was solely based on purported morphological differences, e.g. nucleocytoplasmic ratios. No specific immunohistochemistry was employed. In the latter (1976) study, turkey PGC were once again transferred to "sterilized" chick embryo recipients. In this instance, the chick embryos survived to hatching. Subsequent semen collections obtained from these chickens failed to fertilize turkey eggs, suggesting that functional turkey sperm were absent.

II. POTENTIAL APPLICATIONS OF GERMLINE CHIMERAS

The technology associated with the production of germline chimeras could be utilized to develop systems that may allow for the enhanced production of male offspring in poultry and to potentially produce viable spermatozoa in another poultry species e.g. turkey spermatozoa produced by the chicken testes. The ability to produce a disproportionate number of male offspring would have major implications for the poultry industry. In the production of poultry meat, males exhibit growth, conformational, and yield patterns that are generally superior to that of females. The economic value of producing an increase in the percentage of male broiler chickens or turkeys would be significant.

Presented in Figure 1 is a conceptual mating scheme that could enhance the production of male offspring. In this system, BPR chicken embryos would be incubated until stage 27-28 (Hamburger and Hamilton, 1951, [H&H]) and PGC collected. Male BPR embryos would be utilized as a color marker because they are homozygous recessive (ii) at the *I* locus and express pigment in their plumage. Sex determination of the embryos could be accomplished by utilizing the polymerase chain reaction method of Petitte and Kegelmeyer (1995). Donor PGC cell viability could be estimated using trypan blue exclusion. Aliquots of the cell suspension would be stained with SSEA-1 antibody to determine the number of PGCs injected. Approximately 2-3 µl of cell suspension, containing 100-500 PGCs, would be injected into the blood vessels of White Leghorn (WL) embryos at stages 14-17 (H&H) of development. The WL embryos would serve as recipients because they are known to be homozygous dominant (II). This genotype codes for an absence of pigment in the plumage. At hatching the phenotypic WL chicks would be banded and subsequently grown to sexual maturity. The following test matings would be conducted to determine if germline chimeras existed: male BPR X female WL (BPR PGC) (Figure 1) and the reciprocal cross- male WL (BPR PGC) X female BPR. The offspring from these test matings would be subsequently evaluated to determine if male BPR gonadal PGC were incorporated into the WL. Since only

male BPR embryos were used as donors, all "black" chicks (BPR-phenotype) derived from the male BPR X female WL (BPR PGC) test matings would be male.

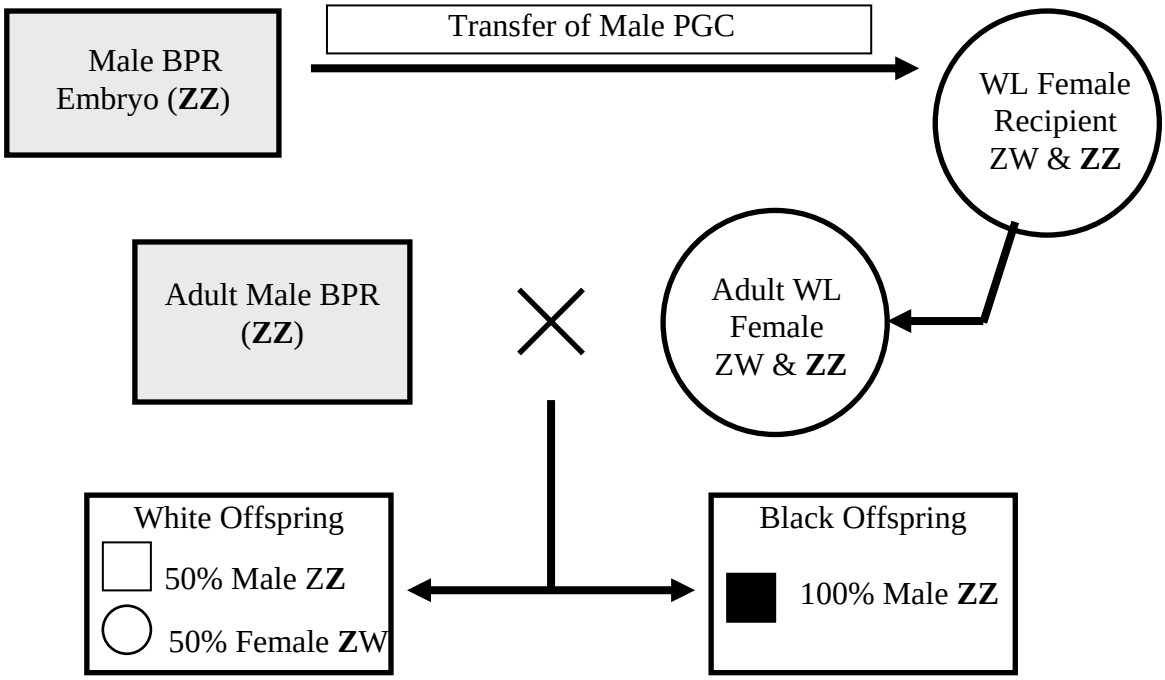


Figure 1. Conceptual mating scheme. BPR = Barred Plymouth Rock, WL = White Leghorn

Producing turkey spermatozoa in a chicken system has the potential to dramatically reduce the time required to produce viable turkey spermatozoa. For example, utilization of Leghorn chicken surrogate embryos, which achieve sexual maturity at 18-20 weeks of age, could potentially produce turkey spermatozoa approximately three months earlier than commercial strains of modern turkeys. A significant savings in the production costs associated with the rearing of breeder flocks could also be achieved. Adult Leghorn males weigh approximately 1/10th of the mature weight of male turkeys. Marked reductions in feed and housing costs could result. Because feed accounts for over 50% of the cost of rearing replacement breeder flocks, inter-specific sperm production could have a profound economic impact. Finally, methods involving PGC transfer have the potential to enhance the survival of endangered species. PGC from endangered avian species could be transferred to chicken embryo hosts to increase the availability of these limited gametes.

Many challenges remain that will determine the rate at which the aforementioned goals are achieved. The development of long-term culture techniques for PGC is paramount, along with the consistent reduction of endogenous PGC. An abundant supply of PGC will allow for the introduction of greater numbers of donor PGC into the recipient embryos. Current methods to reduce the endogenous PGC in recipient embryos also require refinement. Finally, automated systems for the introduction of donor PGC must be developed if large-scale applications are to be feasible.

REFERENCES

Aige-Gil, V. and Simkiss, K. (1991). *British Poultry Science*, **32**:427-438.

Bhagwatwar, H.P., Phadungpojna, S., Chow, D.S.L. and Andersson, B.S. (1996). *Cancer, Chemotherapy and Pharmacology,* **37**:401-408.

Barzolot, C.L., Petitte, J.N., Etches, R.J., Verrinder Gibbins A. (1991). *Molecular Reproduction and Development*, **30**:304-312.

Bresler, J., Behnam, J., Luke, G. and Simkiss, K. (1994). *British Poultry Science*, **35**:241-247.

Carsience, R.S., Clark, M.E., Gibbins, A.M. and Etches, R.J. (1993). *Development,* **117**:669-675.

Chang, I.K., Jeong, D.K., Hong, Y.O., Park, T.S., Moon, Y.K., Ohno, T. and Han, J.Y. (1997). *Cell Biology International*, **21**(8):495-499.

D'Costa, S. (1999). Characterization of turkey primordial germ cells and the production of interspecific embryonic chimeras. PhD Dissertation. NCSU, Raleigh, NC.

D'Costa, S., Pardue, S.L. and Petitte, J.N. (2001). *Poultry and Avian Biology Reviews,* **12 (4)**:151-168.

Eyal-Giladi, H. and Kochav, S. (1976). *Developmental Biology*, **49**:321-337.

Eyal-Giladi, H., Ginsburg M. and Farbarov A. (1981). *Journal of Embryology and Experimental Morphology*, **65**:139-147.

Ginsburg, M and Eyal-Giladi, H. (1987). *Development*, **101**:209-219.

Hallett, J.S. and Wentworth, B.C., (1991). *Poultry Science*, **70**:1619-1623.

Hamburger, V. and Hamilton, H.L. (1951). *Journal of Morphology*, **88**:49-92.

Hemsworth, B.N. and Jackson, H., (1963). *Journal of Reproduction and Fertility*, **6**:229-233.

Kagami, H., Clark, M.E., Verrinder Gibbins, A.M. and Etches, R.J. (1995). *Molecular Reproduction and Development*, **42**:379-387.

Kagami, H., Tagami, T., Matsubara, Y., Harumi, T., Hanada, H., Maruyama, K., Sakurai, M. and Kuwana, T. (1997). *Molecular Reproduction and Development*, **48**:501-510.

Karagenc L., Cinnamon Y., Ginsburg M. and Petitte, J.N. (1996). *Development Genetics*, **19**:290-301.

Kino, K., Pain, B., Leibo, S.P., Cochran, M., Clark, M.E. and Etches, R.J. (1997). *Poultry Science*, **76**:753-760.

Maeda, T., Clark, M.E. and Etches, R.J. (1998). *Poultry Science*, **77**:905-907.

Meyer, D.B. (1964). The migration of primordial germ cells in the chick embryo. *Developmental Biology*, **10**:154-190.

Mraz, F.R. and Woody, M.C. (1973). *Radiation Research*, **54**:63-68.

Naito, M., Watanabe, M., Kinutani, M., Nirasawa, M. and Oishi, T. (1991). *British Poultry Science,* **32**:79-86.

Naito, M., Tajima, A., Yasuda, Y. and Kuwana, T. (1994a). *Molecular Reproduction and Development*. **39**:153-161.

Naito, M., Tajima, A., Tagami, T., Yasuda, Y. and Kuwana, T. (1994b). *Journal of Reproduction and Fertility*, **102**:321-325.

Naito, M., Tajima, A., Yasuda, Y. and Kuwana, T. (1998). *British Poultry Science*, **39**:20-23.

Naito, M., Matsubara, Y., Harumi, T., Tagami, T., Kagami, H., Sakurai, M. and Kuwana, T. (1999). *Journal of Reproduction and Fertility*, **117**:291-8.

Petitte, J.N., Clark, M.E., Liu, G., Verrinder Gibbins, A. M. and Etches, R.J. (1990). *Development*, **108**:185-189.

Petitte, J.N., Brazolot, C.L., Clark, M.E., Liu, G., Verrinder Gibbins, A. M. and Etches, R.J. (1993). In: *Manipulation of the Avian Genome*, R.J. Etches and A. M. Verrinder Gibbins (ed) Boca Raton, CRC Press, pp 81-101.

Petitte, J.N. and Kegelmeyer, A.E. (1995). *Animal Biotechnology*, **6**:19-30.

Reynaud, G. (1969). *Journal of Embryology and Experimental Morphology*, **21**:485-507.

Reynaud, G. (1976). *Roux's Archives of Developmental Biology*, **179**:85-110.
Singh, R.P. and Meyer, D.B. 1967). *Science*, **156**:1503-1504.
Sutasurya, L.A., Yasugi, S. and Mizuno, T. (1983). *Development, Growth and Differentiation*, **25**:517-521.
Swartz, W.J. (1980). *Teratology*. **21**:1-8.
Swartz, W.J. and Domm, L.V. (1972). *American Journal of Anatomy*, **135**:51-70.
Swift, C.H. (1914). *American Journal of Anatomy*, **15**:483-516.
Tajima, A., Naito, M., Yasuda, Y. and Kuwana, T. (1993). *Theriogenology*, **40**:509-519.
Tajima, A., Naito, M., Yasuda, Y., Kuwana, T**.** (1998). *Journal of Experimental Zoology*, **280**:265-267.
Thoraval, P., Lasserre, F., Coudert, F. and Dambrine, G. (1994). *Poultry Science*, **73**:1897-1905.
Vick, L., Luke, G. and Simkiss, K. (1993). *Journal of Reproduction and Fertility*, **98**:637-641.
Wentworth, B.C., Tsai, T., Hallett, J.H., Gonzales, D.S. and Rajcic-Spasojevic, G. (1989). *Poultry Science*. **68**:999-1010.
Yasuda, Y. Tajima, A., Fujimoto, T. and Kuwana, T. (1992). *Journal of Reproduction and Fertility*, **96**:521-528.

BONE BREAKAGE AND OSTEOPOROSIS IN LAYING HENS: CAUSES AND SOLUTIONS

C.C. WHITEHEAD

Summary

Osteoporosis arises from a loss of structural bone during the laying period, resulting in increased bone fragility and susceptibility to fracture. Lower structural bone content at the onset of lay, higher rate of resorption during lay and less cross linking in the collagen matrix all contribute to the bone weakness. Nutritional, husbandry and genetic factors can help to alleviate the problem. The problem is not normally caused by calcium deficiency, but providing a particulate source of calcium can be beneficial. Bone strength is improved by keeping birds in husbandry systems that permit exercise, but this does not necessarily decrease fracture incidences. Selection for improved bone strength is an effective way of increasing resistance to osteoporosis and is likely to provide the best long-term solution to the problem.

I. INTRODUCTION

Osteoporosis is the major cause of skeletal problems in laying hens. It results from a progressive loss of fully mineralised structural bone that leads to increased fragility. It contrasts with another cause of bone mineral loss, osteomalacia, where there is defective mineralisation of bone tissue, with thick seams of poorly mineralised organic matrix. Osteomalacia arises from nutritional deficiencies of calcium, phosphorus or cholecalciferol and is likely to lead ultimately to greater severity of osteoporosis. However, there is no evidence that avoidance of osteomalacia can prevent the development of osteoporosis.

The bone fragility induced by osteoporosis results in increased susceptibility to fracture. Gregory and Wilkins (1989), in a survey of end-of-lay battery hens in UK, reported that 29% of hens had one or more broken bones during their lifetimes. The fractures occurred during the time in cages or during depopulation, transport to a processing factory and hanging on shackles. By the time they reached the end of the evisceration line, 98% of carcasses were found to contain broken bones. A subsequent survey of a number of European flocks confirmed these findings, showing that, on average, fractures were received by 10% of hens during their time in batteries and a further 17% during depopulation and transport (Gregory *et al.,* 1994). Ischium, humerus, keel and furculum showed highest fracture incidences, with pubis, ulna, coracoid and femur also breaking frequently. Different fractures can occur at different stages in the birds life. In a survey of European flocks, Gregory *et al*. (1994) reported that 17% of birds experienced fractures during battery cage life (old breaks) and 10% during depopulation, transport and hanging on shackles (new breaks). Fractures to the humerus and ulna are frequent old breaks. Damage to the keel can arise from contact with the cage front during depopulation and femoral fractures arise commonly during shackling.

Cage layer fatigue has also been associated with osteoporosis. In some cases it is an extreme consequence of a loss of structural bone in the vertebrae that leads to spinal bone collapse and paralysis (Bell and Siller, 1962; Urist and Deutsch, 1960). However, there may also be another, more transient, form of cage layer fatigue perhaps related more to an intracellular calcium imbalance that impairs neuromuscular function. This may occur when calcium intake is inadequate in relation to the productive needs of the bird, resulting in loss of

Roslin Institute, Roslin, Midlothian EH25 9PS, UK

egg production, paralysis and increased mortality. It may also be associated with subsequent osteoporosis (Cransberg *et al*., 2001). The biochemical rather than skeletal cause of this latter form of cage layer fatigue can be confirmed by the quite rapid alleviation of the paralysis by injections of calcium gluconate. This form of cage layer fatigue contrasts with the more typical chronic osteoporosis, the severity of which does not affect egg output (Rennie *et al*., 1997).

II. CAUSES OF OSTEOPOROSIS

The fundamental cause of osteoporosis is a lack of structural bone formation during the laying period, combined with continued structural bone resorption. Structural bone is formed by osteoblasts during the growing period, but at sexual maturity the large increase in oestrogen stimulates osteoblasts to switch to formation of medullary bone This type of woven bone occurs as spicules within structural bone cavities or as a lining on structural bone surfaces. It serves as a labile source of calcium for shell formation and has little intrinsic strength, although it can contribute to overall fracture resistance of bones (Fleming *et al*., 1998a). Osteoclasts, the bone resorbing cells, act mainly on medullary bone but also resorb some structural bone, resulting in progressive loss of structural bone throughout the skeleton. When hens go out of lay, oestrogen levels fall, medullary bone is resorbed and structural bone formation can recommence.

Comparisons between hens selected for resistance or susceptibility to osteoporosis in a genetic study described in Section V have shown that factors related to both the mineral and matrix compositions of bone can influence the severity of osteoporosis at the end of lay. Firstly increased bone formation during rearing can increase peak structural bone mass at the start of lay. This gives the resistant hens greater bone reserves to sustain them against structural bone loss during the laying period. Secondly, resistant hens show a lower rate of bone resorption during the laying period. This is manifest in a slower loss of cortical bone thickness and may be related to the lower numbers of osteoclasts measured in these birds (Fleming *et al*., 2001). The resistant birds also have more medullary bone, and the more complete lining of structural bone surfaces with medullary bone may also decrease the numbers of osteoclasts that come directly into contact with structural bone. A third factor affecting the biomechanical properties of bone is the nature of the organic matrix. Observations of greater cross linking, particularly pyrrolic cross linking, in the collagen matrix of bones suggest that superior matrix quality may also contribute to the better bone strength in the resistant hens (Sparke *et al*., 2002).

A comparison of the relative impacts of the above factors on the overall severity of osteoporosis suggests that the rate of bone resorption during the laying period has the most profound effect. Oestrogen is known to affect the coupling between bone formation and resorption and the higher circulating concentrations of this hormone in the resistant hens (Fleming *et al*., 2001) may indicate a systemic factor influencing avian osteoporosis. This suggests an important genetic regulation of osteoporosis, but nutritional and environmental factors can also be involved.

III. NUTRITIONAL FACTORS IN OSTEOPOROSIS

Dietary deficiency, particularly of calcium or vitamin D, can ultimately accelerate the onset of osteoporosis, but most recent studies have investigated how dietary improvement might alleviate the condition. Rennie *et al*. (1997) reported that none of the dietary factors tested in an experiment over a laying year had any effect on the proportions of cancellous bone in spinal or leg bones but that treatments involving a particulate source of calcium

(oystershell) or supplementation with fluoride increased the proportions of medullary bone. Providing calcium in particulate form extends the period of calcium absorption later into the night when shell formation is taking place and has been shown to have beneficial effects on shell quality. The finding that provision of calcium with improved digestive characteristics can increase the amount of medullary bone without having much impact on the loss of structural bone shows that calcium deficiency is not a primary cause of osteoporosis. However, increases in medullary content may nonetheless have a beneficial effect on bone quality, as discussed earlier. Confirmation of the practical benefits of particulate calcium sources has been provided by Fleming *et al.* (1998b) who found that feeding limestone particles resulted in improved bone strength in end-of-lay hens (Table 1). This effect was probably attributable to the observed increase in medullary bone formation because the treatment again had little effect on cancellous bone volumes, though a subsequent study has suggested that cortical bone characteristics may be improved by particulate calcium.

Table 1. Effect of providing limestone as powder or particles (3mm diameter) on bone characteristics in end-of-lay hens.

Trait	Limestone type		
	Powder	Particles	P value
Proximal tarsometatarsus			
Total bone (%)	23.4	28.6	<0.001
Cancellous bone (%)	6.6	7.3	NS
Medullary bone (%)	16.8	21.4	<0.05
Free thoracic vertebra			
Cancellous bone (%)	10.4	10.2	NS
Tibia			
Radiographic density (mm Al)	1.96	2.26	<0.01
Breaking strength (kg)	19.5	23.6	<0.05
Humerus			
Radiographic density (mm Al)	0.73	0.78	NS
Breaking strength (kg)	11.8	12.1	NS
Keel			
Radiographic density (mm Al)	0.61	0.68	<0.01

(Fleming *et al.,* 1998b)

These observations are consistent with the hypothesis that osteoporosis in hens arises as a result of cellular processes rather than inadequate nutrient supply. The balance of cellular activity can be altered by feeding bisphosphonate, a drug used in human medicine to combat post-menopausal osteoporosis. This acts by inhibiting the action of osteoclasts and has been shown to slow the loss of cancellous bone in hens (Thorp *et al.*, 1993). However, use of bisphosphonate is unlikely to be a practical solution for laying hen osteoporosis.

The main nutritional recommendations for minimizing osteoporosis are:

- Avoid deficiency of calcium, phosphorus, vitamin D.
- Provide a prelay diet containing increased calcium content.
- Provide a particulate source of calcium (e.g. oystershell or limestone particles up to 3mm diameter).
- Do not withdraw feed a few days before depopulation. This will hasten the decline in bone strength at the time of exposure to greatest physical trauma.

IV. ENVIRONMENTAL FACTORS IN OSTEOPOROSIS

The effects of load bearing and biomechanical forces in stimulating bone formation and remodelling are well established. Induced inactivity has been shown to accelerate osteoporosis in birds (Nightingale *et al.*, 1972) and the relative lack of activity of battery caged hens accounts for the severity of the problem in these birds. Effects of exercise and alternative housing systems have been widely studied as potential means of alleviating osteoporosis. Effects of exercise as a way of stimulating bone growth during rearing have been studied, but neither housing birds in pens nor giving extra exercise through use of a carousel have improved bone quality at start of lay compared to cage-rearing (Whitehead and Wilson, 1992).

Changes in bone quality during the laying period are influenced by the nature of the exercise involved. Housing hens in pens has resulted in little change in spinal trabecular bone (Wilson *et al.*, 1993). Fitting perches to cages resulted in small improvements in PTM trabecular bone volume, but no benefit in tibia strength (Hughes *et al*., 1993). More vigorous exercise than is obtained by walking or hopping onto low perches is needed to markedly improve bone quality. This was demonstrated by Knowles and Broom (1990) who found superior tibia and humerus breaking strengths in birds housed in terrace or perchery systems rather than in cages. The improvement in humerus strength was particularly apparent in the perchery system which allowed birds to fly. Confirmation of these findings came from a more detailed study by Fleming *et al.* (1994). It can be concluded that, as in other species, biomechanical effects on individual bones in hens are dependent upon the degree of strain experienced by the bone.

There is little information on the mechanism by which exercise improves bone characteristics in the hen. However, the finding by Newman and Leeson (1998) that tibial strength increased within 20 days of transfer of hens from cages to an aviary suggests that the mechanism may involve stimulation of structural bone formation, rather than inhibition of resorption.

There have been several studies to determine the welfare impact of the improved bone strength of birds kept in alternative housing systems. Lower incidences of new breaks have been found in birds depopulated from aviary or free-range systems compared to battery cages (Gregory *et al*., 1990; Van Niekerk and Reuvekamp, 1994). However, the incidences of old breaks, particularly in the furculum and keel, were higher with aviary and free-range systems (Gregory *et al*., 1990). It may be concluded that allowing birds more exercise in alternative systems will improve bone strength, but this does not necessarily improve bird welfare in proportion.

V. GENETIC FACTORS IN OSTEOPOROSIS

A strong genetic component in osteoporosis has been demonstrated by Bishop *et al.* (2000) who studied the inheritance of characteristics related to osteoporosis over 5 generations in a commercial pure line of White Leghorns previously selected for high egg production. Initially, measurements were made on a range of morphometric, radiological and strength characteristics of different bones in hens at the end of the laying period to determine heritabilities. Morphometric traits involving cancellous and medullary bone volumes were found to be poorly heritable (FTV cancellous bone volume, $h^2 = 0.19$; PTM cancellous bone volume, $h^2 = 0.0$). This was considered surprising in view of the use of cancellous bone to assess severity of human postmenopausal osteoporosis and as a criterion in earlier laying hen studies (Whitehead and Wilson 1992; Wilson *et al.*, 1993; Rennie *et al.*, 1997). In contrast, heritabilities of other characteristics were higher (tibia strength, $h^2 = 0.45$; humerus strength,

h^2 = 0.30; keel radiographic density, h^2 = 0.39). There was also a positive correlation between body weight and bone strength.

On the basis of the above heritabilities, it has proved possible to select hens for stronger bones (Bishop *et al.*, 2000). A restricted selection index designed to improve bone characteristics, yet hold body weight (BW) constant, was derived from genetic parameters obtained from the preliminary analyses, using standard selection index theory. Three biologically meaningful and moderately to highly heritable traits that could be measured in a short period of time on a large number of hens at the end of lay were included in the bone index (BI), namely keel radiographic density (KRD), humerus strength (HSTR) and tibia strength (TSTR). By including characteristics of wing, leg and axial skeleton, this index gave a wide representation of the overall skeleton. The initial index was: BI = 0.27 x KRD + 0.37 x HSTR + 0.61 x TSTR - 0.25 x BW. The coefficient for bodyweight was later increased to 0.35 to counter a slight divergence in this trait that started to appear between the lines. Males were selected on the basis of family breeding values. Selection was performed retrospectively each year, with chickens hatched and raised from all available hens in the experiment

Genetic parameters for the traits in the BI, and the BI itself, showed that all traits were moderately to highly inherited throughout the study, with the heritability of the BI being 0.4. The genetic and phenotypic correlations also show that the three bone measurements in the index were moderately to strongly correlated with each other. Finally, the bone measurements were all positively correlated with body weight, indicating that selection for improved bone strength characteristics alone, without the restriction placed on body weight, would have resulted in considerably heavier birds. Different mean values in the bone strength measurements in different years indicated that these traits were strongly affected by environmental factors, raising the possibility of genotype by environment interactions. However, comparison of full sib flocks reared on different locations gave little evidence for genotype by environment interactions, within the range of environments investigated.

From the first year of selection using the index, the high (H) and low (L) BI lines diverged progressively for KRD, HSTR, TSTR and the BI in the desired direction. Bone characteristics of the two lines are shown in Table 2. For the hens, the lines differed by 17% for KRD, 30% for HSTR and 60% for TSTR after 5 generations. Although selection was based on measurements made on hens, selection was found to also affect bone strength in males as well, with H line males being superior to L line males for all traits. The differences between the lines in the 4th generation were: KRD 13%, HSTR 26% and TSTR 19%.

Table 2. Bone characteristics and body weights at the end of the laying period in female and male chickens divergently selected for high (H) or low (L) bone index. Data for females are from 5th generation of selection, for males from 4th generation.

Trait	Females			Males		
	Line		P value	Line		P value
	H	L		H	L	
Body weight (kg)	1.80	1.79	NS	2.25	2.21	NS
Keel radiographic density (mm Al equivalent)	0.41	0.35	<0.001	0.70	0.62	<0.001
Tibia strength (kg)	38.2	23.7	<0.001	60.6	51.0	<0.001
Humerus strength (kg)	17.9	13.6	<0.001	36.8	29.2	<0.001

Bodyweight at slaughter did not differ between the lines in either sex. All bone measurements were strongly correlated with the presence/absence of breakages; the incidences of humeral fractures in hens occurring during the production period and depopulation showed a 6-fold difference between the lines after 4 generations. These findings confirm that genetically improving bone strength will indeed decrease the incidence of bone fractures. Effects of selection on other bone characteristics have been described in Section II. From these various differences it seems likely that laying hen osteoporosis is polygenic in origin.

Production characteristics are shown in Table 3. Daily feed intake did not differ between the selected lines. Likewise, measurements of rate of egg production and egg weight did not show any differences between the lines. These genetic observations complement the observations of Rennie *et al*. (1997) that the large individual variation observed in the bone characteristics of hens at the end of lay was phenotypically unrelated to egg production in a flock of highly productive hens.

The main production difference between the lines was in shell quality, with H line hens laying eggs that had thinner, weaker shells. This was reflected in a greater incidence of cracked and second quality eggs. The difference in shell output per day was very small (~0.1g), but over the laying life of the hen this could represent a difference in calcium output of 12g. This is large in relation to the total body bone calcium content of a hen (about 80g) and could be expected to have an impact on bone content. Negative phenotypic correlations between shell quality and bone (tibia) strength have been reported previously by Orban and Roland (1990). The poorer shell quality in the H line may be a consequence of the lower rate of osteoclastic bone resorption failing to mobilize sufficient calcium from bone sources to meet the calcium needs for shell formation.

Table 3. Performance and shell characteristics of H and L line hens

	Line		P value
	H	L	
Rate of lay (%)	86.9	87.3	NS
Egg mass (g/h/d)	51.5	51.8	NS
Feed Intake (g/h/d)	105.2	106.2	NS
2nd grade eggs (%)	2.93	2.30	NS
Candling cracks (%)	3.1	2.6	<0.05
Shell wt (mg/cm^2)	79.5	80.5	<0.05

VI. SOLUTIONS TO OSTEOPOROSIS

Osteoporosis seems to result from the long-term cellular effects of bone resorption during the egg laying period. It does not appear to be a consequence of high egg output *per se*, but rather of the length of time during which the hen is continuously in reproductive condition. The condition is not caused primarily by inadequate dietary calcium concentration, though suboptimal calcium intake, resulting from low feed intakes and/or dietary calcium concentrations can lead to short-term production problems and more severe osteoporosis in the longer-term. Nevertheless, nutrition, as well as husbandry and genetics, can contribute to alleviating the severity of osteoporosis.

The relative effectiveness of these different approaches to combating osteoporosis can be compared using the three-point breaking strength of the tibia as criterion. The best

nutritional approach identified so far, providing calcium in particulate rather than powdered form, has been shown to increase tibia strength by 21% (Fleming *et al*., 1998b). However, including particulate calcium sources in feed is already widely used as a means of optimizing shell quality, so the information on bone responses is more a comfirmation of existing good practice than an opportunity for a major improvement in bone quality. The largest improvement in tibia strength (31%) comes from housing birds in an aviary rather than a battery cage (Fleming *et al*., 1994). However, the exposure of birds in alternative systems to greater physical trauma (collisions, crash landings, falling off perches, etc) means that greater bone strength is not necessarily accompanied by fewer fractures (Gregory *et al*., 1990). Most evaluations of effects of alternative systems on bones have measured bone strength rather than fracture incidence, so direct information on fracture incidence is needed before the skeletal welfare characteristics of different systems can be properly compared. Genetics represents perhaps the most effective long-term solution to the problem. An improvement in tibial strength of 23% (H line relative to the H and L line average, Table 2) over 5 generations shows that bone strength is highly responsive to selection. Although the birds were selected in cages, findings from an earlier generation showed that the differences in bone strength between the lines were maintained when birds were housed in an aviary. The genetic approach thus seems to offer a permanent improvement in bone quality in hens whether housed in conventional cages or alternative systems.

The selection method involved retrospective selection on the basis of post mortem data from end-of-lay hens, and so was not very suitable for commercial application. A predictive method would be more efficient. A procedure based on digitized fluoroscopy (DF) has shown that it is possible to predict end-of-lay bone characteristics by radiographic methods (Fleming *et al*., 2000). Radiographic measures are best made on the humerus because this bone is usually pneumatized and thus free from medullary bone. The DF results showed that measurements made as early as 40 weeks gave a good indication of end-of-lay humeral strength and also some indication of tibia strength. *In vivo* selection on the basis of radiological measures could thus be expected to result in improved bone quality. The equipment used for the DF measurements was rather too bulky for easy use on-farm. Newer, more portable x-ray scanners are becoming available for use in human osteoporosis assessment but initial evaluation of these has suggested that the need for immobilisation of a bird over a greater time than was required for the DF method would be a disadvantage.

The future direction of poultry breeding is moving towards the use of marker-assisted selection (MAS). This method of selection depends upon the identification of genetic markers for the desired trait and can be implemented very early in the life of the young chick. As suggested above, it is likely that laying hen osteoporosis is polygenic in origin. Identification of the specific genes associated with osteoporosis and their association with quantitative trait loci for bone quality could lead to the development of markers that could form the basis for commercial MAS for resistance to osteoporosis. It will also be important to establish a method, either genetic or nutritional, of overcoming the apparent inverse relationship between bone and shell quality to encourage poultry breeders to implement this selection.

REFERENCES

Bell, D.J. and Siller, W. (1962). *Research in Veterinary Science,* **3**: 219-230.

Bishop, S.C., Fleming R.H., McCormack, H.A., Flock D.K. and Whitehead, C.C. (2000). *British Poultry Science,* **41**: 33-40.

Cransberg, P.H., Parkinson, G.B., Wilson, S. and Thorp, B.H. (2001). *British Poultry Science,* **42**: 260-265.

Couch, J.R. (1955). *Feed Age,* **5**: 55-57.

Fleming, R.H., Whitehead, C.C., Alvey, D, Gregory, N.G. and Wilkins, L.J. (1994). *British Poultry Science,* **35**: 651-662.

Fleming, R.H., McCormack, H.A., McTeir L. and Whitehead, C.C. (1998a). *Research in Veterinary Science,* **64**: 63-67.

Fleming, R.H., McCormack H.A. and Whitehead, C.C. (1998b). *British Poultry Science,* **39**: 434-440.

Fleming, R.H., McCormack H.A. and Whitehead, C.C. (2000). *Calcified Tissue International,* **67**: 309-313.

Fleming, R.F., McCormack, H.A. and Whitehead, C.C. (2001). *Proceedings of 3rd International Satellite Symposium on Comparative Endocrinology of Calcium Metabolism,* p 7.

Gregory, N.G. and Wilkins, L.J. (1989). *British Poultry Science,* **30**: 555-562.

Gregory, N.G., Wilkins, L.J., Eleperuma, S.D., Ballantyne A.J. and Overfield, N.D. (1990). *British Poultry Science,* **31**: 59-69.

Gregory, N.G., Wilkins, L.J., Knowles, T.G., Sorensen P. and Van Niekerk, T. (1994) *Proceedings of the 9th European Poultry Conference,* **II**: 126-128.

Hughes, B.O., Wilson, S., Appleby M.C. and Smith, S.F. (1993). *Research in Veterinary Science,* **54**: 202-206.

Newman, S. and Leeson, S. (1998). *Poultry Science,* **77**: 1492-1496.

Knowles, T.G. and Broom, D.G. (1990). *Veterinary Record,* **126**: 354-356.

Nightingale, T.E., Littlefield L.H. and Merkley, L.W. (1972). *Poultry Science,* **51**: 1844-1845.

Orban, J.I. and Roland, D.A. (1990). *Poultry Science,* **69**: 2068-2073.

Randall, C.J. and Duff, S.R.I. (1988) Avulsion of the patellar ligament in osteopenic laying fowl. *Veterinary Record* **128**: 397-399.

Rennie, J.S., Fleming, R.H., McCormack, H.A., McCorquodale C.C. and Whitehead, C.C. (1997). *British Poultry Science,* **38**: 417-424.

Sparke, A.J., Sims, T.J., Avery, N.C., Bailey, A.J., Fleming, R.H. and Whitehead, C.C. (2002). *British Poultry Science* (in press).

Thorp, B.H., Wilson, S., Rennie S. and Solomon, S.E. (1993). *Avian Pathology,* **22**: 671-682.

Urist, M.R. and Deutsch, N.M. (1960). *Endocrinology,* **66**: 377-391.

Van Niekerk, T.G.C.M. and Reuvekamp, B.F.J. (1994). *Proceedings of 9th European Poultry Conference,* **II**: 133-136.

Whitehead, C.C. and Wilson, S. (1992) Characteristics of osteopenia in hens. In: *Bone Biology and Skeletal Disorders in Poultry.* Whitehead, C.C. (ed.), Carfax Publishing Co., Abingdon, UK, pp 265-280.

Wilson, S, Hughes, B.O., Appleby, M.C. and Smith, S.F. (1993) Effects of perches on trabecular bone volume in laying hens. *Research in Veterinary Science,* **54**: 207-211.

DO OSTEOPOROSIS AND SKELETAL UNDERMINERALISATION LIMIT EGG PRODUCTION ?

G. PARKINSON and P. CRANSBERG

Summary

Problems of weight loss in the pre- and post-peak phases of egg production have been identified in many commercial and experimental flocks in Australia. These periods of weight loss have been associated with loss of egg production, muscle paralysis in some birds and the development of skeletal abnormalities characteristic of osteoporosis. The weight loss and sub-optimal production do not appear to respond to conventional dietary supplementation with energy and/or protein.

These phenomena may be consequences of environmental stress. Further research is needed to identify the metabolic changes that lead to osteoporosis and the relationship of these changes to the depletion of skeletal mineral reserves, the decline in structural bone volume, and the break in the continuity of egg production.

I. INTRODUCTION

(a) Historical descriptions of cage layer fatigue

In his review of skeletal diseases of poultry, Riddell (1981) suggested that cage layer osteoporosis underlies both cage layer fatigue and the bone breakage which occurs when hens are culled at the end of lay. Recent research on induced osteoporosis in the laying hen is compatible with this hypothesis, and the possibility remains that modern laying strains still experience sub-clinical cage layer fatigue in association with osteoporosis.

The clinical signs of cage layer fatigue include muscle paralysis, sternal deformation, sigmoidal shaped ribs, and infolding of the ribs caused by small fractures at the costochondral junctions. Cortical bone is thin and medullary bone mass is decreased. The paralysis is believed to be due to compression fractures of the fourth and fifth vertebrae (Bell and Siller, 1962) and in some birds that recover, these vertebral fractures are thought to have healed. In some cases, cage layer fatigue appears to be associated with hypocalcaemia (Whitehead, 2001).

Cage layer fatigue is more frequently found in high producing leghorns near peak production (Couch, 1955; Riddell, 1981), and can be accentuated in underweight pullets coming into lay in summer (Grumbles, 1959). The disease appears to have been in decline since the 1980's when Riddell (1981) recorded a prevalence as high as 0.5% per month in some case studies of commercial layers.

Clinical signs of cage layer fatigue will clearly be evident in single bird cage selection of elite breeders, and should also be reflected in lower annual hen housed egg production. Hence there should be some selection pressure against cage layer fatigue, particularly if the breeding companies have been able to select full sisters in both a single bird cage environment and under commercial conditions. Bell and Siller (1962) have suggested that the highly productive laying hen may be on the threshold of its minimum endogenous calcium requirements, and may lack a mechanism to decrease egg production when calcium supply is insufficient.

Through ongoing genetic selection processes, modern strains, in contrast to standard layer breeds, may have a greater resistance to cage layer fatigue by being able to balance

Victorian Institute of Animal Science, Attwood, Victoria 3049

continuous egg production with skeletal integrity. If this is so, the modern birds may still use their skeletal mineral reserves to maintain egg production, but may have the ability to pause egg production to prevent the development of cage layer fatigue.

(b) Induction of osteoporosis in laying hens

Recent research on induction of osteoporosis in the laying hen has found that there is a loss of structural bone in early lay and that this precedes the accumulation of medullary bone under the influence of oestrogen. It is believed that laying hens cannot produce structural bone during egg production (Rennie, 1997), but in hens that are out of lay with lower oestrogen levels, the medullary bone is resorbed and structural bone formation can recommence.

Experimental studies indicate that the erosion of structural bone begins by about 20 weeks of age and then stabilises between 30-40 weeks of age (Wilson and Whitehead, 1992; Thorpe *et al.*, 1993). Within a flock there appears to be almost no further loss of structural bone between 40 weeks of age and 68-72 weeks of age and the bone density at 40 weeks of age is the same as that at the end of the laying cycle (Fleming *et al.*, 2000; Whitehead, 2001).

It has been postulated that the development of osteoporosis in highly productive strains is associated with the length of time that eggs are continuously produced rather than with the actual number of eggs produced. Birds that cease production or have a pause in egg production may be able to regenerate structural bone (Rennie *et al.*, 1997). The time of continuous egg production required to erode structural bone, and the length of time of a pause in production needed to restore structural bone remain to be defined.

Clearly clinical cage layer fatigue compromises egg production and shell quality. The questions still needing answers are firstly whether cage layer fatigue is still occurring in commercial flocks, and secondly, whether moderate erosion of the skeleton in modern layer strains can trigger a pause in egg production or induce a lower rate of egg production as protective mechanisms to maintain skeletal strength.

(c) Weight loss and emaciation in layers

Summers (1983) has suggested that in pullets with low reserves of energy at sexual maturity, protein and calcium are unable to meet the demand for egg production when the intake of these nutrients is low. Furthermore, Leeson (1990) has described problems at peak production of low appetite, weight loss and a subsequent marked slump in egg production in the post-peak period. It seems likely therefore that the appetite of birds in commercial flocks may be inadequate during early egg laying, so that with the drain of production, weight loss and eventually diminished production are inevitable. The relationship of these phenomena to skeletal development and osteoporosis has been poorly described.

Gregory and Devine (1999) have reported that many commercial flocks contain emaciated birds by the end of the production cycle. They speculate that the metabolic demand on birds during egg production induces tissue catabolism. At present we have little knowledge about either the physiological consequences of this emaciation, or the ages at which it occurs in the modern bird.

Australian research has identified problems of sub-optimal growth in early egg production that appears to be correlated with defects in skeletal structure and also with muscular paralysis.

II. BODY WEIGHT LOSS IN EARLY EGG PRODUCTION

Experimental studies in Australia under controlled environmental conditions, with birds fed on commercial diets, have found periods of depressed growth in layers between 20 to 26 weeks of age (Figure 1), similar to the observations of Leeson (1990). Groups of birds have been identified with significant weight losses over a 1-2 week period. Approximately 14% of birds in the flock lost 100 grams or more live weight, whereas the breed standards predict that birds should gain, on average, 80 to 150 grams live weight throughout the same period. Clearly a significant proportion of the flock is undergoing marked tissue catabolism in the early lay period and this is associated with lower egg production performance (Figures 1 and 2).

With tissue catabolism of this extent, it is possible that the processes of bone growth and development are also disrupted. The normal pattern is for medullary bone reserves to be laid down at the same time as structural bone is being resorbed. Significant tissue catabolism would be likely to affect both these processes. Furthermore, these problems of early weight loss may be contributing to the emaciation observed by Gregory (1999) in older birds because the decreased body weight can become a chronic problem (Figure 1).

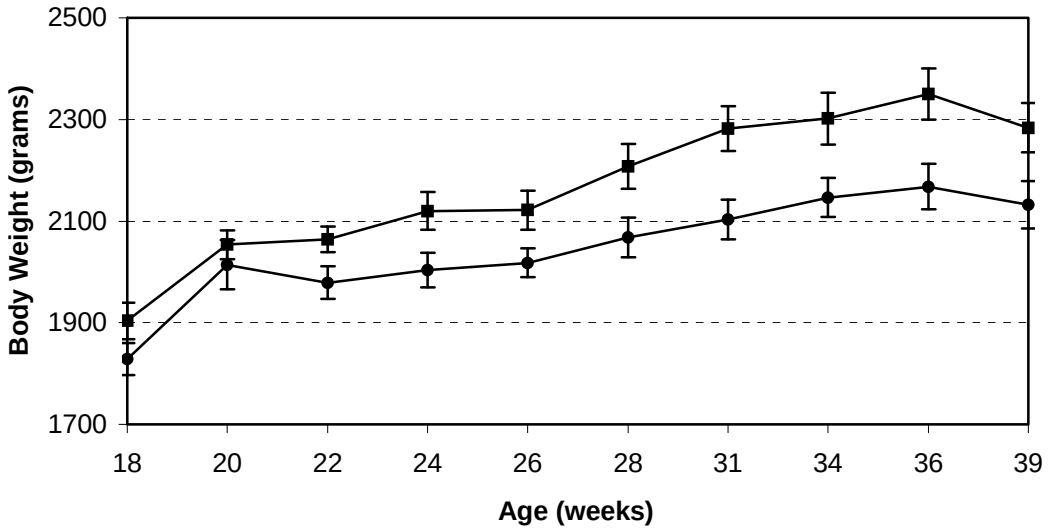


Figure 1. Average flock body weights in two groups of birds (ν - high egg production (> 96%), λ - low egg production (<88%)).

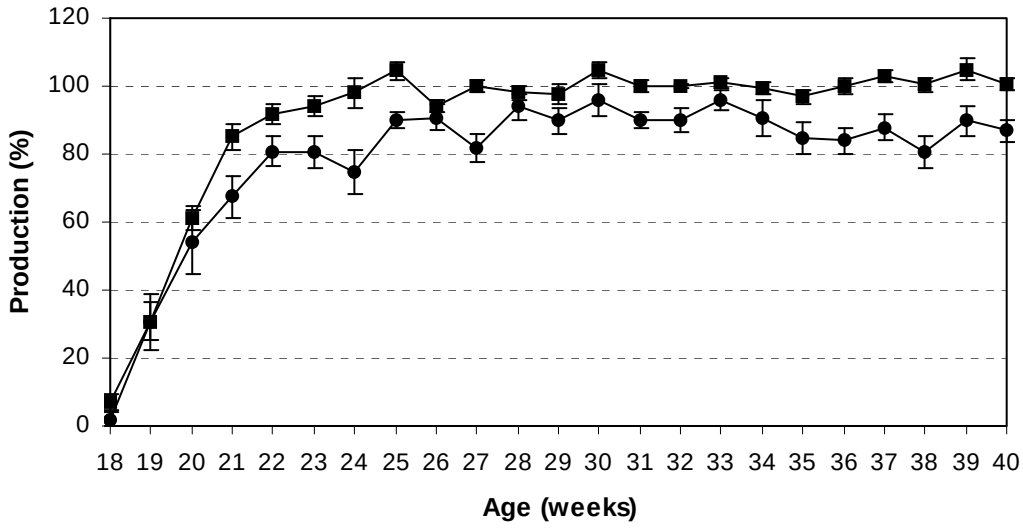


Figure 2. Percentage of days that eggs were produced versus age, in two groups of birds (ν - high egg production (> 96%), λ - low egg production (<88%)).

III. TRANSITORY PARALYSIS IN HIGH PRODUCING BROWN EGG-LAYERS

Birds of a brown-feathered egg-laying strain, aged between 17 and 45 weeks, were fed a commercial laying diet under controlled environmental conditions. The flock achieved both body weight and egg weight standards and had above average egg production performance (99% peak egg production) (Figures 3 and 4). The ratio of egg mass output to body weight was 3.0 grams egg weight/kg live weight for the commercial brown egg-layer at 45 weeks of age.

Between 26 to 30 weeks of age the flock growth rate declined against the breed standards and there was almost no growth between 29-30 weeks of age. At 28 weeks of age, 4% of the birds showed muscle paralysis similar to that seen in cage layer fatigue. A period of weight loss of between 50 to 150 grams over 1 to 2 weeks preceded the paralysis and eventually the affected birds ceased to produce eggs. After a further 1 to 2 weeks, these birds regained both muscle function and body weight and resumed laying at their previous production rates.

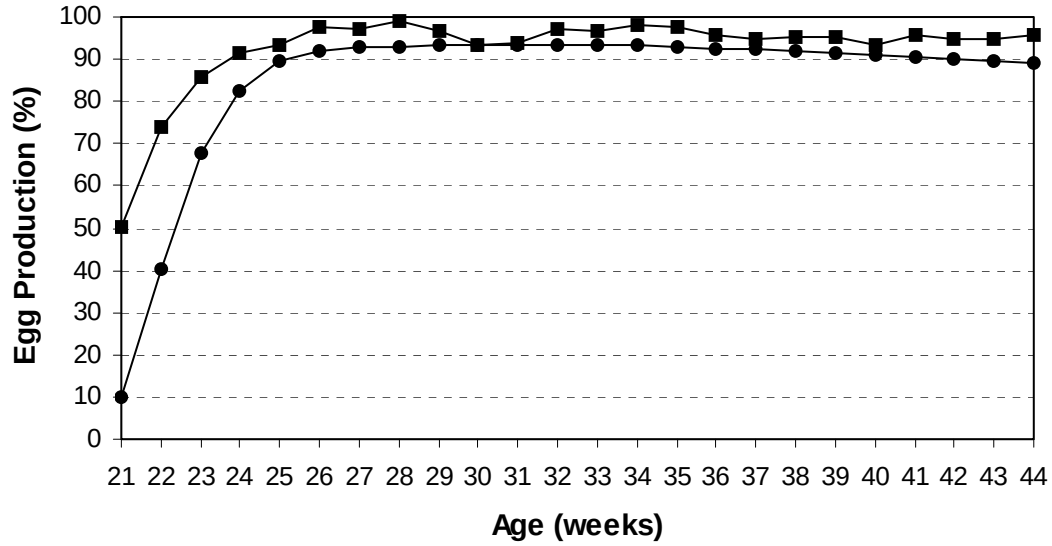


Figure 3. Percentage of days that eggs were produced versus age in a flock of commercial brown egg-layers between 21-45 weeks of age (λ - breed standard).

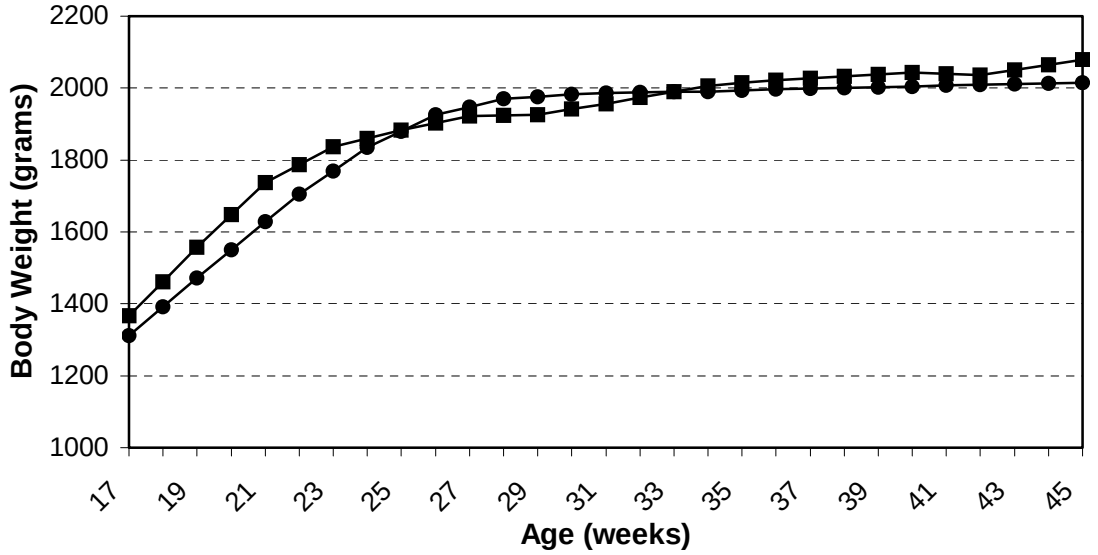


Figure 4. Average body weight in commercial brown egg layer flock between 17 to 45 weeks of age (λ - breed standard).

IV. SKELETAL ABNORMALITIES IN HIGH PRODUCING BROWN EGG LAYERS

Assessment of deformity and swelling of the costochondral junction of the rib cage was done at 45 weeks of age in the highly productive commercial brown egg-laying flock described in figures 3 and 4. The scoring scale ranged from 0 to 5, with 5 indicating very severe lesions and 0 indicating no lesions. Across the whole flock, 57% of birds had a rib abnormality score of 1-5, whilst 29% had a score of 3-5. A retrospective analysis of the flock growth patterns of two groups (those not affected or only mildly affected (score 0-2) against those which were severely affected (score 3-5)) revealed that the birds developing the severe rib abnormalities had a loss of body weight between 29 to 31 weeks of age, which corresponded to the period of transitory paralysis in 4% of birds. A partial recovery from the

loss of body weight eventually occurred between 31 and 34 weeks of age, but these birds remained about 100 grams lighter than the unaffected birds. The loss of body weight was associated with a 15% decrease in egg production, however production eventually recovered as the birds began to gain weight.

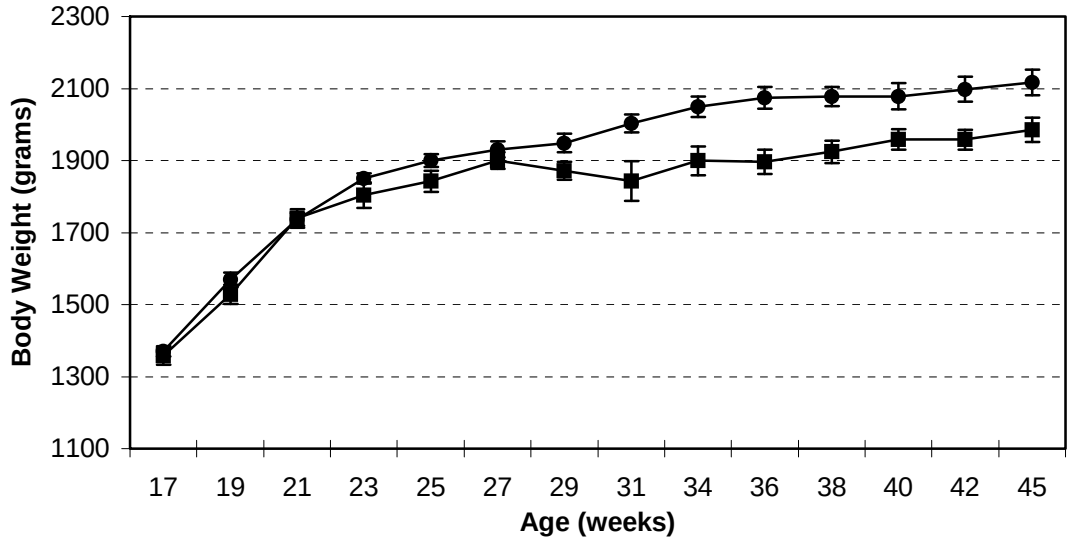


Figure 5. Average body weights of groups severely effected (ν) and those not or mildly effected (λ) with rib abnormalities

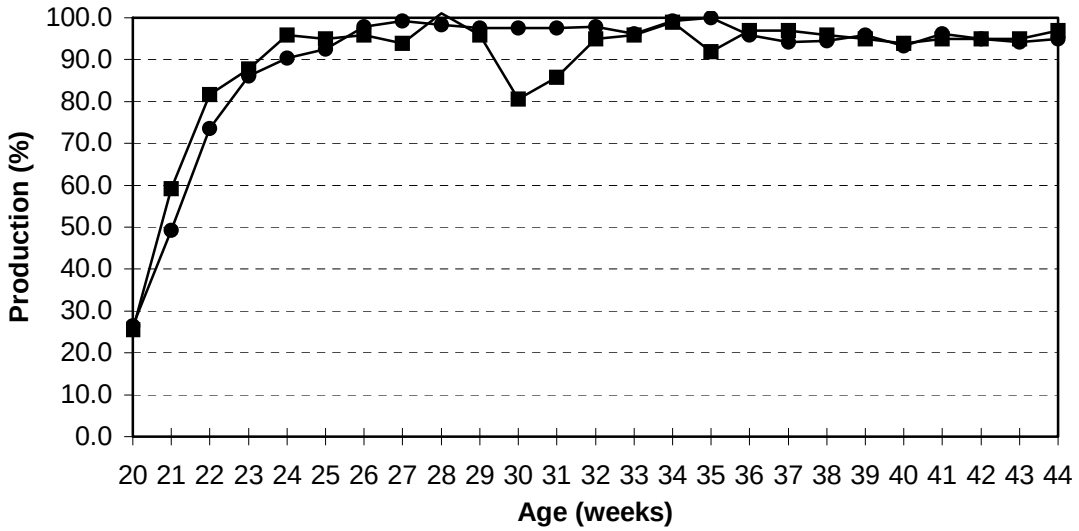


Figure 6. Percentage of days that eggs were produced versus age of groups severely affected (ν) and those not affected or only mildly affected (λ) with rib abnormalities

V. MOULTING AND RECOVERY IN STRUCTURAL BONE MASS IN BROWN EGG-LAYERS

Research to date indicates a poor correlation between egg production and structural bone volume in a modern commercial strain (Rennie, 1997), but it is apparent that standard egg-laying breeds, with lower egg production performance, have higher structural bone volumes at the end of the egg production cycle than those strains with higher egg yields.

Within the modern strains, a high egg production rate and erosion of structural bone may interact with an ability of birds to regenerate structural bone by ceasing or pausing egg production. Research in moulting hens indicates that trabecular bone volume can increase from 5.7% to 21.5% over an 8-week period of recovery (Table 1).

Table 1. Changes in the volumes of proximal tarsometatarsal trabecular bone (TBV%) and medullary bone (MBV%) in brown egg-layers during the tissue recovery process following a moult ending at 72 weeks of age (mean (SE))

Age (weeks)	TBV%	MBV%
72	5.7 (1.0)	5.9 (1.3)
80	21.5 (1.2)	1.5 (0.2)
107	13.7 (1.1)	2.9 (0.6)

VI. CONCLUSIONS

An important question to be resolved is whether the body weight loss, muscular paralysis and skeletal abnormalities described in this paper, are linked to the mechanisms that induce osteoporosis in commercial layers. The induction of osteoporosis in individual birds may occur independently of, but nevertheless interact with, the depletion of reserves of energy, protein and calcium from high rates of egg production.

If the bone density and bone strength at 40 weeks of age, are strongly correlated with the bone density and strength at the end of lay, then an examination of environmental interactions which occur between the onset of lay and the peak of egg production will help in devising management strategies to prevent the problem of osteoporosis.

Experiments with individual birds should also be done to investigate whether small decreases in body weight and feed intake are predisposing factors in the excessive erosion of skeletal mineral reserves. Because the ability of birds to pause in egg production allows structural bone mass to regenerate, the importance of skeletal mineral reserves in sustaining egg production and shell quality should not be underrated.

REFERENCES

Couch, J.R. (1955). *Feed Age*, **5:** 55-57.

Bell, D.J. and Siller, W. (1962). *Research in Veterinary Science*, **3:** 219-230.

Fleming, R.F., McCormack, H.A. and Whitehead, C.C. (2000). *Calcified Tissue International*, **67:** 309-313.

Gregory, N.G. and Devine, C.D. (1999). *Veterinary Record*, **145:** 49.

Grumbles, L.C. (1959). *Avian Diseases,* **3:** 122-125.

Leeson, S. (1990a). *Feed Management,* **41:** 27-31.

Rennie, J.S., Fleming, R.H., McCormack, H.A., McCorquodale, C.C. and Whitehead, C.C. (1997). *British Poultry Science,* **38:** 417-424.

Riddell, C. (1981). *Advances in Veterinary Science and Comparative Medicine*, **25:** 277-310.

Summers, J.D. (1983). *Shaver Focus* ,**12:** 1.

Thorp, B.H., Wilson, S., Rennie, S. and Solomon, S.E. (1993). *Avian Pathology*, **22:** 671-682.

Whitehead, C.C. and Wilson, S. (1992). In: *Bone Biology and Skeletal Disorders in Poultry.* Whitehead C.C. (ed), Carfax Publishing Co., Abingdon, UK pp 265-280.

VIRULENT NEWCASTLE DISEASE IN AUSTRALIA WHAT DID WE LEARN? WHAT CAN WE EXPECT?

H. WESTBURY

Summary

Australia experienced outbreaks of virulent Newcastle disease (ND) in chickens in New South Wales in 1998, 1999 and 2000. Genetic analysis of the outbreak viruses demonstrated that they were of Australian origin, evolving, in fact, from a low virulence virus known to be quite widespread in poultry, at least in NSW. The outbreaks of virulent disease were controlled using a "stamping out" program supported by targeted use of ND vaccines, and the disease has not recurred. However the so-called progenitor virus, i.e. the virus, from which the virulent virus arose, is still present in poultry. This allows the possibility, if conditions are favourable, for the re-emergence of virulent virus and disease. Options for control of re-emergent virulent ND are discussed.

I. INTRODUCTION

Australia experienced virulent outbreaks of Newcastle disease (ND) in and near Melbourne in 1930 and 1932 (Johnstone, 1933; Albiston and Gorrie, 1942) with stamping-out programs being used on both occasions to eradicate the disease. These outbreaks occurred soon after the initial description of the disease by Kraneveld (1926) and Doyle (1927) and were confirmed by laboratory testing (Doyle, 1933). The virus that caused these outbreaks is called the Albiston-Gorrie (AG) (or Australia-Victoria (AuV)) strain and is held in various ND virus repositories around the world. Australia's national poultry flock was considered to be free of NDV infection from 1933 until 1966 when Simmons (1967) reported the isolation of an non-virulent strain of the virus, designated V4, from chickens in the state of Queensland. Serological testing subsequent to this isolation showed that infection was widespread throughout the country, though there was no evidence of a history of ND-like disease in these serologically positive flocks (Anon 1966). An earlier serological survey in 1964 of 1425 sera collected from 17 hatcheries in four states of Australia had revealed no detectable antibody to the virus (French, 1964). This information, together with other investigations of flocks and hatcheries, led McIntosh (1964) to conclude that the Australia national poultry flock was free of infection with NDV at that time. However, there are some doubts about the validity of this claim given current perspectives of statistically based sampling. Nevertheless this information provides a convenient starting point (i.e. mid 1960's) for this paper.

Further isolations of the virus were made in subsequent years (Westbury and Geering, 1977) though all these viruses were demonstrated to be, or were considered to be, non-virulent, like V4 following non-parenteral infection of chickens (Hall *et al.,* 1967; French *et al.,* 1967; Kim *et al.,* 1978; Westbury, 1979; Hooper *et al.,* 1999). This established the myth in Australia that all Australian isolates during this period were V4. This was, in fact, not the case as both Turner and Kovesdy (1974) and Westbury (1979) demonstrated variation among these non-virulent virus strains in some of their biological characteristics.

Australian Animal Health Laboratory, PO Bag 24, Geelong, Victoria 3220

Isolates of NDV able to induce respiratory disease in experimentally infected chickens were detected in Australian poultry during the 1980's and 1990's (Hooper *et al.*, 1999; Hansson *et al.*, 1999) though not all viruses isolated during this time behaved this way (Hooper *et al.,* 1999). These low virulence strains induced lesions in the respiratory tract qualitatively the same as those induced by three commercial La Sota vaccines (Hansson *et al*., 1999).

The ND situation in Australia up to 1998 can thus be summarised as follows:

- Australian poultry free of infection with NDV as judged by a serological survey in 1964.
- non-virulent virus (strain V4) detected in chickens in 1966 and infection with NDV found to be widespread, but no ND-like disease.
- emerging diversity among Australian isolates from 1967 to the early 1980's detected, as judged by nucleotide sequencing of the genes encoding the HN and F proteins, and testing of some biological characteristics, though the virus continued to be non-virulent.
- detection from mid 1980's, or thereabouts, of isolates able to induce respiratory disease in chickens equivalent to that induced by typical lentogenic strains of the virus e.g. LaSota.
- increased concern in the poultry industry during the 1990's about the impact strains able to induce respiratory disease were having on chicken farming, particularly their role in the so-called "late respiratory disease". This is a complex respiratory disease of older broilers seen in some parts of the country during summer months.
- continued concern that the presence of endemic strains of the virus would severely compromise attempts to control incursions of virulent ND.

II. OUTBREAKS OF VIRULENT NEWCASTLE DISEASE

Outbreaks of virulent ND occurred in the Sydney, Mangrove Mountain and Tamworth areas of NSW between 1998 and 2000. An official ND control and eradication scheme was implemented to control and eradicate the disease. This involved the compulsory slaughter of chickens on affected premises, the depopulation of chickens on many farms through orderly abattoir processing, controls on the movement of poultry and poultry products, the disinfection of premises and, finally, the use of ND vaccines. The control program caused severe financial loss and hardship to the NSW poultry industry, and, as a result, there was much critical comment and discussion about the effectiveness of the disease control strategy used, and the implementation of the strategy. This paper will not add to that commentary but rather concentrate on the origin of the virulent viruses responsible for the outbreaks.

Planning for emergency outbreaks of virulent Newcastle disease (ND) in Australia has been done over many years on the supposition that such an outbreak would be caused by an exotic strain derived from a foreign source. It was therefore a complete surprise to virologists when genetic analysis of the virus responsible for the 1998 outbreak of virulent ND, and subsequent outbreaks, indicated that is was an Australian derived virus (Gould *et al*., 2001a). Indeed, phylogenetic analysis indicated that the virulent viruses causing the outbreaks were very closely related to a low virulence ND virus strain called Peat's Ridge, and, most likely, derived from it The term *progenitor strain* is sometimes used to describe this virus strain. The Peat's Ridge virus was, in turn, closely related to two other viruses (NSW 12/86 and Qld 1/87) isolated in Australia in 1986 and 1987 (Gould *et al.,* 2001a). The strains have a family lineage or tree that can be defined by genetic analysis of at least two genes of the ND virus called the HN and F genes. The NSW 12/86, Qld 1/87, Peat's Ridge and the outbreak virulent viruses belong to the HN/9 lineage based on HN gene sequence analysis, and are similarly grouped on the basis of their F gene relationship.

Sequence analysis of a specific section of the F gene also provides a means of predicting whether a ND virus is virulent or not virulent. This is because the F protein of ND virus must have a specific amino acid (the building blocks of proteins) sequence at the so-called cleavage site of the F protein for the virus to be virulent. If it does not have this amino acid sequence (the amino acid sequence can be predicted from the gene sequence) then the virus is not able to induce virulent disease in chickens. Thus sequence analysis of the F gene is a powerful tool because it provides information about relationships among ND virus strains as well as the ability to predict the capacity of a virus strain to cause disease. Analysis of a large number of Australian ND virus strains has allowed Gould *et al* (2001a), Gould *et al.* (2001b) and Stevens *et al.* (2001) to cluster Australian ND virus strains into a number of lineages, such as HN/45, HN/14, HN/9, or HN/7, etc., and to determine that within at least some of these lineages that there is phenotypic (e.g. pathotype) variation. Thus virulent and non-virulent viruses, and virus strains that are in the transitional stages between non-virulent and virulent have been demonstrated within the HN/9 lineage. Virulent virus has also been detected as a sub-population within viruses in the HN/7 lineage (AR Gould unpublished data). Indeed genetic analysis demonstrates that all so-called ND virus strains are, in reality, mixtures of closely related viruses, within which there will be a predominant phenotype, but also a collection of sub-ordinate phenotypes. The idea of mixtures of viruses within a particular virus strain is embraced within the general concept of viral quasi-species (Eigen 1993), a concept that provides a more expansive and dynamic view of viral populations (particularly viruses that have RNA as their genetic material). The quasi-species idea implies that any ND virus strain actually represent a heterogenous population of viruses, with the major phenotype within the population being determined by evolutionary selection pressures within the environment within which the virus is growing eg: a chicken. How does this occur?

III. VIRUS REPLICATION

This paper was “spell-checked” by a computer program before it was submitted for publication in an attempt to ensure that random errors in keyboarding and inadvertent errors in grammatical style were reduced to a minimum. Viruses, particularly those that use DNA to encode their genetic inheritance, also proof read during replication in an attempt to ensure that the progeny of a replication cycle or cylces, are identical or near identical to the original virus. Viruses that use RNA as their genetic material, such as ND virus, have no such proof reading capacity and so when they multiply, random errors occur in the sequence of the replicated RNA of progeny viruses. This ensures genetic diversity by creating swarms of ND viruses that vary randomly and slightly from the original virus. Some of these random changes will confer no survival advantage or disadvantage, some will be inimical to the virus, while others may confer a survival advantage on the progeny viruses, under given sets of circumstances. Thus the virus population arising following replication of the virus will consist mainly of the virus sub-populations within the swarm that are best adapted to that environment, as in any evolutionary process. Thus the phenotype, or biological characteristics, of a ND virus strain can be altered by selection pressures that confer advantage to variants within the population swarm. That selection pressure allows advantaged members of the swarm to out-compete the more disadvantaged members, and consequently become the predominant phenotype. Conversely if the selection pressures remain static then the structure of the population remains essentially unchanged. These twin phenomena of viral genetic diversity created by lack of proof reading, and evolutionary selection for fitness to survive, underpin the concept of viral quasi-species. Can the quasi-species concept be used to better explain and understand what happened with ND virus in Australia?

IV. NEWCASTLE DISEASE VIRUS IN AUSTRALIA – THE QUASI-SPECIES CONCEPT IN ACTION?

The situation with ND virus in Australia up to 1988 as described in the introduction to this paper, and the subsequent outbreaks of virulent ND caused by Australian derived virus, suggest a slow evolution of virulence characteristics in our ND viruses. This began in the 1960's with the detection of non-virulent viruses, followed by the appearance of ND viruses able to induce mild respiratory disease in the 1980's and eventually the detection of virulent virus in 1998. Molecular epidemiological studies have demonstrated that the changes in the virus genome necessary to drive this evolution have slowly accumulated within the genome over this period and that this has occurred within defined lineages of Australian viruses eg: the HN/9 and the HN/7 lineages or groups. The changes occurred in endemic strains that naturally circulated in the Australian poultry population, not as a consequence of the sudden appearance of exotic (foreign) strains of the virus. Molecular studies also point to the fact that these changes have not arisen because some Australian ND viruses lineages are inherently "unstable" and prone to mutation. Rather the changes have occurred at random and slowly accumulated, as quasi-species theory would suggest, as it is chance that determines whether a change in the genome impacts on the phenotype (virulence) of the virus, and then whether this confers some selective advantage. The situation of the molecular evolution of ND virus within Australia is the best recorded example anywhere in the world, in my opinion, of the emergence of virulence within a native population of ND viruses.

The quasi-species concept also requires selection for fitness, implying that something changed in the poultry world to assist the emergence of disease causing ability within our ND viruses. Those concerned with poultry health have conjectured about what may have driven or influenced this process. This conjecture includes:

- the role of international breeds of meat and egg-laying chickens that have replaced local breeds in the national marketplace during this time
- ongoing intensification of the integrated poultry industry, though many poorly managed multi-aged egg-laying farms remain on the fringe of large cities, particularly Sydney
- the appearance of virulent Marek's disease virus in Australia. Proponents of this idea suggest that the immunosuppressive impact of such strains in some way encouraged to emergence of pathogenic strains of ND virus.
- natural infection with endemic strains of NDV continued to induce immunity in chickens, a process that may have encouraged the selection of strains better able to survive in the presence of such immunity

There may be other possibilities.

Can the quasi-species concept also help explain the small numbers of chickens that developed clinical signs of ND on some farms infected with virulent virus. This puzzled many involved in the outbreak, to the extent that there were concerns expressed by some people about the real significance of the virulent virus as a disease causing agent. This issue is quite complex, largely because we have little to no information about the prior exposure of chickens on some of these farms to ND virus. Without the information it is difficult to assess what the real susceptibility of these flocks was to challenge with virulent virus. Nevertheless on a significant number of farms in was possible to detect a mixture of virus isolates within the same flock. Thus Peat's Ridge virus, virulent virus and strains with a transitional

genotype between low virulence and full virulence were detected on the same farm. The pattern of detection was that the virulent virus was detected in the flock following the earlier detection of Peat's Ridge virus in that flock i.e. in a sequential study of the viruses circulating on the farm. Thus it is conceivable that on farms where there were a mixture of viruses that some birds, and perhaps most, were exposed to virus strains that caused mild disease, but induced immunity to subsequent infection with virulent virus. Other birds, however, might be exposed only to virulent virus, and so develop virulent disease. This might be particularly so if the transitional virus strains, and the virulent viruses, evolved during the multiple replication cycles of the virus that occurred within the one flock following its initial infection with the Peat's Ridge virus. Does this represent quasi-species in action?

V. THE FUTURE

Viruses from which virulent ND virus evolved or emerged are still present in the Australian poultry industry – they were detected a number of times during the first six months of 2001. The theoretical possibility of outbreaks of virulent ND, predicted following the first detection of ND viruses that had accumulated genomic changes in critical segments of the viral genome such that full virulence could easily develop, became reality in 1998. Whether the separate outbreaks of the disease in Sydney, Mangrove Mountain and Tamworth resulted from the spread of a virulent virus between these sites, or resulted from the emergence of virulent virus in each region or farm, or from a combination of such events, is not known. What is known is that the progenitor virus was present in each of the outbreak areas, and therefore there was the potential for virulent virus to evolve in each region. This is, perhaps, irrelevant since it is perfectly feasible that virulent virus could emerge again, given sufficient time and favourable conditions (whatever they may be). The presence of virulent virus within Australian ND viruses has now been detected in two Australian ND virus lineages (HN/9 and HN/7), although only viruses within HN/9 have actually caused virulent disease outbreaks at this time. Clearly there remains a risk of further outbreaks and this risk is increased the more prevalent viruses that require fewer genomic changes for virulence to emerge become in the Australian poultry industry. This leads to the almost inevitable conclusion that it is necessary, in some way, to control these types of viruses (essentially low virulence viruses). Vaccination seems to be the only practical option.

VI. WHAT DID WE LEARN? WHAT CAN WE EXPECT?

The poultry industry and community experienced the largest emergency animal disease control effort in the history of Australian animal health. All involved with disease control in the poultry industry learnt from the experience and it is likely that a future control program would be different. Indeed this experience is being used to refine and further develop plans for national emergency animal disease control programs, in particular the role that vaccination can have and should have in emergency animal disease control. Issues such as the biosecurity of farming enterprises have also become paramount.

The outbreak also provided a unique insight into the biology of ND virus and, in particular, the now real threat of the emergence of virulent ND viruses from populations of naturally circulating endemic low virulence ND viruses. The Australian poultry scene has, perhaps, been a giant field laboratory in which the virus has had the opportunity to develop and evolve over many years. Newcastle disease virologists now include emergence as a means by which outbreaks of virulent ND can arise (Alexander, 2000). This requires animal health professionals to think about the need for – and the necessity of - disease control strategies when there is a background of the presence of potentially virulent virus in a

livestock population. The situation with virulent avian influenza in turkeys in Italy (Banks *et al.*, 2001) provides considerable support to this need.

What can we expect if the present situation with regard to ND viruses remains the same? More outbreaks of virulent ND, in an indeterminate timeframe.

REFERENCES

Albiston, H.E. and Gorrie, C.J.R. (1942). Newcastle disease in Victoria. *Aust.Vet.J.* **18:** 75-79.

Alexander, D.J. (2000). Newcastle disease and other avian paramyxoviruses. Rev.sci.tech.Off.int.Epiz. **19:** 443-462.

Anon. (1966) Newcastle disease in poultry. *Aust.Vet.J.* **42:** 353-354.

Banks, J., Speidel, E.S., Moore, E., Plowright, L., Piccirillo, A., Capua, I., Co Fiorett, A. and Alexander, D.J. (2001). Changes in the haemagglutinin and neuraminidase genes in the emergence of highly pathogenic H7N1 avian influenza virus in Italy. *Arch. Virol.* **146** (5): 963-973.

Doyle, T.M. (1927). A hitherto unrecorded disease of fowls due to a filter-passing virus. *J.Comp.Pathol.* **40:** 144-169.

Doyle, T.M. (1933). cited by Albiston, H.E. and Gorrie, C.J.R. (1942) Newcastle disease in Victoria. *Aust.Vet.J.* **18:** 75-79.

Eigen, M. (1993) Viral quasispecies. *Scientific American* **269:** 32-39.

French, E.L. (1964). Evidence of freedom of Australian flocks from infection with Newcastle disease virus. *Aust.Vet.J.* **40:** 119 – 120.

French, E.L., St George, T.D. and Percy, J.J. (1967). Infection of chicks with recently isolated Newcastle disease virus of low virulence. *Aust.Vet.J.* **43:** 404-409.

Gould, A.R., Kattenbeldt, J.A., Selleck, P., Hansson, E., Della-Porta, A.J. and Westbury, H.A. (2001a). Virulent Newcastle in Australia: Molecular analysis of viruses isolated prior to and during the outbreak of 1998-2000. *Virus Res.* **77**:51-60.

Gould, A.R., Kattenbeldt, J.A., Stevens, M. and Selleck,P. (2001b). The molecular basis for the mergence of virulent NDV in Australia: Current trends in circulating NDV. Proc 3rd Virology in Australia Conf. Univ. Syd. Sept 26-28.

Hall, W.T.K., Rosenfeld, L.E. and Simmons, G.C. (1967). Studies on the serological response and pathogenicity to chickens of a Queensland isolate of Newcastle disease virus. *Aust.Vet.J.* **43:** 400-404.

Hansson, E., Young, J.G., Hooper, P.T. and Della-Porta, A.J. (1999). Virulence and transmissibility of some Australian and exotic strains of Newcastle disease virus used in some vaccines. *Aust.Vet.J.* **77:** 51-53.

Hooper, P.T., Hansson, E., Young, J.G., Russell, G.M. and Della-Porta, A.J. (1999). Lesions in the upper respiratory tract in chickens experimentally infected with Newcastle disease viruses isolated in Australia. *Aust.Vet.J.* **77:** 50-51.

Johnstone, R.N. (1933). Pseudo poultry plague. The second outbreak. *J.Dept.Agric.Vic.* **31:** 80-82.

Kim, S.J., Spradbrow, P.B. and Chung, Y.S. (1978). The serological response of chickens to Australian lentogenic strains of Newcastle disease virus. *Aust.Vet.J.* **54:** 430-436.

Kraneveld, F.C. (1926). Over een Ned-Indie heerschende Ziekte onder het Pluimves. Nederland Ind. *Bladen Diergeneesk* **38:** 448-456.

McIntosh, K.S. (1964) Official statement on the health status of Australian day-old chicks. *Aust.Vet.J.* **40:** 119.

Simmons, G.C. (1967). The isolation of Newcastle disease virus in Queensland. *Aust.Vet.J.* **43:** 29-30.

Stevens, M.,Kattenbeldt, J.A. and Gould, A.R. (2001). Quasispecies analysis of recent NDV isolates in Australia. Proc 3rd Virology in Australia Conf. Univ.Syd. Sept.26-28.

Turner, A.J. and Kovesdy, L. (1974). Studies on the epizootiology of infection with non-virulent Newcastle disease virus in broiler flocks in Victoria. *Aust.Vet.J.* **50:** 155-158.

Westbury, H.A. (1979). Newcastle disease virus – some properties of Australian strains. *Avian Dis.* **23:** 564-570.

Westbury, H.A. and Geering, W.A. (1977). Newcastle disease virus infection in Australia. *Bull.Off.int.Epiz.* **87:** 815-820.

ENVIRONMENTAL DETERMINANTS OF BROILER ASCITES SYNDROME

P.J. GROVES

Summary

Three studies looking at the influence of micro-environmental control in broiler sheds on the occurrence of the ascites syndrome are summarized. The importance of temperature control in the first two weeks of broiler life on later ascites is emphasized. It appears that the duration of early sub-optimal temperature exposure in the first two weeks predisposes susceptible birds to later clinical ascites. The possibility of the existence of two separate ascites syndromes, delineated by age and sex, is hypothesized, with different causations.

I. INTRODUCTION

The broiler ascites syndrome is a complex problem characterised by congestive right-sided heart failure as a result of pulmonary hypertension. It has been a significant problem in the Australian broiler industry since the late 1980's. In the main, the disease affects male broilers predominantly over four weeks of age. In an attempt to delineate the effects of different management factors on the occurrence of ascites ("key determinants"), an epidemiological study was undertaken (Groves, 1999). Several key determinants were studied but the focus of this paper is on micro-environmental control, in particular brooding management. Three studies are summarised here.

II. METHODS

At the time these studies were undertaken, environmental control in most broiler sheds was achieved using natural ventilation and the supplemental heat sources for brooding were gas-fired hover style radiant brooders. The general effect from these brooders was to create a "hot spot" under the brooder and a wider circle where temperature declines with distance from the centre. The chicks were usually confined in this area for the brooding period, depending upon individual management. The standard target temperatures at day old were 31-32^{o}C, decreasing by one degree every second day until 21^{o}C was reached by 21 days. Hot air brooding systems, using space heaters to heat the entire brooding area to a more or less constant temperature were becoming more popular at the time. It was also common practice to provide artificial light such that the effective scotoperiod was only one hour per day, to maximize time for feed consumption.

(a) Field case study

Five broiler sheds growing HiChick meat strain broilers were selected throughout a winter period (chicks placed May through July) and were closely monitored during grow out. The selected sheds represented different areas of Sydney and different qualities of housing. Differences in roof insulation was a major factor, varying from none to full fibreglass/foil. All sheds in this study employed a 23 hour photoperiod throughout broiler grow out. A continuously recording drum thermograph (Seikosha^{TM}) was placed in each shed at bird height, approximately 2 metres from a brooder. Temperature records were analysed by

Baiada Poultry Pty Limited, Pendle Hill NSW 2145.

determining the number of hours that the chosen location in each shed was below 13^{o}, 15^{o}, 17^{o} or 19^{o} C on each day. Mortality was recorded daily. All birds dying on the day of a weekly visit by the author were necropsied. The necropsy results were used to estimate weekly losses due to the ascites syndrome by extrapolation from the total weekly figures (assuming relative loss rates were similar for the 3 days preceding and following each visit).

(b) Field observation study

Twenty-five broiler sheds, all housing HiChick meat broilers, were monitored over a winter period (chicks placed July through August). Sheds were visited weekly by the author and all mortalities for the day were necropsied, post mortem findings and sex being recorded. Daily mortalities were recorded by the farmer. A maximum-minimum thermometer was positioned at bird height 2 metres from a brooder in each shed and daily readings were recorded. Changes in shed environment, including brooding area size adjustments and lighting programs were recorded. The farmer weighed a sample of birds in each shed every 7 days as part of normal bird management procedures. All sheds in this study used hover brooders (spot brooding) as a heat source for the chicks. The approximate altitude of each farm was obtained from New South Wales Department of Lands.

(c) Brooding experiment

An experiment was conducted to evaluate the association between brooding method (hot air versus spot), and the incidence of the ascites syndrome in broiler chickens.

One thousand, one hundred and twenty male and 1120 female day old Cobb 500 broiler chicks were obtained from a commercial hatchery in early October. The chicks were instrument sexed and vaccinated for infectious bronchitis (Webster Vic-S strain IB vaccine) by coarse aerosol at the hatchery and then transported to a floorpen facility. The chicks were randomly placed in 28 pens at 80 birds of one sex per pen so that 14 pens in each end of the shed contained 7 pens of each sex (randomised block design). A plastic curtain was erected from floor to ceiling across the shed, separating each group of 14 pens.

One end of the shed was brooded using a small radiant gas brooder (SBM 1ZNRF) within each pen plus a large hover brooder suspended in the middle of the section to provide some space heating. This section was defined as “Spot Brooded” and mimicked the common brooding form used by the industry in this geographic location. In the other end of the shed, a gas-fired fan-assisted space heater (Jetfire^{TM} 72A) was installed and used as the only source of heat for this section. This was defined as “Hot Air Brooded”.

Brooders in both sections were controlled by thermostats. The brooders were left operational from day 0 through day 30 in both sections of the shed. This was longer than commercially practiced but the trial shed contained much empty space that would have been occupied by chickens in a commercially run shed. Hence, there would not have been the body heat production from the available biomass to maintain shed temperature similar to commercial conditions.

A continuously recording thermograph (Seikosha^{TM}) was placed in each section of the shed and maximum-minimum thermometers were also placed on the edge of certain pens in similar positions in each section. The latter were recorded daily as were mortalities, and all dead birds were necropsied.

III. RESULTS

(a) Field case study

Temperature records were analysed by determining the number of hours spent below 19ºC for the first three weeks and below 15ºC for the following weeks in each shed. Pearson correlation coefficients (**r**) were calculated for each week's estimated ascites losses and total hours that the temperature was recorded below the specified point (Table 1). There were strong correlations between period spent below a specified temperature in the early weeks and later ascites losses.

Table 1. Pearson correlation coefficients between ascites losses in given periods with time spent during the same or earlier periods at temperatures below specified levels. (field case study).

Period of exposure to low temps.	Spec. temp. (^{0} C)	Period of ascites measure						
		Days 1-7	Days 8-14	Days 15-21	Days 22-28	Days 29-35	Days 36-42	Days 0-42
Days								
1-7	19	-0.42	-0.03	0.58	**0.95**	**0.97**	0.71	**0.93**
8-14	19		-0.20	0.65	**0.99**	**0.89**	0.83	**0.98**
15-21	19			0.13	0.47	0.05	**0.81**	0.49
22-28	15				**0.91**	0.68	**0.98**	**0.91**
29-35	15					0.50	0.59	0.51
36-42	15						-0.09	-0.08
1-42	15							**0.97**

Correlation coefficients in bold type are significant (P<0.05)

(b) Field observation study

Over the first 21 days, 58% of birds dying from ascites were female, whereas after this age, 72% of ascites cases were male.

Each factor considered was initially analysed using a univariate analysis (correlation analysis between each factor and ascites attack rates over different age periods – data not shown). "Attack rate" is defined as the proportion of a population affected by a disease during a prescribed, short period (Blood and Studdert, 1999). Altitude varied only between 14 and 168 m above sea level and gave no meaningful correlation with ascites rates. Neither did cumulative weight density (progressive kg liveweight per m^2). There were moderate positive correlations between cumulative photoperiod over the first 28 days and attack rates of ascites after 29 days. Higher bird weights at 14 and 21 days of age were moderately positively correlated with ascites attack rates over most ages. Higher number of chicks placed per brooder was negatively correlated with ascites rates but this factor was also strongly negatively correlated with bird weights at 14 and 21 days, which may have confounded the former observation. Average weekly minimum shed temperatures measured 2m from the brooder in the first 3 weeks of life and between 36 and 42 days were moderately negatively correlated with ascites attack rates over the same periods (Table 2).

Table 2. Correlation between weekly minimum temperatures and ascites attack rates across subsequent time intervals.

Period of temp. Measure	Pearson correlation coefficients Period of ascites attack rate measure Days15-21	Days 21-28	Days 29-35	Days 36-42	Days 1-42
Days 1-7	-0.04	**-0.55**	-0.33	**-0.43**	**-0.42**
Days 8-14	-0.11	**-0.50**	-0.17	-0.19	-0.24
Days 15-21	-0.16	**-0.44**	-0.17	-0.19	-0.24
Days 22-28		-0.27	-0.22	-0.32	-0.31
Days 29-35			0.04	0.07	0.04
Days 36-42				**-0.42**	**-0.44**

Correlation coefficients in bold type are significant (P<0.05)

Interactions were also assessed and a forward stepwise multiple linear regression analysis undertaken to identify the most important contributors to variation in ascites attack rate between days 1 to 42. The results of this analysis are shown in Table 3.

Table 3. Multiple forward stepwise linear regression analysis of the field study of 25 broiler flocks.

Dependent Variable: Ascites Attack Rate days 1-42 Independent Variables	β	Standard error of coefficient	R squared	P
Intercept				0.02
Interaction: Minimum average temperature days 1-7 and chicks per brooder	-0.42	0.22	0.36	0.07
Interaction: Light hours days 1-28 and weight at 14 days	0.23	0.22	0.36	0.31

Regression values: r = 0.58, r^2 = 0.34, adjusted r^2 = 0.28, F (2,22) = 5.62, P < 0.01, Std error of estimate= 7.41

This model suggested that 28% of the variation in overall ascites attack rate could be explained by interactions between minimum shed temperatures in the first 7 days of life with number of chicks placed per hover brooder and the interaction between early photoperiod and 14d liveweight.

(c) Brooding experiment

Minimum temperatures using hot air brooding were generally well maintained near target while those achieved by the spot brooding technique fell well below target. Mortality due to ascites is shown in Table 4.

Table 4. Mortality from ascites syndrome in the floor-pen brooding study

		Number of birds with ascites			Ascites %
Brooding method	Sex	Days 0-22	Days 23-43	Days 0-43	Days 0-43[1]
Hot Air	Male	0	27	27	4.82[a]
Spot	Male	5	40	45	8.04[b]
Hot Air	Female	5	11	16	2.85[s]
Spot	Female	6	26	32	5.71[t]
Main Effects					
Brooding	Hot air	5	38	43	3.84[x]
	Spot	11	66	77	6.88[y]
Sex	Male	5	67	72	6.43[p]
	Female	11	37	48	4.28[q]

[1] means with different superscripts within effects differ significantly ($P < 0.05$)

Mortality from ascites over the first 22 days was minimal but was higher in the spot brooded than hot air group. Ascites incidence over this early period was higher in females and there was no effect of brooding method on ascites losses in females over this period. After 23 days, male losses from ascites greatly exceeded that in females and hot air brooding methods proved to be protective in both sexes.

IV. DISCUSSION

The studies described here highlight the importance of temperature in the pathogenesis of the ascites syndrome. The findings from case and field studies indicated that early temperature control was extremely important in subsequent ascites incidence. Moreover it was apparent that it was more the length of time that temperatures were sub-optimal rather than the minimum temperature that was reached that was important. Correlations indicate that exposure to sub-optimal temperatures may lead to ascites mortalities about two weeks later. Sub-optimal temperatures after week three appear to be less critical. It appears that prolonged sub-optimal temperatures in the first two weeks of life may predispose susceptible birds to ascites development and subsequent exposure to periods of sub-optimal temperatures in week four or later may promote the clinical expression of the syndrome in these predisposed birds. High early growth rates also contribute to a higher incidence of ascites. The manipulation of the latter factor by increasing the scotoperiod, in most cases to a level similar to natural day length, has provided a useful tool in the management of this syndrome.

Differences in sex-specific ascites rates at different ages were demonstrated (Table 4), with females contributing to the majority of cases when birds were less than three weeks of age (11 cf. 5) while males predominated thereafter (67 cf. 37). It is also noteworthy that space heating failed to protect against this early loss in females. This sex difference with age has been consistently found across a number of the studies conducted by the author (Groves, 1999). Traditionally, males have been reported as more susceptible (Hoerr, 1988; Wideman, 1988; Hargis and Odom, 1990; Maxwell, 1990; Matthiu, 1991) and the findings here support this. However there is no mention of earlier cases being mostly female in the literature, probably because losses at this age are usually quite low. Reasons for our observation are

unclear but it does suggest a difference in pathogenesis of the syndrome at different ages. Wideman (1988) commented that "cull" chicks (weak chicks in their first week) had a much higher incidence of ascites in Mexico and Coleman (1991) claimed that ascites could be modulated by manipulating hatchery management. It is also documented that aspergillosis induces ascites (Julian, 1990) and flocks we have seen with this pattern tended to have higher losses in females (60%) in the first two to three weeks. Thus the early, predominantly female ascites cases may be due to poor chick quality, hatchery influences, early respiratory infection or other factors, leading to losses of mainly poorly grown "cull" type chicks in the first three weeks. This aetiology may be quite different to the more commonly recognized male-predominant ascites cases in well-grown birds generally after 28 days of age. The latter is more likely to be a metabolic syndrome related to conditions conducive to higher oxygen requirements, such as sub-optimal temperature and rapid growth rate.

REFERENCES

Blood, D.C and Studdert, V.P. (1999). Saunders Comprehensive Veterinary Dictionary, 2nd Ed. WB Saunders, London.

Coleman, M.A. (1991). *Misset-World Poultry*, **7**:33-35.

Groves, P.J. (1999). The ascites syndrome in broiler chickens: identification of epidemiological risk factors associated with the syndrome and examination of their nature. PhD Thesis, University of Sydney.

Hargis, B.M. and Odom, T.W. (1990). *Zootechnia International*, February, pp 60-61

Hoerr, F.J. (1988). *Zootechnia International*, September, pp 60-61.

Mathiu, J.K. (1991). Studies on the ascites syndrome of broiler chickens. Master's Thesis, University of Sydney, Sydney.

Maxwell, M.H. (1990). *Poultry International*, Feb. pp.32-38.

Julian, R.J. (1990). *Feedstuffs*, January 29. pp19-22, 78.

Wideman, R.F., (1988). *Monsanto Nutrition Update*, Vol. 6, No2, August.

USE OF OILSEED MEALS IN BROILER DIETS: EFFECTS OF FEED ENZYMES

A. KOCHER

Summary

The removal of animal protein sources from poultry diets combined with increased production of oilseeds for human consumption has placed greater emphasis on the use of oilseed meals in broiler diets. This paper reviews the nutritive value of oilseed meals, such as soyabean meal, canola meal and sunflower meal, and presents data on the efficacy of feed enzymes to enhance their nutritive value. In particular, the effects of feed enzymes on the utilisation of non-starch polysaccharides are discussed.

I. INTRODUCTION

Oilseeds are grown primarily for the production of vegetable oil for the human food and animal feed industries. The shift from animal protein and fat sources in human nutrition to vegetable products saw a substantial increase in the production of the major oilseed commodities like soyabean (154 million tonnes in 1999), canola (42 million tonnes) and sunflower (28 million tonnes) (FAOSTAT, 2001). Subsequently large amounts of oilseed meals (OSM), as by-products from the oil extraction process, are available for use in animal feed.

Oilseed meals are already widely used as the main protein source in pig and poultry diets. The recent ban in Europe on the inclusion of feed ingredients of animal origin in feed for monogastric animals has placed even greater emphasis on the inclusion of vegetable proteins. The composition and nutritive value of OSM for animal production depends not only on the cultivar and growing conditions but also heavily on the processing conditions. In addition, OSM contain a variety of anti-nutritive factors (ANF), which interfere with digestion and absorption of nutrients or have toxic properties (Warenham *et al.*, 1994).

This review will discuss the nutritive value of OSM for broiler diets, the effects of ANF and possible solutions to enhance the nutritive value of OSM. In particular, the effects of supplemental carbohydrases in broiler diets containing high levels of OSM will be discussed.

II. NUTRITIVE VALUE OF OILSEED MEALS IN POULTRY DIETS

The chemical composition of OSM and their inclusion in broiler diets at elevated levels have been the subject of various studies (Dale, 1996, Thorpe and Beal, 2000). The average crude protein content of OSM varies from 32% in sunflower meal (SFM) to over 48% in some soybean meals (SBM). In contrast to cereal grains, OSM are generally rich in lysine but have low levels of sulphur-containing amino acids (AA), methionine and cystine. One notable exemption is SFM, which is comparatively rich in methionine but contains only moderate levels of lysine (van Kempen and Jansman, 1994).

The energy content of OSM depends largely on the degree of oil extraction. The preferred method is a pre-press solvent extraction process, which involves mechanical oil removal with a screw press as well as solvent extraction with hexane. The oil content in pre-pressed solvent extracted meal is less than 1%. However, other extraction methods, such as mechanical screw press extraction or continuous solvent extraction, leave between 3-8% of the oil in the meal (Senkoylo and Dale, 1999). Although there are no strict guidelines concerning the amount of residual oil, the oilseed industry has published its recommendations

on the actual oil contents of meals to guarantee a standard energy content (Bell and Hickling, 1997; Swick, 1998).

III. ANTI-NUTRITIVE FACTORS IN OILSEED MEALS

Oilseeds and subsequently OSM have specific ANF depending on the species. Soyabeans are particular high in trypsin and chymotrypsin inhibitors, which interfere with the digestibility of dietary protein. In addition soyabeans have high levels of lectins, which have the ability to bind to the epithelial cells lining the small intestine, leading to interference with nutrient absorption (Liener, 1989). Canola contains tannins, which forms complexes with dietary protein and carbohydrates as well as inhibits the activity of various digestive enzymes and, to a lesser extent, glucosinolates a precursor for toxic breakdown products produced by myrosinase activity (Warenham *et al.*, 1994). On the other hand, sunflower seeds are not known to have any specific ANF (Senkoylo and Dale, 1999). Common ANF in all OSM are phytates (Thorpe and Beal, 2000). Phytate is the major storage form of phosphorus in plants. The lack of endogenous phytases makes phytate phosphorus unavailable to monogastric animals, consequently inorganic phosphorus has to be added to broiler diets. Unavailable excess phosphorus is simply excreted and results in a serious phosphorus pollution problem. Furthermore, phytate has also the potential to form indigestible complexes with cations (Mg, Ca, Fe) and bind with protein (Reddy *et al.*, 1982), which leads to a reduced availability of these nutrients.

Perhaps the most important component that influences the nutritive value of OSM are indigestible carbohydrates. Oilseed meals are particular rich in galacto-oligosaccharides and pectic polysaccharides. Although there is no clear evidence on the nutritive or anti-nutritive value of these oligosaccharides (OS) and non-starch polysaccharides (NSP), they are generally considered being ANF. It is well known that OS and NSP cannot be hydrolysed by pancreatic enzymes of the chicken in the small intestine, and subsequently will undergo microbial fermentation in the large intestine and caeca. Bacterial degradation of α-galactosides in the hindgut can lead to increased fluid retention, increased hydrogen production, and can impair the utilisation of nutrients (Saini, 1989). Similar, it is known that increased levels of soluble NSP will increase fermentation in the upper gut which is detrimental to the overall performance as well as to the bird's health (Choct *et al.*, 1996). Bacterial fermentation of these carbohydrates is highly effective in poultry and although the energy contribution via the end-products of bacterial fermentation, mainly lactose and volatile fatty acids, is less effective that the direct absorption of glucose, these products can provide some energy to the chickens.

IV. REDUCTION OR REMOVAL OF ANTI-NUTRITIVE FACTORS IN OILSEED MEALS

The feed value of OSM can be greatly enhanced if ANF are eliminated or their contents reduced. Several methods have been described in the literature to reduce these ANF.

a) Physical and chemical processing

Dehulling: Quality and the nutritive value of OSM are influenced by several variables during the oil extraction procedure. A simple and very effective method to reduce the concentration of NSP in vegetable proteins is the removal of the seed coat. Mechanical removal of hulls will therefore directly decrease the level of NSP and increase the level of protein and energy in the dehulled product. It was estimated that the difference between dehulled and non-

dehulled SBM is between 0.7-0.9 MJ/kg (Swick, 1998). Similarly, dehulling of canola prior to oil extraction will result in improved ME levels, considering that the hull represents about 16% of the seed weight and about 30% of the meal weight, respectively (Bell, 1993).

Heat treatment: Commercial heat processing is widely used to inactivate heat labile ANF like trypsin inhibitors and lectins. The effect of heat treatment on the overall nutritive value depends on the combination of processing temperature and heating time. The precise amount of toasting after oil extraction with solvent is of utmost importance where heat labile ANF have to be deactivated without lowering the protein availability. Excessive heating of meal will reduce the nutritive value, by decreasing the digestibility of amino acids, in particular lysine (Anderson-Haferman *et al.*, 1993; Parsons *et al.*, 1992; Zhang and Parsons, 1994).

Chemical treatment: Chemical treatments can be used to extract specific fractions of seeds. Ethanol extraction has been successfully used to remove 97% of the OS from SBM (Coon *et al.*, 1990) or 96% from canola meal (CM) (Slominski *et al.*, 1994). The removal of OS from SBM using ethanol extraction increased nitrogen corrected true metabolisable energy (TME_N) (Coon *et al.* 1990; Leske *et al.*, 1991; Leske *et al.* 1993). These authors acknowledged the high digestibility of OS and concluded that the increased microbial fermentation of OS led to a reduction in transit time and increased acidity of the caeca, resulting in a significant decrease of true dry-matter (DM) digestibility. The removal of OS therefore resulted in increased true DM digestibility and in a greater caecal digestion of other carbohydrates, mainly cellulose. The hypothesis that the removal of OS enhances fibre digestion was confirmed by Slominski *et al.* (1994), who found the removal of OS from CM did improve NSP digestibility.

b) Use of exogenous enzymes: Phytase

The best example of the successful elimination of ANF through the addition of exogenous enzymes is the successful development of feed phytases to hydrolyse plant phytate. There are many reviews published discussing the efficacy of phytase in animal feed (Bedford, 2000, Beudeker, 1996; Touchburn *et al.*, 1999). Most of the research is focused on improvements in growth performance or amino acid digestibility when phytase was added to corn/SBM based diets (Biehl and Baker, 1997; Sebastian *et al.*, 1997; van der Klis *et al.*, 1997). The benefits of phytase addition to diets based on other OSM, however is less clear. Whereas the addition of phytase to semipurified CM based diets had a tendency to improve broiler performance (Simbaya *et al.*, 1996), no such benefits were seen in diets based on peanut meal (Biehl and Baker, 1997). It was suggested that the variation in response might be related to the actual storage sites of phytate in the meal (Bedford, 2000). Differences in the susceptibility of native phytate to exogenous phytase in SBM where phytate is associated with the protein bodies compared to peanuts where it is concentrated in the crystalloids may explain such differences.

c) Use of exogenous enzymes: Galactosidases

Studies *in vitro* showed that fungal α-galactosidase could be successfully used to hydrolyse galacto-oligosaccharides of SBM and CM (Angel *et al.*, 1988; Slominski *et al.*, 1992). These findings were confirmed *in vivo* with caecectomised laying hens when it was shown that the addition of this enzyme to CM was highly effective in hydrolysing raffinose and stachyose in the gastrointestinal tract of chickens (Slominski *et al.*, 1994). However, to date there is no evidence in the literature that under practical conditions the addition of α-

galactosidase actually improves performance or energy utilisation in broilers fed diets with SBM (Angel *et al.*, 1988; Irish *et al.*, 1995) or CM (Slominski, 1994).

d) Use of exogenous enzymes: Glycanases

The benefits of glycanases, such as xylanase and β-glucanase in broiler diets based on cereal gains are well documented. More recently a new generation of feed enzymes including polygalacturonase (pectinase) has been developed to target pectic NSP in OSM. Evaluation of 9 commercial carbohydrases recommended for OSM *in vitro* showed overall low activities (Simbaya *et al.*, 1996). Even more, the optimum pH level of these enzymes is at pH 5.3 rather than pH 7.0 as found in the small intestine. The authors of this study seriously questioned the effectiveness of such enzyme products *in vivo*. Nevertheless a number of studies have reported positive effects of carbohydrases on the NSP fraction of OSM in diets fed to broilers. The enzymes used in these studies were multi-activity glycanases targeting pectic polysaccharides as well as neutral NSP.

Soyabean meal: Marsman *et al.* (1997) and Zanella *et al.* (1999) demonstrated that the addition of commercial enzymes to a corn/SBM broiler diet significantly improved weight gain and feed conversion ratio (FCR). The increase in performance was related to an increase in ileal digestibility of crude protein (CP), starch and fat (Zanella *et al.*, 1999) as well as the improvement in ileal digestibility of NSP (Marsman *et al.*, 1997). The improvement of ileal NSP and protein digestibility could be a direct result of the partial depolymerisation of NSP in SBM. The disruption of the cell wall matrix led to the release of entrapped protein and to easier access of endogenous proteolytic enzymes (Bedford, 1996). Furthermore, studies *in vitro* revealed that the added enzyme products not only had cell wall degrading activities but also exhibited protease activity, which most likely also contributed to the improvement in nutrient digestibility. Our recent study showed that the effects of enzyme addition not only depended on the enzyme product itself but also on the inclusion level (Kocher *et al.*, 2001). The inclusion of a multi activity commercial enzyme product with hemicellulase, pectinase and cellulase activities at the supplier's recommended dosage (400ppm) had no effect on FCR, AME, CP and NSP digestibility. However, when the same enzyme was added at five times the recommended dosage level (2000ppm) a significant improvement in AME_N, protein and NSP digestibility was observed (Table 1). Analysis of NSP content in ileal digesta revealed a significant increase in free sugars correlated to a significant decrease in insoluble NSP, indicating a partial depolymerisation of the main NSP of SBM by the enzyme. In the same study the effects of an experimental product with mainly galactanase activities was tested. Addition of the enzyme at both low and high levels resulted in a significant increase in AME_N. Analysis of monosaccharides in the jejunum and ileum clearly showed a significant reduction in soluble and insoluble galactose with a corresponding increase in galactose in the free sugar fraction. The increase in the concentration of volatile fatty acids (VFA) in the caeca would suggest that this enzyme preparation effectively depolymerised part of the arabinogalactan in the ileum, making an increased amount of smaller NSP molecules available to the caecal microflora. Although the energy contribution via caecal fermentation is less effective than absorption of glucose in the upper intestine, VFA as the end product of bacterial fermentation can provide some energy to the birds (Carré *et al.*, 1995), a fact which can be seen in the increased AME_N. In contrast, Irish and Balnave (1993) found, that the addition of two multi-activity enzyme preparations to corn/wheat-SBM diets resulted in a significantly poorer growth compared to an unsupplemented control diet. These authors concluded that NSP were broken down into smaller fragments, which resulted in increased fluid retention in the small intestine and adversely affected the absorption of nutrients.

Canola meal: Leeson *et al.* (1987) showed that CM could replace up to 100% of dietary SBM. However the reduced level of crude protein as well as the increased levels of indigestible carbohydrates make CM a less competitive alternative when used at high levels in broiler diets. Slominski and Campbell (1990) and Simbaya *et al.* (1996) showed the potential of exogenous enzymes high in polygalacturonase to enhance the digestion of the NSP of CM *in vitro*, by rapidly hydrolysing the soluble NSP fraction followed by a slow degradation of the insoluble NSP fraction. These finding could be confirmed when the same enzyme was added to a laying hen diet with 40% CM resulting in a significant increase in the NSP digestibility in the ileum (Slominski and Campbell, 1990). Similarly, we found a reduction in soluble and insoluble NSP in the jejunum and a tendency towards improved FCR when adding the same glycanase (Kocher *et al.*, 2000). It was suggested that the enzyme depolymerised soluble NSP in the upper intestine and possibly disrupted the cell-wall matrix leading to easy access of endogenous proteolytic enzymes to digest entrapped proteins. A second study (Kocher *et al.*, 2001) showed that the inclusion of CM with or without enzymes in place of SBM had no effect on broiler performance. However, birds fed the CM control diet had significantly reduced eviscerated weight and breast meat as well as increased drip loss compared to the SBM based control diet (Table 2). These effects could be overcome when a multi-activity enzyme preparation with pectinase activities was added to the diet.

Table 1. Nitrogen corrected AME (AME_N), ileal protein digestibility and VFA concentration in the caeca of broiler chickens fed on diets containing soyabean meal with or without enzyme supplementation

	AME_N MJ/kg DM	Ileal protein digestibility	Caecal VFA concentration mMol/bird
Diet			
Control	12.84 [b]	0.82 [b]	568 [b]
[1]Enzyme A normal	12.89 [b]	0.80 [bc]	522 [b]
Enzyme A (5x)	13.04 [a]	0.86 [a]	575 [b]
[2]Enzyme B normal	13.05 [a]	0.81 [bc]	754 [a]
Enzyme B (5x)	13.07 [a]	0.81 [c]	744 [a]
SEM	0.05	0.008	
Probability of greater *F* value in analysis of variance [3]			
Diet	***	***	**

[1] Enzyme A: commercial multi-activity glycanase
[2] Enzyme B: experimental product with mainly galactanase activities
[a,b,c,] Values with unlike superscripts differ significantly ($P<0.05$)
[3] ** $P<0.01$, *** $P<0.001$

Sunflower meal (SFM): Non-starch polysaccharides of SFM are highly susceptible to enzymatic degradation under controlled condition *in vitro* (Düsterhöft *et al.*, 1993). However, reports in the literature failed to show any improvements in broiler performance or AME when commercially available multi activity enzymes were included in broiler diets (Rebole *et al.*, 1999; Sherif *et al.*, 1997). In our study (Kocher *et al.*, 2000), we demonstrated that the addition of enzyme preparations based on pectinase and hemicellulase to broiler diets significantly improved the digestibility of NSP in the jejunum and the digestibility of protein in the ileum. The lack of response in growth performance and AME in this study was

explained by the fact that birds fed SFM regardless of enzyme addition were close to their genetic growth potential.

Table 2. Eviscerated weight, breast meat yield and drip loss of selected broiler chickens fed soyabean meal diets and canola meal diets supplemented with enzymes measured over 38 days (mean ± standard deviation)

Diet	Eviscerated weight (g/bird)	Breast yield (g/bird)	Drip loss %
SBM control	1493.6 ± 125.6[a]	381.8 ± 46.4[a]	1.44 ± 0.6[c]
CM control	1423.1 ± 121.1[b]	345.0 ± 42.4[b]	2.23 ± 1.1[a]
CM+Enzyme A	1428.3 ± 131.5[b]	367.1 ± 47.6[a]	1.91 ± 0.7[ab]
CM+Enzyme B	1481.5 ± 149.2[ab]	372.2 ± 43.2[a]	1.56 ± 1.0[bc]
Mean	1456.8	366.4	1.8
	Probability of greater *F* value in analysis of variance [3]		
Diet	*	**	**

[1] Enzyme A: commercial multi-activity glycanase (without pectinase)
[2] Enzyme B: commercial multi-activity glycanase (with pectinase)
[a,b,c,] Values with unlike superscripts differ significantly ($P<0.05$)
[3] * $P<0.05$, ** $P<0.01$

Non-conventional oilseeds: Beside the OSM discussed earlier there are a range of so-called non-conventional OSM for use as feed ingredients. However, there are only very limited data available on their nutritive value or the effects of feed enzymes on their NSP fraction. Palm kernel meal is a common ingredient in Asia. This meal contains up to 70% NSP, mainly insoluble linear mannans (Omar and Hamdan, 1998). Although it has been shown that *in vitro* 20-50% of these mannans can be hydrolysed depending on enzyme composition, with the monomer and dimer of mannose as major end-products (Düsterhöft *et al.*, 1993), there are no conclusive data available on the effect of such enzymes *in vivo*. On the other hand, Pluske *et al.* (1997) reported that the addition of a commercially available mannanase to diets including 20% copra meal improved weight gain of broilers measured over a 42d period.

e) Use of exogenous enzymes: Proteases and amylase

The classical approach to eliminate proteinaceous ANF, primarily trypsin inhibitors and lectins, is to use thermal processing. However, pre-treatment with protease has the potential to improve the availability and digestion of SBM protein (Caine *et al.*, 1998). It has also been shown that the addition of protease and amylase to diets containing SBM or CM can improve the utilization of protein and assist the endosperm starch digestion in the upper gastrointestinal tract of broilers (Simbaya *et al.*, 1996; Zanella *et al.*, 1999). These authors speculated that some of the positive effects of protease supplementation were related to improved protein hydrolysis in the crop where protein digestion is normally minimal. Furthermore there is some evidence that protease, amylase and pectinase have synergistic effects, which would imply that exogenous enzymes are more effective when used in combination rather than individually (Simbaya *et al.*, 1996).

V. CONCLUSIONS

The nutritive value of OSM varies widely depending on species, processing conditions and occurrence of anti-nutrients. This review has highlighted several avenues to improve the nutritive value of OSM in broiler diets. Elimination of heat labile protease inhibitors and reduction of phytate by adding microbial phytase increase the availability of nutrients. It is also evident that the inclusion of exogenous enzymes has some effects on the utilisation of protein and NSP in OSM. The depolymerisation of pectins by exogenous enzymes in the upper intestine will give the bird access to intracellular entrapped nutrients and provide a more efficient energy utilisation, in comparison to the microbial fermentation.

There is a need for future research to pursue the understanding of the complex structural changes of individual pectic polysaccharides of OSM that occur in the gastrointestinal tract of the chicken with or without enzymes. The precise chemical structure of each polymer and its quantity in the meal have to be elucidated before highly effective enzymes for use in diets containing high levels of OSM can be produced and utilised.

REFERENCES

Anderson-Haferman, J.C., Zhang, Y. and Parson, C.M. (1993). *Poultry Science,* **72:** 326-333.

Angel, C.R., Sell, J.L. and Zimmermann, D.R. (1988). *Journal of Agricultural and Food Chemistry,* **36:** 542-546.

Bedford, M.R. (2000). *Animal Feed Science and Technology,* **86:** 1-13.

Bedford, M.R. (1996). In: *Proc Finnfeeds International/PharmoChem Australia Seminar,* pp. 13-23.

Bell, J.M. (1993). *Canadian Journal of Animal Science,* **73:** 679-697.

Bell, J.M. and Hickling, D.R. (1997). http:/www.canola-council.org/meal/composit.mtm, 29/9/98.

Beudeker, R.F. (1996). *International Poultry Production,* **4:** 13-15.

Biehl, R.R. and Baker, D.H. (1997). *Poultry Science,* **76:** 355-360.

Caine, W.R., Verstegen, M.W.A., Sauer, W.C., Tamminga, S. and Schulze, H. (1998). *Animal Feed Science and Technology,* **71:** 177-183.

Carré, B., Gomez, J. and Chagneau, A.M. (1995). *British Poultry Science,* **36:** 611-629.

Choct, M., Hughes, R.J., Wang, J., Bedford, M., Morgan, A.J. and Annison, G. (1996). *British Poultry Science,* **37:** 609-621.

Coon, C.N., Leske, K.L., Akavanichan, O. and Cheng, T.K. (1990). *Poultry Science,* **69:** 787-793.

Dale, N. (1996). *Animal Feed Science and Technology,* **59:** 129-135.

Düsterhöft, M., Bonte, A.W. and Voragen, A.G.J. (1993). *Journal of the Science of Food and Agriculture,* **63:** 211-220.

FAOSTAT (2001). http://apps.fao.org/default.htm, 17/10/2001.

Irish, G.G. and Balnave, D. (1993). *Australian Journal of Agricultural Research,* **44:** 1483-1499.

Irish, G.G., Barbour, G.W., Classen, H.L., Tyler, R.T. and Bedford, M.R. (1995). *Poultry Science,* **74:** 1484-1494.

Kocher, A., Choct, M., Morrisroe, L. and Broz, J. (2001). *Australian Journal of Agricultural Research,* **54:** 447-452.

Kocher, A., Choct, M., Porter, M.D. and Broz, J. (2000). *Poultry Science,* **79:** 1767-1774.

Kocher, A., Choct, M., Porter, M.D. and Broz, J. (2001). *British Poultry Science,* **42:** 606-615.

Leeson, S., Atteh, J.O. and Summers, J.D. (1987). *Canadian Journal of Animal Science,* **67:** 151-158.

Leske, K.L., Akavanichan, O., Cheng, T.K. and Coon, C.N. (1991). *Poultry Science,* **70:** 892-895.

Leske, K.L., Jevne, C.J. and Coon, C.N. (1993). *Poultry Science,* **72:** 664-668.

Liener, I.E. (1989). In: *Proc Recent Advances of Research in Antinutritional Factors in Legume Seeds,* Eds. J. Huisman, A.F.B. van der Poel and J.E. Liener, pp. 6-13.

Marsman, G.J., Gruppen, H., van der Poel, A.F., Kwakkel, R.P., Verstegen, M.W. and Voragen, A.G. (1997). *Poultry Science,* **76:** 864-872.

Omar, M.A. and Hamdan, A.M. (1998). In: *Proc 8th World Conference for Animal Production, Pre-Conference Symposia,* pp. 306-312.

Parsons, C.M., Hashimoto, K., Wedekind, K.J., Han, Y. and Baker, D.H. (1992). *Poultry Science,* **71:** 133-140.

Pluske, J.R., Moughan, P.J., Thomas, D.V., Kumar, A. and Dingle, J. (1997). In: *Proc Alltech's 13th Annual Symposium,* Eds. T.P. Lyons and K.A. Jacques, pp. 81-93.

Rebole, A., Rodriguez, M.L., Alzueta, C., Ortiz, L.T. and Trevino, J. (1999). *Animal Feed Science and Technology*, **78:** 153-158.

Reddy, N.R., Sathe, S.K. and Salunkhe, D.K. (1982). *Advances in food research,* **28:** 1-91.

Saini, H.S. (1989). In: *Proc Recent Advances of Research in Antinutritional Factors in Legume Seeds,* Eds. J. Huisnan, T.F.B. van der Poel and I.E. Liener, pp. 329-341.

Sebastian, S., Touchburn, S.P., Chavez, E.R. and Lague, P.C. (1997). *Poultry Science,* **76:** 1760-1769.

Senkoylo, N. and Dale, N. (1999). *World's Poultry Science Journal,* **56:** 153-174.

Sherif, K.E., Gerendai, D. and Gippert, T. (1997). *Archiv für Geflügelkunde,* **61:** 8-14.

Simbaya, J., Slominski, B.A., Guenter, W., Morgan, A. and Campbell, L.D. (1996). *Animal Feed Science and Technology*, **61:** 219-234.

Slominski, B.A. (1994). *Journal of the Science of Food and Agriculture,* **65:** 323-330.

Slominski, B.A. and Campbell, L.D. (1990). *Journal of Agricultural and Food Chemistry,* **53:** 175-184.

Slominski, B.A., Campbell, L.D. and Guenter, W. (1994). *Poultry Science,* **73:** 156-162.

Slominski, B.A., Campbell, L.D. and Guenter, W. (1992). In: *Proc 19th World's Poultry Congress,* pp. 241-245.

Swick, R.A. (1998). http://www.pacweb.net.sg/asa/technical/an14-1998.html, 13/8/99.

Thorpe, J. and Beal, J.D. (2000). In: *Enzymes in farm animal nutrition.* Eds. M.R. Bedford and G.G. Partridge, CABI Publishing, Wallingford; UK, pp. 125-143.

Touchburn, S.P., Sebastian, S. and Chavez, E.R. (1999). In: *Recent Advances in Animal Nutrition,* Eds. P.C. Garnsworthy and J. Wiseman, University of Nottingham, Nottingham, pp. 147-164.

van der Klis, J.D., Versteegh, H.A.J., Simons, P.C.M., Kies, A.K. and Van der Klis, J.D. (1997). *Poultry Science,* **76:** 1535-1542.

van Kempen, G.J.M. and Jansman, A.J.M. (1994). In: *Recent Advances in Animal Nutrition.* Eds. P.C. Gransworthy and D.J.A. Cole, Nottingham University Press, Nottingham, pp. 31-56.

Warenham, C.N., Wiseman, J. and Cole, D.J.A. (1994). In: *Principles of Pig Science,* Eds. D.J.A. Cole, J. Wiseman and M.A. Varley, Nottingham Press, Nottingham, pp. 141-167.

Zanella, I., Sakomura, N.K., Silversides, F.G., Fiqueirdo, A. and Pack, M. (1999). *Poultry Science,* **78:** 561-568.

Zhang, Y. and Parsons, C.M. (1994). *Poultry Science,* **73:** 436-442.

BROILER BREEDER NUTRITION FROM PHOTOSTIMULATION THROUGH PEAK EGG PRODUCTION

M.S. LILBURN

Summary

The time interval between photostimulation (PS) and peak egg production represents a very challenging period with respect to feed allocations for broiler breeder hens. This age period represents both the terminal stage of pullet growth and the onset of sexual maturity and we need to accommodate the nutritional requirements for both developmental processes. The pullet-rearing program will, to a large extent, govern the relative allocation of nutrients to growth versus reproductive development during the first weeks of egg production. Differences in body weight and carcass composition at PS will significantly influence the onset of lay, the intensity of early production, and the feeding regimen required to optimise peak egg production.

I. INTRODUCTION

Over the last 20 years, selection for growth and meat yield in commercial broiler strains has reduced days to whole-bird market weight (2.0 kg) by approximately a week, and has also resulted in strains that can be grown to heavy body weights (> 3.0 kg) for maximal breast meat yield. Over this same time period, the genetic changes in growth and carcass development have been accomplished with no discernible reductions in either hatching egg numbers (165 eggs/hen) or chick numbers (140 – 145 chicks/hen). Egg production and hatch have been aided greatly by changes in management (i.e. dark out pullet rearing, separate sex feeding), but the fact remains that we are having success with completely different genetic products. One factor that has facilitated our overall management of breeder pullets is the practice of photostimulating hens at 21 to 22 weeks versus 18 to 19 weeks, which had been the norm. The later age allows for a more uniform body weight together with an increase in the relative proportions of carcass protein and lipid at PS. The end result is uniform sexual development after PS, resulting in earlier production and the potential for higher peak egg production.

II. EFFECT OF BODY WEIGHT

Renema *et al*. (1999a,b) conducted an interesting study in which they divided pullets into three body weight categories (Low, Standard and High) and continued to restrict feed (RF) the hens after photostimulation (21 weeks), or allowed them *ad libitum* access to feed. There was an effect of body weight at PS on carcass lipid ($P<0.05$), which closely followed the body weight differences (Table 1).

Table 1. Effects of body weight (g) on carcass composition (g/kg) at 21 weeks.

	Low	Std	High	Pooled SEM
Body weight	1639 [a]	1995 [b]	2394 [c]	27
Carcass protein	200	210	203	3.0
Carcass lipid	63 [a]	89 [b]	101 [b]	6.0

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

Department of Animal Sciences, OARDC, Wooster, OH 44691, USA

There were no effects of rearing treatment on either carcass protein or ash. Within the ad libitum treatment, there was an approximate 8-day spread (21.5 to 29.1 days) within body weight groups between PS and sexual maturity (SM=first egg). Within the RF treatment, however, the spread was 24 days (27 to 51 days). While this represents an exaggerated view of what can happen when underweight pullets are photostimulated, the negative correlation between underweight pullets and delayed SM is common in commercial production. It is interesting that at the onset of SM, there were significant differences between the ad libitum and RF treatments in breast muscle and abdominal fat percentage, but no effects due to pullet body weight. (Table 2). This same trend was observed for the distribution of large yellow follicles within the ovary (Renema et al., 1999 b).

Table 2. The effect of body weight at PS or feeding regime from PS to first egg on carcass composition (g/kg) and ovary development.

Treatment	Breast yield	Abdominal. fat yield	Large YF	Small YF
Feeding regime				
Ad libitum	146	36.8	11.0	12.6
Restricted	162	19.6	7.1	11.7
Probability	*P<.0001*	*P<.0001*	*P<.0001*	*P<.60*
Body weight				
Low	155	28.2	8.8	13.3
Standard	151	26.8	9.5	10.8
High	156	29.6	8.9	12.4
Probability	*P<.48*	*P<.44*	*P<.37*	*P<.44*

Large YF = large yellow follicles, >10 mm; Small YF = 5-10 mm
(Renema *et al*., 1999 a,b).

III. EFFECT OF INCREASE IN FEEDING RATE

In a recent study (Lilburn, unpublished data), hens from two commercial strains were reared and fed similarly through rearing and SM. From 25 weeks through peak egg production, however, feed allocations were increased at different rates resulting in a peak feed allocation (159 g/hen) at 27 weeks (Fast) versus 29 weeks (Slow). Fat deposition ($P<0.10$) and oviduct weight ($P<0.05$) were greater in the Fast treatment group and ovary weight was greater ($P<0.05$) in Strain 1 than in Strain 2 (Table 3).

Table 3. Effects of Fast versus Slow feed allocations on body, carcass and reproductive organ weights (g) in two strains of broiler breeder hens.

Strain	Treatment	Bodyweight	Pectoralis Major	Abdominal fat	Ovary	Oviduct
1		3120	171.5	96.3	43.0 ^{b}	47.4
2		3070	172.3	96.9	32.5 ^{a}	51.5
	Fast	3075	174.0	106.8 ^{q}	38.4	55.2 ^{b}
	Slow	3115	169.8	86.3 ^{p}	37.1	43.7 ^{a}
Pooled SEM		92	6.0	9.1	3.33	3.27

Means within effects within columns with different superscripts, are significantly different (^{a,b} $P<0.05$; ^{p,q} $P<0.10$).

There were no significant treatment differences in egg production or egg weight from 26 to 30 or from 30 to 34 weeks. The data suggest that conditions being equal with respect to body weight and carcass composition coming out of the rearing period, accelerated or aggressive feed adjustments early in the production cycle will only enhance excessive body weight gain and fat deposition without any beneficial effect on egg production.

REFERENCES

Renema, R.A., Robinson, F.E., Newcombe, M. and McKay, R.I. (1999a). *Poult. Sci.* **78:**619-628.

Renema, R.A., Robinson, F.E., Proudman, J.A., Newcombe, M. and. McKay, R.I (1999b). *Poult. Sci.* **78:**629-639.

SENSORY EVALUATION OF EGGS FROM HENS FED ON CANOLA MEAL AND COTTONSEED MEAL BASED DIETS

G.E. BELL[1], S.M. NOTTINGHAM[1] and R.A. PEREZ-MALDONADO[2]

Previously, fishy type odours and flavours have been detected in eggs predominately from brown-egg-producing hens given diets containing canola meal (CM). This paper reports on the incidence and level of several odours and flavours (including fishy) in eggs from both brown- and white-egg-producing hens given CM and cottonseed meal (CSM) diets. Eggs (twenty per treatment) were assessed from Hy-line brown and Hy-line W36 hens given diets containing 120 or 200 g/kg CM sourced from plants at Newcastle, Melbourne, Numurkah and Pinjarra. Eggs (nineteen per treatment) were also assessed from Hy-line brown and Hy-line W36 hens given diets containing 120 or 200 g/kg high- and low-protein CSM. In addition, control eggs from Hy-line brown and W36 hens that had no added CM or CSM in their diet were assessed.

For the assessment on raw eggs, three trained panellists assessed the level of fishy odour of one raw egg from each hen in all treatments (total of 550 eggs). For the assessment of cooked eggs, preliminary odour assessments on all raw eggs identified those hens that were likely to produce fishy tainted eggs and therefore could be combined, prior to cooking, without diluting the fishy taint. Only some brown-egg hens on the CM diets were identified as potential fishy taint egg producers. Subsequently, two separate sets of eggs from these hens were blended and cooked for assessment. For the remaining treatments (where no eggs had been identified as being fishy), all eggs from each treatment were split into two sets, blended and cooked for assessment. Twelve trained panellists assessed the cooked eggs (served at between 18 and 22 ^{o}C) for seven odour and six flavour attributes using a standard rating test (AS 2542.2.3, 1988).

In the raw eggs, the incidence of fishy odour found in brown CM eggs, ranged from 15% (Numurkah 120g/kg) to 40% (Numurkah 200g/kg) with an average of 28%. A fishy odour was also detected in some white CM eggs (range 10%-30%, mean 18%). No brown CSM eggs and only one white CSM egg was identified as having a fishy odour.

Analysis of variance on the mean sensory scores for the cooked egg assessment showed brown eggs from hens fed Melbourne CM 200g/kg, Newcastle CM 200g/kg and Numurkah CM 120g/kg had significantly higher ($P<0.05$) overall odour intensities than the brown control eggs. There was no significant ($P>0.05$) difference in overall odour intensity between the white treatment and control eggs. As expected, the brown eggs from CM fed hens had a significantly ($P<0.05$) higher level of prawny odour than brown and white control eggs and white eggs from CM fed hens. Even though a prawny odour was present, the levels of seafoody flavour detected in all treatments of cooked CM eggs were very low.

No significant differences ($P>0.05$) were found in any of the odour attributes between the control eggs and any of the CSM treatment eggs. The only flavour difference across all CSM and control eggs was found in the level of yolk flavour, where low protein CSM eggs had more yolk flavour than the control eggs.

In conclusion, the addition of CM, even at 120g/kg to the diet, will produce an increased incidence of fishy odour in raw eggs and an increased level of prawny and overall odour in cooked eggs, particularly in brown eggs. The addition of cottonseed meal to the diet is unlikely to affect the sensory properties (apart from yolk flavour) of brown or white eggs.

[1] Centre for Food Technology, Department of Primary Industries, Hamilton, Qld 4007.
[2] Queensland Poultry Research and Development Centre, Department of Primary Industries, Alexandria Hills, Qld 4161.

AGE DEPENDENT RESPONSES OF CHICKENS TO ENZYMES IN WHEAT AND BARLEY DIETS

R.R. CARTER[1] and R.J. HUGHES[2]

The development of the digestive system with age assumes higher nutrient digestibility coefficients for older animals. The relationship between age of chicken and enzyme supplementation on the digestibility of the dietary dry matter (DM) and grain apparent metabolisable energy (AME) value was examined over one week intervals.

The basal, cold-pelleted diets consisted of 800g/kg of wheat or barley, 152g/kg casein, 20g/kg dicalcium phosphate, 11g/kg limestone, 7g/kg DL-methionine, 5g/kg mineral-vitamin premix, 3g/kg salt and 2g/kg choline chloride. The xylanase/β-glucanase based enzyme (Kemzyme® W Dry) was included at 1kg/t of diet. Each treatment was fed to 3 cages of male chickens and 3 cages of female chickens in a randomised block design (5 birds/cage). A separate cohort of birds from the same flock was used at each age. The first 3 days of each week were adaptation periods followed by 4 day collection periods. The results are summarised in the table below with statistical comparisons to show the combined effects of age of chicken, grain and enzyme.

Age (days)	Grain	Feed intake (g/bird/day)		Grain AME (MJ/kg DM)		DM digestibility coefficient	
		Control	Enzyme	Control	Enzyme	Control	Enzyme
15-22	Wheat	71.2 ef	72.4 ef	15.4 b	15.7 a	0.768 a	0.773 a
	Barley	67.5 f	73.2 e	13.2 f	13.6 e	0.668 g	0.689 de
22-29	Wheat	95.2 c	90.3 cd	14.6 d	15.1 c	0.742 b	0.765 a
	Barley	87.2 d	94.4 c	13.1 f	13.6 e	0.676 fg	0.695 d
29-36	Wheat	108.5 b	114.6 a	14.4 d	15.2 bc	0.711 c	0.744 b
	Barley	104.5 b	109.0 b	12.8 g	13.5 e	0.654 h	0.681 ef
Pooled SEM		1.9		0.09		0.004	

Means with a common letter are not significantly different at the 5% level (P>0.05)

The effects of age and enzyme were significant for feed intake, grain AME, DM digestibility and excreta moisture (P<0.01). There was a significant interaction between the effects of age and grain on grain AME and DM digestibility (P<0.001). The AME and DM digestibility differences between wheat and barley were larger in the first week than the following weeks. There was also a significant interaction between the effects of age and enzyme on grain AME (P<0.01) and DM digestibility (P<0.001). The increase in grain AME and DM digestibility due to the enzyme became larger as the birds aged. The decrease in grain AME with age of chicken was contrary to expectation. There were large increases in hydrogen and methane contents of breath with age (data not shown) which suggest increased amounts of undigested feed escaping into the hindgut which is consistent with the reduction in dry matter digestibility with age.

Despite a decrease in grain AME with age, the increase in feed intake over this period ensures an increasing energy intake for the growing bird. The effects of the enzyme will also assist bird performance by arresting the decline in grain AME and DM digestibility.

[1]Kemin (Aust.) Pty. Ltd, 1.02/10 Edgeworth David Ave., Hornsby, NSW, 2077.
[2]SARDI, Pig and Poultry Production Institute, Adelaide University, Roseworthy, SA, 5371.

ACCREDITING BEAK TRIMMERS

P.C. GLATZ[1], M. BOURKE[2], J.L. BARNETT[3] and K.L. CRITCHLEY[4]

The State and Territory Agriculture Ministers recommended that beak trimmers in Australia be accredited. As a result the Egg Program of the Rural Industries Research and Development Corporation (RIRDC) provided funds for a project to develop Quality Assurance (QA) documentation and a training manual for beak trimmers working in the Australian egg industry. A project management committee comprising RIRDC representatives, beak trimmers, egg producers and researchers is directing the project. The management group has completed the QA documentation for all aspects of beak trimming and currently are overseeing the development of the training manual to address the following issues; 1) background to beak trimming, 2) biosecurity, 3) handling birds, 4) setting up beak trimming equipment, 5) trimming birds 6) assessing quality of beak trimming and 7) record keeping. The manual will be written in plain English and kept simple to meet the needs of those with low literacy skills. Case studies, exercises, photographs and graphics will enhance the learning process.

An accredited, competency-based beak trimming course will be designed to be delivered as a short course or as workplace training. Workplace training is flexible, cheap to deliver, minimises time spent off the job and can be customised to suit each enterprise. It is expected that workplace training will be the main delivery mechanism with experienced beak trimmers providing instruction and assessment. Experienced beak trimmers will be assessed to ensure that they meet required standards before they commence workplace training. It is suggested that they satisfactorily complete appropriate trainer/assessor qualifications before they can deliver training to or assess other beak trimmers.

Accredited training is nationally recognised and increases the opportunities for gaining funding for course delivery. Under this system the beak trimming course will be assessed through a Registered Training Organisation (RTO). In some cases the RTO may be a poultry company the trimmers work for, or it might be an educational institution. All RTOs throughout Australia submit training records to a central database that can be interrogated to provide industry with a profile of the training that has occurred.

Accreditation of beak trimmers is likely to lead to improved standards of beak trimming and bird welfare in the Australian egg industry. The accreditation process will ensure that minimum standards are achieved and best practice is promoted.

---

[1]Pig and Poultry Production Institute, South Australian Research and Development Institute, Roseworthy, South Australia, 5371.
[2]NSW Agriculture, Locked Bag 21, Orange, NSW, 2800.
[3]Animal Welfare Centre, Victorian Institute of Animal Science, Department of Natural Resources and Environment, 600 Sneydes Road, Werribee, Victoria, 3030.
[4] PIRSA, 33 Fleming St, Glenside, South Australia, 5065.

N-3 AND N-6 FATTY ACIDS: DIETARY LEVELS, GROWTH PERFORMANCE AND CARCASS COMPOSITION OF BROILERS

R.E. NEWMAN[1,2], S.J. WILKINSON[1], J.A. DOWNING[1,2] and W.L. BRYDEN[1,2]

In previous studies we have shown that feeding broilers diets containing 80g/kg of fish oil or sunflower oil reduced the abdominal fat pad mass when compared to feeding tallow (Newman *et al*., 1998). The dietary fatty acid concentration used in these studies may not have been optimal and in excess of the threshold required to reduce carcass fat content. In the present study, the growth performance and abdominal fat pad mass of broilers fed different levels of n-3 and n-6 fatty acids were assessed.

Day-old broiler chickens were randomly divided into groups and allocated to one of 9 experimental diets (6 birds/pen and 9 replicates/treatment). The chicks were reared in a brooder and fed experimental starter diets for 18 days and then transferred to grower cages and fed experimental finisher diets for a further 24 days. Diets were based on wheat and sorghum but differed in the percentage and type of dietary fat. To accommodate for the differing levels of fat, the quantity of the dietary ingredients were adjusted so that each diet gave a similar calculated protein and ME value. Fish oil, sunflower oil and tallow were added (25, 50 or 75 g/kg) giving diets enriched in n-3, n-6 or saturated fatty acids respectively. The birds were fed *ad libitum* throughout the experiment and their feed intakes and body weights measured weekly. At day 42 of the experiment, 12 birds from each treatment group were selected close to the mean body weight of all experimental treatments, slaughtered and their abdominal fat pads removed and weighed. Statistical examination of treatment effects was determined by ANOVA and Tukey-Kramer multi comparison test.

Treatment	Body weight (g/bird)	Feed intake (g/bird)	FCR	Fat pad (g/kg body wt)
25 g/kg Tallow	2265 ± 47^{bc}	3285 ± 157^{a}	1.74 ± 0.04^{a}	14.6 ± 0.9^{a}
25 g/kg Sunflower Oil	2202 ± 30^{c}	3047 ± 91^{bc}	1.71 ± 0.01^{ab}	11.9 ± 0.9^{ab}
25 g/kg Fish Oil	2205 ± 25^{c}	3041 ± 81^{c}	1.72 ± 0.01^{ab}	14.4 ± 1.2^{ab}
50 g/kg Tallow	2240 ± 20^{bc}	3097 ± 74^{abc}	1.64 ± 0.02^{abc}	13.9 ± 0.9^{ab}
50 g/kg Sunflower Oil	2352 ± 32^{abc}	3130 ± 95^{abc}	1.57 ± 0.03^{c}	11.6 ± 1.1^{ab}
50 g/kg Fish Oil	2298 ± 55^{abc}	3037 ± 106^{c}	1.66 ± 0.01^{abc}	11.6 ± 0.8^{ab}
75 g/kg Tallow	2450 ± 50^{a}	3271 ± 80^{ab}	1.61 ± 0.03^{abc}	14.0 ± 0.5^{ab}
75 g/kg Sunflower Oil	2400 ± 33^{ab}	3040 ± 45^{c}	1.55 ± 0.02^{c}	11.9 ± 0.9^{ab}
75 g/kg Fish Oil	2310 ± 25^{abc}	3099 ± 86^{abc}	1.60 ± 0.04^{b}	10.4 ± 0.7^{b}

Means (± SEM) in a column without a common superscript are significantly different $P<0.05$

Both body weight and feed efficiency showed a general improvement with increased inclusion of oil in the diet, with highest feed efficiency on the 50 and 75 g/kg sunflower oil diets. Unlike tallow or sunflower oil, however, increased levels of fish oil resulted in a linear decrease in abdominal fat pad proportion. Abdominal fat pad proportion was lower in chickens fed fish and sunflower oil than in those fed tallow, but only significantly for birds fed 75 g/kg of fish oil. The data suggests that fat deposition is dependent on both fatty acid sub-type and dietary concentration.

Newman, R.E., Downing, J.A., Bryden, W.L., Fleck, E., Buttemer, W.A. and Storlien, L.H. (1998). *Proc. Nutr. Soc. Aust.* **22**: 54.

[1]Faculty of Veterinary Science, The University of Sydney, Camden NSW 2570.
[2]Affiliated with the Smart Foods Centre, University of Wollongong, Wollongong NSW 2522.

EFFECT OF DIET COMPOSITION AND FEED FORM ON THE BEHAVIOUR OF ISA BROWN LAYING HENS

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

Summary

The effect of dietary fibre on the behaviour of laying hens was investigated by offering two diets, one with a lower fibre and one with a higher fibre concentration. Rice hulls were included at 100 g/kg of the high fibre diet and protein meal was about 180 g/kg of the low fibre diet. Each of the diets was offered in two forms, mash or pellets. Feeding, pecking, escape and freeze behaviours were altered by diet and feed-form and the interactions suggest that time spent feeding declines with increased energy concentration of the diet. The lowest incidence of feeding was observed with the low-fibre pelleted treatment. A lower incidence of feeding was paralleled by an increase in pecking and associated behaviours, although factors other than diet clearly contributed to some of the variation in social pecking. Pecking activity directed towards coloured objects did not appear to be affected by colour. These observations suggest that earlier reports of changes in cannibalism due to dietary manipulations are linked to reciprocal changes in the time that birds spend on feeding relative to their other daily activities.

I. INTRODUCTION

Increases in fibre levels in the diet have been reported to reduce mortality due to pecking and cannibalism (Esmail, 1997) and this was confirmed in one of our recent studies (Hartini *et al*., 2001) in which high-fibre diets significantly reduced cannibalism mortality. However, these studies did not identify mechanisms whereby added fibre may alter pecking behaviour. The use of a mash as an alternative to pelleted feed may also reduce the likelihood of an outbreak of cannibalism (Linberg and Nicol, 1994). The present experiment aimed to examine the effects of differences in fibre and metabolisable energy concentrations of diets, and feed form, on the behaviour of laying hens and to test the hypothesis that increased energy intake per peck will reduce feeding time and increase the incidence of social pecking and associated behaviours.

II. METHODS

ISA Brown birds (n=216) at 70 weeks of age were used. The design was a 2 x 2 factorial array with 6 replicates of 54 birds per treatment. The treatments included high (100 g/kg rice hulls) or low (about 180 g/kg sunflower and soybean meal) fibre diet sources (D) and mash vs. pelleted diet forms (F). All diets were formulated and produced at a commercial mill in Tamworth and the composition is shown in Table 1. Birds used had been on the treatment diets (D) for at least 4 weeks prior to the behavioural experiment. Two birds in each cage were long-term residents and an additional 'unfamiliar' bird was added just prior to the commencement of behavioural observations to induce a disturbance to social structure. Scanning observations were done in two sessions (a.m. and p.m.) with three replications at hourly intervals per session. Each cage was observed for 5 min with instantaneous recordings made every 30 s. Behaviours recorded (yes or no) included: pecking of other

School of Rural Science and Agriculture, University of New England, Armidale, NSW 2351.

birds (social pecking), feeding, moving, preening and drinking. Escape or 'freeze' behaviour in birds being pecked was also recorded. A generalized linear model using a binomial distribution was used to analyse the proportion of birds involved in each behaviour and to determine the effects of diet (D), feed form (F), time of day (a.m. vs p.m.) and interactions.

An "coloured object pecking" test was also performed on birds from each treatment at the start and end of the experiment (4 wks later) to determine the intensity of pecking orientated toward red (blood) or feather (white/brown) colours. Rice grains glued to a board and dyed brown, white or red were used as the pecked objects. The board was placed over the feed trough in front of each cage for 10 min and the number of pecks at each coloured grain was recorded. Apart from the periods when this test was conducted feed and water were provided *ad libitum*. Data from the pecking test were analysed using least squares analysis of variance procedures.

Table 1. Ingredient and calculated nutrient composition of the experimental diets (g/kg)[1]

Ingredient	High fibre diet	Low fibre diet
Wheat 12.5%	607.5	683.1
Oil	-	10
Meat meal 50%	100	-
Sunflower meal 30%	50	68.5
Cottonseed meal 37%	5	-
Soybean meal 48%	8.5	110
Rice pollard	63	37.5
Rice hulls 2%	100	-
Limestone	59.5	75
Kynofos	-	10
Sodium Chloride	1	1.25
Choline chloride 75%	0.3	0.3
DL-methionine	0.6	0.9
L-Lysine	1.1	-
Layer premix	2	2
Synthetic yolk colour premix	1.5	1.5
Nutrient		
ME (MJ/kg)	10.40	11.36
Protein	164.5	165.5
Fat	35.4	32.7
Fibre	74.2	39.6
Methionine	3.3	3.5
Lysine	7.3	6.8
Calcium	32.0	30.8
Available Phosphorus	4.8	3.5

[1]Diets were formulated and produced by Ridley Agriproducts Pty Ltd.,Tamworth

III. RESULTS

The time spent feeding was greater for birds given high-fibre rather than low-fibre diets ($P<0.01$) and an interaction ($P<0.01$) showed that pelleting reduced the time spent feeding by birds only in the low-fibre group (Table 2). Feeding frequency was higher during the afternoon observations ($P<0.001$) but there was no D x F interaction.

Social pecking behaviour of the hens was not influenced by D or time of day but was influenced by F (P< 0.001), with pecking incidence being higher for the pelleted diet. There was an interaction between D and F (P<0.001) with birds on the low-fibre diet showing a significant difference in pecking frequency for the two feed forms whereas, for birds on the high-fibre diets, the effect of feed form was reversed (Table 2).

Escape behaviour was influenced by both D and F (P<0.01), being lowest for the high-fibre diet in mash form. A D x F interaction (P<0.001) was due to a much higher incidence of escape behaviour of birds on the pelleted than mash low-fibre diet, but the reverse was true for the high fibre diet (Table 2). The incidence of escape behaviour was higher (P<0.01) during the morning but again there was no interaction between time of day and treatment.

Freeze incidence was low but influenced by D (P<0.001) and F (P<0.05). A D x F interaction (P<0.001) was due to a similar relative response to the four treatments as observed for escape behaviour, although the overall effect of diet was different with higher freeze behaviour on the high fibre diet (Table 2).

Table 2. Mean percentage of cages exhibiting a behaviour in any minute of observation for different diets and feed forms

Behaviour	Low-Fibre		High-Fibre		Interaction
	Mash	Pellet	Mash	Pellet	P<
Feeding	16.2^{b}	9.4^{a}	16.0^{b}	17.3^{b}	0.01
Social pecking	10.6^{a}	32.0^{c}	26.8^{c}	19.5^{b}	0.001
Escape	7.2^{a}	23.7^{b}	16.0^{b}	9.1^{a}	0.001
Freeze	1.2^{a}	4.9^{b}	12.6^{c}	5.7^{b}	0.001
Moving	27.1	26.3	28.1	26.8	NS
Drinking	5.4^{a}	5.1^{a}	4.4^{a}	7.4^{b}	0.05

Means in a row sharing the same superscripts do not differ significantly (P<0.05).

Preening behaviour incidence was low (mean 1.8%). Moving behaviour was not influenced by D or F. Drinking frequency was higher during the afternoon (P<0.01) and higher (P<0.05) on the pelleted high-fibre diet than on the other dietary treatments (Table 2).

In the pecking test, birds preferred to peck white grains (42.5%) than grains of other colours (P<0.01). The red (blood) colour was least pecked (26%). Birds given the low-fibre diets tended (P=0.1078) to peck the white grains more frequently (48%) than birds given the high-fibre diets (37%). Total number of pecks was unaffected by D (28.8 vs 28.1) but was higher for birds fed pellets rather than mash (35.0 vs 21.9, P<0.08).

IV. DISCUSSION

There was no effect of fibre content on feeding behaviour of layers given mash diets but on low fibre diets, birds given pellets spent significantly less time feeding than when given a mash diet. This confirms our original hypothesis that a more energy dense (MJ/kgDM) diet would lead to a lower feeding time as fibre increases the bulk of the diet. Feeding pelleted, high fibre diets to birds did not decrease the time spent feeding, suggesting that the time spent on feeding in birds fed high fibre diets may develop as a response to increased fibre concentration over a longer period of time.

With social pecking frequency, the D x F interaction suggests that the time spent on feeding is indeed very important. For the low-fibre diets, birds given pelleted diets spent less time feeding and more time pecking each other. On the other hand, a comparable response of

higher pecking behaviour was observed for birds given the high fibre, mash diet. This indicates that probably birds on higher fibre diets cannot so readily satisfy their energy and nutrient requirements when given their feed in mash rather than pelleted form and so they increase social pecking behaviour. However, further studies are needed to clarify the effects of D and F on pecking frequency and pecking time. The implication for management of cannibalism by manipulation of dietary fibre level is that there will be a fine balance between increasing birds' feeding time to reduce social pecking whilst still meeting their nutritional requirements.

Escape and freeze behaviours are considered to be responses to social pecking (Appleby *et al*., 1989). In the current experiment, escape incidence essentially mirrored pecking behaviour incidence. However, it is possible that the disturbance caused by escape behaviour also triggers pecking behaviour. The incidence of freeze behaviour is normally associated with fear and can be a response to increased aggression. Consequently, it was assumed that the incidence of this behaviour would parallel escape behaviour. Within D, F did have the same relative effect on both freeze and escape behaviour. Thus, freeze behaviour was (relatively) more frequent on the high fibre diet. Close examination of cage variability, however, revealed that two cages contributed appreciably to the elevated mean of this treatment and consequently it seems likely that a fear response other than one associated with aggression may have confounded these data.

There is no clear explanation for the treatment differences in the other behaviours recorded. Preening behaviour has been considered to be an indicator of social stability (Glatz, 2000). The very low incidence of preening behaviour (1.8%) and variability in incidence within a day suggest that more frequent observations would be needed before preening could reliably be used as an indicator of social stability. Furthermore, there was no evidence that either D or F altered the bird's colour preference. The birds preferred pecking white as opposed to red objects which is contrary to a popular belief that cannibalistic birds are specifically attracted to the red colour of blood.

This study indicates that fibre level and the form in which a diet is fed (mash vs pellets) can influence the behaviour of laying hens. The incidences of feeding, pecking, escape and freeze behaviours may all be useful indicators of factors leading to the onset of cannibalism. Clearly changing diets can alter the incidence of social pecking behaviour and to a degree this is associated with changes in the proportion of time spent feeding. Further studies are needed to further quantify the relative effect of D and F on pecking and cannibalism but initial indications are that feed form is of practical significance only when birds are given low-fibre diets.

ACKNOWLEDGEMENTS

We wish to thank the Egg Program of the Rural Industries Research and Development Corporation for providing funding for this study.

REFERENCES

Appleby, M.C., Hughes, B.O. and Hogarth, G.S. (1989). *British Poultry Science*, **30**: 545-553.

Esmail. H.M. (1997). *Poultry International,* **July**: 31-34.

Glatz, P.C. (2000). *A review of beak-trimming methods*. www.rirdc.gov.au

Hartini,S., Choct, M., Hinch, G. and Nolan, J.V. (2001). *Australian Poultry Science Symposium*, Ed. D. Balnave. **13**: 216-219.

Linberg, A.C. and Nicol, C.J. (1994). *Applied Animal Behaviour Science*, **41**: 211-227.

BREATH TESTS FOR ESTIMATING DIGESTA TRANSIT TIME IN CHICKENS

R.J. HUGHES[1], D.R. TIVEY[2] and R.N. BUTLER[3]

Summary

The lactulose breath test is a non-invasive diagnostic procedure for estimation of digesta transit time in human subjects. A similar procedure applied to ten broiler chickens yielded a mean and standard error for oro-caecal transit time of 166 ± 9 mins. In comparison, whole tract transit time for ferric oxide administered in a gelatine capsule was 162 ± 9 mins. The sensitivity of the breath hydrogen test was improved by fasting chickens for 3 h prior to dosing with lactulose. The gut microflora of chickens produced large increases in breath hydrogen from fermentation of lactulose without the need for prior dosing. Finally, it may be possible to devise a breath test for chickens based on fermentation of naturally-occurring complex carbohydrates in commercial chicken feed.

I. INTRODUCTION

Transit time, or inversely the period of residence of digesta in the gastrointestinal tract, influences the rates of digestion and absorption of nutrients in chickens (van der Klis and van Voorst 1993; Uni *et al*., 1995). Transit time in human subjects is measured by a non-invasive diagnostic procedure involving rise in hydrogen concentration in breath following dosing with the synthetic disaccharide lactulose. The test relies on the production of hydrogen by hindgut fermentation of lactulose which is not absorbed in the small intestine (Wutzke *et al*., 1997). Hydrogen not otherwise utilised as a food source by other bacteria in the gut diffuses through the gut wall to the bloodstream and is then expired via the lungs. Recent studies by Hughes *et al*. (2001) point to the usefulness of breath tests as non-invasive methods for studying gastrointestinal function in chickens.

This paper describes two experiments with chickens to develop a hydrogen breath test for non-invasive measurement of transit time of digesta. The first experiment examined whether prior dosing with lactulose was necessary to prime gut microflora and whether fasting prior to test dosing with lactulose improved the sensitivity of the test. The second experiment compared oro-caecal transit time (OCTT) determined by rise in breath hydrogen with whole tract transit time (WTTT) determined by appearance of ferric oxide in excreta.

II. MATERIALS AND METHODS

(a) Experiment 1 - Prior dosing with lactulose and fasting

Male broiler chickens (Ross breed) were reared from hatch under electric brooders in a floor pen in a controlled temperature room. The chickens had free access to commercial starter crumbles and water. At 20 days of age chickens were transferred to single-bird metabolism cages in a room kept at 25-27^{o}C and given commercial finisher pellets. Commencing at 26 days of age, four chickens entered the testing cycle which ran over two days. The cycle was repeated with separate sets of four chickens on the following two days. On day 1 of each cycle, two chickens were given a priming dose of lactulose at 1100 h. Each chicken was treated with approximately 130 mg lactulose in 5 mL of water. The solution was administered via a disposable syringe fitted with a soft plastic tube which was inserted 4 cm into the oesophagus. On day 2, two chickens (one prime-dosed and one not dosed) were fasted for 3 h from 0800 h. The other two chickens (one prime-dosed and one not dosed) had free access to feed. Commencing at 1100 h, all four chickens were test-dosed with approximately 130 mg lactulose in 5 mL of water. Following the test dosing with lactulose

chickens had free access to feed and water. Serial breath testing of each chicken commenced immediately before test-dosing at 1100 h then at 60, 90, 105, 120, 135, 150, 165, 180, 210, 240 and 300 minutes thereafter. A 50 mL gas sample from the head space was taken 15 sec after a prototype helmet was placed over the head of the chicken and held firmly against the shoulders to minimise loss of expired gas, as described previously by Hughes *et al*. (2000 and 2001).

(b) Experiment 2 – Measurement of oro-caecal and whole tract transit time

Male and female broiler chickens (Ross) were reared from hatch under electric brooders in separate floor pens in a controlled temperature room. The chickens had free access to commercial broiler starter crumbles and water. At 14 days of age, chickens were transferred to single-bird metabolism cages in a room kept at 25-27^{o}C and given commercial starter crumbles. Commencing at 18 days of age, 14 chickens in total were fasted for 3 h starting at 0800 h. At 1100 h, all chickens were administered with a gelatine capsule containing ferric oxide (Fe_2O_3 200 mg/kg live weight) as described by Iskander and Pym (1987). Then ten chickens (six male and four female) were dosed with approximately 130 mg lactulose in 5 mL of water, as described in (a) above. The remaining four chickens (two of each sex) not dosed with lactulose provided a measure of base-line variation in hydrogen production from undigested carbohydrate by gut microflora. Serial breath testing of each chicken commenced immediately before test-dosing at 1100 h then at 120, 150, 165, 180, 195, 210 and 240 minutes thereafter. Breath samples were collected as described in (a) above. Excreta trays were examined frequently for signs of ferric oxide in voided droppings.

III. RESULTS AND DISCUSSION

(a) Experiment 1 - Prior dosing with lactulose and fasting

All chickens showed an increase in breath hydrogen after dosing with lactulose whether or not they received a priming dose on the previous day. Fasting prior to dosing appeared to reduce variation in hydrogen concentration between chickens at the same time after test dosing, and to also reduce the within-chicken variation. Results of serial breath sampling of fasted chickens are shown in Figure 1. Estimates of oro-caecal transit time for lactulose following fasting ranged from 165 mins for chicken E, 180 mins for chickens B and F, to 210 mins for chickens A, C and D. The mean and standard error for transit time were 193 ± 8 mins. The above estimate is likely to be biased upwards because the interval between serial breath samples was 15 minutes up to 180 minutes post dosing, then 30 minutes thereafter. That is, mean transit time occurred after the period of most frequent sampling.

The hydrogen profile for chicken D with two peaks in hydrogen concentration at 180 and 240 minutes (Figure 1) is very similar in appearance to breath profiles observed in rats and humans with small bowel bacterial overgrowth (proliferation of facultative anaerobes in the small intestine). Choct *et al*. (1996) observed microbial proliferation in the small intestine of chickens associated with an increase in digesta transit time as a result of the gelling properties of soluble non-starch polysaccharides in wheat.

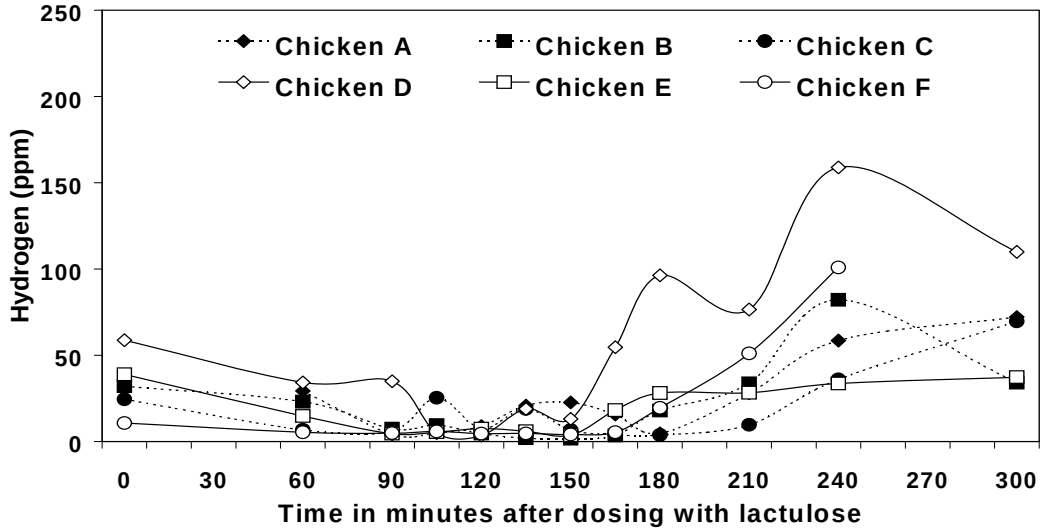


Figure 1. Breath hydrogen concentration (in ppm) in male chickens fasted for 3 h prior to test dosing with lactulose (130 mg in 5mL water) in Experiment 1.

(b) Experiment 2 – Measurement of oro-caecal and whole tract transit time

Oro-caecal transit time and standard error were 165 ± 12 mins and 164 ± 13 mins for whole tract transit time in male chickens (Figure 2). For female chickens (Figure 3), the corresponding values were 158 ± 13 mins for OCTT and 166 ± 112 mins for WTTT. That is, there was no difference due to sex of the chicken for either measurement. OCTT and WTTT were significantly correlated (r=0.76, P< 0.05) with WTTT being generally shorter than OCTT. Possible explanations for this anomalous observation are (a) lactulose and ferric oxide move in a different manner through different sections of the alimentary tract, or (b) there is a pause when undigested carbohydrate reaches the caeca in fasted animals while bacterial fermentation gets under way.

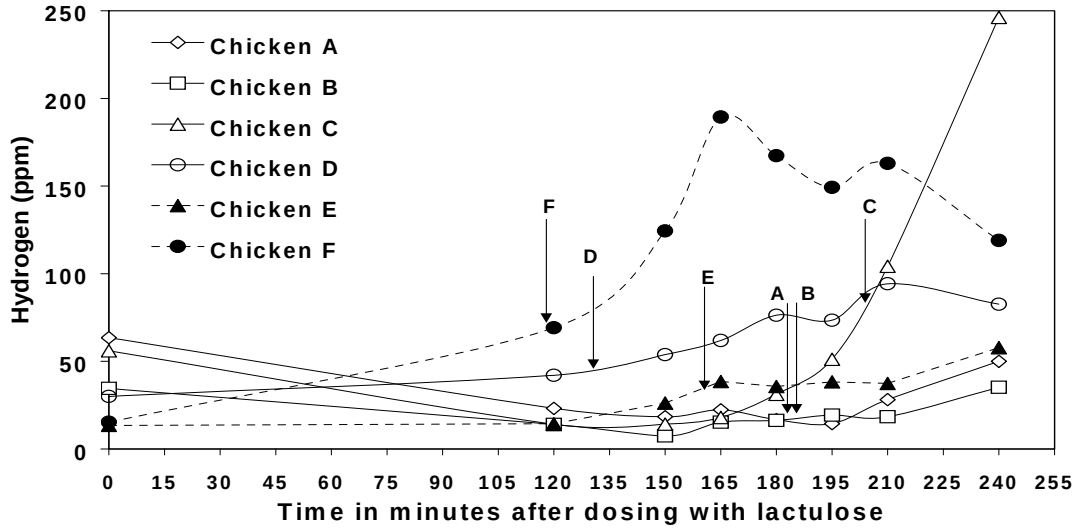


Figure 2. Breath hydrogen concentration (in ppm) in male chickens fasted for 3 h prior to test dosing with lactulose (130 mg in 5mL water) in Experiment 2. The vertical arrows indicate whole tract transit time for ferric oxide marker.

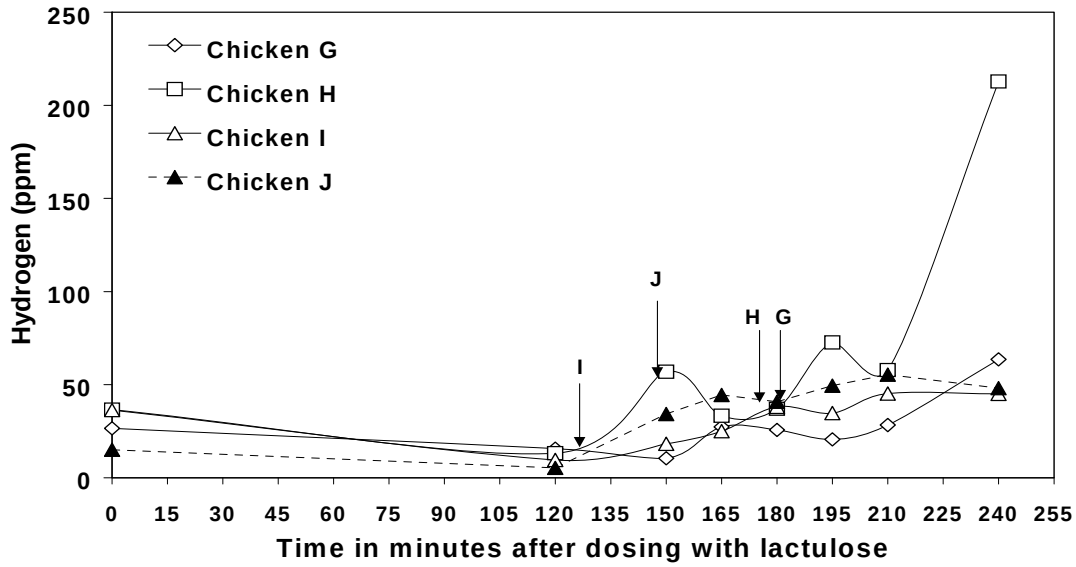


Figure 3. Breath hydrogen concentration (in ppm) in female chickens fasted for 3 h prior to test dosing with lactulose (130 mg in 5mL water) In Experiment 2. The vertical arrows indicate whole tract transit time for ferric oxide marker.

Finally, it is possible that a non-invasive breath test for estimation of oro-caecal transit time can be devised without the need to dose chickens with lactulose. OCTT and WTTT were 184 ± 8 mins and 172 ± 5 mins, respectively, in four chickens fasted for 3 h but not dosed with lactulose. That is, caecal microflora may produce enough hydrogen from normal levels of complex carbohydrates in commercial broiler feed.

IV. CONCLUSIONS

Serial breath testing of fasted chickens dosed with lactulose can be used to measure oro-caecal transit time in chickens. There may be a sufficient level of dietary fibre in normal broiler diets to avoid the need for lactulose in field testing of commercial flocks.

V. ACKNOWLEDGMENTS

We gratefully acknowledge RIRDC Chicken Meat Program for financial support and the dedicated efforts of the poultry research staff at PPPI and the staff of the Gastroenterology Unit at the Women's and Children's Hospital, Adelaide.

REFERENCES

Choct, M., Hughes, R., J., Wang, J., Bedford, M.R., Morgan, A.J. and Annison, G. (1996). *British Poultry Science*, **37**: 609-621.

Hughes, R.J., Tivey, D.R. and Butler, R.N. (2001). *Proceedings of the Australian Poultry Science Symposium* (Ed. D. Balnave), **13**: 168-171.

Iskander, S. and Pym, R.A.E. (1987). *Recent Advances in Animal Nutrition* (Ed. D. Farrell), University of New England, pp.252-259.

Uni, Z., Noy, Y. and Sklan, D. (1995). *Poultry Science*, **74**: 1622-1629.

van der Klis, J.D. and van Voorst, A. (1993). *Poultry Science*, **72**: 503-512.

Wutzke, K.D., Heine, W.E., Plath, C., Leitzmann, P., Radke, M., Mohr, C., Richter, I., Gülzow, H.U. and Hobusch, D. (1997). *European Journal of Clinical Nutrition*, **51**: 11-19.

KIDNEY HISTOLOGY OF VACCINATED AND UNVACCINATED COCKERELS EXPOSED TO T-STRAIN INFECTIOUS BRONCHITIS VIRUS

M. JOLLY, J.R.ROBERTS and W.BALL

Histological examination was conducted of kidneys made available from a previous experiment, which investigated different infectious bronchitis virus (IBV) vaccination protocols for laying hens (Sulaiman *et al*., 2001). Histological changes were used as a possible measure of the level of protection afforded by different vaccination protocols.

Kidneys were examined from ISA Brown cockerels that were vaccinated at day old or two weeks by coarse spray, eye-drop or drinking water and then revaccinated by the same routes at 8 weeks. A control group of unvaccinated birds was also maintained. Ten birds from each group were challenged at 11 weeks of age with T-strain infectious bronchitis virus. Five birds from each group were euthanased at one, two and three weeks post exposure and the kidneys preserved for histological examination. At each time interval following exposure to T-strain IBV, kidney sections were prepared from the birds with the largest and smallest kidneys (as a percentage of body weight), stained with haematoxylin and eosin, and examined for histological changes (a total of 6 birds per treatment, 2 kidneys per bird, 3 divisions per kidney). Also, the cranial divisions were examined for all 5 birds in vaccinated groups that showed histopathology.

The unvaccinated birds, at one week post-infection, all showed some degree of histopathology, although the severity of the lesions varied between individuals. The histopathological signs displayed by the birds in this group ranged from minimal monocyte infiltration, to dense widespread lymphocyte infiltration and necrosis of renal tubules. The majority of infiltration was observed in medullary regions of the kidney. The collecting ducts in the most affected areas of the kidney were distended and contained granulocytic casts. Although it varied between birds, generally the pathological signs were observed equally in all three divisions of both the right and left kidney. Unvaccinated birds that were euthanased at two and three weeks after exposure to the T-strain virus showed an initial increase in the area of dense monocyte infiltration into cortical regions and then regression at 3 weeks to the medullary areas. This suggests a progression of the nephritis that can be used as a guide to indicate severity and duration of an infection.

None of the vaccinated birds exhibited clinical signs following exposure to T-strain IBV. Based on histological examination, all of the vaccination protocols appear to have protected the birds against kidney lesions to some degree. Individual birds in most treatments showed varying levels of minor monocyte infiltration but no further lesions, indicating a mild reaction that had been halted by the birds' primed immune systems. However, a reaction comparable in severity to those observed in the unvaccinated birds was seen in two of the vaccinated groups, vaccination by drinking water at day-old (4 out of 5 birds) and vaccination by coarse spray at two weeks (2 out of 5 birds). The lack of protection in the water-vaccinated birds may result from the birds not drinking sufficiently to receive an adequate dose of active vaccine virus. The response of the spray-vaccinated birds may be due to individual variation in the susceptibility of the birds, or to some other factor that compromised the birds' immune status.

Sulaiman, A., Roberts, J.R. and Ball, W. (2001). *Proceedings of the Queensland Poultry Science Symposium*. **10**: 10.1-10.7.

Animal Physiology, School of Rural Science, Univ. of New England, Armidale, NSW 2351.

ENDOGENOUS NITROGEN FLOW IN CHICKENS AS INFLUENCED BY DIETARY LEVELS OF VISCOUS AND NON-VISCOUS NON-STARCH POLYSACCHARIDES

R.M. PADILLA[1], P.C.H. MOREL[1], V. RAVINDRAN[1] and G.D. COLES[2]

Summary

The influence of two types of soluble non-starch polysaccharides (barley β-glucan and maize arabinoxylan) at two inclusion levels (30 and 60 g/kg) on endogenous ileal nitrogen flow in broiler chickens was investigated, using the peptide alimentation method. Ileal nitrogen contents (as g/kg titanium marker) and endogenous nitrogen flow (as μg/g dry matter intake) were increased with the addition of non-starch polysaccharides, but the differences were not statistically significant ($P>0.05$). The results indicate that the type and level of soluble non-starch polysaccharides may influence the extent of ileal flow of nitrogen in chickens.

I. INTRODUCTION

Non-starch polysaccharides (NSP) assume considerable practical significance in poultry nutrition due to the anti-nutritive effects elicited by soluble pentosan components (arabinoxylan and β-glucan) and their effects on bird performance (Choct, 1997). The detrimental effects of NSP are believed to be due to its viscous nature that has physiological, morphological and microbiological effects on the digestive tract. These events negatively impact nutrient digestion and also cause wet and sticky droppings (Bedford and Schulze, 1998).

It is well established that soluble NSP in wheat influence the apparent ileal digestibility of nutrients, including nitrogen (Choct and Annison, 1992). The reduction in nitrogen digestibility can be attributed to impaired digestion, inhibition of amino acid absorption or increased secretion of endogenous protein derived from gut secretion and sloughed off epithelium, or increased mucin (glycosaminoglycan) secretion. Using the guanidation method, Angkanaporn *et al*. (1994) found that the addition of isolated pentosans equivalent to 15 and 35 g wheat arabinoxylans per kg diet significantly increased endogenous amino acid flow and lowered overall digestibility of amino acids. In the present study, the influence of different types and levels of NSP on endogenous ileal nitrogen losses in broiler chickens was examined, using the peptide alimentation method (Moughan *et al*., 1990). Two types of soluble NSP, namely barley β-glucan (viscous) and maize arabinoxylan (non-viscous), were compared.

II. MATERIALS AND METHODS

Five diets were prepared, including a control diet and test diets that contained 30 and 60 g/kg purified maize arabinoxylan or barley β-glucan extract (Glucagel™). The composition of the diets is shown in Table 1. All diets contained 180 g/kg enzymatically hydrolysed casein (EHC) as the source of amino acids and peptides, and 5 g/kg titanium oxide as an inert internal marker.

[1] Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.
[2] Crop & Food Research, Christchurch, New Zealand.

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

Ingredient	Control	30 g/kg arabinoxylan	60 g/kg arabinoxylan	30 g/kg β-glucan	60 g/kg β-glucan
Maize starch	569.7	539.7	509.7	539.7	509.7
Dextrose	100.0	100.0	100.0	100.0	100.0
EHC[1]	180.0	180.0	180.0	180.0	180.0
Arabinoxylan	-	30.0	60.0	-	-
β-glucan	-	-	-	30.0	60.0
Cellulose	35.0	35.0	35.0	35.0	35.0
Vegetable oil	50.0	50.0	50.0	50.0	50.0
Titanium oxide	5.0	5.0	5.0	5.0	5.0
Common ingredients[2]	60.3	60.3	60.3	60.3	60.3

[1] Enzymatically hydrolysed casein.

[2] Dicalcium phosphate, 24.0; Dipotassium hydrogen phosphate, 14.3; sodium bicarbonate, 12.0; magnesium oxide, 2.0; salt, 2.0; trace mineral premix, 5.0 and vitamin premix, 1.0.

Three-week old male broilers (Ross) were selected on the basis of body weight (1.3 to 1.7 kg) and allocated to 25 colony cages (4 birds per cage) so that all cages had a similar average weight. The cages were then assigned at random to the five dietary treatments so that there were five replicate cages per group.

The cages were housed in an electrically heated grower shed (22 – 24 ^{0}C) during the trial. All birds had *ad libitum* access to feed and water. The birds were given a mash commercial-type diet till Day 27. Following overnight fasting on Day 27, a casein-based diet was introduced and fed for the next three days. The casein-based diet was similar to the control diet (Table 1) except that casein was used in place of EHC. The use of casein-based diet enabled the birds to adjust to the change-over from the commercial mash diet to a purified diet. Previous studies in our laboratory have shown that this adjustment is necessary to maintain satisfactory feed intake levels when EHC-diets are introduced. The casein-based diet was withdrawn on the evening of Day 30 and the test diets (Table 1) were introduced on the morning of Day 31. The test diets were offered for 36 hours and records of feed intake during this period were maintained. The birds were then euthanased by an intracardial injection of sodium pentabarbitone and the contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. The ileum was defined as that portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileo-caecal junction. The digesta were pooled from birds within a cage, frozen immediately after collection and subsequently freeze-dried. Diet and dried ileal digesta samples were ground to pass through a 0.5 mm sieve and subsequently analysed for titanium and nitrogen. Nitrogen was determined following Kjeldahl digestion by colorimetric auto-analysis (Technicon, 1973). Titanium was analysed according to the procedures of Short *et al*. (1996).

The ileal nitrogen flow (related to the ingestion of 1 g of dry matter; the units are μg/g dry matter intake) was calculated using the following equation (Moughan *et al*., 1992).

$$\text{Ileal nitrogen flow} = \text{Nitrogen concentration in digesta} \times \frac{\text{Titanium concentration in diet}}{\text{Titanium concentration in digesta}}$$

III. RESULTS AND DISCUSSION

In the peptide alimentation method (Moughan *et al*., 1990), the animal is fed a purified diet containing enzymically hydrolysed casein (composed of free amino acids and peptides with a molecular weight of less than 10,000 Da) as the sole source of nitrogen. The digesta are then collected from the animal and the endogenous protein (molecular weight, > 10,000 Da) is separated from unabsorbed free amino acids and peptides by centrifugation and ultrafiltration. In the present study, because of the presence of soluble NSP, difficulties were experienced with the ultrafiltration step. The endogenous flow was therefore calculated assuming a digestibility value of 100% for EHC. This assumption is based on previous work where digestion of amino acids in EHC by broiler chickens was found to be complete (Ravindran, unpublished data). A similar approach has been previously used by Leterme *et al*. (1994) who employed the peptide alimentation method, but without the ultrafiltration of digesta. It should be noted, however, this approach may result in overestimation of the ileal flow if the EHC is not completely digested.

The results are summarised in Table 2. Dry matter digestibility was not influenced by the dietary inclusion of arabinoxylan or 30 g/kg β-glucan, but was lowered ($P<0.05$) with 60 g/kg inclusion of β-glucan. Ileal nitrogen contents (as g/g titanium) and endogenous nitrogen flow (as μg/g dry matter intake) were numerically increased with the addition of soluble NSP, but the differences were not statistically significant ($P>0.05$) due to high variation among replicates.

Table 2. Influence of type and level of NSP on endogenous nitrogen flow in broilers.

	Dry matter digestibility	Ileal N (g/g titanium)	Endogenous N flow (μg/kg DMI)
Control	0.878[a]	0.457	2383
30 g/kg Arabinoxylan	0.882[a]	0.552	2549
60 g/kg Arabinoxylan	0.879[a]	0.605	2625
30 g/kg β-glucan	0.884[a]	0.506	2372
60 g/kg β-glucan	0.856[b]	0.535	2700
Pooled SEM	0.006	0.055	269

[a, b] Values in the same column with different superscripts are significantly different ($P<0.05$).

The lack of significant treatment effects in respect to endogenous N flow was in contrast to the results of Angkanaporn *et al.* (1994) who found marked increases in endogenous amino acid losses when birds were fed diets containing levels of wheat arabinoxylans lower (15 and 35 g/kg diet) than those used in the present study. The guanidination technique, based on the homoarginine marker (Siriwan *et al*., 1994), was used by these researchers, whereas the peptide alimentation technique was used in the present study. However, the methodologies employed could not have caused the observed discrepancy since it has been shown that, under similar dietary protein intakes, these two techniques produce comparable results in terms of endogenous nitrogen and amino acid flow in the pig (Hodgkinson, 1999) and chickens (Ravindran *et al*., 2000). Differences in viscosity in the NSP extracts may explain, at least part of, this discrepancy. It is possible that the effect of barley β-glucan extract on intestinal digesta viscosity may have been lower than that assumed. Although *in vivo* digesta viscosity was not determined in our study, previous *in vitro* studies (Maqueda, 1999) have shown only small increases in extract viscosity with diets containing 50 g/kg barley β-glucan extract.

The present data suggest that soluble NSP may influence the extent of the increase in ileal flow of nitrogen in chickens. The exact causes of the increased nitrogen flow with high NSP levels are not known. It could be due to increased secretion of endogenous proteins into the gut, decreased reabsorption of endogenous proteins or a combination of both effects. As suggested by Angkanaporn *et al.* (1994), it is also possible that soluble NSP interacts with the gut wall modifying the action of peptide hormones, which regulate gut functions including stimulation of secretion of endogenous protein. Finally, the increased nitrogen flow may also reflect, in part, the increased secretion of mucins as an allergic/antigen response, and this may help to explain the high variability found within treatments. Further studies are clearly required to examine the influence of sources and levels of NSP on mucin secretion in the gut. A proportion of the unexplained variation may arise from differences in extract and intestinal digesta viscosities, and these should be examined in future studies.

ACKNOWLEDGEMENTS

This study was funded by the New Zealand Foundation for Research, Science and Technology. Maize arabinoxylan was the kind gift of Dr Alain Bonjean, LimaGrain SA, Clermont-Ferrand, France. Glucagel ™ was supplied by GraceLinc Ltd, Lower Hutt, New Zealand.

REFERENCES

Angkanaporn, K., Choct, M., Bryden, W.L., Annison, E.F. and Annison, G. (1994). *Journal of the Science of Food and Agriculture*, **66**: 399-404.

Bedford, M.R. and Schulze, H. (1998). *Nutrition Research Reviews,* **11**: 91-114.

Choct, M. (1997). *Feed Non-starch Polysaccharides: Chemical Structures and Nutritional Significance*, American Soybean Association, Singapore.

Choct, M. and Annison, G. (1992). *British Journal of Nutrition*, **67**: 123-132.

Hodgkinson, S.M.. (1999). *Endogenous Protein Flow in the Gut of the Simple-stomached Animal*. Ph.D Thesis, Massey University, Palmerston North, New Zealand.

Leterme, P., Monmart, T., Morandi, P. and Thewis, A. (1994). In: *Proceedings of the VIth Symposium on Digestive Physiology* in Pigs. Eds W.B.Souffrant and H. Hagemeister, EAAP Publication No. 80, Germany, pp. 60-63.

Maqueda, L. (1999). *Evaluation of the Anti-nutritive and Hypocholesterolemic Effects of a Beta-glucan Preparation Extracted from New Zealand Barley*. M.Sc. Thesis, Massey University, Palmerston North, New Zealand.

Moughan, P.J., Darragh, A.J., Smith, W.C. and Butts, C.A. (1990). *Journal of the Science of Food and Agriculture*, **52**: 13-21.

Moughan, P.J., Schuttert, G. and Leenaars, M. (1992). *Journal of the Science of Food and Agriculture*, **60**: 437-442.

Ravindran, V., Hew, L.I. and Bryden, W.L. (2000). In: *Proceedings of the Australian Poultry Science Symposium*, Ed R.A.E.Pym, **12**: 197.

Siriwan, P., Bryden, W.L. and Annison, E.F. (1994). *British Journal of Nutrition,* **71**: 515-529.

Short, F.J., Gorton, P., Wiseman, J. and Boorman, K.N. (1996). *Animal Feed Science and Technology*, **59**: 215-221.

Technicon (1973). *Industrial Method No. 98/70w*, Technicon, Tarrytown, New York.

THE EFFECTS OF VACCINE STRAIN, ROUTE OF ADMINISTRATION OF IB VACCINE AND REVACCINATION ON EGG PRODUCTION AND EGG QUALITY IN LAYING HENS

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

Summary

Different vaccination protocols for infectious bronchitis (IB) virus were administered to Isa Brown laying hens. Half the birds were revaccinated regularly during lay whereas the other birds were not vaccinated beyond 14 weeks of age. Body weight, production, blood haematocrit and plasma electrolyte concentrations were not affected by vaccination treatment. However, egg and egg shell quality differed between birds which were revaccinated regularly and those which were not. In general, egg shell quality was better in the birds which were not revaccinated at regular intervals. The birds which were vaccinated initially with the A3 vaccine tended to have lower albumen height and Haugh Units than the other treatment groups. The IB antibody titres were greatest at 6 and 16 weeks for both revaccinated and non-revaccinated birds. However, regular revaccination of birds beyond 14 weeks of age had no significant effect on IB antibody titre levels. These results suggest that there may be no advantage in regular revaccination of birds for IB, provided that birds have been properly vaccinated during rearing. However, more information is required about the correlation between blood IB titre levels and protection against intercurrent IB infection before recommendations can be made to the Australian industry.

I. INTRODUCTION

Infectious Bronchitis (IB) is a contagious viral disease that affects the respiratory system, oviduct, and kidneys of chickens. The disease has the potential for serious economic impacts on layers where it may cause a reduction in the quantity and quality of egg production (Jordan, 1996). In Australia, at the present time, all the commercially available vaccines are live virus vaccines but in the future the availability of inactive vaccine viruses is a possibility (Cavanagh and Naqi, 1997). Clearly, vaccination programs will remain the cornerstone of the strategy for IB control, using a combination of live and inactivated vaccines targeted at the IB strains or variants active in a particular geographical area (Lister, 2001). Results from a previous experiment, using Webster's VicS IB vaccine strain with ISA brown cockerels, indicated that vaccination at either day-old or two weeks of age, by eyedrop, coarse spray or water vaccination, protected birds against the effects of exposure to T strain IB virus (Sulaiman, Roberts, and Ball, 2001).

This experiment investigated the effect of strain of vaccine, route of vaccine administration and regular revaccination for IB, on production performance in laying hens

II. METHODS

Day-old ISA Brown hens (625) were purchased from the Winton Hatchery near Tamworth, NSW and transferred to isolation pens (geographically isolated in relation to natural wind directions) at the University of New England, Armidale, NSW. The birds were reared according to standard commercial practice. There were seven experimental groups,

Animal Physiology, School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351.

each of 89 birds: Control (No vaccination), VicS eye (VicS vaccine by eye drop at day old), VicS spray (VicS by coarse spray at day old), VicS water (VicS in water at day old), A3 eye (A3 vaccine strain by eye drop at day old), A3 spray (A3 by coarse spray at day old), and A3 water (A3 in water at day old). Blood samples were taken from ten birds from each group at 4 weeks of age and birds were then revaccinated with the other strain of vaccine to that used at day-old, via the same routes as day old. The Control Group remained unvaccinated. Blood samples were taken from ten birds per group at 6 weeks of age. At 14 weeks of age, all birds (including the Control birds) were revaccinated with VicS vaccine strain by eye drop. At 15 weeks of age, all birds were transferred to two poultry isolation sheds equipped with 3-bird commercial-style cages. One-half of the birds from each treatment group were allocated to each shed, 2 birds per cage. The birds in one shed were revaccinated every 8 weeks with VicS vaccine strain by coarse spray, whereas the birds in the other shed were not revaccinated beyond 14 weeks of age. For each group, body weight (BW) was recorded regularly. Egg production, egg weight and the external appearance of the eggs were recorded daily. Faecal moisture was measured 1 and 2 weeks post revaccination. Every 4 weeks, 21 eggs of each group from each shed were collected for egg and egg shell quality measurements (a total of 294 eggs). Blood samples were taken from 5 birds from each group, in each shed, 3 weeks after revaccination for determination of the plasma electrolytes Na^+, K^+ and Ca^{++}, haematocrit, and antibody titres (Birling Avian Laboratories: ProFLOK IBV ELISA kit). Clinical signs and mortality were recorded if observed and all mortalities autopsied.

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

III. RESULTS

Body Weight

There was no effect of vaccination treatment on the body weight of the birds. Body weights were within the target range recommended by the breeder company.

Hen-day Production

Hen-day production of non-revaccinated birds and revaccinated birds is shown in Figures 1 and 2. There was no significant effect of vaccination treatment on egg production between 18 and 40 weeks of age.

Egg and Egg Shell Quality

The effects of treatments and revaccination for IB on egg and egg shell quality measurements: shell deformation (μm), shell breaking strength (Newtons), shell reflectivity (%), egg weight (g), albumen height (mm), Haugh units (HU), yolk colour (Roche Scale), shell weight (g), percentage shell (%) and shell thickness (μm); were determined at 20, 24, 28, 32, 36 and 40 weeks of age. At 24 weeks of age (2 weeks post-vaccination), birds in the revaccinated group showed lower shell breaking strength, yolk colour, shell weight, percentage shell and shell thickness, as compared with birds which were not revaccinated. In addition, albumen height and Haugh units differed among treatment groups, being lowest for the A3 water group.

Figure 1. Hen-day Production of Non-revaccinated Birds

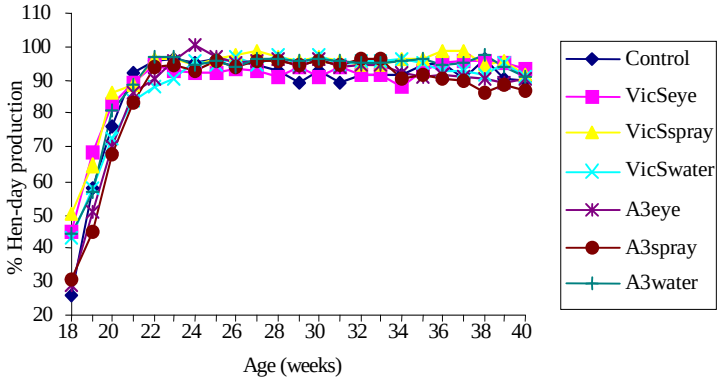
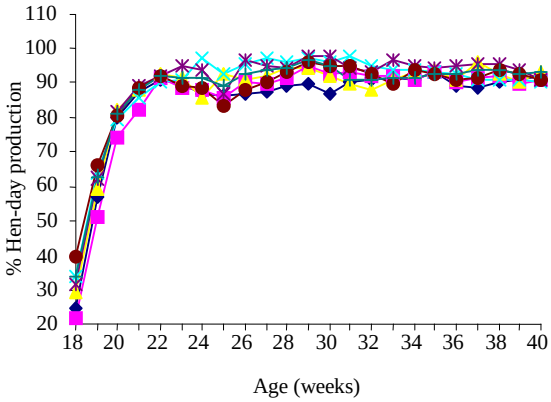


Figure 2. Hen-day Production of Revaccinated Birds



There were few significant effects of vaccination treatment on egg quality at 28 weeks (6 weeks postvaccination) with yolk colour being higher in the revaccinated birds and shell reflectivity lowest for the A3 groups. At 32 weeks of age (2 weeks postvaccination), birds in the revaccinated groups had lower shell breaking strength and percentage shell and higher egg weight and shell weight. At 36 weeks of age (6 weeks postvaccination), the revaccinated birds had lower shell breaking strength, percentage shell and shell thickness. There were also significant effects of treatment group on albumen height and Haugh Units with the A3 groups being lowest. At 40 weeks of age (2 weeks postvaccination), percentage shell was lower for the revaccinated birds. In addition, albumen height and Haugh Units were lowest for the A3 treatment groups.

Faecal Moisture

Faecal moisture was measured in samples collected over a 24 hour period, one and two weeks following re-vaccination. The control group, which had not been vaccinated prior to 14 weeks of age, tended (p=.0544) to have wetter faeces than the other groups at 16 weeks of age, two weeks following vaccination for the first time in that group (Table 1).

Table 1. Faecal moisture of control and vaccinated birds at 16 weeks of age

Treatment Group	Control	VicS eye	VicS spray	VicS Water	A3 eye	A3 spray	A3 water
Faecal Moisture %	74.7 ± 2.3	67.4 ± 2.2	68.2 ± 0.9	71.5 ± 1.1	70.6 ± 2.3	67.8 ± 2.1	71.1 ± 1.7

IB Titre Levels, Blood and Plasma Measurements

Titre levels of non-revaccinated and revaccinated birds are shown in Figures 3 and 4. There was no significant effect of vaccination treatment on titre levels. However, titre levels varied with the age of bird, being greatest at 6-16 weeks of age.

There were no significant effects of vaccination treatment on blood and plasma measurements (haematocit and concentrations of Na, K, Ca^{++}).

Figure 3. The Titres of Non-revaccinated Birds

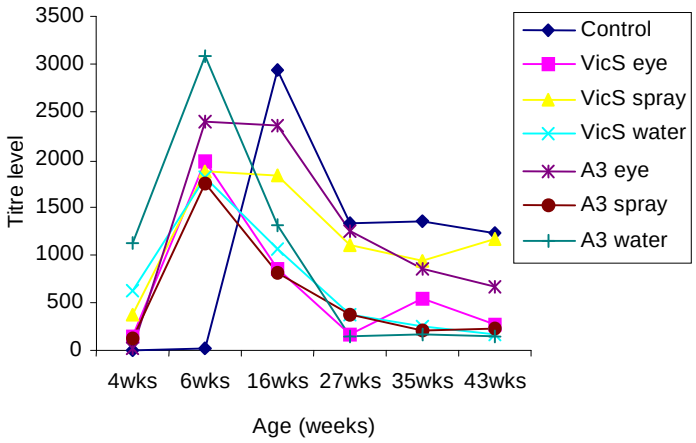
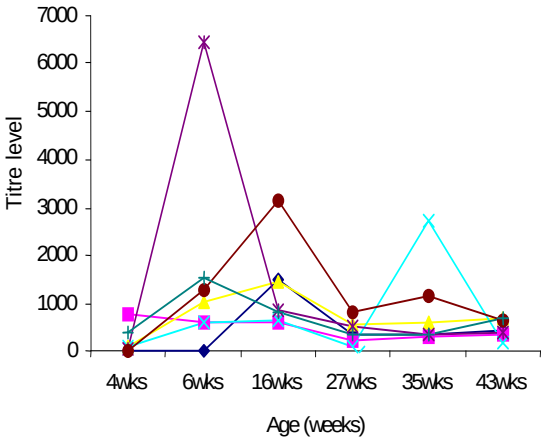


Figure 4. The Titres of Revaccinated Birds



IV. DISCUSSION and CONCLUSIONS

The results of the present study indicate that vaccination treatment, including regular revaccination for IB does not affect body weight, egg production, faecal moisture, titre level, haematocrit or plasma concentrations of Na, K or Ca^{++}. However, regular revaccination had some deleterious effects on egg shell quality. In addition, the groups of birds which had been vaccinated at day-old with A3 strain IB vaccine tended to have lower albumen height and Haugh Units. Although this study is on-going, results to date suggest that there is little advantage in regularly revaccinating laying hens for IB virus, provided that they have received appropriate vaccination during the rearing phase. However, more information is required about the correlation between blood IB titre levels and protection against intercurrent IB infection before recommendations can be made to the Australian industry.

V. ACKNOWLEDGEMENTS

The support of the Rural Industries Research and Development Corporation Egg Program for this study is gratefully acknowledged. We thank Associate Professor Rob Cumming and Dr. Roger Chubb for their assistance and stimulating discussions and Mr. Gary Taylor for assistance with maintenance of the birds.

REFERENCES

Cavanagh, D. and Naqi, S.A. (1997). In: *Diseases of Poultry, 10th Ed*. Eds B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald, Y.M. Saif, Iowa State Univ Press, Iowa. p 511-526.

Jordan, F.T.W. (1996). *Infectious Bronchitis* in *Poultry Diseases*. 4th Ed., Jordan, F.T.W. and Pattison, M. (eds). W.B. Saunders. London

Lister, S.A. (2001). Infectious Bronchitis - A Significant World Problem. Antec International Ltd. http://www.antecint.co.uk./main/infectbr.htm (Accessed September 2001)

Sulaiman, A., Roberts. J.R. and Ball, W. (2001). *Proceedings of the Australian Poultry Science Symposium*, Ed. D. Balnave. **13:** 237.

DIETARY PROTEIN EFFECTS UPON GROWTH AND BODY COMPOSITION IN SELECTED LINES OF JAPANESE QUAIL

H. SUTEDJO, L. KNOTT AND R.A.E. PYM

Summary

The effect of dietary protein (120 v 240 g/kg) in isoenergetic (13 MJ AME/kg) diets on 17-31 d growth rate, food intake and FCR and 32 d body moisture, fat and protein was measured in approximately 32 birds from each of five lines of Japanese quail selected for increased weight gain (line HW) or body fat (line HF) or decreased FCR (line FE) or body fat (line LF). A randomly selected control (line C) was included. There were significant ($P<0.001$) effects of Line and Diet on all traits measured, but also a significant ($P<0.001$) interaction between Line and Diet for growth rate, food intake and FCR. Whilst birds in all lines given the low protein diet grew slower, ate less and converted food less efficiently than their counterparts given the high protein diet, the relative performance of the lines on the two diets varied considerably. The Diet effect was much greater in the FE than in the HF line, with the three other lines intermediate. Birds in each line given the high protein diet had more carcass moisture and protein and less fat than their counterparts given the low protein diet. The interaction between Line and Diet for all carcass traits was not significant ($P>0.05$).

I. INTRODUCTION

In recent years considerably greater emphasis has been given in commercial broiler breeding programs to body composition and food utilisation efficiency. Emphasis on the former is associated with increasing the proportion of more expensive carcass components (e.g. breast meat) and in reducing body fat, since it is regarded as undesirable by consumers and is energetically expensive to deposit. Index selection incorporating individual growth rate and feed efficiency has been shown to result in a significant improvement in overall economic response relative to selection for growth rate alone (Pym and James, 1979). The direct and correlated responses to selection for growth rate, body composition or feed efficiency have been identified in separate selection experiments in chickens (e.g; Sorensen, 1984; Whitehead and Griffin, 1984; Pym, 1985; Cahaner *et al.,* 1986; Pym, 1987; Leenstra and Pit, 1987; Leclercq, 1988; Pym *et al.*, 1998), but a contemporaneous comparison of responses to selection for all three traits in the one experiment, has not been reported. Responses to selection can be significantly affected by founder population and selection procedures which bring into question any interpretation of relative responses across experiments. At the last Symposium, (Sutedjo *et al.,* 2001a) we reported on selection responses in lines of Japanese quail selected in the one experiment for different aspects of growth, feed efficiency and body composition.

In developing strains through different selection procedures, a critical element determining the viability and use of the strains so derived, is the impact on amino acid utilisation and requirements. We recently reported on the effects on growth rate and feed efficiency in the quail lines after six generations of selection to variation in dietary protein levels from 120 to 240 g/kg (Sutedjo *et al.*, 2001b). This was followed by an experiment, reported here, on the effect of such variation using the two extreme diets, on growth, feed efficiency and carcass chemical composition.

School of Veterinary Science, University of Queensland, St Lucia, Qld 4072.

II. METHODS

Birds used in the study came from the sixth generation of the five lines listed in Table 1 and were derived from a mating between 10 males and 30 females per line. Following hatching, the birds were wingbanded and placed in group brooding cages in a temperature controlled room, where they were held until 16 d. During this period, all birds were given a crumbled broiler starter diet containing 220 g CP and 12.5 MJ AME/kg. At 17 d all birds were moved to single cages with individual feeders and the experiment commenced.

Table 1. Selection lines and selection criteria.

Line HW	Increased liveweight at 35d of age
Line HF	Increased 35d abdominal fatness – by sib selection
Line LF	Decreased 35d abdominal fatness – by sib selection
Line FE	Decreased 15-30d FCR – in individual cages
Line C	Unselected control

A factorial design was used with the five lines, two sexes and two diets containing either 120 or 240 g/kg protein. The diets were based on sorghum, wheat, soyabean meal and meatmeal and were isoenergetic with 13 MJ ME/kg. Amino acid composition was essentially proportional to the relative crude protein contents. At 17 d, 16 birds of each sex from each of the five lines were allocated randomly to individual cages in a controlled temperature room. Half of the birds were given the high protein diet and the other half, the low protein diet to 31 d. Birds were weighed at 17 and 31 d and food intake from 17 to 31 d was measured. At day 32, all birds were fasted for 12 h before being killed by cervical dislocation and then stored at -20^{o}C for subsequent carcass analysis of moisture, fat and nitrogen.

Data on all measured traits were analysed by analysis of variance with main effects of Line, Sex and Diet using the General Linear Models procedure of SAS® (SAS Institute, 1997). Where effects were significant, differences between main and interaction effect means were determined using Least Significant Difference.

III. RESULTS

Although main effects of Line and Diet for 17-31 d weight gain, food intake and FCR were highly significant (P<0.001, Table 2), there were highly significant (P<0.001) interactions between these two factors, which constrained inferences drawn from the Line and Diet main effect comparisons for these traits.

Table 2. The effect of dietary protein (H=240 g/kg, L=120 g/kg) on 17-31 d weight gain (g), food intake (g) and FCR in the five lines

	Weight gain		Food intake		FCR	
Line	H	L	H	L	H	L
C	88.5 ^{ef}	42.2 ^{b}	273 ^{d}	205 ^{b}	3.13 ^{b}	5.07 ^{f}
FE	94.6 ^{fg}	35.2 ^{a}	245 ^{c}	164 ^{a}	2.61 ^{a}	4.88 ^{ef}
HF	85.5 ^{e}	60.1 ^{d}	304 ^{e}	269 ^{d}	3.58 ^{c}	4.54 ^{de}
LF	95.8 ^{g}	50.2 ^{c}	276 ^{d}	215 ^{b}	2.90 ^{ab}	4.30 ^{d}
HW	106.3 ^{h}	60.4 ^{d}	331 ^{f}	274 ^{d}	3.14 ^{b}	4.59 ^{de}
LSD $_{0.05}$	7.0		19		0.44	
Mean	94.1 ^{y}	49.6 ^{x}	286 ^{y}	226 ^{x}	3.07 ^{x}	4.68 ^{y}

Means within traits with different superscripts are significantly different ($^{a-h}$ P<0.05, ^{x,y} P<0.001)

On the high protein diet the HW line grew faster and ate more than all other lines but had intermediate FCR, whereas the FE line had intermediate growth rate but the lowest food consumption, and as a result, the lowest FCR of all lines. Although the HF line birds grew significantly slower than their LF line counterparts, they ate significantly more and as a result had the highest FCR of all lines. On the low protein diet, whilst all lines grew slower, ate less and had higher FCR than on the high protein diet, the relative performance of the individual lines changed considerably. Growth rate, food intake and FCR in the HF line was essentially similar to the HW line, whilst the FE line grew slower and ate less than all other lines and as a result, had the second highest FCR next to the Control line.

The principal contribution to the significant Line X Diet interaction in each case was the relative performance of the high fat-selected (HF) and feed efficiency-selected (FE) lines on the two diets. In each case, the difference in performance of the birds on the two diets was much smaller in the HF line than in the FE line. Differences in the three other lines were intermediate. Although females grew faster than males (5.22 cf 5.05 g/d, $P<0.05$), there were no differences between the sexes for food intake or FCR.

The effects of Diet and Line on the body composition measures in the five lines are shown in Table 3.

Table 3. The effect of dietary protein (H=240 g/kg, L=120 g/kg) on 32 d body moisture, fat and protein (g/kg) in the five lines

	Moisture		Fat		Protein	
Line	H	L	H	L	H	L
C	662 ^{cd}	659 ^{c}	81 ^{bc}	103 ^{de}	213 ^{cd}	197 ^{ab}
FE	700 ^{e}	683 ^{de}	55 ^{a}	87 ^{cd}	203 ^{abc}	190 ^{a}
HF	628 ^{ab}	607 ^{a}	106 ^{e}	139 ^{f}	222 ^{de}	215 ^{cd}
LF	676 ^{cd}	669 ^{cd}	66 ^{ab}	89 ^{cde}	215 ^{cd}	205 ^{bc}
HW	637 ^{b}	635 ^{b}	85 ^{cd}	108 ^{e}	229 ^{e}	212 ^{cd}
$LSD_{0.05}$	22		18		14	
Mean	661 ^{y}	651 ^{x}	79 ^{x}	105 ^{y}	216 ^{y}	204 ^{x}

Means within traits with different superscripts are significantly different ($^{a-f}$ $P<0.05$, ^{x,y} $P<0.001$)

Across lines and sexes, birds given the high protein diet had higher carcass moisture and protein and less carcass fat than their counterparts given the low protein diet ($P<0.001$). Across diets and sexes, the HF line birds were fatter and had less moisture than all other lines ($P<0.05$), but paradoxically, had more ($P<0.05$) protein than the C or FE lines. The FE line birds had more ($P<0.05$) carcass moisture than all other lines but less ($P<0.05$) fat than all lines other than the LF line and more ($P<0.05$) protein than all lines other than the C line. There was no effect of sex on any of the carcass traits, nor were there any significant ($P>0.05$) interactions between Line, Diet or Sex for these traits.

IV. DISCUSSION

The profound difference in relative performance of the FE line on the two diets suggests a much greater sensitivity to low dietary protein levels in this line and possibly, a higher protein requirement than in the other lines, as shown in the earlier study (Sutedjo *et al.* 2001b). The high-protein diet was essentially similar to the selection diet and the impressive direct selection response in the FE line appears to be only achieved in a high protein environment. Differences in fat deposition (Table 3) evidently contributed relatively little to

the diet-related responses in FCR in the FE line, although overall fat deposition in the FE line was lower than in all other lines (not significantly from the LF line).

Notwithstanding the indicated high protein requirement of the FE line, the proportion of protein in the carcass of the FE birds was lower than all other lines on both diets, whilst the proportion of moisture was highest. A comparison of the body composition and performance of the FE and LF lines provides an insight into the relative physiological responses to selection. On the high-protein diet, there was essentially no difference in growth rate over the test period between the two lines, but FCR and carcass fat and protein were lower and carcass moisture higher in the FE than the LF line. Individual selection for feed efficiency was thus at least as effective in reducing carcass fat as sib-selection for low fat; the lower energetic cost of body tissue (i.e high moisture) deposition in the FE line contributing to the lower FCR in this line. On the low-protein diet, growth rate was severely compromised in the FE line, which despite a reduction in food intake, resulted in a dramatic increase in FCR in this line. The considerable reduction in food intake in all lines on the low protein diet suggests that the amino-static mechanism of food intake regulation, is relatively weak.

Selection for increased fatness would appear to have reduced sensitivity to dietary protein level and possibly also to have reduced protein requirements. On the other hand, selection for reduced fatness does not appear to have affected sensitivity to dietary protein level as indicated by the relatively similar responses of the LF and Control lines. Similarly, selection for increased growth rate in the HW line does not appear to have affected sensitivity to dietary protein, although the earlier study (Sutedjo *et al.*, 2001b) indicated, surprisingly, a somewhat lower protein requirement in this line. Further study is underway of dietary protein utilisation in the lines.

In the case of selection for feed efficiency, the results pose the question of the effect of amino acid composition of the selection diet on response to variation in dietary protein. In most poultry species, commercial breeders are usually selected on high nutrient density starter/ grower diets, although results from selection studies for growth rate (e.g. Pym and Solvyns, 1981, using quail), question this approach and suggest that selection should perhaps be practiced on low nutrient density or low protein diets to optimise amino acid utilisation.

REFERENCES

Cahaner, A., Nitsan, Z. and Nir, I. (1986). *Poultry Science*, **65**:215-222.

Leclercq, B. (1988). In: *Leanness in Domestic Birds: Genetic, Metabolic and Hormonal Asapects.* Eds B leclercq and C.C. Whitehead, Butterworths, London, Pp 25-40.

Leenstra, F.R. and Pit, R. (1987). *Poultry Science*, **66**:193-202.

Pym, R.A.E. (1985). In: *Poultry Genetics and Breeding.* Eds W.G. Hill, J.M. Manson and D. Hewitt. *British Poultry Science Symposium 18*. Pp 97-112.

Pym, R.A.E. (1987). In: *Recent Advances in Animal Nutrition in Australia*, 1987. Ed D.J. Farrell, The University of New England, Armidale. Pp 222-227.

Pym, R.A.E. and James, J. (1979). *British Poultry Science*, **20**:99-107.

Pym, R.A.E., Popovic, B. and Bodero, D.A.V. (1998). *Proceedings of the 6th World's Congress on Genetics Applied to Livestock Production*, **24**:290-293.

Pym, R.A.E. and Solvyns, A.J. (1981*). Proceedings of the 4th Australasian Poultry and Stock Feed Convention, October 1981, Perth. Pp 242-247.*

Sorensen, P. (1984). *Ann. Agric. Fenn*. **23**:238-246.

Sutedjo, H., Knott, L., and Pym, R.A.E. (2001a.) *Proceedings of the Australian Poultry Science Symposium*, Ed D. Balnave. **13**:175-178.

Sutedjo, H., Knott, L., and Pym, R.A.E. (2001b). *Proceedings of the Queensland Poultry Science Symposium*, **10**: 8.1-8.6.

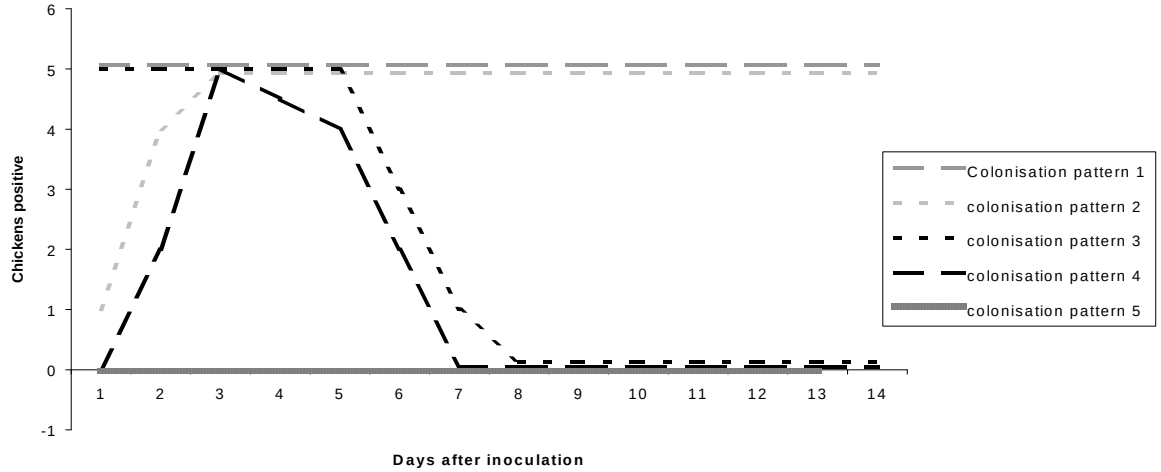
Whitehead, C.C. and Griffin, H.D. (1984). *British Poultry Science,* **25**:573-582.

FIVE DIFFERENT COLONISATION PATTERNS OF *CAMPYLOBACTER JEJUNI*

D.D. RINGOIR, D. SZYLO and V. KOROLIK

Ability to colonise is important in order for bacteria to be able to maintain themselves and propagate in the host. Chickens, which have encountered C. *jejuni*, maintain *C. jejuni* as part of their intestinal flora though it does not cause clinical disease. *C. jejuni* is recognised as the major cause of enteritis in humans, which is frequently related to the consumption of contaminated poultry (Blaser, 1997; Korolik *et al.*, 1998).

Various *C. jejuni* strains (27) isolated from chicken droppings and from patients suffering from enteritis were screened using a 2-day-old chicken model to determine colonisation patterns of the strains tested. Duplicate groups of 5 chickens were inoculated with 5×10^7 viable bacteria per chicken, and colonisation was screened by taking cloacal swabs and a final *post mortem* caecal sample. There were 5 different colonisation types observed, 1) immediate colonisation and prolonged excretion of viable *C. jejuni* bacteria (9 isolates: 3 chicken, 6 human), delayed colonisation and prolonged excretion of viable *C. jejuni* after several days (9 isolates: 3 chicken, 6 human), 3) immediate colonisation and slowly clearing excretion of viable *C. jejuni* bacteria (1 human isolate), 4) delayed colonisation and slowly clearing excretion of viable *C. jejuni* bacteria (3 isolates: 2 human 1 chicken), and 5) no colonisation of the intestines with *C. jejuni* bacteria (5 isolates: 4 human 1 chicken).



Colonisation type 1 and 2 led to sustained colonisation of the intestines of the chickens and, apart from pattern 3 expressed by only one isolate, none of the colonisation patterns was restricted to only one isolation source. In addition, the maximum caecal colonisation of various *C. jejuni* strains before and following a passage *in vivo* was determined. An increase in colonisation potential of 1000 fold was observed after a single passage *in vivo*, which is consistent with a previous study by Cawthraw *et. al*. (1996) The colonisation pattern of passaged strains were determined and the pattern remained the same. Enhanced colonisation potential may therefore account for the rapid rate of transmission within large flocks.

Blaser, M.J. (1997). *J Infect Dis*. **176:(**suppl**)** s103-105.

Cawthraw, S.A., Wassenaar, T.M., Ayling, R. and Newell, D.G. (1996). *Epidemiol Infect*. **117:** 213-215.

Korolik, V., Alderton, M.R., Smith, S.C., Chang, J. and Coloe, P.J. (1998). *Vet Microbiol*. **60:** 239-249.

School of Health Science, Griffith University Gold Coast Campus, Qld 9276.

STRUCTURAL ORGANISATION OF EMU SKIN: INFLUENCE OF SEX AND BODY REGION

K.A. WEIR AND C.A. LUNAM

The aim of this work was to describe the histological structure of emu skin and assess the influence of both sex and body region on skin structure. This was performed as part of a larger study which aims to determine how structural variation in skin affects the quality of the tanned product. Skins from 10 male and 10 female emus approximately 18 months of age were examined (mean carcass weight 17.4 kg). Emus were euthanised by electrical stunning and bled from the carotid artery. Feathers were plucked and skin was sampled from the rump, back and wing regions of each emu. Skin sections (5µm thick) were then stained for light microscopy. The thickness of dermal and epidermal layers were measured along with the density and orientation of collagen and elastic fibres within the dermis.

Emu skin thickness varied considerably between each region and within each skin sample. Frequent undulations of the skin surface, variation in the number of cell layers in the epidermis and variation in dermal thickness were observed. The epidermis consisted of a single layer of cuboidal cells known as the *stratum basale* plus two or more living cell layers, the *stratum intermedium*, *stratum transitivum* and the keratinous *stratum corneum*. The dermis may be divided into the *stratum superficiale* and *stratum profundum*. The *stratum superficiale* consisted of a layer of thin bundles of collagen fibres which lie predominantly parallel to the epidermis. The *stratum profundum* was comprised of a dense layer of connective tissue (*stratum compactum*) and an underlying layer of fat containing connective tissue, large blood vessels and the bulk of feather follicles and their associated muscle.

Emu skin thickness (µm)

	Male			Female		
	Rump	Back	Wing	Rump	Back	Wing
Whole skin	556 ±113	630 ±135	550 ±103	544 ±174	613 ±139	484 ±102
Epidermis	22 ±14	22 ±15	20 ±9	17 ±8	19 ±11	15 ±10
Keratin	6 ±5	6 ±8	5 ±4	5 ±4	5 ±6	5 ±5
Str. germinativum	15 ±11	15 ±8	15 ±6	12 ±6	14 ±6	11 ±6
Dermis	536 ±109	607 ±130	527 ±101	519 ±166	593 ±138	468 ±99
Str. superficiale	36 ±16	41 ±26	39 ±16	32 ±14	34 ±14	33 ±10
Str. compactum	500 ±102	569 ±120	487 ±96	486 ±161	559 ±137	434 ±95

(values are means ±SD)

Skin thickness data were examined by repeated measures analysis of variance (SPSS Inc). Males had significantly thicker ($P<0.05$) *stratum germinativum* and *stratum superficiale* than females. The thickness of whole skin, dermis and *stratum compactum* varied with body region and this was independent of the sex of the emu. In each case the back skin was significantly thicker than from both the rump and wing. Similar differences in skin thickness between these regions were described by Coleman and Dingle (1998) in tanned emu skin.

SPSS Inc. Chigaco USA.
Coleman and Dingle (1998) *Proc. Aust. Poult. Sci. Sym.* Ed. R.A.E. Pym.**10**: 184–187.
This work was supported by an Australian Postgraduate Award (Industry) – C09942100.
Department of Anatomy and Histology, Flinders University, Bedford Park, SA 5062.

INFLUENCE OF SEX ON NUTRIENT UTILISATION IN BROILERS FED DIETS WITH LOW OR ADEQUATE DIETARY PHOSPHORUS LEVELS

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

Summary

Energy utilisation and ileal nitrogen digestibility in male and female broilers fed diets deficient or adequate in phosphorus content were compared. Sex of broilers had no effect on the apparent metabolisable energy values determined during week 3. During week 6, the apparent metabolisable energy values for male broilers were higher ($P<0.01$) than those for the females. The differences ($P<0.01$) in favour of males remained, even after correction for zero-nitrogen retention. An interaction ($P<0.05$) between P level x sex was also observed where the apparent metabolisable energy value determined with male broilers was lower in the adequate phosphorus diet, whereas no effect of P level was observed in females. Female broilers tended ($P<0.10$) have a higher ileal nitrogen digestibility than the males. Significant ($P<0.01$) P level x sex interaction indicated that the ileal nitrogen digestibility differed between sexes at each phosphorus level. Males receiving the adequate-phosphorus diet had a lower nitrogen digestibility than those receiving the deficient diet, whereas the opposite was true in the females.

I. INTRODUCTION

Nutrient utilisation in broiler chickens is influenced by a number of factors that are related to the bird, feed, environment and husbandry. The bird-related factors that are relevant include genotype, age, sex and physiological status. Limited published data are available on the influence of sex on the nutrient utilisation in broiler chickens, but there is an increasing interest in this topic (Hughes, 2001; Hughes *et al*., 2001). Hughes (2001), in studies with 21-day old broiler chickens, found that the females were superior in their ability to digest and absorb energy. It was proposed that sex-related differences in gut morphology and gut microflora may be responsible for this superiority. In the present study, the apparent metabolisable energy (AME) and nitrogen digestibility in male and female broiler chickens fed diets containing deficient or adequate levels of phosphorus (P) were compared.

II. MATERIALS AND METHODS

Day-old broiler (Ross) chicks, females and males, were obtained from a commercial hatchery and randomly assigned to 20 pens (8 birds/ pen) in 3-tier electrically heated battery brooders. Within each sex (female or male), the two dietary treatments (low or adequate P levels) were randomly assigned to five pens of eight chicks each. The birds were transferred to colony cages in an environmentally controlled room on day 14. Room temperature was maintained at 32 ^{0}C during the first week and gradually decreased to 24 ^{0}C by the end of the third week.

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

For the broiler starter phase (1-21 d), the P-deficient diet was formulated to meet or exceed recommended specifications for all nutrients, except P and calcium (Table 1). The non-phytate P levels in the deficient and adequate diets were maintained at 3.0 and 4.8 g/kg, respectively. During the broiler finisher phase (22-42 d), the corresponding non-phytate P levels were 2.0 and 3.8 g/kg, respectively. The Ca: total P ratio was maintained at 1.4:1 in all diets. Titanium oxide was included in finisher diets as a dietary marker.

Table 1. Ingredient composition and calculated analysis (g/kg) of broiler starter diets.

Ingredient	Low P diet	Adequate P diet
Wheat	671.7	671.7
Soyabean meal	200.1	200.1
Canola meal	50.0	50.0
Monocalcium phosphate	7.3	15.8
Limestone	15.3	18.4
Sand	11.6	-
Common ingredients[1]	44.0	44.0
Calculated analysis		
AME (MJ/kg)	13.0	13.0
Lysine	11.5	11.5
Methionine + cysteine	9.4	9.4
Calcium	8.1	10.6
Total P	5.8	7.6
Non-phytate P	3.0	4.8

[1] Vegetable oil, 30.0; maize starch, 2.0; lysine.HCl, 3.5; DL-methionine, 4.0; salt, 2.5; trace mineral premix, 1.5 and vitamin premix, 0.5.

Feed was offered *ad libitum* and water was freely available at all times during the 42-day trial period. Body weights and feed intake were recorded on a pen basis at weekly intervals. During the third week (day 18-21) and sixth week (day 36-39), total collection of excreta was carried out for the determination of AME and nitrogen retention. On day 42, all surviving birds were euthanased and digesta contents from the lower half of the ileum were collected. Toe samples were also obtained for toe ash measurements. Apparent ileal nitrogen digestibility (AIND) values were calculated using the ratio of marker in the diet and digesta. The nitrogen-corrected AME (AME_n) values were calculated using a factor of 36.52 kJ per gram nitrogen retained in the body (Hill and Anderson, 1958). The data were treated by 2 x 2 factorial analysis within the GLM procedure of SAS for the effects of P level, sex and their interaction.

III. RESULTS AND DISCUSSION

Dietary nP levels influenced broiler performance and toe ash contents (Table 2). As expected, weight gains and toe ash contents of birds fed the adequate P diet were higher ($P< 0.001$) and the feed/gain were lower ($P<0.01$) than those fed the P-deficient diet. Weight gains were higher ($P<0.001$) and feed/gain was lower ($P<0.05$) in male broilers than in the females. Toe ash content, which is a good indicator of bone mineralisation (Potter, 1988), was similar ($P>0.05$) in the two sexes. P level x sex interactions were not significant ($P> 0.05$) for performance parameters or toe ash contents.

Though both diets were formulated to contain similar levels of AME, the determined values during week 3 showed that the AME of the P-adequate diet for broilers was lower ($P< 0.05$) than that of the P-deficient diet (Table 3). It is unclear why increasing the dietary P

level adversely affects energy utilisation, but similar observations have been previously reported (Ravindran *et al*., 1999). Perhaps the high molar ratio of calcium to phytate in adequate non-phytate P diets leads to the formation of insoluble calcium-phytate complexes, thereby contributing to the observed effects, but how the calcium-phytate complex lowers AME is difficult to explain. Ravindran *et al*. (1999) postulated that calcium-phytate may complex with fatty acids in the gut lumen to form insoluble soaps, thereby lowering fat digestibility and AME. Dietary P level had no effect on the AME values determined during week 6.

Table 2. Weight gain, feed/gain and toe ash contents of male and female broilers (1-42 days post-hatching) fed diets containing deficient or adequate levels of non-phytate phosphorus.

Treatment	Weight gain, g	Feed/gain, g/g	Toe ash, % dry basis
Males			
P-deficient diet	2452	1.911	9.68
P-adequate diet	2627	1.832	10.70
Females			
P-deficient diet	2139	1.944	9.71
P-adequate diet	2324	1.885	11.16
Pooled SEM	41.4	0.018	0.20
Source of variation			
P level	***	**	***
Sex	***	*	NS
P level x sex	NS	NS	NS

*** P<0.001; ** P<0.01; * P<0.05; NS Not significant.

Table 3. Apparent metabolisable energy (AME; MJ/kg DM), nitrogen-corrected AME (AME_n; MJ/kg DM) and apparent ileal nitrogen digestibility (AIND) of diets containing deficient or adequate levels of nP for male and female broilers.

Treatment	AME, 17-21 d	AME, 38-41 d	AME_n, 17-21 d	AME_n, 38-41 d	AIND
Males					
P-deficient diet	13.32	13.45	12.58	12.82	0.794
P-adequate diet	13.05	13.08	12.33	12.48	0.777
Females					
P-deficient diet	13.34	12.81	12.57	12.27	0.788
P-adequate diet	13.27	12.91	12.53	12.33	0.810
Pooled SEM	0.08	0.13	0.07	0.12	0.005
Source of variation					
P level	*	NS	†	NS	NS
Sex	NS	**	NS	*	†
P level x sex	NS	†	NS	NS	**

*** P<0.001; ** P<0.01; * P<0.05; † P<0.10; NS Not significant.

Sex of broilers had no effect on AME values determined during week 3. During week 6, the AME values for female broilers were lower (P<0.01) than those for the males. This finding is in contrast to previous reports where females were shown to utilise the feed energy

better than the males. Ten Doeschate *et al*. (1993) reported that female broilers showed a small, but significantly, better energy metabolisability than those of males (0.74 *vs* 0.73). Hughes *et al*. (2001) similarly reported that the energy value of wheat-based diets is influenced by sex, with female broilers showing significantly higher AME values (14.6 *vs* 14.9 MJ/kg DM). On the other hand, Wallis and Balnave (1984) found that sex had no major effect on metabolisable energy of a wheat-based diet for broiler chickens from 30 to 50 days of age. Guirguis (1975; 1976) found that sex had no significant effect on the AME of a range of feedstuffs, the exceptions being oats, tallow and fish meal where AME values were higher for females. In the present study, an interaction ($P<0.05$) between P level x sex was also observed; the AME values in male broilers tended to decrease when dietary P level was increased, whereas no effect was observed in females.

Correction for zero-nitrogen retention was carried out to exclude the possibility that the observed sex differences in energy utilisation during week 6 was the result of differences in nitrogen retention. Nitrogen correction had little effect on the magnitude of differences between the males and females, with differences ($P < 0.01$) in favour of males remaining.

It is difficult to propose a biological hypothesis that would explain the unexpected decreases in AME values determined with females as they get older. On the contrary, one would have expected the metabolisability to increase with age (Ten Doeschate *et al*., 1993).

Female broilers had a slightly better ($P<0.10$) ileal nitrogen digestibility than the males (Table 2). These findings are in agreement with those reported by Ten Doeschate *et al*. (1993) who found that female broilers showed nitrogen digestibility coefficients that were, in general, 3% higher than those of male birds. However, Wallis and Balnave (1984) reported that amino acid digestibilities were not influenced by the sex of broilers. The significant ($P< 0.01$) P level x sex interaction indicated that the AIND differed between sexes at each P level. Males receiving the adequate-P diet had a lower AIND than those receiving the deficient diet, whereas the reverse was true in the females.

The present data, when considered along with previous studies, suggest that the effect of sex on energy utilisation and nutrient digestibility in broiler chickens is inconsistent and inconclusive. In the present study, AME values were similar between sexes during week 3, but favoured the males during week 6. Ileal nitrogen digestibility, on the other hand, tended to favour the females.

REFERENCES

Guirguis, N. (1975). *Australian Journal of Experimental Agriculture and Animal Husbandry*, **15**: 773-779.

Guirguis, N. (1976). *Australian Journal of Experimental Agriculture and Animal Husbandry*, **16**: 691-695.

Hill, F.W. and Anderson, D.L. (1958). *Journal of Nutrition*, **64**: 587-603.

Hughes, R.J. (2001). In: *Recent Advances in Animal Nutrition in Australia*. Ed J.L.Corbett, **13**: 153-161.

Hughes, R.J., Choct, M. and Van Barneveld, R.J. (2001). In: *Proceedings of the Australian Poultry Science Symposium*, Ed D. Balnave. **13**: 30-38.

Potter, L.M. (1988). *Poultry Science*, **67**: 96-102.

Ravindran, V., Cabahug, S., Ravindran, G., Bryden, W.L. and Selle, P.H. (1999). *British Poultry Science*, **41**: 193-200.

Ten Doeschate, R.A.H.M., Scheele, C.W., Schreurs, V.V.A.M and Van der Klis, J.D. (1993). *British Poultry Science*, **34**: 131-146.

Wallis, I.R and Balnave, D. (1984). *British Poultry Science*, **25**: 401-407.

NUTRITIVE VALUE OF TRITICALE FOR BROILER CHICKENS IS AFFECTED BY VARIETY, WEATHER CONDITIONS AND GROWTH SITE

R.J. HUGHES[1] and K.V. COOPER[2]

Summary

The nutritive value of seven Australian triticale varieties was assessed in three energy balance studies each of 7-days duration with broiler chickens 22-29 days of age. Across all samples, apparent metabolisable energy (AME) of triticale averaged 14.15 MJ/kg dry matter and ranged from 13.83 to 14.62. Some varieties faired poorer than others due to unfavourable weather conditions during the grain-filling period in 2000/01. However, all varieties were relatively high in AME with no indication of a severe reduction in AME to less than 13 MJ/kg dry matter as a result of warm, dry conditions as has been observed for wheat in several studies in Australia in the past 20 years.

I. INTRODUCTION

The usefulness of triticale (genus *X Triticosecale)*, a synthetic cross between wheat and rye, as an ingredient in diets for broilers and layers was clearly established over 20 years ago by McKenzie and Farrell (1980). Since then, Farrell (1983), Johnson and Eason (1988) and Jones *et al*. (2000) have confirmed that the nutritive value of Australian triticale varieties has not diminished as a result of genetic selection for improved agronomic characteristics.

Hughes and Choct (1999) noted that variety, seasonal effects and growth site can significantly affect the nutritive value of cereal grains. In the case of triticale, there is little information available on the relative importance of these factors. This paper summarises the results of three experiments with chickens to evaluate the nutritive value of a total of seven different varieties of triticale grown in three separate localities in South Australia in the 1999/2000 and 2000/2001 seasons.

II. MATERIALS AND METHODS

All but two of the 22 samples of grains examined in these three experiments were obtained from breeding trial sites at Cleve, Lameroo and Callington in South Australia managed by the National Triticale Improvement Program based at Adelaide University. The exceptions were a sample of Tahara from the same paddock as the trial site at Cleve, and a sample of Tahara from a local producer in the Lameroo region in 2000/01. The average annual rainfalls for Cleve, Lameroo and Callington are 375, 400 and 400 mm, respectively. One of the sites, Cleve on eastern Eyre Peninsula, experienced warm, dry conditions during the grain-filling period in 2000/01. This resulted in an overall reduction in test weight (67.5 kg/hL) and starch content (656 g/kg) compared with Lameroo (76.1 kg/hL and 705 g/kg) in the Murray Mallee and Callington (76.8 kg/hL and 698 g/kg) in the eastern Mount Lofty Ranges. The grain yields in 2000/01 for the check variety Tahara were 2051, 2915 and 2901 kg/hectare at Cleve, Lameroo and Callington, respectively.

The AME values of triticale were determined in conventional energy balance experiments involving measurements of feed intake and excreta output as described by Mollah *et al.* (1983) with minor modifications, and subsequent measurement of gross energy values of

[1] SARDI, Pig and Poultry Production Institute, Adelaide University, Roseworthy, SA 5371.
[2] Department of Plant Science, Adelaide University, Urrbrae, SA 5064.

feed and excreta by bomb calorimetry. Day-old feather-sexed broiler chickens were raised in floor pens on a commercial broiler diet to 3 weeks of age then transferred in single-sex groups of five to metabolism cages in controlled temperature rooms. Experimental diets contained (per kg) 800 g triticale, 152 g casein, 20 g dicalcium phosphate, 11 g limestone, 7 g DL-methionine, 5 g mineral and vitamin premix, 3 g salt and 2 g choline chloride (60%). Dietary treatments were replicated four times (two cages of males and two cages of females) in Experiment 1 and six times (three cages of males and three cages of females) in Experiments 2 and 3. Cold-pressed diets were fed for seven days. The first three days enabled the chickens to adapt to the cages and feeds. During the following four days, all excreta were collected and dried at 85^{o}C. Moisture content of excreta voided over a 24 h period was measured. Feed intake was measured during the adaptation and collection phases of the study. Birds were weighed at the start and end of the 7-day period. Dry matter (DM) contents of samples of pelleted and milled feeds were measured. Gross energy values of dried excreta and milled feeds were measured with a Parr isoperibol bomb calorimeter. AME of the grain was calculated by subtracting from the total energy intake the energy contribution of casein, which was assumed to be 20.1 MJ/kg dry matter (Annison *et al*., 1994*).*

III. RESULTS AND DISCUSSION

The results of Experiment 1 which compared varieties selected for high test weight of grain (Treat and Everest) with lower test weight varieties (Tahara, Credit and Tickit) are summarised in Table 1. The largest difference in AME due to variety was 0.44 MJ/kg. There were no significant differences in feed intake or growth rate.

Table 1. Effects of triticale variety grown at Lameroo during 1999/2000 on grain test weight (TW, kg/hL), feed intake (FI, g/bird), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of triticale, and dry matter digestibility (DMD, g retained/g eaten) in Experiment 1.

Variety	TW	FI	GR	FCR	AME	DMD
Tahara	72.6	105	369	1.99 ^{a}	14.18 ^{b}	0.717 ^{b}
Tickit	71.4	104	378	1.93 ^{ab}	14.35 ^{ab}	0.725 ^{ab}
Treat	76.0	103	386	1.87 ^{bc}	14.62 ^{a}	0.737 ^{a}
Everest	74.5	104	392	1.86 ^{bc}	14.62 ^{a}	0.738 ^{a}
Credit	69.3	102	394	1.81 ^{c}	14.38 ^{ab}	0.725 ^{ab}
Pooled SEM		2	10	0.03	0.14	0.006

Means in a column having a common letter are not significantly different (P>0.05).

The effect of growth site on AME of triticale is shown in Table 2. The Cleve site had good rainfall during June and July leading to initiation of many tillers with high seed number, which were then unable to fill properly due to a dry August and warm, dry September. This provided an opportunity to compare a range of varieties grown under stressful conditions at Cleve and without stress at Lameroo during the grain filling period. AME values for triticale samples from Lameroo were generally higher than those from Cleve with the differences being significant (P < 0.05) for Treat and Everest, the varieties selected for high test weight. The largest difference in AME due to growth site was 0.56 MJ/kg for Everest, and the largest varietal differences were 0.44 MJ/kg at Cleve and 0.56 MJ/kg at Lameroo.

Table 2. Effects of triticale variety and growth site during 2000/01 on grain test weight (TW, kg/hL), starch content (SC, g/kg), feed intake (FI, g/bird), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of triticale, and dry matter digestibility (DMD, g retained/g eaten) in Experiment 2.

Variety	Site	TW	SC	FI	GR	FCR	AME	DMD
TX93-19-2	Cleve	63.0	-	99 ^{abc}	365 ^{bcd}	1.90 ^{c}	14.00 ^{bc}	0.710 ^{c}
	Lameroo	71.6	-	101 ^{ab}	373 ^{bc}	1.90 ^{c}	14.25 ^{ab}	0.728 ^{ab}
Tickit	Cleve	68.2	64.5	99 ^{abc}	356 ^{cd}	1.94 ^{bc}	13.92 ^{cd}	0.703 ^{cd}
	Lameroo	74.2	69.0	101 ^{ab}	361 ^{bcd}	1.95 ^{bc}	14.04 ^{bc}	0.716 ^{bc}
Treat	Cleve	73.2	63.9	99 ^{abc}	370 ^{bc}	1.87 ^{cd}	14.08 ^{bc}	0.711 ^{c}
	Lameroo	79.4	68.8	100 ^{abc}	342 ^{d}	2.04 ^{b}	14.49 ^{a}	0.737 ^{a}
Credit	Cleve	63.1	63.2	96 ^{c}	380 ^{abc}	1.77 ^{e}	13.64 ^{d}	0.691 ^{d}
	Lameroo	72.2	71.5	101 ^{ab}	363 ^{bcd}	1.96 ^{bc}	13.86 ^{cd}	0.712 ^{c}
Everest	Cleve	68.8	68.8	100 ^{ab}	401 ^{a}	1.75 ^{e}	13.86 ^{cd}	0.693 ^{d}
	Lameroo	79.0	-	102 ^{a}	376 ^{abc}	1.91 ^{c}	14.42 ^{a}	0.733 ^{a}
Tahara	Cleve	64.4	-	98 ^{bc}	382 ^{ab}	1.80 ^{de}	13.99 ^{bc}	0.694 ^{d}
	Lameroo	-	-	89 ^{d}	279 ^{e}	2.24 ^{a}	13.88 ^{cd}	0.716 ^{bc}
		Pooled SEM		1	9	0.03	0.10	0.015

Means in a column having a common letter are not significantly different (P>0.05).

The influence of seasonal effects on AME of triticale is evident by comparing AME values for the varieties Tickit, Treat, Credit and Everest grown at Lameroo in 1999/2000 (Table 1) and 2000/2001 (Table 2). The largest difference was 0.54 MJ/kg for the variety Credit. However, some of this difference could be attributable to quality of chickens used in different experiments which can affect AME as pointed out by Hughes *et al.* (2001). The effect of differences between batches of chickens can also be seen by comparing AME values for the Lameroo site in Table 2 with the corresponding values in Table 3. The same samples of each triticale variety were used in both experiments but were determined with different batches of chickens reared some months apart at PPPI.

Table 3. Effects of triticale variety and growth site during 2000/01 on grain test weight (TW, kg/hL), starch content (SC, g/kg), feed intake (FI, g/bird), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of triticale, and dry matter digestibility (DMD, g retained/g eaten) in Experiment 3.

Variety	Site	TW	SC	FI	GR	FCR	AME	DMD
TX94-46-1	Callington	80.6	69.5	93	327	2.00	14.47 ^{a}	0.750 ^{a}
	Lameroo	78.6	71.6	97	345	1.97	14.37 ^{ab}	0.745 ^{ab}
Tahara	Callington	76.6	70.6	93	317	2.06	14.01 ^{de}	0.730 ^{cde}
	Lameroo	-	71.6	90	284	2.29	13.81 ^{e}	0.725 ^{de}
Tickit	Callington	70.0	70.9	94	321	2.06	13.83 ^{e}	0.721 ^{e}
	Lameroo	74.2	69.0	93	322	2.03	14.16 ^{bcd}	0.736 ^{bcd}
Treat	Callington	80.0	71.2	93	311	2.10	14.06 ^{cde}	0.737 ^{bc}
	Lameroo	79.4	68.8	96	327	2.07	14.28 ^{abc}	0.743 ^{ab}
		Pooled SEM		2	15	0.07	0.09	0.004

Means in a column having a common letter are not significantly different (P>0.05).

AME and DMD for the variety Tickit differed between samples from Lameroo and Callington growing sites despite each sample having similar starch content (Table 3). This tends to suggest that other characteristics of the grain such as the soluble non-starch

polysaccharide content may have influenced the result. AME of other varieties did not differ due to site in this particular experiment.

The variety TX94-46-1 (Table 3), a breeding line under consideration for commercial release, has a relatively small, shiny grain as a result of selection for high test weight. Preliminary investigations by Rajinder Sharma (personal communication) indicate that this line has starch of lower amylose content, a property of grain associated with higher digestibility in pigs (van Barneveld, 1999) and possibly other monogastric animals. This variety had significantly higher AME and DMD than any other variety grown at Callington in 2000/01 (Table 3). Amylose content of starch was 216 g/kg in TX94-46-1 compared with 241, 249 and 263 g/kg in Tickit, Tahara and Treat, respectively.

IV. CONCLUSIONS

The results of three 7-day AME studies with broiler chickens 22-29 days of age indicate that variety, seasonal effects and growth site have significant influence on the nutritive value of Australian triticale. Furthermore, the largest differences observed between any two samples of triticale due to variety, season and locality were 0.64, 0.52 and 0.56 MJ/kg, respectively. However, these limited data suggest that triticale is less susceptible than wheat to adverse weather conditions experienced during the grain filling period which can result in a reduction in starch content and, hence, AME value of the grain.

V. ACKNOWLEDGMENTS

We gratefully acknowledge the technical support provided by Derek Schultz, Evelyn Daniels and Christine Adley (SARDI), and Michael Kroehn and Rajinder Sharma (Adelaide University). The Grains Research and Development Corporation provided financial support for the triticale breeding program and chicken studies.

REFERENCES

Annison, G., Choct, M. and Hughes, R.J. (1994). *Proceedings Australian Poultry Science Symposium,* (Ed. R.J. Johnson), **6**: 92-96.

Farrell, D.J. (1983). In: Feeding Standards for Australian Livestock – Poultry. Standing Committee on Agriculture technical Report. SCA, Canberra ACT.

Hughes, R.J. and Choct, M. (1999). *Australian Journal of Agricultural Research,* **50**: 689-701.

Hughes, R.J., Choct, M. and van Barneveld, R.J. (2001). *Proceedings Australian Poultry Science Symposium, (*Ed. D. Balnave), **13**: 30-38.

Johnson, R. and Eason, P. (1988). *Journal of the Science of Food and Agriculture,* **42**:95-108.

Jones, G.P.D., Jessop, R.S. and Cooper, K.V. (2000). *Proceedings Australian Poultry Science Symposium, (*Ed. R.A.E. Pym), **12**: 141-144.

McKenzie, R.J. and Farrell, D.J. (1980). In: Recent Advances in Animal Nutrition in Australia, pp 91-110. Ed. D.J. Farrell, University of New England; Armidale NSW.

Mollah, Y., Bryden, W.L., Wallis, I.R., Balnave, D. and Annison, E.F. (1983). *British Poultry Science,* **24**: 81-89.

Van Barneveld, R.J. (1999). *Australian Journal of Agricultural Research,* **50**: 667-687.

NUTRITIVE VALUE OF DWARF CHICKLING *LATHYRUS CICERA* CV CHALUS FOR LAYING HENS

R.J. HUGHES[1] and C.D. HANBURY[2]

Dwarf chickling *Lathyrus cicera*, a winter grain legume species new to Australia, has considerable potential as an alternative to field pea in rotation with cereals (Siddique *et al.*, 1996; Hanbury *et al.*, 1999). However, the presence in the seed of the neurotoxin 3-(-*N*-oxalyl)-L-2,3-diamino propionic acid (ODAP) restrains potential commercial releases to only lines having low ODAP levels. ODAP has been identified as the causal agent of lathyrism, a paralysis of the lower limbs, in humans and also in animals. Lathyrism occurs following consumption over an extended period of high levels of ODAP, and is most often associated with *L. sativus*. Recent studies have shown that ODAP concentrations in *L. cicera* (mean 1.8 g/kg) are typically lower than in *L. sativus* (mean 3.9 g/kg; Hanbury *et al.*, 1999). Chalus is a recently released cultivar of *L. cicera* with an ODAP concentration less than 0.1 g/kg.

This study examined the laying performance of Hyline Brown hens given diets containing *L. cicera* cv Chalus at 0, 50, 100, 150, 250 and 300 g/kg. Lathyrus replaced some of the wheat, peas and meat and bone meal in the control diet. Diets were formulated to contain (per kg) ME 11.5 MJ, protein 175g, calcium 37.5g, available phosphorus 4g, methionine 4.2 g and lysine 8.4 g. The hens were 26 weeks of age at the start of the 8-week experimental period. Hens were housed in groups of four in cages 50 cm wide and 54.5 cm deep. Two adjacent cages comprised an experimental plot. Each of the six diets was replicated 16 times. Eggs were collected daily, and feed intake was measured weekly. Eggs laid over three consecutive days in week 8 were used for measurements of egg weight, egg mass and shell thickness.

Lathyrus content of diet (g/kg)	Egg production (eggs/100 hen-days)	Feed intake (g/bird/day)	Feed efficiency (g feed/g egg mass)	Egg weight (g/egg)	Shell thickness (μm)
0	92.7	106.4	2.07	58.4	369
50	93.9	106.9	1.96	59.8	375
100	93.2	108.7	2.02	59.7	369
150	94.5	110.0	2.02	59.6	367
250	93.5	110.9	2.08	58.8	372
300	92.8	110.1	2.04	59.2	373
Pooled SEM	1.2	1.3	0.04	0.5	6

Dietary inclusion level of Lathyrus had no significant effects (P>0.05) on any of the measurements shown in the Table. The results indicate that dwarf chickling *L. cicera* cv Chalus is comparable in nutritive value to field peas for laying hens. Preliminary results from other studies at PPPI (data not shown here) suggest that there is no accumulation of ODAP in eggs, breast muscle or brain tissue from hens fed 300 g/kg *L. cicera* cv Chalus for 15 weeks. This will be examined in a 26-week feeding study due to finish in 2001.

Hanbury, C.D., Siddique, K.H.M., Galwey, N.W. and Cocks, P.S. (1999). *Euphytica*, **110**: 45.
Siddique, K.H.M., Loss, S.P., Herwig, S.P. and Wilson, J.M. (1996). *Aust. J. Exp. Agric.*, **36**: 209.

[1] SARDI, Pig and Poultry Production Institute, Adelaide University, Roseworth, SA 5371.
[2] CLIMA, University of Western Australia, Nedlands, WA 6009.

INFLUENCE OF POST-HARVEST STORAGE ON NUTRIENT UTILISATION OF WHEAT

V. RAVINDRAN[1], B.J. CAMDEN[1], D.V. THOMAS[1], G. RAVINDRAN[1] and S. JOHNSTON[2]

The new season grain phenomenon has received considerable attention in recent years and has been documented for Australian grains (Choct and Hughes, 1999). The current study was initiated to examine the post-harvest changes in apparent metabolisable energy (AME), apparent ileal starch digestibility (AISD) and apparent ileal nitrogen digestibility (AIND) of New Zealand wheats for broiler chickens. Five wheat cultivars, from the same general growing area in South Island, were collected at the time of harvest (1999/2000 season). A maize sample from the 1998/1999 harvest was included in the study as a control. The samples were stored at room temperature under well-ventilated conditions, and assayed for AME and nutrient digestibility at 1, 4 and 13 months post-harvesting. Assay diets contained 959 g/kg of the test cereal, supplemented with mineral and vitamin supplements. Titanium oxide (5 g/kg) was included in all diets as an inert marker for the estimation of nutrient digestibility. Each assay diet was fed to four replicate pens (five 28-day old male broilers per pen). Total excreta collection was carried out to determine the AME values and the results (as MJ/kg dry matter) are summarised.

	1 month	4 months	13 months
Wheat 1 (Consort)	13.5 (0.88, 0.78)[1]	13.8 (0.86, 0.80)	14.2 (0.91, 0.83)
Wheat 2 (Reaper)	13.2 (0.89, 0.77)	14.0 (0.93, 0.77)	14.6 (0.97, 0.81)
Wheat 3 (Ardnork)	12.7 (0.81, 0.74)	13.7 (0.86, 0.77)	14.2 (0.90, 0.79)
Wheat 4 (Equinox)	13.6 (0.87, 0.74)	13.8 (0.84, 0.74)	13.7 (0.88, 0.76)
Wheat 5 (Hussar)	13.0 (0.79, 0.76)	13.9 (0.84, 0.77)	13.8 (0.82, 0.78)
Maize	15.7 (0.93, 0.82)	15.7 (0.96, 0.83)	15.9 (0.96, 0.82)

[1] Values in parentheses refer to AISD and AIND values, respectively.

The AME contents of all wheat samples, except one (Equinox), were improved after 3 months of storage. The increments in AME ranged from 0.3 to 1.0 MJ/kg dry matter. Storage for a further 9 months improved the AME contents in three samples, but had no effect on those of Equinox and Hussar. The AME content of the maize sample was unaffected by storage. These results, in general, are consistent with the trends reported for Australian grains (Choct and Hughes, 1999). The AISD and AIND values increased with storage time, except in maize and one wheat (Equinox) sample. These increases generally paralleled improvements in AME contents. Poor ileal starch digestibility in some newly harvested wheats may be due to other factors within the grain that limit starch digestion (Wiseman *et al*., 2000) and the influence of these factors appear to be reduced during extended post-harvest storage.

Choct, M. and Hughes, R. (1999*).* In: Eleventh Australian Poultry and Feed Convention, pp. 233-239. Gold Coast, Qld.

Wiseman, J., Nicol, N.T. and Norton, G. (2001*). Wld's Poult.Sci. J.* **56**: 306.

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

[2] Tegel Foods, Newmarket, Auckland, New Zealand.

THE USE OF FEED ENZYMES IN WHEAT-BASED DIETS FOR LAYING HENS

J.R. ROBERTS, W. BALL and E. SUAWA

Summary

Four different commercial enzyme products were added to standard commercial layer diets, based on two different types of wheat. Wheat A had received adequate water prior to harvest and was "normal" and Wheat B had been water-stressed and was "pinched". Diets were fed to Isa Brown laying hens from twenty-five weeks of age. Measurements of egg and egg shell quality were conducted at 27, 30, 35, 40, 45 and 50 weeks of age. Apparent metabolisable energy and excreta moisture were measured every 5 weeks from 30 to 50 weeks of age. The AME of the diets was similar for the two types of wheat. AME increased to 40 weeks of age, remained relatively stable and then decreased slightly at 50 weeks. Excreta moisture was not affected by enzymes. Egg and egg shell quality varied significantly as the birds grew older and was significantly better for Wheat A than Wheat B. Shell breaking strength was slightly lower for Enzymes 1 and 2, shell reflectivity was higher for Enzymes 3 and 4 and percentage shell and shell thickness were higher for Enzyme 4. Yolk colour showed minor variation among the enzyme treatment groups. Production was not affected by type of wheat or enzymes. Both type of grain and the addition of enzymes have the potential to affect egg and egg shell quality.

I. INTRODUCTION

Enzymes are used in commercial layer diets to increase the digestibility of feed ingredients and reduce the incidence of wet droppings which may result from the presence of non-starch polysaccharides in the diets (Acamovic, 2001; Bedford and Morgan, 1996). Some ingredients present in feed bind other feed components such as phosphorus, calcium and trace minerals. Therefore, use of appropriate enzymes potentially increases the availability of these feed components, many of which influence egg shell quality (Hurwitz, 1987). Concern has been expressed about reduced egg shell quality resulting from the use of enzymes (Richards, 1998). However, a recent study showed that addition of commercial enzyme preparations improved egg shell quality in wheat- and barley-based layer diets but that there were some negative effects on shell colour and Haugh Units (Roberts and Choct, 1999; Roberts et al., 1999). In Australia, wheat is a common ingredient in layer diets. However, the quality and composition of Australian wheats are variable (Choct and Hughes, 1996). The present study was therefore conducted to investigate the effect of dietary enzymes and wheat quality on egg and egg shell quality in Isa Brown laying hens.

II. MATERIALS AND METHODS

Two basal diets were formulated to standard commercial specifications. Each diet contained 670 g/kg of wheat; diet A with "normal" wheat and diet B with water-stressed "pinched" wheat (B). The other ingredients were identical in the two diets. The basal diets were each used to prepare five experimental diets by adding one of four commercial feed enzyme preparations according to the manufacturers' instructions; a control diet of each wheat type had no enzyme added. Enzymes 1 and 2 were xylanase preparations whereas enzymes 3

Animal Physiology, School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351.

and 4 were multi-enzyme preparations. The diets were fed from 25 weeks of age to 760 Isa Brown laying hens which were maintained, three to a cage, in a commercial poultry house at the University of New England "Laureldale" Poultry Farm. The different treatment groups were randomised to avoid effects due to position in the poultry house.

Egg and egg shell quality were assessed at 27, 30, 35, 40, 45 and 50 weeks of age. At each age, 300 eggs were collected, 30 from each of the ten treatment groups. Egg and egg shell quality analyses were completed within 24 hours of the eggs being laid. Measurements taken to assess egg shell quality were egg weight, shell reflectivity (an indication of the colour of the egg shell), egg shell breaking strength (measured by quasi-static compression), deformation (the distance that the egg shell is depressed by the shell breaking strength machine before the shell cracks) and shell weight (Technical Services and Supplies, U.K.). The percentage shell was calculated as the ratio of shell weight to egg weight, expressed as a percentage. The internal quality of the eggs was assessed as albumen height and Haugh Units as well as yolk colour.

Apparent metabolisable energy (AME) and excreta moisture were measured every 5 weeks from 30 to 50 weeks of age. AME was determined by the conventional total collection procedure. Birds had received the experimental diets for at least 5 weeks prior to the AME assays which were conducted over 4 days. Feed intake was measured and all excreta collected daily. Excreta were dried in a fan-forced oven at 80ºC for 36 h and excreta from each replicate were pooled over the collection period for the determination of gross energy (GE). AME of diets was calculated as:

$$\frac{(\text{g of feed eaten} \times \text{GE of feed}) - (\text{g excreta voided} \times \text{GE excreta})}{\text{g feed eaten}}$$

Data were analysed by ANOVA with bird age, wheat type and enzyme treatment as independent variables. Differences between means were assessed by Fisher's (Protected) Least Significance Difference test. Significance was assumed at $P<0.05$.

III. RESULTS

It was anticipated that the two wheats would differ in the levels of non-starch polysaccharides. However, on analysis, the wheats were found to be similar for total, soluble and insoluble non-starch polysaccharides, although Wheat B was higher in free sugars. Protein analysis of both wheats and the control diets prepared from them showed that the crude protein level was higher for Wheat B (178 g/kg in the wheat, 228 g/kg in the final diet) than for Wheat A (149 g/kg in the wheat, 185 g/kg in the final diet). However, the average AME of the two diets from 30 to 50 weeks of age, was similar at 13.52 MJ/kg DM for Wheat A and 13.53 MJ/kg DM for Wheat B. For both diets, AME increased to 40 weeks of age ($p<0.0001$), remained relatively constant and then decreased slightly at 50 weeks (Table 1).

Table 1. Effect of hen age on AME (MJ/kg DM)

Age	30 weeks	35 weeks	40 weeks	45 weeks	50 weeks
AME	^{c}12.98±0.05	^{c}12.99±0.09	^{a}14.43±0.10	^{a}13.99±0.14	^{b}13.53±0.12

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

Feed intake (Wheat A 115.3 g/bird/d; Wheat B 112.7 g/bird/d) and excreta moisture (Wheat A 730 g/kg; Wheat B 715 g/kg) of birds on the two wheat diets were also similar, as was production. The addition of commercial enzyme preparations had no significant effect on AME, excreta moisture or production.

For egg and egg shell quality measurements, there were statistically significant main effects of age on most variables, as shown in Table 2. As hens became older, egg weight, shell weight, shell thickness and yolk colour increased whereas shell colour, shell breaking strength (BS), albumen height, Haugh Units, and percentage shell decreased.

Table 2. Effect of hen age on egg and egg shell quality

Trait	27 weeks	30 weeks	35 weeks	40 weeks	45 weeks	50 weeks
Egg Wt g	^{e}59.2±0.2	^{d}62.1±0.2	^{c}65.0±0.2	^{b}67.2±0.3	^{a}68.5±0.3	^{a}68.6±0.3
B.S. N	^{a}40.3±0.4	^{b}38.9±0.4	^{c}37.5±0.4	^{d}35.5±0.4	^{d}36.3±0.4	^{d}36.1±0.4
Reflect %	^{d}30.2±0.2	^{c}31.6±0.3	^{c}32.0±0.2	^{b}32.2±0.2	^{ab}32.8±0.2	^{a}33.3±0.3
% Shell	^{a}9.70±0.03	^{b}9.51±0.04	^{cd}9.32±.04	^{de}9.25±.04	^{c}9.38±0.04	^{e}9.20±0.05
Shell Wt g	^{f}5.74±0.03	^{e}5.89±0.03	^{d}6.05±0.03	^{c}6.20±0.03	^{a}6.43±0.04	^{b}6.31±0.04
Shell Thick	^{b}403.9±0.3	^{c}397.5±1.4	^{d}385.5±1.3	^{b}406.4±1.4	^{ab}409.5±1.8	^{a}411.8±2.0
Haugh Unit	^{a}98.0±0.3	^{b}95.3±0.5	^{b}94.4±0.4	^{c}91.9±0.4	^{d}89.4±0.4	^{e}87.2±0.4
Yolk Colour	^{c}11.04±.05	^{d}10.82±.05	^{b}11.37±.05	^{b}11.38±.05	^{b}11.40±.05	^{a}11.64±.06

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

Egg laid by birds receiving diets based on Wheat A had greater shell breaking strength, Haugh Units, percentage shell, shell weight and shell thickness, and darker shell colour than those given Wheat B (Table 3).

Table 3. Effect of wheat type on egg and egg shell quality

Wheat Type	Breaking Strength N	Reflectivity %	% Shell	Shell Wt g	Shell Thickness µm	Haugh Units
A	^{a}38.3±0.2	^{b}31.8±0.1	^{a}9.45±0.03	^{a}6.14±0.02	^{a}405.3±0.9	^{a}93.4±0.3
B	^{b}36.6±0.2	^{a}32.2±0.1	^{b}9.34±0.02	^{b}6·07±0.02	^{b}399.6±0.9	^{b}92.0±0.2

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

Enzyme type and/or inclusion had significant effects on shell breaking strength, shell reflectivity, yolk colour, % shell and shell thickness (Table 4). Shell breaking strength was slightly lower on diets with Enzymes 1 and 2 than for the control or Enzymes 3 and 4 diets. Shell reflectivity was slightly but significantly higher for diets with Enzymes 3 and 4. Percentage shell and shell thickness were highest for Enzyme 4 whereas yolk colour varied, being highest for the control and lowest for Enzyme 2.

Table 4. Effect of enzyme treatment on egg and egg shell quality

	Control	Enzyme 1	Enzyme 2	Enzyme 3	Enzyme 4
Breaking Strength N	^{a}37.8±.03	^{b}36.6±0.3	^{b}36.8±0.4	^{a}37.8±0.4	^{a}38.2±0.4
Reflectivity %	^{c}31.6±0.2	^{c}31.4±0.2	^{bc}31.8±0.2	^{b}32.0±0.2	^{a}33.2±0.3
Percentage Shell	^{b}9.35±0.04	^{b}9.30±0.03	^{b}9.35±0.04	^{b}9.40±0.04	^{a}9.57±0.04
Shell Thickness µm	^{b}401.5±1.4	^{b}400.0±1.3	^{b}401.3±1.5	^{b}402.3±1.7	^{a}407.3±1.5
Yolk Colour	^{a}11.48±0.05	^{b}11.25±0.05	^{c}11.03±0.05	^{b}11.27±0.05	^{b}11.35±0.05

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

IV. DISCUSSION

Although the two wheats were grown under different conditions and were different in appearance, they were very similar in total, soluble and insoluble NSP levels and had almost

identical AME values. However the crude protein level of the "pinched" wheat was higher, resulting in a higher crude protein content of the finished feed. The addition of commercial enzyme preparations had no effect on the AME value of either of the wheat-based diets, nor was there any significant effect on excreta moisture levels.

Egg and egg shell quality, in general, deteriorated with the age of the hens. Egg weight and shell weight increased although the increase in shell weight was not proportional to that of egg weight, resulting in a reduction in the percentage shell. Shell thickness decreased from 27 to 35 weeks of age and then increased up to 50 weeks. Haugh Units deteriorated with increasing hen age, and yolk colour increased overall. The age-related changes in egg and egg shell quality are similar to those reported previously (Roberts *et al*., 1997).

The diets based on Wheat A resulted in consistently better shell quality, darker shell colour and higher Haugh Units than those based on Wheat B. The reason for this is unclear. Wheat B had a higher crude protein level which resulted in a higher crude protein level in the finished feed.

The effects of commercial feed enzymes found in this study differ from those reported for a previous study (Roberts and Choct, 1999; Roberts et al., 1999). The improved shell breaking strength observed in the previous study in eggs from birds given enzymes was not found in the present study. This may be due to differences in feed ingredients used in the two experiments. However, the decrease in shell colour in response to dietary enzyme inclusion, was consistent across the two studies. Percentage shell and shell thickness were improved only by Enzyme 4 of the present study. Although yolk colour was at acceptable levels in all groups, it varied somewhat between the enzyme treatments and was highest in the control group.

In conclusion, both type of wheat and addition of commercial feed enzyme preparations had effects on egg and egg shell quality.

V. ACKNOWLEDGEMENTS

The support of the Egg Program of the Rural Industries Research and Development Corporation for this study is gratefully acknowledged. We thank Ridley AgriProducts, Tamworth, Australia for formulation and manufacture of the diets, the enzyme companies for their cooperation in this study, and colleagues and staff of the University of New England for advice and assistance.

REFERENCES

Acamovic, T. (2001). *World's Poultry Science Journal*, **57**: 225-242.

Bedford, M.R. and A.J. Morgan. (1996). *World's Poultry Science Journal*, **52**: 61-68.

Choct, M. and B. Hughes. (1996). *Proceedings of the Queensland Poultry Science Symposium* **5**: 6.1-6.6.

Hurwitz, S. (1987). In: *Egg quality – current problems and recent advances*. pp. 235-254. Ed. R.G. Wells and C.G. Belyavin, Butterworths, London.

Richards, G. (1998). *Proceedings of Alltech's 12th Annual Asia Pacific Lecture Tour*: 35-40.

Roberts, J.R. and M. Choct. (1999). *Proceedings of the VIII European Symposium on the Quality of Eggs and Egg Products*, Bologna, Italy: 113-118.

Roberts, J.R., M. Choct and W. Ball. (1999). *Proceedings of the Australian Poultry Science Symposium*, Ed. D.J. Farrell. **11**: 139-142.

Roberts, J.R., A.M. Leary, W. Ball and J.V. Nolan (1997). *Proceedings of the Australian Poultry Science Symposium*, Ed. D. Balnave. **9**: 126-129.

XYLANASE PLUS PHYTASE SUPPLEMENTATION OF BROILER DIETS BASED ON DIFFERENT WHEATS

P. H. SELLE[1], P. H. PITTOLO[2], R. J. GILL[1] and W. L. BRYDEN[1,3]

Summary

The combined effects of phytase and xylanase supplementation of diets based on two different wheats were investigated in a broiler growth study from 4-24 days post hatch. The diet based on wheat 'A' had a higher phytate content and lower apparent metabolisable energy (AME) than the wheat 'B' diet. Growth performance of birds receiving wheat 'B' diets was superior and was coupled with higher N retention. Xylanase plus phytase supplementation enhanced growth performance, AME, N retention and reduced gut viscosity. There were significant interactions between diets and enzyme inclusion for growth rate, feed efficiency and N retention. Higher phytate and presumably higher arabinoxylan contents of wheat 'A' and their negative effects on protein digestibility illustrate the importance of dietary substrate levels when evaluating broiler responses to feed enzyme supplementation.

I. INTRODUCTION

Wheat contains variable amounts of arabinoxylan and phytate; both components have anti-nutritive properties. Consequently, there is increasing industry interest in the use of phytase and xylanase feed enzymes in combination, to enhance digestion of wheat-based diets by broiler chickens. As wheat is the major cereal used in broiler diets, the possibility that differences in wheat composition may alter bird responses to enzyme supplementation was examined in this study.

II. MATERIALS AND METHODS

Day-old male broiler chicks (Cobb) were housed in battery brooders and offered experimental diets based on two different wheats (Table 1) in duplicated feeding studies. On day 4, birds were weighed and identified, and 48 birds per treatment were allocated to 8 units on the basis of body weight. Birds were weighed at 15 and 24 days post hatch, feed intake recorded and feed efficiency calculated. At 15 days the birds were transferred into wire-floored cages, and from 16-19 days total excreta was collected to determine AME and nitrogen (N) retention. On day 24 two birds closest to average pen weight were euthanased to determine intestinal viscosity. The diets were fed as mash and supplemented with 510 units of phytase (FTU) and 1976 units of xylanase (EXU) activity per kg diet, which are 85% and 36% respectively of standard inclusion rates. Analyses of phytase and xylanase activities of the experimental diets confirmed the accurate addition of the feed enzymes. Wheat 'A' in the first trial had a higher phytate content (8.5 g/kg) and a lower estimated AME content (13.1 MJ/kg DM) than wheat 'B' (6.2 g/kg and 14.8 MJ/kg DM). Data from both trials were pooled and analysed as a 2 x 2 factorial array for diet type and enzyme inclusion, by analyses of variance using general linear model procedures (SPSS Inc., Chicago, IL.).

[1]Faculty of Veterinary Science, The University of Sydney, Camden NSW 2570.
[2]Weston Animal Nutrition, Merrylands, NSW 2160.
[3]School of Animal Studies, The University of Queensland, Gatton, Qld 4343.

Table 1. Composition, specification and analysed values of the basal diets.

Ingredient	g/kg	Specification	g/kg
Wheat ['A' or 'B']	702.05	Protein	216.60
Meat & bone meal	57.50	Fibre	30.40
Soyabean meal	193.00	Calcium	8.40
Tallow	20.00	Total phosphorus	6.27
Sunflower oil	6.00	Available phosphorus	3.93
Limestone	3.50	AME (MJ/kg)	11.99
Salt	1.00	Lysine	12.93
Sodium bicarbonate	4.00	Available lysine	11.95
Lysine HCl	3.50	Methionine	5.40
DL-Methionine	2.35	Methionine + cystine	8.80
Choline chloride	0.35	Threonine	7.12
Potassium carbonate	1.75	Tryptophan	2.52
Mineral vitamin premix	5.00	Isoleucine	8.03
		Arginine	14.01
Analysed values – 'Wheat A' diet		*Analysed values – 'Wheat B'diet*	
Protein	211.9	Protein	223.0
Calcium	10.8	Calcium	8.2
Total phosphorus	7.7	Total phosphorus	6.0
Phytate-P[1]	2.50	Phytate-P[2]	2.13
Phytate[3]	8.87	Phytate[3]	7.55
Phytase activity (FTU/kg)	530	Phytase activity (FTU/kg)	365

[1]Calculated from phytate-P content of wheat (2.40 g/kg), soyabean meal (4.20 g/kg). [2]Calculated from phytate-P content of wheat (1.75 g/kg), soyabean meal (4.65 g/kg). [3]Phytate = 3.546 x Phytate-P.

III. RESULTS

Birds offered diets based on wheat 'B' had significant ($P<0.001$) increases in growth rate, feed intake and feed efficiency from 4-24 days post hatch, in comparison to chickens fed wheat 'A' diets (Table 2). Wheat 'B' diet was associated with higher N retention and AME values but gut viscosity was not significantly different. The combination of xylanase and phytase significantly ($P<0.001$) increased growth rate, feed intake, feed conversion, AME, N retention and reduced gut viscosity. Interactions between treatments were observed for growth rates ($P = 0.06$), feed efficiency ($P = 0.01$) and N retention ($P = 0.05$); enzyme effects were more pronounced in wheat A diets for these parameters. However, interactions were not recorded for feed intake ($P = 0.78$), AME ($P = 0.30$) or intestinal viscosity ($P = 0.78$).

IV. DISCUSSION

Bryden and Ravindran (1998) demonstrated that the simultaneous inclusion of phytase and xylanase in wheat-based broiler diets enhances protein and energy utilisation. Subsequent reports (Ravindran *et al*., 1999; Zyla *et al*., 1999) have supported this approach by showing improvements in growth performance and P utilisation. Responses observed in this study provide further justification for the simultaneous dietary inclusion of phytase and xylanase.

The phytate content of Australian wheat is variable; values of 37 samples ranged from 4.79 to 11.35 g/kg about a mean of 7.8 g/kg (unpublished data). The phytate content of wheats A and B were within this range at 8.5 and 6.2 g/kg respectively. While this difference appears subtle it may be important as increasing phytate levels of broiler diets based on wheat

and sorghum from 10.4 to 13.2 and 15.7 g/kg (Cabahug *et al.*, 1999) significantly reduced growth rate, feed intake and feed efficiency. Improvements in feed efficiency following phytase addition to weaner pig rations appear related to dietary substrate levels (Cadogan *et al.*, 1997; Selle *et al.*, 1997). The possibility that substrate levels are mediating responses to phytase supplementation emphasises the need to establish dietary phytate levels. On the other hand, variations in the AME content (Mollah *et al.*, 1983) and the relationship of this phenomenon to the non-starch polysaccharide content of wheat (Annison and Choct, 1991) are well recognised.

Table 2. Effects of diets based on different wheats with phytase[1] (510 FTU/kg) plus xylanase[2] (1,976 EXU/kg) supplementation on growth performance, intestinal viscosity, AME and N retention of broiler chickens from 4-24 days post hatch.

Treatments	Growth performance			Intestinal viscosity (cPs)	AME (MJ/kg DM)	N retention (%)
	Growth (g/bird)	Intake (g/bird)	Feed: Gain			
Wheat A diet						
Nil	767	1204	1.57	8.79	13.0	53.3
Phytase + xylanase	885	1290	1.46	3.73	13.9	58.4
Wheat B diet						
Nil	906	1326	1.46	10.21	14.2	61.0
Phytase + xylanase	957	1394	1.46	4.66	14.8	62.5
SEM	17.01	29.73	0.019	0.866	0.130	0.888
Significance						
Wheat-based diet	0.000	0.001	0.006	0.186	0.000	0.000
Phytase + xylanase	0.000	0.015	0.003	0.000	0.000	0.001
Interaction	0.060	0.777	0.009	0.780	0.302	0.053

[1]Natuphos®, [2]Natugrain® Blend, supplied by BASF Aktiengesellschaft.

Interactions between wheat composition and enzyme supplementation were observed for growth rate, feed conversion and N retention in this study. As this was not the case for AME and gut viscosity, it appears that the significant interactions were 'protein driven'. Phytate (Selle *et al.*, 2000) and arabinoxylan (Choct and Annison, 1992; Angkanaporn *et al.*, 1994) have a negative influence on amino acid utilisation and phytase (Ravindran *et al.*, 1999a) and xylanase (Hew *et al.*, 1998) have been shown to increase apparent ileal digestibility of amino acids. The higher phytate content and presumably higher arabinoxylan content (lower AME) of wheat A may have had a greater impact on protein digestibility. Thus more pronounced responses in growth, feed conversion and N retention following the inclusion of enzymes were observed with wheat A diets. It is relevant that phytase and xylanase have been shown to increase digestibilities of certain amino acids in a synergistic manner (Ravindran *et al.*, 1999b).

It is noteworthy that nearly three-quarters of phytate present in bread dough made from wheat has been detected in the soluble fibre fraction and not in the insoluble fibre fraction (Frolich and Asp, 1984). Also, there is indirect, *in vitro* evidence that xylanase increases the access of phytase to its substrate in the aleurone layer of wheat (Parkkonen *et al.*, 1997), where phytate is concentrated. Thus phytase and xylanase feed enzymes may facilitate access to their respective substrates. Moreover, phytase and xylanase may enhance

apparent ileal digestibility of amino acids by complementary modes of action by increasing digestibility of dietary protein and reducing endogenous amino acid losses. The simultaneous use of phytase and xylanase in wheat-based diets holds distinct promise, although responses to inclusion will be influenced by between batch variations in the phytate and arabinoxylan contents of wheat.

REFERENCES

Angkanaporn, K., Choct, M., Bryden, W.L., Annison, E.F. and Annison, G. (1994). *Journal of the Science of Food and Agriculture,* **66**: 399-404.

Annison, G. and Choct, M. (1991). *World's Poultry Science*, **47**: 232-242.

Bryden, W.L. and Ravindran, V. (1998). *Proceedings of the Nutrition Society of Australia*, **22**: 180.

Cabahug, S., Ravindran, V., Selle, P.H., and Bryden, W.L. (1999). *British Poultry Science*, **40**: 660-666.

Cadogan, D.J., Selle, P.H., Campbell, R.G., and Walker, A.R. (1997). *Manipulating Pig Production*, **6**: 245.

Choct, M. and Annison, G. (1992), *British Journal of Nutrition*, **67**: 123-132.

Frolich, W. and Asp, N-G. (1984). *Cereal Chemistry*, **62**: 238-242.

Hew, L.I., Ravindran, V., Mollah, Y. and Bryden, W.L. (1998). *Animal Feed Science and Technology,* **75**: 83-92.

Mollah, Y., Bryden, W.L., Wallis, I.R., Balnave, D and Annison, E.F. (1983). *British Poultry Science,* **24**: 81-89.

Parkkonen, T., Tervila-Wilo, A., Hopeakoski-Nurminen, M., Morgan, A., Poutanen K. and Autio, K. (1997). *Acta Agriculturae Scandinavica Section B,* **47**: 43-47.

Ravindran, V., Cabahug, S., Ravindran, G. and Bryden, W.L. (1999a). *Poultry Science,* **78**: 699-706.

Ravindran, V., Selle, P.H. and Bryden, W.L. (1999b). *Poultry Science*, **78**: 1588-1595.

Selle, P.H., Cadogan, D.J., Campbell, R.G. and Walker, A.R. (1997). *Manipulating Pig Production*, **6**: 244.

Selle, P.H., Ravindran, V., Caldwell, R.A. and Bryden, W.L. (2000). *Nutrition Research Reviews*, **13**: 255-278.

Zyla, K., Gogol, D., Koreleski, J., Swiatkiewicz, S. and Ledoux, D.R. (1999). *Journal of the Science of Food and Agriculture,* **79**: 1841-1848.

BROILER RESPONSES TO MICROBIAL PHYTASE AS INFLUENCED BY DIET TYPE AND DIETARY NON-PHYTATE PHOSPHORUS LEVELS

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

Summary

The effects of microbial phytase (0 or 500 units/kg diet), diet type (DT; maize- or wheat-based), dietary non-phytate phosphorus level (nP; 3.0 or 4.5 g/kg), and their interactions on performance and nutrient utilisation were investigated. Main effects of DT and phytase were observed for all traits. Main effect of nP was significant only for feed/gain and toe ash contents. The effects of phytase on toe ash contents and apparent nitrogen digestibility were influenced by dietary nP levels. The apparent metabolisable energy responses to added phytase were observed only in wheat-based diets. The lack of a significant DT x phytase interaction for most traits indicates that the influence of microbial phytase is similar in both diet types.

I. INTRODUCTION

Responses in broiler chickens to microbial phytase addition are influenced by *inter alia* dietary levels of calcium, non-phytate phosphorus (nP) and vitamin D (Ravindran *et al*., 1995). Efficacy of microbial phytase is probably different in different diet types, due to inherent variations in the concentration and structural or chemical properties of phytic acid. This aspect, however, has not been investigated. There have been numerous studies evaluating the efficacy of microbial phytase for broilers fed maize- and wheat-based diets (Coelho and Kornegay, 1999), but none in which the effects on the two diet types were examined in the same study. In the present study, the influence of phytase (Allzyme® phytase; Alltech Inc., Nicholasville, USA) addition on the performance, toe ash contents and nutrient utilisation of broilers fed diets based on maize or wheat was examined. This phytase product is produced in a solid-state fermentation process where natural *Aspergillus niger* organisms are grown on water-insoluble substrates in the presence of minimal free-flowing water.

II. MATERIALS AND METHODS

The experiment was conducted as 2 x 2 x 2 factorial arrangement of treatments. Two diet types (DT; maize-soy or wheat-soy) containing two levels of nP (3.0 or 4.5 g/kg) were evaluated and each level of nP was supplemented with either 0 or 500 PU phytase/kg diet. The nP level was increased by the addition of monocalcium phosphate. All diets were formulated to contain National Research Council (1994) recommendations for major nutrients, except the low-nP diets which had lower levels of calcium and nP. The Ca: total P ratio was maintained at around 1.4:1 in all diets. After mixing, the diets were cold pelleted (65 ^{0}C).

[1]Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.
[2] Alltech, Inc., Nicholasville, Kentucky, USA.

Each of the eight dietary treatments was fed to six pens of eight male broilers (Ross) from day 1 to 21 post-hatching. Body weights and feed intake were recorded on a pen basis at

weekly intervals. Feed conversion ratios were calculated by dividing total feed intake by weight of live plus dead birds.

During the third week, food intake and excreta outputs were measured quantitatively per pen over four consecutive days (day 17-21) for the determination of apparent metabolisable energy (AME). On day 21, all birds were sacrificed by intravenous injection of sodium pentobarbitone and, toe and digesta samples were obtained. Apparent ileal nitrogen digestibility (AIND) coefficients were calculated using the ratio of inert titanium marker in the diet and digesta. The data were analysed by the General Linear Models procedure of the SAS® (SAS Institute, 1997) with pen means as the experimental unit.

III. RESULTS AND DISCUSSION

Significant (P<0.05) effects of DT and phytase were observed for all traits, but the effect of nP was significant (P<0.05) only for feed/gain and toe ash contents (Table 1). Two-way interactions were significant (P<0.05) for a number of traits, but the three-way interaction was non-significant (P>0.05) for all traits.

Table 1. Influence of diet type, non-phytate phosphorus level and microbial phytase on the performance, toe ash contents and nutrient utilisation in broilers.

Dietary nP, g/kg	Phytase	Weight gain, g/bird[1,3,4]	Feed/gain, g/g[1,2,3]	Toe ash, % DM[1,2,3,5]	AME, MJ/kg DM[1,3,6]	AIND[1,3,7]
Maize-soy diet						
3.0	-	855	1.34	10.5	14.57	0.81
	+	899	1.32	11.6	14.64	0.83
4.5	-	883	1.31	11.7	14.53	0.81
	+	914	1.30	12.0	14.55	0.82
Wheat-soy diet						
3.0	-	793	1.49	11.0	13.62	0.74
	+	858	1.47	11.9	13.81	0.78
4.5	-	796	1.49	12.1	13.65	0.75
	+	805	1.46	12.2	13.93	0.78
Pooled SEM		13.4	0.01	0.13	0.06	0.007
LSD, P < 0.05		38.2	0.03	0.37	0.18	0.02

[1] DT effect (P<0.05), [2] nP effect (P<0.05), [3] Phytase effect (P<0.05), [4] DT x nP (P<0.05), [5] nP x phytase (P<0.05); [6] DT x phytase (P<0.05), [7] nP x phytase (P<0.10).

Birds fed the maize-based diets had greater (P<0.05) weight gains and better (P<0.05) feed efficiency than those fed the wheat-based diets. This is to be expected, partly because the wheat-based diets were not supplemented with exogenous xylanases. In general, weight gains and feed efficiency were improved by increase in dietary inorganic phosphorus and by phytase addition. However, a significant (P<0.05) DT x nP interaction was observed for weight gain. This was due to an increase in weight gain with increasing nP levels on the maize-based diets, but the opposite response on the wheat-based diets.

As might be expected, increasing dietary nP and phytase addition increased (P<0.05) the toe ash content of broilers, but the response to phytase addition was seen only in P-deficient diets, which resulted in a significant (P<0.05) nP x phytase interaction. Phytase addition improved AME values of wheat-based diets, but had no effect on AME of maize-

based diets resulting in a DT x phytase interaction ($P<0.05$). Responses in AIND to added phytase tended to be higher in P-deficient diets, as shown by a nP x phytase interaction ($P<0.10$).

The main aim of the current study was to test the proposition that broilers fed different diet types (maize-soy and wheat-soy) would respond similarly, in terms of performance and nutrient utilisation parameters, to phytase addition. The lack of significant DT x phytase interaction for most parameters indicates that the efficacy of microbial phytase was similar in both diet types. The only exception was the AME data that showed wheat-based diets to be more responsive to phytase supplementation than maize-based diets. Improvements in the AME of wheat and wheat-based diets with added phytase are consistent with previous reports (Ravindran *et al*., 1999). The exact mechanism of this AME effect is unclear, but improved digestibility of protein and starch may be responsible, at least in part, for these responses (Selle *et al*., 2000). Based on the observation that phytate is an integral component of the cell wall matrix in wheat (Frolich, 1990),) it has been also postulated that microbial phytase may be acting in a manner similar to that of exogenous xylanase, by disrupting cell wall matrix and enhancing contact between digestive enzymes and cell contents (Ravindran *et al*., 1999).

REFERENCES

Coelho, M.B. and Kornegay, E.T. (1999). *Phytases in Animal Nutrition and Management: A Practical Reference Manual*, Second Revised Edition, BASF Monograph Series. BASF Corporation, Parsippany, New Jersey.

Frolich, W. (1990). In: *New Developments in Dietary Fibre*, Eds I.Furda and C.J.Brine, Plenum Press, NY. pp. 83-93.

National Research Council. (1994). *Nutrient Requirements of Domestic Animals. Nutrient Requirements of Poultry*. National Academy Press, Washington, DC.

Ravindran, V., Bryden, W.L. and Kornegay, E.T. (1995). *Poultry and Avian Biology Reviews*, **6**: 125-143.

Ravindran, V., Selle, P.H. and Bryden, W.L. (1999). *Poultry Science*, **78:** 1588-1595.

SAS Institute. (1997). *SAS/STAT® User's Guide: Statistics*. Version 6.12. SAS Institute Inc., Cary, North Carolina.

Selle, P.H., Ravindran, V., Caldwell, R.A. and Bryden, W.L. (2000). *Nutrition Research Reviews*, **13**: 255-278.

THE USE OF THE AVIAN MODEL TO STUDY THE IMPACT OF MATERNAL DIETARY ω-3 FATTY ACIDS COMPOSITION ON THE BRAIN, HEART AND SPLEEN **C22:6ω3** STATUS OF GROWING BROILER CHICKENS

A.O. AJUYAH [1], Y. WANG [2], G. CHERIAN [3], H. SUNWOO [4] and J.S. SIM [4]

Summary

Broiler breeder hens were fed three diets that were low, moderate or high in ω3 fatty acids. The diets were based on the addition of 50 g/kg sunflower oil (diet1), 25 g/kg each of sunflower oil and fish oil (diet2), and 50 g/kg fish oil (diet3) to a wheat-soybean meal basal diet. Samples of brain, heart and spleen were taken at hatch and at 2 weeks of age from broiler chicks hatched from eggs produced by these hens. The trend for C22:6ω3 accretion were brain > heart > spleen at hatch and brain > spleen > heart at 2 weeks of age and diet 3 > diet 2 > diet 1($P<0.05$). In contrast, the trends for C20:5ω3 were spleen > heart > brain at hatch, and heart > spleen > brain at 2 weeks of age, but again diet3 > diet2 > diet1 ($P<0.05$). The pattern of incorporation of C22:6ω3 and C20:5ω3 reflected essentiality (structural and energetic) and maternal dietary composition. The practical implications for these observations are related to the role of these fatty acids in brain development, cardiovascular disease and immune response.

I. INTRODUCTION

In recent years there has been an upsurge in the use of the avian and animal models to study metabolic and physiological events that could be of benefit to humans and commercial livestock and poultry producers. An example is the study of fatty acid (FA) synthesis, accretion and utilization during embryonic or fetal development and growth. Most of these studies have provided additional information on the nutritional essentiality and importance of ω3-FA (Noble and Sand, 1985; Bowen and Clandinin, 2000). The ω3-FA, in particular docosahexaenoic acid (DHA, C22:6ω3) and the precursors of eicosapentaenoic acid (EPA, C20:5ω3) have been implicated with the prevention of vascular diseases, platelet function, inflammation, immunology, visual process, mental acuity, reproductive function and tumor inhibition in humans and animals (Sim, 1998; Cunnane *et al.,* 2000).

The usefulness of the avian model encompasses the following: (i) conducting experiments/ clinical procedures that cannot be performed using humans, (ii) short generation interval, (iii) multi-generation studies, (iv) self-contained nutritional environment of the egg, (v) low experimental cost, and (vi) anatomical and physiological similarities between the avian and mammalian species in the utilization and requirement for long chain polyunsaturated fatty acids (Cherian *et al.,* 1997; Innis, 2000).

Therefore based on the avian model, this research was designed to investigate pre and post maternal dietary effects on the accretion of EPA and DHA into the brain, heart and spleen of growing broiler chickens.

[1] The University of the South Pacific, School of Agriculture, Alafua Campus, Samoa.

[2] School of Dietetics and Human Nutrition, McGill University, Macdonald Campus, Quebec, Canada.

[3] Department of Animal Science, Oregon State University, Corvallis, Oregon, USA.

[4] Department of Agriculture, Food and Nutrition Science, University of Alberta, Edmonton, Alberta, Canada.

II. MATERIALS AND METHODS

This study was part of a larger study wherein fertile eggs were collected over a one-week period from artificially inseminated broiler breeder hens that were fed *ad-libitum* three experimental diets. The diets were: a wheat-soybean basal diet containing 50 g/kg sunflower oil, 25 g/kg sunflower oil plus 25 g/kg fish oil, and 50 g/kg fish oil as diets 1, 2 and 3 respectively. The eggs were incubated and one hundred newly hatched chicks from each treatment were distributed to floor pens and offered the same commercial broiler starter and finisher diets from hatch to 6 weeks of age (to delineate maternal dietary fatty acids residuum). Brain, heart and spleen tissues were collected at 0 (hatch), 2, 4 and 6 weeks of age from 5 broiler chicks per treatment which were killed by cervical decapitation, and all the samples were stored at –20^{o}C for future chemical analysis. All the tissue, feed and egg yolk samples were analyzed for fatty acid composition, using the direct methylation method described by Wang *et al.* (2000). The data were all analyzed by ANOVA, using the general linear model procedure of SAS. The remaining chickens were used to study maternal effects on selected economic and physical traits, including mortality from sudden death syndrome (data not shown).

III. RESULTS

The values for total ω6-FA composition (%) of the maternal dietary fatty acids were 65, 42 and 19, and for total ω3-FA 2, 10 and 19 for diets 1, 2 and 3 respectively. The major ω3-FA were EPA and DHA for diets 2 and 3, and C18:3ω3 for diet 1. The commercial broiler starter and finisher diets contained similar proportions of total ω6-FA (~28%) mainly C18:2ω6, and total ω3-FA or C18:3ω3 (~7%) from canola oil. Total ω6-FA concentrations in the fertile eggs (mg/g yolk) were 73, 50 and 23, and total ω3-FA were 3, 18 and 26 for diets 1, 2 and 3 respectively. The most abundant ω6 and ω3-FA in all the egg yolks were C18:2ω6 and C22:6ω3, in-addition to which, eggs from hens given diet 1 now contained other ω3-FA metabolites (C22:5ω3 and C22:6ω3). The DHA composition of the brain, spleen and heart at 0 and 2 weeks of age is shown in Figures 1 and 2. The trend for the organs were brain > heart > spleen at hatch, and brain > spleen > heart at 2 weeks of age. Differences between treatments were significant (P<0.05), with diet3 > diet2 > diet1 at both ages (P<0.05). Regarding EPA the trend was spleen > heart > brain at hatch and heart > spleen > brain at two weeks of age and the differences between treatments were significant (P<0.05), with diet3 > diet2 > diet1 at both ages (Figures 3 and 4).

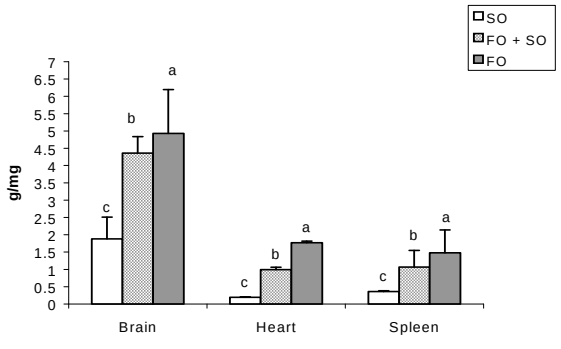


Figure 1. C22:6ω3 FA concentration of the brain, heart and spleen (mg/g) from newly hatched broiler chickens. [a-c] Columns with different superscripts are significant different at P<0.05.
SO = Sunflower Oil FO = Fish Oil

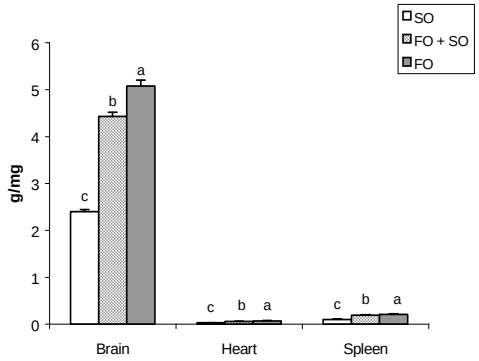


Figure 2. C22:6ω3 FA concentration of the brain, heart and spleen (mg/g) from two weeks old broiler chickens. [a-c] Columns with different superscripts are significantly different at P<0.05
SO = Sunflower Oil FO = Fish Oil

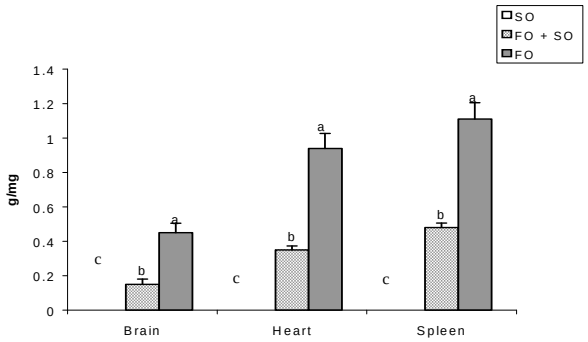


Figure 3. C20:5ω3 FA concentration of the brain, heart and spleen (mg/g) from newly hatched broiler chickens.
[a-c] Columns with different superscripts are significantly different at P<0.05
SO = Sunflower Oil FO = Fish Oil

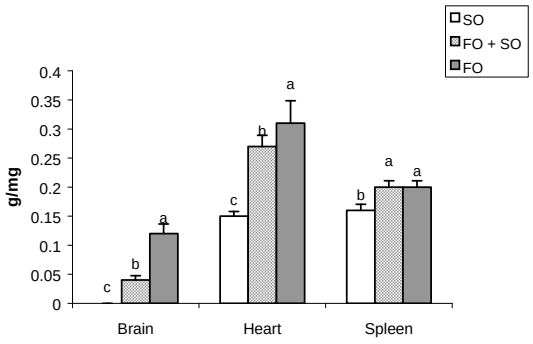


Figure 4. C20:5ω3 FA concentration of the brain, heart and spleen (mg/g) from two weeks old broiler chickens.
[a-c] Columns with different superscripts are significantly different at P<0.05
SO = Sunflower Oil FO = Fish Oil

IV. DISCUSSION

The egg as the only source of nutrients for the developing embryo, excluded other extraneous nutrient sources from invalidating the use of the avian model in this study. However, there were parallels between maternal dietary fatty acids composition with that of the fertile eggs and all the selected organs from the newly hatched chicks. The enrichment of the hen's egg with nutritionally desirable and function-specific fatty acids is a well-established concept, and this observation further confirms previous studies (Cherian *et al.*, 1997). The high concentration of DHA in the brain at 0 and 2 weeks of age, and wide variations between the brain, heart and spleen indicate the major structural role of DHA as opposed to an energetic role in the heart and spleen. The wide variation observed between treatments for brain DHA concentration is contrary to the observation of Crawford *et al.* (1989), who observed similar DHA levels across mammalian species despite large dietary variations. The large variation in brain DHA observed from our study was directly related to the wide ω6:ω3-FA ratio of the maternal diets. Post hatch residuum of DHA in the chick's brain up to 2 weeks of age underpins the importance of maternal dietary DHA status for brain growth and development. It has earlier been reported that brain accretion of DHA in humans increases dramatically during the last trimester of pregnancy and plateaus 2 years postpartum (Martinez, 1991), this is because 30% of the structural lipid of the cerebral gray matter is DHA. The heart and spleen seem to have greater requirements for EPA compared to the brain, which might indicate essentiality or requirements for ecosaniods production. The progeny diets were totally deficient in C20:4ω6, C20:5ω3 and C22:6ω3 because vegetable oil was the major fat source, therefore the chicks would have relied on their precursors (C18:2ω6 and C18:3ω3) for their production. Results show that at two weeks of age, chicks from DHA deficient parents incorporated EPA into the heart and spleen tissues, which indicates the ability of the chick to synthesise DHA from C18:3ω3. In contrast, no EPA was incorporated into the brain, which further supports the essentiality or specificity for DHA in the brain.

In summary, the brain, heart and spleen are major organs of the body that are involved primarily in mental acuity, blood circulation and immunity respectively. Their activity and functions are greatly influenced by membrane fatty acids composition *viz a viz* membrane permeability, which in turn is dependent on dietary fatty acid composition and concentration. The manipulation of dietary fatty acids, especially maternal fatty acids, will invariably affect membrane fatty acid composition of the brain, heart and spleen of the developing fetus or embryo and inevitably modulate certain physiological functions postpartum. Therefore, dietary intervention, in particular with ω3 FA has been proposed by some researchers as a strategy to reduce the incidence of certain diseases, improve mental acuity of infants and

modulate immune responses to inflammation (Sim, 2000). Currently, major sources of ω3 FA in human and animal nutrition include fish and fish products (C20:5ω3 and C22:6ω3), plant products - flax and canola oils (C18:3ω3), and modified poultry and animal products containing C20:5ω3 and C22:6ω3.

V. ACKNOWLEDGMENTS

The authors are grateful to the Natural Sciences and Engineering Research Council of Canada, Ottawa, ON K1A 1H5, the Poultry Industry Council of Canada and Omega-Protein Inc. for research funding supports, and the Omega Protein Inc. Reedville VA for donation of menhaden fish oil. We thank all staff in the Poultry Unit, University of Alberta, Edmonton, AB T6H 2V8 for supplying the basal diet and the assistance in the care and feeding of the animals used in this research. Appreciation is also given to Mr. Kim for laboratory assistance.

REFERENCES

Bowen, R. A. R. and M. T. Clandinin (2000). *Lipids,* **35**:389-394.

Cherian, G., N. Gopalarkrishana, Y. Akiba and J. S. Sim, (1997). *Biology of the Neonate,* **72**: 165-174.

Crawford, M. A., W. Doyle., G. Williams and P. J. Drury, (1989). *In*: The role of fats in human nutrition. Vergroesen, A. J. and M. Crawford, Eds. Academic Press. London.

Cunnane, S. C., V. Francescutti, J. T. Brenna and M. A. Crawford (2000). *Lipids,* 35:105-111.

Innis, S. M. (2000). *American Journal of Clinical Nutrition,* **71**:(1) 238-244.

Martinez, M. (1991). *In*: Simopoulos, A.P., R.R. Keifer, R. E. Martin, S. M. Barlow, eds, Health effects of n-3 polyunsaturated fatty acids in seafoods. *World Review of Nutrition and Dietetics,* **66**: 87-102.

Noble, R. C. and J. H. Shand, (1985). *Lipids,* **20**:278-282.

Sim, J. S. (1998). *World Review of Nutrition and Dietetics*, **83**: 89-101.

Sim, J. S. (2000). Personal Communication.

SAS Institute, 1990. SAS User's Guide: Statistics. Version 6.06 Edition. SAS Institute Inc., Cary, NC.

Wang, Y., H. Sunwoo, G. Cherian and J. S. Sim (2000). *Poultry Science,* **79**:1168-1171.

EFFECTS OF CONJUGATED LINOLEIC ACID ON BROILER GROWTH PERFORMANCE AND CARCASS COMPOSITION

J.A. DOWNING [1,2], S.J. WILKINSON [1], R.E. NEWMAN [1,2], W.L. BRYDEN [1,2] and P.H. SELLE [1]

Conjugated linoleic acid (CLA) acts as a nutrient partitioning agent in pigs, increasing lean tissue deposition and decreasing fat deposition (Ostrowska *et al*., 1999). In the present study we have investigated the effects of CLA on growth performance, breast muscle yield and abdominal fat weight of broilers.

Day-old male broiler chicks were allocated to one of seven dietary treatments, six birds per pen, and nine replicate pens per treatment. They were reared in brooders and fed *ad libitium*, starter diets from days 1-18 and then transferred to grower cages and fed grower diets from days 19- 42. Diets were based on wheat and sorghum with the control diet having 53.8 g/kg 'Sunola' oil. For CLA treatment diets, part or all of the of the 'Sunola oil' was replaced with 3.85, 7.7, 15.4, 23.1, 38.5, 53.8 g/kg of an oil mixture containing 600g/kg CLA isomers. This was equivalent to diets having 2.5, 5, 10, 15, 25 and 35 g/kg CLA respectively. Bodyweight and feed intake were measured weekly. On day 42, 12 birds from each treatment and having bodyweights near the mean for their treatment group were euthanased and the breast muscle and abdominal fat pad removed and weighed. Differences between treatments were assessed by analysis of variance and multiple comparisons made using the Tukey-Kramer test.

Treatment (g/kg CLA)	Body wt (g)	Feed intake (g)	FCR	Breast muscle (g/kg body wt)	Abdominal fat (g/kg body wt)
0	2638 ^{ab}	4277 ^{abc}	1.62 ^{d}	174.9^{a}	15.7
2.5	2586 ^{abc}	4315 ^{abc}	1.67 ^{cd}	183.3 ^{a}	13.7
5	2692 ^{a}	4557 ^{a}	1.69 ^{bcd}	175.6 ^{a}	16.0
10	2514 ^{bcd}	4333 ^{abc}	1.72 ^{abc}	182.7 ^{a}	15.0
15	2422 ^{cd}	4236 ^{bc}	1.75 ^{abc}	177.3 ^{a}	16.2
25	2364 ^{d}	4179 ^{c}	1.77 ^{ab}	181.0 ^{a}	15.0
35	1913 ^{e}	3448 ^{d}	1.80 ^{a}	155.0 ^{b}	15.0
Pooled SEM	39	67	0.022	3.4	1.3

Means in a column without a common superscript are significantly different (P<0.05)

Inclusion of CLA above 5 g/kg depressed bodyweight and food intake, and feed efficiency decreased linearly with increase in CLA. Breast muscle proportion was unaffected by increasing levels of CLA up to 25 g/kg, but dropped significantly in birds given the 35 g/kg CLA diet. Abdominal fat proportion was unaffected by CLA inclusion. The reduction in growth rate and feed efficiency on CLA levels above 5 g/kg suggests a possible toxic effect at these levels.

Ostrowska, E., Muralitharan, M., Cross, R.F., Bauman, D.E. and Dunshea, F.R. (1999*) J. Nutr*. **129**: 2037-2042.

1. Faculty of Veterinary Science, University of Sydney, Camden NSW 2570.
2. Affiliated with 'Smart Foods Centre', Wollongong University, Wollongong, NSW 2522.

EFFECT OF LIPOPOLYSACCHARIDE ADMINISTERED BEFORE SLAUGHTER ON MEAT COLOUR IN BROILERS

N.G. GREGORY[1] and T.A. NAGLE[2]

Summary

The effect of ante-mortem injection of *E. coli* lipopolysaccharide on heat-stable pinkness was examined in cooked chicken meat. The reason for the study was that lipopolysaccharide can induce nitric oxide formation, and its effects mimic the early stages of an infectious disease. Theoretically, if nitric oxide was present in sufficient amounts its derivatives could combine with myoglobin in muscle to form heat-stable pinkness (nitrosomyoglobin), which is objectionable amongst chicken meat consumers. Lipopolysaccharide had no effect on thigh meat colour in comparison with saline controls, but breast meat was slightly paler and less red.

I. INTRODUCTION

Nitrite, nitrate and nitric oxide can have a profound effect on meat colour, and nitrite and nitrate are used commercially during curing to preserve meat and enhance its colour. The effect that they have on colour depends on the redox state of the iron that is in myoglobin. When myoglobin is exposed to 25 ppm nitrite in the meat, metmyoglobin is formed. Metmyoglobin imparts a grey-brown colour, but if the iron is reduced to the ferrous state, it has a bright, heat-stable pink-red colour. The stability of the pinkness is increased at acidic pHs, and its rate of formation is accelerated in the presence of pro-oxidants such as ascorbate. In chilled meat, the formation of the pink colour takes time, and if the meat is cooked before that colour has developed, the meat loses its redness during cooking. In poultry processing plants, heat-stable pink pigments can be formed following exposure to excessive nitrate levels in the water of the spin-chiller. An essential step in this process is the conversion of nitrate to nitrite by ubiquitous bacteria which possess nitrate reductase. This process takes time if the carcasses are chilled, and requires a storage period after slaughter. The nitrite formed by these bacteria combines with myoglobin to form the pink nitrosomyoglobin (Morita *et al.*, 1998).

In the live animal, nitrite and nitrate can be formed under certain circumstances from nitric oxide. Nitric oxide itself is formed from arginine through the action of nitric oxide synthase (NOS), which is present in a wide range of tissues. NOS is present in two isoforms; a constitutive form which is present in nervous tissue, endothelial cells and platelets, and an inducible form which is present in macrophages, hepatocytes and tumour cells (Stuehr and Griffith, 1992). The activity of the inducible form is increased during immune responses through exposure to cytokines. Cytokine production occurs when the live animal is exposed to endotoxins from microorganisms, including lipopolysaccharide, which is a cell membrane extract from Gram negative bacteria. It is likely, therefore, that muscle could be exposed to large amounts of nitric oxide, and its derivatives, during the acute phase response of a generalised infection.

[1] SARDI, PO Box 1571, Flaxley, SA 5153, Australia.
[2] AgResearch, Private Bag 3123, Hamilton, New Zealand.

Inducible NOS is usually activated 4-12 hours after exposure to a toxin or cytokine, and once it is induced it can be stable for days. The induction process can be inhibited by anti-inflammatory agents such as corticosterone and dexamethasone (Johnson *et al*., 1996; Tracey, Tse and Carter, 1995). There are no known regulators of inducible NOS once it has been induced, but the formation of nitric oxide may be limited by the availability of L-arginine, and hence by arginase activity. Overproduction of nitric oxide by inducible NOS can result in dramatic hypotension through its relaxing effect on smooth muscle in blood vessels.

If nitric oxide is important in influencing meat quality, it seems likely that it would have to be formed in relatively large quantities. Nitric oxide is converted rapidly to nitrite and nitrate when in an aerobic aqueous phase, and so muscle or meat is more likely to be exposed to high concentrations of these oxygenated derivatives than to nitric oxide itself (Beckman and Koppenol, 1996).

II. METHODS

Forty-nine Cobb broilers were used in four treatments, with approximately 12 birds per treatment. The treatments were Saline (control), Dexamethasone, Dexamethasone + lipopolysaccharide, and lipopolysaccharide, which were injected before slaughter. The experiment started at 1630 h on Day 1. At that time, the Dexamethasone and the Dexamethasone + lipopolysaccharide-treated birds were weighed and injected intraperitoneally with 5 μM dexamethasone/kg. At 0500 h on Day 2, the lipopolysaccharide and the dexamethasone + lipopolysaccharide birds were weighed and injected with 0.8 mg *E. coli* 05.B55 (Sigma Chemical Co.), and the saline-treated birds were weighed and injected intraperitoneally with an equivalent volume of 0.9% sodium chloride. Feed and water were continuously available up to this time, but thereafter, only water was available. The behaviour of each bird was recorded at 15 minute intervals from 0600 to 0945 h. During this time the birds were in their original pen, which was provided with lighting (165 lux) and shavings as litter. From 1000 h the birds were slaughtered by neck dislocation plus bleeding, scalded, plucked, eviscerated and chilled in a crushed ice slurry. The average time between injection of the lipopolysaccharide and slaughter was 5.25 hours

Twenty hours after slaughter the carcasses were filleted and jointed. The meat was cooked in a waterbath oven to an internal temperature of 75^0C using a water temperature of 85^0C. Meat colour measurements were made with a Minolta L a b meter on a freshly cut surface of the cooked portions. The portions were cooked at 4 and 22 h after slaughter for the breast muscle (*pectoralis major*), and at 5 and 9 days after slaughter for the thighs. The breast fillet colour results were the mean of three measurements made on the same sample. Thigh meat colour was measured in five regions, two of which were red muscles over the lateral aspect of the femur, and the three other regions were predominantly white meat.

III. RESULTS

Two of the birds in the dexamethasone treatment were obviously sick before the dexamethasone was injected, and so they were removed from the study. The forty seven birds weighed 2.27 kg $\pm$ s.e., and there were no significant differences in liveweight between the treatments ($P>0.05$).

During the interval between injection and slaughter, the 47 birds spent approximately 94% of the time sitting on the floor. For 52% of that time the birds adopted a sleep-like posture. They were resting their heads on the ground and/or they had their eyes closed. The

birds that were treated with either lipopolysaccharide or dexamethasone + lipopolysaccharide, showed a higher incidence of sleep-like behaviour than the saline-injected birds (Table 1). The dexamethasone birds showed a similar incidence of sleeping as the saline treated birds.

Table 1. Incidence of sleeping in broilers treated with saline, dexamethasone, dexamethasone plus lipopolysaccharide, and lipopolysaccharide.

Treatment	Incidence of sleeping (%)
Saline	38[a]
Dexamethasone	40[a]
Dexamethasone + lipopolysaccharide	52[b]
Lipopolysaccharide	63[b]

Means with a different superscript letter were significantly different (P<0.05).

Table 2. Effect of the four preslaughter treatments on cooked meat colour.

	L	a	b
Breast – 4h after slaughter			
Saline	84.3 ± 0.2	3.77 ± 0.24 a	12.09 ± 0.15
Dexamethasone	84.3 ± 0.3	3.75 ± 0.36 ab	12.01 ± 0.25
Dexamethasone + LPS	84.0 ± 0.3	3.38 ± 0.16 ab	12.19 ± 0.10
LPS	84.0 ± 0.3	3.05 ± 0.12 b	12.28 ± 0.75
Breast – 22h after slaughter			
Saline	84.5 ± 0.1 a	2.82 ± 0.13	12.42 ± 0.20 a
Dexamethasone	84.6 ± 0.4 ab	3.38 ± 0.42	11.91 ± 0.29 ab
Dexamethasone + LPS	83.8 ± 0.4 ab	3.03 ± 0.23	12.08 ± 0.11 ab
LPS	83.7 ± 0.3 b	2.74 ± 0.17	11.65 ± 0.22 b
Thigh – 5d after slaughter, white and red muscle regions			
Saline	70.3 ± 0.8	10.68 ± 0.55	12.08 ± 0.24
Dexamethasone	71.6 ± 0.5	10.20 ± 0.47	11.61 ± 0.28
Dexamethasone + LPS	71.7 ± 0.8	10.22 ± 0.24	11.77 ± 0.35
LPS	70.7 ± 0.6	10.38 ± 0.41	11.68 ± 0.20
Thigh – 5d after slaughter, red muscle region			
Saline	62.9 ± 1.3	14.26 ± 1.13	11.93 ± 0.37
Dexamethasone	65.7 ± 1.2	13.99 ± 1.00	11.32 ± 0.38
Dexamethasone + LPS	65.2 ± 0.7	13.76 ± 0.62	11.47 ± 0.47
LPS	64.5 ± 0.8	14.31 ± 0.66	11.32 ± 0.26
Thigh – 9d after slaughter, white and red muscle regions			
Saline	70.3 ± 0.6 a	11.06 ± 0.23 a	11.69 ± 0.22 a
Dexamethasone	72.8 ± 0.5 b	9.82 ± 0.28 b	11.89 ± 0.15 ab
Dexamethasone + LPS	70.4 ± 0.4 a	9.99 ± 0.41 b	12.31 ± 0.18 b
LPS	69.6 ± 0.6 a	10.78 ± 0.66 ab	12.19 ± 0.20 ab
Thigh – 9d after slaughter, red muscle regions			
Saline	62.1 ± 0.8 a	15.59 ± 0.43 a	11.25 ± 0.37
Dexamethasone	67.0 ± 0.9 b	13.40 ± 0.50 b	11.43 ± 0.25
Dexamethasone + LPS	63.8 ± 0.8 a	14.30 ± 0.51 ab	11.99 ± 0.27
LPS	62.3 ± 1.2 a	15.28 ± 1.08 ab	12.09 ± 0.29

Means in a column within a cooked meat type which had a different adjacent letter were significantly different (P<0.05).

At 4 hours after slaughter the lipopolysaccharide treated broilers had lower 'a' values in the breast meat, and by 22 hours after slaughter the breast meat was slightly darker (lower 'L' value). These differences were small (Table 2).

In the thigh meat, there were no differences in meat colour by 5 days after slaughter. At 9 days, the dexamethasone treated birds had a paler (higher 'L' value), less red (lower 'a' value) meat, and this effect was largely due to differences in the red muscle region. The dexamethasone + lipopolysaccharide treated birds also had less red meat in comparison with the saline treated controls.

IV. DISCUSSION

At the outset of this study, it was anticipated that lipopolysaccharide would induce the formation of nitric oxide which, in turn, would result in the formation of heat-stable red pigments in the muscle. Nitric oxide formation was not monitored directly in the present study, but, in comparison with studies in other species, a relatively high dose of lipopolysaccharide was used, and the birds were slaughtered at a time interval after injection when nitric oxide formation should have been occurring (Nakamnra *et al,.* 1998; Takahashi *et al*., 1998; Macari *et al.*, 1993).

The meat colour results showed that lipopolysaccharide had no effect on cooked thigh meat colour in comparison with saline controls. The cooked breast meat was slightly paler and less red than the corresponding controls. The behavioural results indicated that the lipopolysaccharide did have an effect on the birds. There was more pronounced sleeping, but this was not reversed by dexamethasone. When dexamethasone was injected on its own, the thigh meat was paler and less red. This effect was particularly evident in the red muscle regions of the thigh, after it had been aged.

In conclusion, this study failed to implicate endogenous NO_x as a potential cause of heat-stable pinkness in chicken meat. Nitrate pick-up from the spin-chiller and under-cooking of the meat still remain the most likely causes of this problem.

REFERENCES

Beckman, J.S. and Koppenol, W.H. (1996). *American Journal of Physiology,* **271**: C1424-C1437.

Johnson, R.W., Propes, M.J. and Shavit, Y. (1996). *American Journal of Physiology,* **270**:R192-R198.

Macari, M., Furlan, R,L, Gregorut, F.P., Secato, E.R. and Guerreiro, J.R. (1993). *British Poultry Science,* **34**:1035-1042.

Morita, H., Sakata, R. and Nagata, Y. (1998). *Journal of Food Science,* **63**: 352-355.

Nakamnra, K, Mitarai, Y., Yoshioka, M., Koizumi, N., Shibahara, T. and Nakajima, Y. (1998). *Poultry Science,* **77**:908-911.

Stuehr, D.J. and Griffith, O.W. (1992). *Advances in Enzymology,* **65**: 287-346.

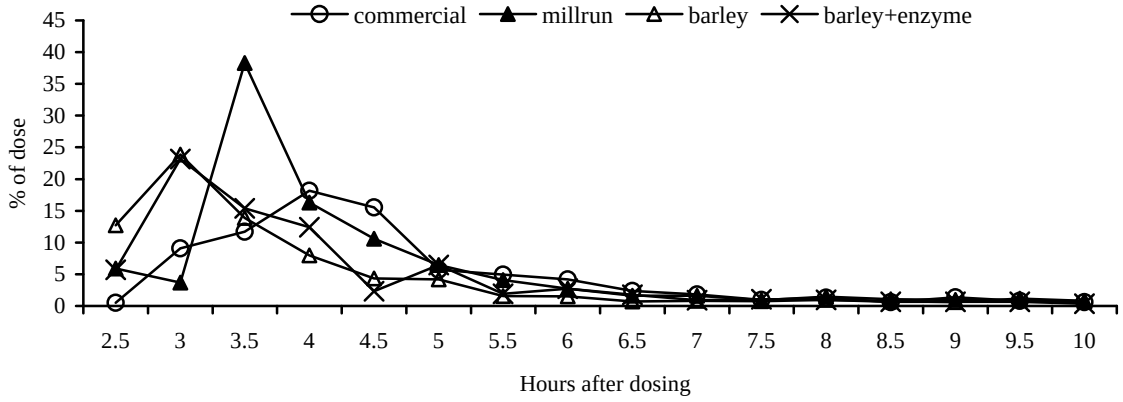
Takahashi, K., Miyake, N., Ohta, T., Akiba, Y. and Tamura, K. (1998). *British Poultry Science,* **39**:152-155.

Tracey, W.R., Tse, J. and Carter, G. (1995). *Journal of Pharmacology and Experimental Therapeutics,* **272**: 1011-1015.

DIETARY PREVENTION OF CANNIBALISM IN LAYERS

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

Severe beak trimming results in chronic pain in laying hens (Lunam and Glatz, 1995). Consequently, some European countries have either imposed a total ban or reduced the severity of beak-trimming. This has led to major outbreaks of cannibalism in birds housed in production systems, such as barn and free range, and welfare-friendly methods of controlling cannibalism, e.g., dietary management and low-light housing, are urgently required. We reported (Hartini *et al.*, 2001) that cannibalism mortality in untrimmed birds fed a commercial diet was 28.9%, but was 14.3%, 15.9% and 17.8%, respectively, in birds fed diets containing higher levels of fibre. To investigate the mechanisms whereby high fibre diets prevent cannibalism, we used the same four diets (Diet 1: commercial; Diet 2: Millrun; Diet 3: Barley, and Diet 4: Barley + enzyme) used by Hartini *et al.* (2001) and determined digesta viscosity and transit time in ISA Brown hens at 42 weeks of lay. Eight birds per diet were kept in individual cages and fed the experimental diets for 7d. On d8, all birds were fasted for 2h and given 10g of their diet containing a digestibility marker (400 mg/kg alkane $C_{36}H_{74}$). After two hours, excreta were collected every 30 min for 8h, alkane concentrations were determined and digesta transit time calculated (Figure). The birds were fed their respective diets for a further 7d and were killed and viscosity measures made on their gut contents. The ileal viscosity values (mPa.s) for Diets 1, 2, 3, and 4, were 8.4, 5.6, 4.8 and 3.5, respectively, and were significantly ($P<0.01$) different from one another.



Digesta transit rate was faster (between 3-3.5h) in birds fed the higher fibre diets than in those fed Diet 1 (4-4.5h). The maximum excretion of the marker from birds fed Diet 2 occurred in a very narrow time frame about 3-4 hours post dosing, whereas it was scattered over a wider window (3-5h after dosing) for those fed Diet 1. It appears that the high level of insoluble fibre in Diet 2 shortened food transit rate, whereas the higher level of soluble fibre in the other diets lengthened it. A slower transit rate of food caused by viscous digesta could induce "gut fill" and satiety before birds completely meet their requirements for essential nutrients and energy. Nutrient deficiencies and extended periods between eating bouts could predispose the birds towards cannibalistic behaviours.

Lunam, C. and Glatz, P.C. (1995). *Aust. Poult. Sci. Symp.* **7:** 176-179.
Hartini, S. Choct, M., Hinch, G. and Nolan, J.V. (2001). *Aust. Poult. Sci. Symp.* **13:** 216-219.

School of Rural Science and Agriculture, University of New England, Armidale, NSW 2351.

BEHAVIOURS OF HENS IN FURNISHED AND CONVENTIONAL CAGE SYSTEMS

C. RUDKIN and G.D. STEWART

Summary

Time-lapse videos of hens' behaviours in "furnished" Edinburgh cages and in conventional Harrison cages were viewed to develop methods for assessing cage space use and relative behavioural welfare status. In Edinburgh cages, hens laid in both the nest and litter box. There was little or no increase in activity before laying. The litter box was used for dustbathing and as a refuge. "Vacuum" dustbathing sometimes occurred outside the litter box on the floor or perch. The perch was used approximately 40% of the time, but the area behind the perch was rarely entered. Before laying in Harrison cages, hens' activity levels increased and they often attempted to creep under cage mates.

There was great variability between hens' behaviours within cages. Individuals varied in how much they sat, preened, fed, drank, and moved about the cage. Feather pecking, stereotypies, and aggressive behaviour were encountered in both cage systems.

I. INTRODUCTION

This pilot study aimed to develop techniques for collecting and analysing data from time-lapse videos of the behaviours of hens in different housing systems to assess cage space use and to determine relative behavioural welfare status. Careful observation could promote better design, and debug myths. Informed recommendations could then be made that would have optimum impact on welfare, and changes that have dubious or minimal benefits would be avoided.

Edinburgh cages provide a perch, nest, and litter box. Since modified cages are specifically designed to improve behavioural welfare, it is necessary to observe how behaviours are affected to determine if these systems do indeed improve welfare status.

This is a report of an ongoing pilot study of the behaviours of two strains of laying hens in Edinburgh and conventional Harrison cages. Data have been gathered from time-lapse videos of behaviours that could enable assessment of how facilities are used, and to indicate whether behaviours indicative of barren housing differ between the two environments.

II. METHODS

Data on the behaviours of laying hens approximately 30 weeks of age were obtained from time-lapse videos, each taken over the entire light period. Four Tegel hens (a heavy black strain) and four Aztec hens (a light white leghorn strain) in four of 18 experimental Edinburgh cages, and two of eight experimental Harrison cages in a shed at Toowoomba have been observed. Lighting was natural and artificial, with a day length of 16 hours. Layer mash was topped up daily. The Harrison cages measured 500 mm across the front, and 450 mm deep. The Edinburgh cages measured 950 mm across and 360 mm deep, contained a perch across the main part of the cage, a nest with a litter box above it at one side of the cage, and 700 mm of feeder space.

Data were obtained from the videos for the first and last half hour of the light period,

School of Animal Studies, The University of Queensland, Gatton, Qld. 4343.

a half hour period every two hours during the light period, and a half hour period following topping up of the food trough. Where egg laying was observed, behaviours and amount of activity (as measured by position changes) of the hen concerned was recorded for approximately the previous 2 hours and the following 30 minutes. The entire tape was viewed for use of the nest and litter box by each individual. Data were collected by scan sampling and by continuous monitoring to note bouts of rarer and more sporadic behaviours (Martin and Bateson, 1986). The behaviours measured by scan sampling included feeding, drinking, preening, sitting, standing, and position changes. Behaviours measured by continuous monitoring included bouts of non-aggressive feather pecking, aggressive pecking, comfort movements (stretch, feather ruffle, bilateral wing raise) and vertical wing shaking associated with dust-bathing.

III. RESULTS

(a) Egg laying

Amount of movement about the cage by hens (particularly Aztecs) in Harrison cages increased before laying an egg (Figure 1a). Close to the time of laying, most hens attempted to creep under cage mates.

Hens in Edinburgh cages laid in either the litter box or the nest and paid most attention (by looking) to the site of choice for some time before laying. Movement about the cage before entering the nest did not increase or increased slightly (Figure 1b). The maximum time a bird occupied the nest or litter box to lay an egg was 2h19min, and the median time was 41min.

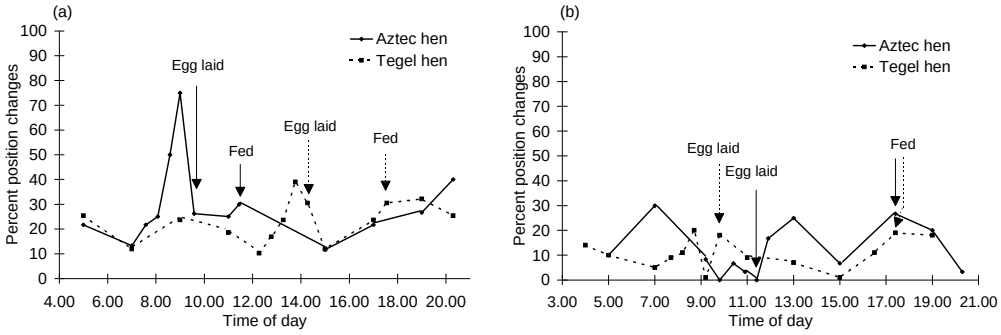


Figure 1. Amount of activity as measured by percent of position changes by an Aztec hen and a Tegel hen in (a) Harrison cages, and (b) Edinburgh cages.

(b) Use of facilities in Edinburgh cages

Perch. Hens generally perched for approximately 40% of the time. At lights on, usually only half the birds were perching. Drinking, and preening were mostly done on the perch, but the hens were unable to reach the food from the perch. Tegel hens in one of the cages occasionally went behind the perch, but this area was not entered by hens in the other three cages.

Litter box. The litter box was sometimes used as a nest, for dust bathing, pecking litter, and as a refuge by bullied individuals (e.g. hen 1 in Figure 2). In one cage, a Tegel hen was apparently "brooding" in the litter box. It was unoccupied for 95% of the day when not used as a refuge or nest. When used as a refuge, it was unoccupied for 34.2% and 46.3% of the day. Hens have been observed "vacuum" dustbathing on the wire floor at the front, or on the perch, even though the litter box was unoccupied. The median time individual hens

occupied the litter box was 2.3% of the day (range 0% to a maximum of 83.2% by the "broody hen.

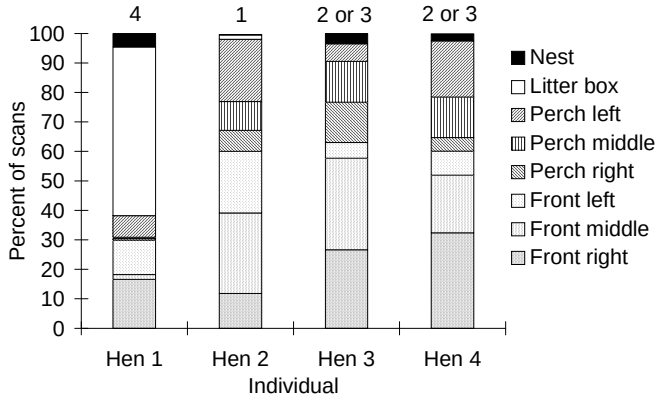


Figure 2. Percent of scans that Aztec hens were in different positions in the Edinburgh cages 1. The number at the top of the columns indicates social status as far as could be determined

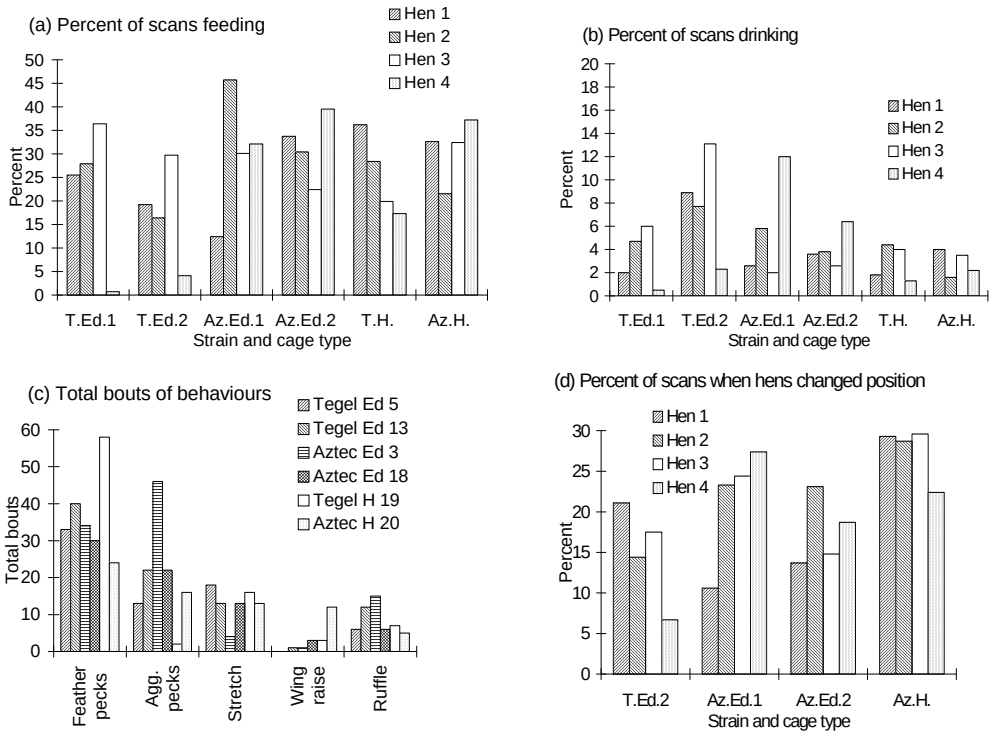


Figure 3. Behaviour rates in different cages. (T., Tegel hens, Az., Aztec hens; Ed, Edinburgh cage; H., Harrison cage).

Nest. The nest was used primarily for egg laying and was entered rarely at other times (Figure 2). Individual hens occupied the nest for 0% to 25.7% of the day, with a median of 4.9% of the day. The nest was unoccupied for 53% to 95% of the day depending on the group.

(c) Behaviour rates

Feeding, drinking, feather pecking, comfort behaviours, and amount of movement varied considerably amongst individuals within cages (Figure 3a,b,d). Some individuals

showed stereotyped behaviour associated with excess drinking in Edinburgh cages (Figure 3b). Feather pecking, aggressive pecking, stretching, partial wing raise, and feather ruffling occurred in both cage types (Figure 3c).

IV. DISCUSSION

Behaviours indicative of frustration around laying time were not seen in Edinburgh cages. These preliminary data indicated that the welfare of individual birds with a nest was improved compared to birds without a nest for around 5% of the day. The perch was the site of choice for preening and drinking. Not all birds were on the perch at lights on, and it reduced use of the entire cage space. Free-range adult hens rarely rest or perch during the day (Rudkin, 1998).

Hens could be prevented from using the litter box as a nest by keeping it closed at night and morning as is usually recommended. However, bullied hens were unable to access food or water from this site (see Tegel hen 4 in Edinburgh cage 2, and Aztec hen 1 in Edinburgh cage 1, Figure 3). Apart from use by bullied hens and as a nest, it remained empty for much of the day. Some individuals "vacuum" dustbathed outside the litter box.

The time spent at the food trough, and the incidence of stereotypies and feather pecking are known to be greater in unenriched environments (Appleby *et al.* 1989; Blokhuis *et al.*, 1993; Rudkin, 1998). However, hens in Edinburgh cages spent a large part of the time at the food trough, they feather pecked, and some showed stereotyped behaviour associated with drinking to excess. These preliminary data indicated that feeding, drinking, feather pecking, aggression, moving about, and comfort behaviours vary considerably between individuals, in both the Edinburgh and Harrison cages.

Data so far gathered indicate that the presence of a nest helped prevent pre-lay frustration, but in other ways the Edinburgh cages were not noticeably "enriching" as compared to Harrison cages. This study has developed techniques to obtain data from time-lapse videos of the behaviours of laying hens and their interaction with cage resources in conventional and furnished cages. Such data can be utilised to better understand the welfare of laying hens and how hens use resources in conventional and modified cages.

V. ACKNOWLEDGEMENTS

The work was carried out under a research grant from the RIRDC, and supported by the School of Animal Studies at the University of Queensland Gatton.

REFERENCES

Appleby, M.C., Hughes, B.O., and Hogarth, G.S. (1989). *British Poultry Science,* **30:** 545-553.

Blokhuis, H.J., Beuving, G., and Rommers, J.M. (1993). In: Savory, C.J. and Hughes B.O. (eds.), *Fourth European Symposium on Poultry Welfare* pp 19-26. Universities Federation for Animal Welfare: Potters Bar, Great Britain.

Martin, Paul, and Bateson, Patrick (1986). Measuring Behaviour. Cambridge University Press, Cambridge.

Rudkin, Clare (1998). Feather pecking, exploratory behaviour, and environmental enrichment of chickens. *A Thesis for the degree of Doctor of Philosophy, University of Queensland Gatton.*

EVALUATION OF A WELFARE AUDIT FOR BROILER GROWERS

J.L. BARNETT[1], P.H. HEMSWORTH[2], E. SELBY[1] and A. ALMOND[3]

As part of a project to develop a welfare audit for all sectors of the Australian chicken meat industry an evaluation was undertaken of part of the documentation to determine if there were any improvements in welfare. Twenty-four broiler farms contracted to one company and located about 100 km south west of Melbourne were used in the study. The company provided production data and the farms were ranked according to their Performance Indicator Factor (PIF) at pick-up (42 days). This factor is used by the company to rank performance of their contracted farms and was calculated by combining the feed conversion ratio, mortality and growth rate for each batch into a single value - PIF= growth rate/FCR x Live% x 100, where growth rate=weight(kg)/age(days), FCR=feed conversion ratio (kg feed/kg liveweight) and Live%=number of birds alive at the end of grow out/number of birds housed x 100. Based on the PIF score, the farms were ranked from best to worst and farms with similar rankings for the previous 3 batches of birds were paired and randomly allocated to either the treatment group or the control group. The 12 treatment farms received the broiler audit document and were asked to complete the recording sheets while the 12 control farms did not receive a copy of the audit and were asked to continue recording what they normally would have done, such as mortalities, culls, feed supplied and body weight. During a visit to the farm at the start of the study, all farmers were informed an audit, using the recently prepared audit documentation, would be conducted at the end of the study. At the end of the third batch of birds, the audit was conducted at all farms for the period from 2-5 weeks of age; this time period was chosen to avoid variation due to pick-up schedules. The production data provided for each batch of birds by the company were analysed for treatment effects by analysis of variance using the data from the previous 3 batches of birds as the co-variate.

The data showed that mortality to day 7, was significantly lower ($P<0.01$) on the treatment farms than the control farms, although there was no effect on subsequent mortality ($P>0.05$).

Variable	Farm		
	Treatment	Control	$LSD_{(P=0.05)}$
Mortality to day 7 (%)	1.37^{p}	1.74^{q}	0.176
Total mortality (%)	5.05	5.35	0.475
FCR*	1.88	1.87	0.021
Growth rate index**	41.2	42.5	2.13
PIF*	251.4	253.2	4.19

^{pq} different letters denote a significant difference at $P<0.01$; * PIF and FCR (see text for definitions); ** number of days to reach 2.1 kg.

Introduction of a welfare audit resulted in both production and welfare benefits, based on a significant reduction in mortality in the first 7 days at the treatment farms. It is considered that the closer attention to detail required by stockpeople undertaking the audit procedure may have been in part responsible for the lower mortality.

[1]Animal Welfare Centre, DNRE, Victorian Institute of Animal Science, Werribee, Victoria 3030.
[2]Animal Welfare Centre, University of Melbourne, Victorian Institute of Animal Science, Werribee, Victoria 3030.
[3]University of Melbourne, ILFR, Longeronong College, Horsham, Victoria 3401.

INTERACTIONS BETWEEN AMINO ACID TRANSPORT SYSTEMS AND INTESTINAL BACTERIA: IMPLICATIONS FOR THE FORMULATION OF BROILER CHICKEN DIETS

M.D. DREW[1], A.E. ESTRADA[1], A.G. VAN KESSEL[1] and D.D MAENZ[2]

Summary

The brush border membrane of the intestinal epithelium possesses a host of amino acid transport systems that rapidly remove available amino acids from the intestinal lumen before they are taken up and metabolized by bacteria. Differences in the substrate affinity and transport velocity of these amino acid transport systems may affect practical formulation of broiler diets. One example of this is the absorption of methionine (Met) and methionine hydroxy-analogue (MHA). Previous studies in our laboratory have shown that absorption of Met takes place via the System B amino acid transport system while MHA is absorbed by the Lactate transport system. The lactate transport system has a lower transport velocity and substrate affinity than System B. This lower efficiency of intestinal absorption of MHA relative to Met will increase exposure of MHA to intestinal bacteria which may then take up and metabolize the analog. To test this hypothesis we compared the absorption of Met and MHA in germ-free and conventional broiler chickens. Two diets containing 2.36g/kg of added Met or MHA were fed *ad libitum* for 21 days to broiler chickens. On day 21 of the experiment, the birds were fasted overnight and then re-fed the experimental diets to which 300 uCi of ^{3}H-DL-MHA or ^{3}H-DL-Met per Kg of feed had been added. The small intestines were removed, partitioned into 6 sections and the specific activity of the feed and digesta samples were measured. The residual specific activity of MHA and Met in the distal ileum of the conventional broilers was 10.2% and 3.7%, respectively while in the germ-free broilers, the residual specific activity MHA and Met in the distal ileum was 4.7% and 3.0%. The residual specific activity of MHA in the distal ileum of germ-free broilers was significantly lower than in their conventional counterparts ($P<0.05$). This study demonstrates that absorption of MHA by intestinal bacteria is significant and is at least partly responsible for the lower bioefficacy of MHA compared to Met.

I. INTRODUCTION

The intestinal epithelium is the primary interface between the internal environment of the host and the nutrient-rich external environment of the intestinal lumen. The brush border membrane of the intestinal epithelium consists of a lipid bilayer which provides a substantial energy barrier to the diffusion of hydrophilic molecules such as amino acids, resulting is a slow rate of absorption by passive diffusion. In the absence of intestinal microorganisms, absorption of nutrients from the intestinal tract via passive diffusion would probably suffice. However, the intestinal tract is not sterile and contains up to 10 billion viable bacteria per gram of contents that are in competition with the host for the nutrients present in the intestinal lumen. To out-compete the intestinal microflora for nutrients, the vertebrate host has developed a highly efficient set of membrane nutrient transporters to remove available substrates such as amino acids from the intestinal lumen before they are taken up and metabolized by bacteria. The uptake of amino acids by active transport systems therefore has

[1] Department of Animal and Poultry Science, University of Saskatchewan, 51 Campus Drive, Saskatoon SK Canada S7N 5A8.
[2] MCN Bioproducts Inc., PO. Box 22114, RPO Wildwood, Saskatoon SK Canada S7H 5P1.

important implications for the formulation of diets for broiler chickens to maximize growth and reduce the incidence of intestinal bacterial overgrowth. This paper will review the transport of amino acids across the intestinal epithelium and recent work in our laboratory characterizing intestinal transport systems for methionine and its hydroxy-analogue and implications of this research for practical broiler diet formulation.

II. AMINO ACID TRANSPORT SYSTEMS OF THE SMALL INTESTINE

Amino acid metabolism begins with the uptake of amino acids from ingested feed present in the small intestine. Although we formulate diets to meet the broiler chicken's requirement for amino acids, these amino acids must be absorbed across the intestinal epithelium for the bird to utilize them for growth and metabolism. Absorption of amino acids and di and tri peptides from the lumen of the small intestine into the body requires two sets of transport systems. The first set of amino acid transporters is present on the intestinal epithelium brush border. They transport free amino acids and di and tri peptides from the lumen of the intestine to the interior of the epithelial cell. The second set of amino acid transporters is present on the basolateral membrane of the epithelial cell and transports amino acids into the serosa where they enter the systemic circulation (Table 1). Many of the amino acid transporters present on the brush border are unique to this site while those on the basolateral membranes are common to most cell types in the body (Mailliard *et al*., 1995).

The amino acid transporters of the brush border can be broadly categorized into 3 systems: 1) Na^+-dependent systems, 2) Na^+-independent systems and 3) H^+-dependent systems. The Na^+-dependent amino acid transporter systems in the intestinal brush border include the B, X_{AG} and IMINO systems. System B may be thought of as a bulk absorptive mechanism for the uptake of the neutral amino acids. It has a high or intermediate affinity for all neutral amino acids (met, thr, ala, gly, val, ser, ile, leu, phe, his, tyr, cys, asn). It also has a low affinity for acidic, basic and imino acids (glu, asp, lys, arg, pro, OH-pro). The substrate specificity of the B and most other amino acid transport systems is relatively weak and the structure of the side chain has little affect on transporter affinity (Chistensen, 1990). System X_{AG} is another Na^+-dependent amino acid transport system found on virtually all cell types of the body (Stevens, 1992) and is responsible for the transport of glutamate and aspartate. The COO- group found of the side chains of these amino acids appears to be required for uptake by this transport system. A third Na^+-dependent amino acid transporter system is the IMINO system which has substrate specificity restricted to the imino acids proline and hydroxy-proline. System IMINO is found only on the intestinal brush border (Stevens, 1992).

Na^+-independent transport systems present on the brush border include systems y^+ and L. System y^+ has specificity restricted to lysine and arginine and provides the mechanism for the bulk absorption of these basic amino acids. The transport mechanism is electrogenic and the + charge on the amino acid is transported into the cell during the cycling of the transporter. The transport is driven by the interior negative electrical potential across the cell membrane. System L has substrate specificity for long and branched-chain neutral amino acids.

H^+-dependent transport systems transport di and tripeptides as well as hydroxy-analogues of amino acids. The peptide transport system is responsible for the transport of approximately 20-30% of absorbed dietary amino acids. It consists of 2 H^+ dependent systems: 1) HA, a high affinity, low capacity system and 2) LA, a low affinity, high capacity system. The LA system is responsible for approximately 80% of the peptide flux. Another transport system, the lactate transporter, is responsible for the transport of hydroxy-analogues of amino acids.

Table 1. Amino acid transport systems present in the broiler chicken (Christensen, 1990).

System	Specificity	Distribution
1. Na^+-dependent systems		
A	Short and medium chain linear neutral amino acids	All cell types except intestinal brush border
ASC	Short and medium chain linear neutral amino acids	All cell types except intestinal brush border
N	Gln, His, Asn	Liver
B	Neutral amino acids (DL-Met)	Intestinal brush border
$B^{o,+}$	Neutral and basic amino acids	Intestinal brush border
X_{AG}	Glu, Asp	Ubiquitous
IMINO	Pro, OH-Pro	Intestinal brush border
Gly	Gly	Liver, CNS, RBC
2. Na^+-independent Systems		
L	Long and branched-chain neutral amino acids	Ubiquitous
y^+	Lys, Arg	Ubiquitous
$b^{o,+}$	Neutral and basic amino acids	Oocytes and blastocytes
x_c^-	Glu, Cys	Liver
3. H^+-dependent systems		
Lactate	Hydroxy analogues of amino acids (DL-MHA)	Ubiquitous
Peptide	Di and tri peptides	Intestinal brush border

In addition to the amino acid transport systems above, passive diffusion has been reported to be an important route for the uptake of amino acids and nutrients. Immediately following a meal, the concentration of amino acids can rise to 1-25 mM in the lumen of the small intestine. At these high concentrations, there have been reports of substantial diffusion of amino acids across the intestinal epithelium (Stevens *et al*., 1984; Dibner *et al*., 1992). However, several studies in our laboratory on the uptake of L-threonine (Maenz and Patience, 1992) and L-methionine/L-methionine hydroxy analogue (Maenz and Engele-Schaan, 1996a) did not detect any significant uptake of these amino via low affinity pathways including diffusion and uptake of these compounds was mediated entirely by a saturable transport process. Further study on the importance of passive diffusion of amino acids relative to active transport in the whole animal are necessary to determine the relative importance of these two mechanisms. The amino acid transport mechanisms of the intestinal brush border are shown in Figure 1.

III. DEVELOPMENT OF AMINO ACID TRANSPORT AFTER HATCH

At hatch, the organs of supply (gastrointestinal tract, liver, pancreas) are undersized and underdeveloped relative to the organs of demand (heart, lungs, skeletal system etc.). The gut is underdeveloped such that the bird eats very little in the first few days after hatch and is dependent on nutrients in the residual yolk sac. Rapid growth of the organs of supply relative to total body mass during the first week of life results in huge increases in the nutrient assimilation capacity which facilitates an increased rate of growth in the animal. The rate of

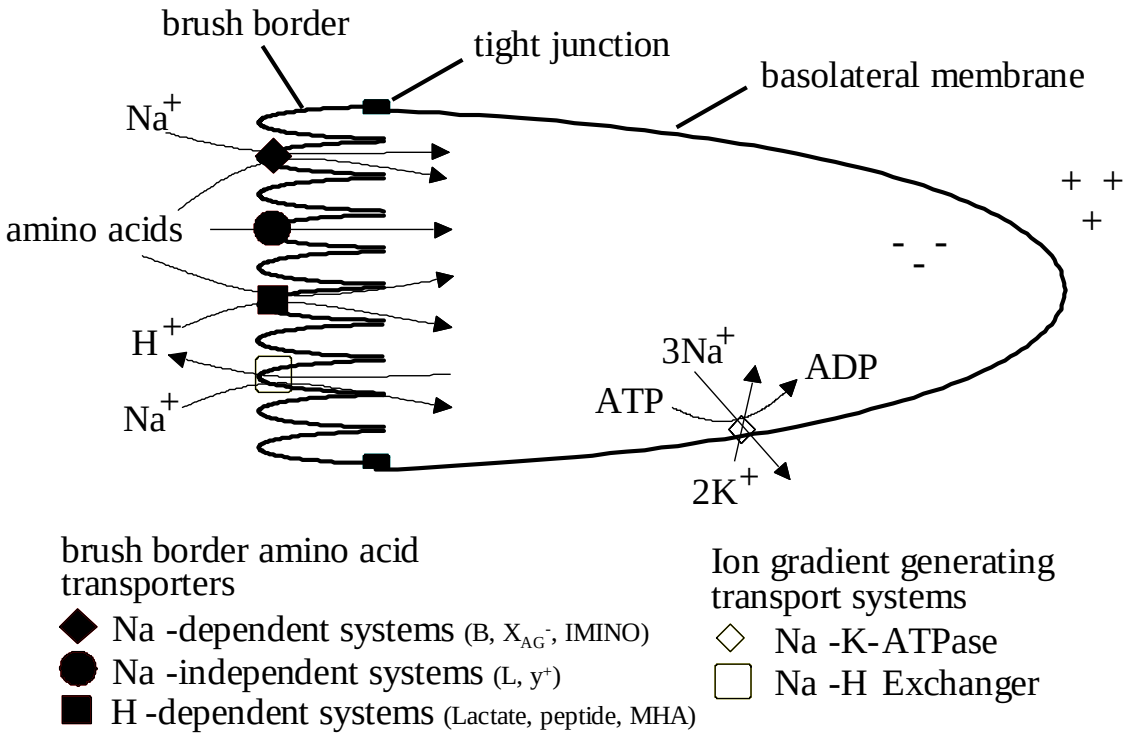


Figure 1. Amino acid transport in the brush border membrane.

growth of the small intestine is 4 fold greater than the rate of total body growth (Mitjans *et al*., 1997). This is associated with rapid structural maturation of the intestine including increased villus height and differentiation of epithelial cells into absorptive columnar cell types during migration up the villus (Diamond, 1991; Mitjans *et al*., 1997). In addition to increases in cell numbers, the number of amino acid transporters present on the brush border membrane is increased by exposure to amino acids, di and tripeptides on the lumenal side of the epithelial cell (Stevens *et al*., 1992). These two mechanisms significantly increase the net amino acid transport capacity per unit of intestinal length in the first few days after hatch.

The intestinal tract is sterile at hatch but is rapidly colonized by ingestion of feed. Rapid development and maturation of gut function may in part be triggered by events occurring in response to initial bacterial colonization. Hooper et al. (2001) reported that colonization of the mouse intestinal tract by *Bacillus iotathetaomicron* increased the expression of Na+glucose cotransporter by a factor of 2.4 and colipase expression by a factor of 9.4 compared to germ-free controls. They did not report the effect of bacterial colonization on the expression of amino acid transport systems and further studies in this area are required.

IV. IMPLICATIONS OF AMINO ACID TRANSPORT SYSTEMS FOR BROILER DIET FORMULATION

The mechanism of amino acid transport in the small intestine has a significant impact on practical diet formulation for broiler chickens. L-methionine (L-Met) is a limiting amino acid in broiler production which is commonly supplemented in commercial diets as either DL-Met or as the hydroxy analogue (DL-MHA) (Figure 2). In recent years a substantial body of research has consistently shown that on a molar basis the bioefficacy of the hydroxy analog of methionine (MHA) is 65-80% of that of L-methionine (Huyghebaert, 1993;

Rostagno and Barbosa, 1995; Thomas *et al*., 1991). The reasons for this difference may be due to either reduced intestinal absorption of MHA, inefficient conversion of MHA to methionine after absorption or a combination of both.

In recent years a substantial body of research has been performed on the relative absorption efficiencies of DL-Met and DL-MHA. Based on differences in the transport mechanism of these two commercial sources of methionine it is reasonable to hypothesize that this might affect their overall absorption from the gut. Previous studies comparing the efficiency of absorption of MHA and methionine have yielded conflicting results. Han et al. (1990) determined that absorption of DL-Met and DL-MHA were equal using a true-digestibility-balance assay using caecectomized broiler chickens. This method measures the non-absorbed MHA and methionine present in digesta after intestinal passage. However, metabolism of these compounds by intestinal bacteria would result in an overestimate of the absorption of MHA and methionine. Other studies (Lingens and Molnar, 1996; Esteve-Garcia and Austic, 1993) used radiolabelled DL-Met and DL-MHA to estimate intestinal absorption. This method would account for bacterial uptake of MHA and methionine since the radioactivity would remain in the intestinal tract. Both studies reported 10-20% of the original radiolabelled MHA activity in the feed was present in the distal sections of the small intestine compared to 4-5% for methionine. Moreover, HPLC analysis of gut contents from the distal ileum showed that the residual radioactivity was not associated with MHA demonstrating that the compound had been metabolized during intestinal transit. However, Esteve-Garcia and Austic (1994) later retracted their results after reverse-phase HPLC analysis of the ^{14}C-MHA used in their study revealed the presence of significant amounts of non-MHA ^{14}C-labelled compounds.

To further complicate the picture, several studies have reported that heat-stressed broiler chickens have impaired absorption of DL-Met compared to thermoneutral controls while absorption of DL-MHA is increased in heat-stressed birds (Dibner *et al*., 1992; Knight *et al*., 1994). However, Mitchell and Carlisle (1992) found that L-methionine absorption per gram of jejunum was greater in heat stressed birds. Furthermore, balance studies in broiler maintained under heat-stressed conditions during the hot summer in Brazil showed 97.2% DL-Met and 90.8% DL-MHA retention (Rostagno and Barbosa, 1995).

We performed a similar experiment to examine the disappearance of highly purified ^{3}H-L-Met and ^{3}H-L-MHA during passage down the length of the small intestine and to determine the effect of 48-h exposure to conditions of 32^{o}C, 50% humidity on initial rates of ^{3}H-L-Met and ^{3}H-L-MHA absorption across small intestinal brush border vesicles and on in vivo small intestinal passage of the nutrients (Maenz and Engele-Schaan, 1996b). We found that 2.5-3.5% of the ^{3}H-activity associated with L-Met remained in the distal small intestine compared to 15% of ^{3}H-activity associated with L-MHA. HPLC analysis showed that only 10% of the radiolabelled material remaining in the terminal ileum eluted at the time expected for L-MHA. Heat exposure did not affect in vivo intestinal passage or in vitro transport of L-Met and L-MHA across intestinal brush border membrane vesicles.

Based on this study we hypothesized that differences in the efficiency of absorption in the proximal small intestine result in a prolonged passage time for L-MHA relative to L-Met. A prolonged passage time will increase exposure to intestinal bacteria which may then take up and metabolize the analog. This hypothesis would explain the partial conversion of L-MHA to other compounds that remain in the digesta. Microbial uptake and metabolism of nutrients during intestinal passage will not occur in germ-free birds. By comparing intestinal passage of DL-Met and DL-MHA in conventional and germ-free birds it is possible to determine the effects of gut microbes on the fate of these nutrients in the digesta.

To test this hypothesis we compared the absorption of ^{3}H-labelled DL-Met and DL-MHA in germ-free and conventional broiler chickens (Drew and Maenz, 2001). The two diets

contained 2.36g/kg of added DL-Met or DL-MHA. Nineteen germ-free broilers were maintained in isolators and fed diets that had been sterilized by gamma irradiation (50 KGy). Twenty conventional birds were reared in batteries and received non-irradiated feed. The diets were fed ad libitum for 3 weeks. On day 21 of the experiment, the birds were fasted overnight and then refed the experimental diets to which 300 uCi of ^{3}H-DL-MHA or ^{3}H-DL-Met per Kg of feed had been added. 300 uCi of $^{51}CrCl_3$ per Kg of feed was added as an indigestible marker. The chickens were fed the radiolabelled diets for 3 hours and then euthanized. The small intestine of the birds were removed and partitioned into 6 sections and the specific activity of the feed and digesta samples was calculated as the ratio of ^{3}H: ^{51}Cr in each sample. The residual specific activities of MHA and Met in the 6 sections of the intestinal tract are shown in Figure 2.

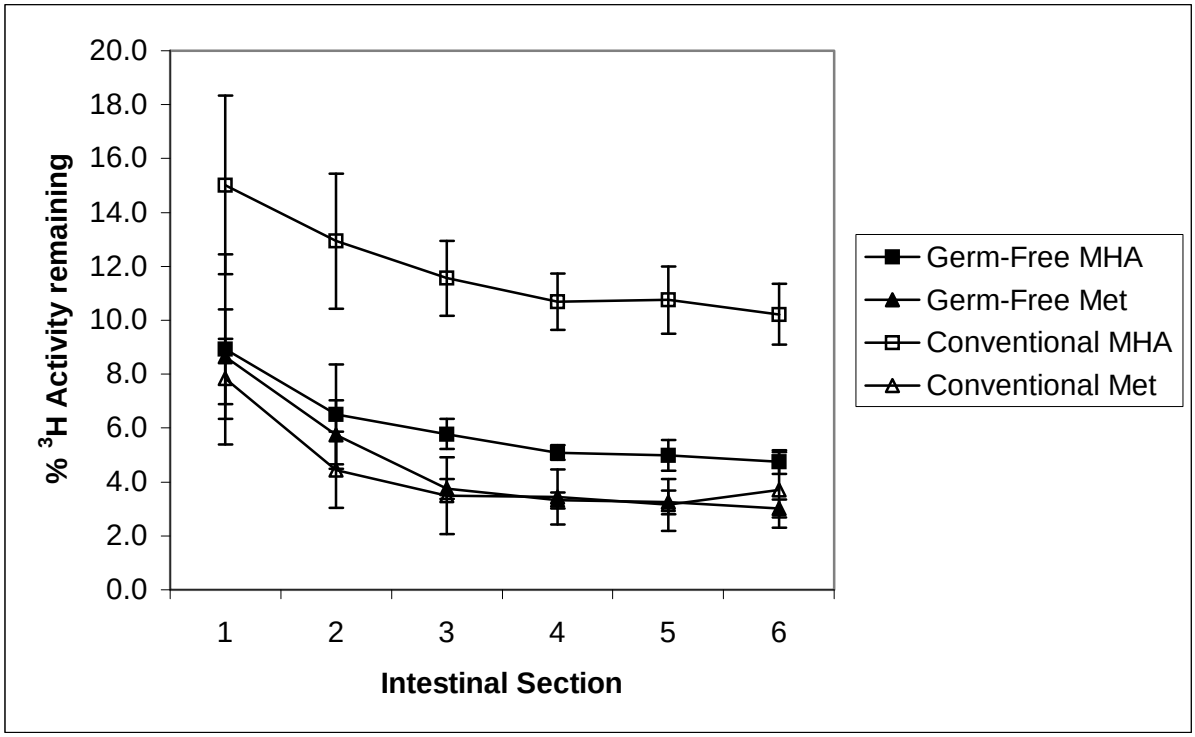


Figure 2. Residual ^{3}H-DL-Methionine and ^{3}H-DL-MHA digestibility in chickens reared in conventional or germ-free environments.

In the conventional broilers the residual activity remaining in the distal ileum in the DL-MHA and DL-Met fed birds was 10.2% and 3.7%, respectively. In the germ-free broilers, the residual specific activity DL-MHA and DL-Met in the distal ileum was 4.7% and 3.0%, respectively. The residual specific activity of DL-MHA in the distal ileum of germ-free broilers was significantly higher than in their conventional counterparts ($P<0.05$). This study demonstrates that the difference in intestinal amino acid transport systems used for the uptake of DL-Met and DL-MHA result in greater uptake and metabolism of DL-MHA by intestinal bacteria.

V. CONCLUSIONS

There are few reports on the competition for nutrients between the host and intestinal bacterial populations. Bacterial metabolism has a significant impact on the requirement for taurine in cats (Anantharaman-Barr *et al*., 1994; Backus *et al*., 1994) but we are aware of no other reports on the effects of bacterial metabolism on the uptake of nutrients from the small intestine. Not all bacteria have the ability to utilized MHA. Hegedus *et al*. (1993) examined the ability of 3 bacteria (*Lactobacillus plantarum, Leuconostoc mesenteroides* and *Lactobacillus casei)* to utilize MHA for growth. None of these bacteria could utilize MHA although they all could use methionine. This suggests a limited number of bacterial species may be responsible for the uptake and metabolism of MHA. Determining the species of bacteria that compete for nutrients with the host and developing strategies to limit their populations in the small intestine may result in increases in feed efficiency. Bacteria also have a significant effect on the expression of nutrient transporters in the small intestine (Hooper *et al*., 2001). Determining which species of bacteria significantly up-regulate the expression of amino acid transport systems in the brush border membrane may allow us to promote earlier maturation of amino acid uptake in broilers and enhanced growth and efficiency by controlling intestinal bacterial populations. New information provided by research in this area will permit a greater understanding of control of amino acid transport for more efficient production of broilers.

REFERENCES

Anantharaman-Barr, G., Ballevre, O., Gicquello, P., Bracco-Hammer, I., Vuichoud, J., Montigon, F. and Fern, E. (1994). Fecal bile acid excretion and taurine status in cats fed canned and dry diets. *Journal of Nutrition,* **124**:2546S-2551S.

Backus, R., Rogers, Q. and Morris, J. (1994). Microbial degradation of taurine in fecal cultures from cats given commercial and purified diets. *Journal of Nutrition* ,**124**:2540S-2545S.

Christensen, H.N. (1990). Role of amino acid transport and countertransport in nutrition and metabolism. *Physiological Reviews*, **70**:43-77.

Diamond, J.M. (1991). Evolutionary design of intestinal nutrient absorption: enough but not too much. *News in Physiological Sciences,* **6**:92-96.

Dibner, J.J., Atwell, C.A. and Ivey, F.J. (1992). Effect of heat stress on 2-hydroxy-4-(methylthio)butanoic acid and DL-methionine absorption measured in vitro. *Poultry Science,* **71**:1900-1910.

Drew, M.D. and Maenz, D.D. (2001). The effect of intestinal bacteria on the absorption of methionine and 2-hydroxy-4-methylthiobutoanic acid in germ-free and conventional broiler chickens. International Poultry Scientific Forum. Atlanta GA Jan 15-16 2001 pp. 5.

Esteve-Garcia, E. and Austic, R.E. (1993). Intestinal absorption and renal excretion of dietary methionine sources by the growing chicken. *Journal of Nutrition and Biochemistry,* **4**:576-587.

Esteve-Garcia, E. and Austic, R.E. (1994). Digestibility of methionine hydroxy analog (MHA) in broiler chicks. *Poultry Science,* **66** (Suppl. 1):14.

Han, Y., Castanon, F., Parsons, C.M. and Baker, D.H. (1990). Absorption and bioavailability of DL-methionine hydroxy analogue compared to DL-methionine. *Poultry Science* **69**:281-287.

Hegedus, M., Andrasofszky, E., Brydll, E., Veresegyhazy, T., Tamas, J. (1993). Biological activity of methionine derivatives: I. Microbiological activity of methionine derivatives. *Magyar Allatorvosok Lapja,* **48**:527-532.

Hooper, L.V., Wong, M.H., Thelin, A., Hannson, L., Falk, P.G. and Gordon, J.I. (2001). Molecular analysis of commensal host-microbial relationships in the intestine. *Science,* **291**:881-884.

Huyghebaert, G. (1993). Comparison of DL-methionine and methionine hydroxy analogue-free acid in broilers by using multi-exponential regression models. *British Poultry Science,* **34**:351-359.

Knight, C.D., Wuelling, C.W., Atwell, C.A. and Dibner, J.J. (1994). Effect of intermittent periods of high environmental temperature on broiler performance responses to sources of methionine activity. *Poultry Science*, **73**:627-639.

Lingens G. and Molnar, S. (1996). Studies on metabolism of broilers by using ^{14}C-labelled DL-methionine and DL-methionine hydroxy analogue Ca-salt. *Archiv fur Tierernahrung,* **49**:113-124.

Maenz, D.D., and Patience, J.F. (1992). L-threonine transport in pig jejunal brush border membrane vesicles. *Journal of Biological Chemistry,* **267:**22079-22086.

Maenz D.D. and Engele-Schaan C.M. (1996a). Methionine and 2-hydroxy-4-methylthiobutanoic acid are transported by distinct Na+-dependent and H+-dependent systems in the brush border membrane of the chick intestinal epithelium. *Journal of Nutrition.* **126**:529-536.

Maenz D.D. and Engele-Schaan C.M. (1996b). Methionine and 2-hydroxy-4-methyltiobutoanic acid are partially converted to nonabsorbed compounds during passage through the small intestine and heat exposure does not affect small intestinal absorption of methionine sources in broiler chicks. *Journal of Nutrition,* **126**: 1438-1444.

Mailliard, M.E., Stevens, B.R. and Mann, G.E. (1995). Amino acid transport by the small intestinal, hepatic and pancreatic epithelia. *Gastroenterology,* **108**:888-910.

Masters, C. and Holmes, R. (1977). Peroxisomes: new aspects of cell physiology and biochemistry. *Physiological Reviews,* **57**:816-882.

Mitchell, M.A. and Carlisle, A.J. (1992). The effect of chronic exposure to elevated environmental temperature in intestinal morphology and nutrient absorption in the domestic fowl (*gallus domesticus*). *Comparative Biochemistry and Physiology,* **101A**:137-142.

Mitjans, M., Barniol, G. and Ferrer, R. (1997). Mucosal surface area in chicken small intestine during development. *Cell and Tissue Research,* **290**:71-78.

Rostagno, H.S. and Barbosa, W.A. (1995). Biological efficacy and absorption of DL-methionine hydroxy analogue free acid compared to DL-methionine in chickens as affected by heat stress. *British Poultry Science,* **36**:303-312.

Stevens, B.R., Kaunitz, J.D., and Wright, E.M. (1984). Intestinal transport of amino acids and sugars: Advances using membrane vesicles. *Annual Review of Physiology,* **46**:417-433.

Stevens, B.R. (1992). Amino acid transport in intestine. In. *Mammalian Amino Acid Transport: Mechanisms and Control,* Ed. M.S. Kilberg and D. Haussinger. pp. 149-163. Plenum Press, New York.

Thomas, O.P., Tamplin, C., Crissey, S.D., Bossard, E. and Zuckerman, A. (1991). An evaluation of methionine hydroxyanalogue free acid using nonlinear (exponential) bioassay. *Poultry Science,* **70**:605-610.

FACTORS INFLUENCING DIETARY THREONINE REQUIREMENT OF GROWING MEAT CHICKENS

M. PEISKER[1], M. RIMBACH[2] and F. LIEBERT [2]

Summary

Several factors such as age of chicks, quality and level of dietary protein, dry matter intake, dietary nutrient balance and daily protein deposition influence the threonine requirement of growing meat chickens. This may explain different findings on threonine requirements in the literature. Using an exponential equation for estimation of amino acid utilisation, dietary threonine requirements were calculated for different age groups depending on feed intake and protein deposition. Varying these parameters by ±5% resulted in significant differences in dietary threonine requirements. Variation in protein deposition had a greater effect on requirements than variation in feed intake. These data show that the results obtained in classical requirement studies should be considered strictly in relation to the experimental conditions such as performance level, expected feed intake and efficiency of threonine utilisation.

I. INTRODUCTION

Threonine is typically the third most limiting amino acid in broiler diets. Since threonine is available in crystalline amino acid form, fine-tuning of broiler diets with lower crude protein content is possible. Therefore the knowledge of minimum dietary threonine in least cost formulation becomes crucial so that performance is not sacrificed against least feed cost. Dietary threonine requirements reported in the literature for the age period 1 - 3 weeks vary from 5.3 g/kg (true ileal digestible; Batal *et al*., 2001) to 8.0 g/kg (total; NRC, 1994) and for the age period 3 - 6 weeks from 5.2 g/kg (true ileal digestible; Webel *et al*., 1996) to 7.4 g/kg (total; NRC, 1994). In contrast to other amino acids, dietary threonine requirement for feed efficiency is not higher than for weight gain.

Classical dose-response studies suffer from the limitation that the results obtained are only valid under the given circumstances applied in the experiment. i.e. the basal diet, the genotype or protein deposition, the efficiency of utilisation of the amino acid under study and the actual feed intake. These parameters clearly influence the results and in turn account for the wide differences in amino acid requirements found in the literature. An effort has been made to describe the relationship of feed protein quality, amino acid intake and nitrogen balance in an exponential equation (Liebert *et al*., 2000). Knowledge of the maximum protein deposition of birds of a given genotype and age (Peisker *et al*., 2000) allows the usage of the equation to calculate amino acid requirements depending on age period, level of protein deposition, efficiency of utilisation of the amino acid and feed intake.

To generate the parameters needed for calculation of threonine requirements with the exponential equation, a study was carried out to determine the threonine efficiency in a wheat-wheat gluten based diet as well as apparent ileal threonine digestibility, mean body weights, dry matter intake and protein deposition data. This study simultaneously allowed the determination of dietary threonine requirement in the classical way.

[1] ADM BioProducts, 55001 Mainz, Germany.

[2] University of Goettingen, Institute Anim Physiology and Nutrition, 37077 Goettingen, Germany.

II. MATERIALS AND METHODS

A growth trial with triplicate groups of 10 male chicks (Cobb 500) per treatment was conducted in three-tier battery cages in a fully climatised room on 24-h light regime. Feeding was ad libitum and temperature settings were maintained according to the breeder's recommendation. Body weights and feed intake were determined at weekly intervals. Body composition was determined by analysing three chicks per treatment at the end of each age period. Digesta samples from seven chicks (in treatments A and C) were taken from the distal ileum to determine ileal amino acid digestibility at day 17, 31 and 45. Acid-insoluble ash (Celite) served as the inert marker. The results were analysed using STATISTICA 5.1.

The basal diet (Table 1) was formulated to be limiting in threonine and L-threonine was added incrementally at the expense of wheat starch in the experimental diets. The amino acid profile, except for threonine, was identical in all diets. Glycine was added to avoid any limitation of amino acids, glycine + serine, which may influence threonine requirement (Heger and Pack, 1996). The other essential amino acids were set according to the Illinois ideal chick protein ratio (Baker and Han, 1994). The amino acid content of the experimental diets was calculated based on the analysis of wheat and wheat gluten and on the results of the digestibility trial at day 31, including the amino acid supplements.

Table 1. Composition (g/kg) of the experimental diets

Treatment	A	B	C	D	E
Wheat	739.2	739.2	739.2	739.2	739.2
Wheat gluten	118.3	118.3	118.3	118.3	118.3
Soybean oil	40.0	40.0	40.0	40.0	40.0
Wheat starch	30.1	29.3	28.6	27.8	27.0
Celite	10.0	10.0	10.0	10.0	10.0
Common minor ingredients[1]	44.0	44.0	44.0	44.0	44.0
L-Lysine-HCl	7.71	7.71	7.71	7.71	7.71
DL-Methionine	1.87	1.87	1.87	1.87	1.87
L-Threonine	-	0.76	1.53	2.30	3.06
L-Tryptophan	0.30	0.30	0.30	0.30	0.30
L-Glycine	3.75	3.75	3.75	3.75	3.75
L-Arginine	3.55	3.55	3.55	3.55	3.55
L-Isoleucine	1.20	1.20	1.20	1.20	1.20
Calculated composition					
Crude protein	254.3	254.3	254.3	254.3	254.3
ME, MJ/kg DM	15.40	15.40	15.40	15.40	15.40
Lysine[2]	11.32	11.32	11.32	11.32	11.32
Methionine[2]	4.00	4.00	4.00	4.00	4.00
Cystine[2]	3.89	3.89	3.89	3.89	3.89
Threonine[2]	4.48	5.23	6.00	6.77	7.53
Isoleucine[2]	7.77	7.77	7.77	7.77	7.77
Valine[2]	9.15	9.15	9.15	9.15	9.15
Tryptophan[2]	2.03	2.03	2.03	2.03	2.03

[1] Cellulose, 10.0; Monocalcium phosphate, 18.0; Limestone, 13.0; Salt, 3.0 and vitamin/trace mineral premix, 10.0.
[2] Ileal digestible, as fed (920 g DM/kg).

III. RESULTS AND DISCUSSION

Threonine digestibility in treatment diets A and C at different broiler ages is shown in Table 2. With increasing age, apparent ileal threonine digestibility was lowered. This trend was also observed for lysine. It is assumed that this observation could be due to increased losses of endogenous threonine and lysine with higher dry matter intake in older birds. However, these results should not be interpreted as a general trend due to the limitations of the indicator-method used. Threonine digestibility was lower in the deficient diet (Treatment A) compared to a more balanced, threonine supplemented diet (Treatment C).

Table 2. Influence of age of broilers on the apparent ileal threonine digestibility (%) in selected treatment diets.

Age of broilers (days)	Treatment A	(SD)	Treatment C	(SD)
17	88.5	0.0	92.1	0.0
31	84.9	4.85	88.9	1.69
45	80.7	4.34	85.9	1.46

The results from the dose-response trial with incremental threonine levels are shown in Table 3.

Table 3. Influence of graded levels of threonine on the body weight gain (BWG), feed conversion ratio (FCR) and protein deposition (PD) of broilers.

	Treatment	A	B	C	D	E
	Digestible threonine (g/kg as fed)	4.48	5.23	6.00	6.77	7.53
Wk. 1 -2	BWG (g/d)	13.8^{a}	23.3^{b}	21.4^{b}	23.7^{b}	21.7^{b}
	FCR (g/g)	1.53^{a}	1.32^{b}	1.36^{b}	1.27^{b}	1.35^{b}
	PD (g/d)	2.4^{a}	3.7^{b}	3.9^{b}	4.0^{b}	3.7^{b}
Wk. 3 - 4	BWG (g/d)	57.9^{a}	74.7^{b}	74.7^{b}	74.8^{b}	75.5^{b}
	FCR (g/g)	1.67^{a}	1.50^{b}	1.50^{b}	1.54^{b}	1.56^{b}
	PD (g/d)	11.0^{a}	12.8^{b}	13.8^{c}	14.3^{c}	14.1^{c}
Wk. 5 - 6	BWG (g/d)	67.7^{a}	78.7^{b}	72.7^{ab}	76.3^{b}	75.4^{ab}
	FCR (g/g)	2.07^{a}	1.88^{b}	1.94^{ab}	1.89^{b}	1.93^{ab}
	PD (g/d)	13.2^{a}	15.2^{b}	14.7^{ab}	15.4^{b}	15.1^{b}

^{a,b,c} Means in the same row with different superscripts differ significantly ($P<0.05$).

From the results in Table 3, it is apparent that digestible dietary threonine levels higher than that used in Treatment B and C do not cause further responses in body weight gain and feed conversion. However, protein deposition improved numerically in 1-2 week-old broilers from treatment B to D and significantly in 3-4 week-old broilers from treatment B to C, D and E with increasing dietary threonine concentration respectively. These results suggest that higher dietary threonine levels can support increased protein deposition without increasing bodyweight gain.

The data from this growth trial (mean body weight, DM intake, protein deposition and efficiency of threonine utilisation) were used to calculate the dietary threonine requirement, using the exponential equation described by Liebert *et al*. (2000). Theoretical upper limit for protein deposition ($PD_{max}T$) values were taken from previous trials for this broiler genotype

(Peisker *et al*., 2000). Using this model, total threonine requirements for different age groups were calculated under simulated experimental conditions. In the example shown in Table 4, the efficiency of utilisation of threonine was assumed to remain unchanged, but feed intake and protein deposition data were varied by ±5%,

Table 4. Total dietary threonine requirement (g/kg diet, as fed) of growing meat chickens as influenced by varying levels of feed intake and protein deposition.

Age (Weeks)	Current study	Feed intake		Protein deposition	
		- 5%	+ 5%	- 5%	+ 5%
1 - 2	6.6	6.9	6.3	6.2	7.0
3 - 4	6.7	7.1	6.4	5.9	7.8
5 - 6	6.6	6.9	6.3	6.1	7.1

The indicated dietary requirement data in Table 4 on the basis of "total" threonine comprise the efficiency of threonine utilisation (absorption plus post-absorptive utilisation) of the given crude protein source. Correction using the threonine digestibility figures given in table 2 to obtain requirements for digestible threonine, would be inappropriate since this would mean that "digestibility" is considered twice.

It is clear that variation in protein deposition had a greater effect on dietary threonine requirements than variation in feed intake. These calculations demonstrate that defining the correct dietary amino acid requirement is a rather complex task. Results from dose-response trials are valid only under the experimental conditions and caution is necessary when applying such results under practical situations. Approaches to establish amino acid requirement data should therefore consider the various factors that impact on the results. The exponential equation approach employed in this paper represents a viable solution since it takes the major factors into account. However, further data is needed on the efficiency of threonine utilisation from different feed ingredients and its variability to make the best use of this approach.

REFERENCES

Baker, D.H. and Han, Y. (1994). *Poultry Science*,**73**: 1441-1447.

Batal, A.B., Parr, T.M., Augspurger, N.R., Parsons, C.M. and Baker, D.H. (2001). *Poultry Science,* **80** (Supplement 1): 48.

Heger, J. and Pack, M (1996). *Agribiol. Res.* 49, **4**: 257-265.

Liebert, F., Rimbach, M. and Peisker, M. (2000). *Proceedings of the Australian Poultry Science Symposium*, Ed. R.A.E. Pym. **12:** 88-92.

NRC (1994). National Academy of Science Press, Washington, D.C.

Peisker, M., Rimbach, M. and Liebert, F. (2000). *Proceedings of the Australian Poultry Science Symposium*, Ed. R.A.E. Pym. **12**: 93-96.

Webel, D.M., Fernandez, S.R., Parsons, C.M. and Baker, D.H. (1996). *Poultry Science* **75**: 1253-1257.

EFFICIENCY OF PROTEIN UTILIZATION IN MALE AND FEMALE BROILER CHICKENS

LI LI and J.V. NOLAN

Summary

The efficiency of dietary protein utilisation in broiler chickens was studied by conventional balance techniques and by the digestion and metabolism of ^{15}N-labelled protein in the diet. Between 16 and 30 days of age, male chickens tended to be heavier and to ingest more feed than female chickens: however, female chickens tended to absorb and utilize dietary protein more efficiently than male chickens. Whole-body synthesis rate did not differ between sexes and was 6.7-7.5 times the protein absorption rate. Only about 10% of the protein synthesized was deposited in tissue gain whereas 90% was degraded. Fractional protein synthesis rates were determined from the decline in enrichment in small intestinal, liver and breast muscle samples taken sequentially from birds that had earlier ingested ^{15}N-labelled feed. FSRs declined with age but did not differ between sexes and were 44-52, 23-29 and 15-18 %/d, respectively.

I. INTRODUCTION

The efficiency of dietary protein deposition into meat in broiler birds affects the profitability of the broiler industry. Breast muscle (*pectoralis major*) synthesised from absorbed amino acids accounts for up to 30% of the edible meat and as much as 50% of the edible protein in the carcass (Summers et al. 1988) and is often a major focus for studies of whole-body protein metabolism in meat chickens (Remignon, 1994). Protein accretion rate in breast muscle tissue is the net effect of rates of protein synthesis (PS) and protein degradation (PD) that may both be several-fold higher than the deposition itself. Owing to their very high fractional PS rates, the liver and small intestine are other major contributors to whole-body PS (Tesseraud, 1996). There are few studies that describe the changes in rates of PS and PD that occur in the tissues of broilers as they age. This experiment was designed to examine age-related changes in whole-body protein PS and PD in growing male and female broilers as well as protein turnover in specific tissues, namely, breast muscle, liver and small intestine.

II. MATERIALS AND METHODS

Day-old Cobb strain broiler chickens (n =108) with equal numbers of the two sexes were obtained from the Biaida Hatchery in Kootingal, NSW. They were housed in single-sex groups in battery brooders and offered a commercial broiler starter diet *ad libitum* (Ridley Agriproducts Pty. Ltd., Tamworth, NSW) until they were 14 days' old.

Two experimental diets were formulated to conventional broiler starter/grower standards (12.4 MJ ME/kg (calculated) and crude protein content 21.6% (determined by the Kjeldahl method) using wheat (35%), sorghum (28%), soybean meal (20%), meat and bone meal (7%), cottonseed meal (2%) and minor amounts of oil, salt, synthetic amino acids and mineral-vitamin mix. Both diets also contained 5% dried duckweed, one with unlabelled (Diet 1) and the other with ^{15}N-labelled duckweed (Diet 2). At 14 days of age, 15 female and 15 male birds were randomly selected and weighed, and placed into individual cages and offered Diet 1 *ad libitum* during a 2 d period of adaptation. Feed and excreta samples were collected from 3 female and 3 male birds during this period to enable background ^{15}N enrichments to be determined later.

School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351.

At 16 days of age, these birds were weighed and at 08.00 h switched to Diet 2 which was then freely available until 20.00 h when Diet 2 was replaced by Diet 1 for a further 60 h. Excreta were collected over the 24-h period from the time Diet 2 was first offered.

To enable protein concentrations and fractional synthesis rates in small intestinal, liver and breast muscle tissues to be estimated, groups of 3 female and 3 male birds were weighed and killed sequentially at 14, 18, 24, 48 and 72 h from the time the ^{15}N-enriched diet was first offered to the birds. The liver and small intestine were excised from each bird, cleaned, washed with physiological saline and blotted with tissue paper before their weights were recorded. Samples of these tissues were digested immediately by the Kjeldahl method and enrichments of the resulting ammonia-N were determined on a mass spectrometer (TRACERMASS, Stable Isotope Analyser; Europa Scientific, Pfeiffer/Balzers) Feed and excreta samples were also analysed for total N by the Kjeldahl procedures. Uric acid concentration in excreta was used to predict total daily urinary and faecal N excretion. Faecal N excretion was then calculated (by subtracting the resulting calculation of urinary N excretion from the total N output in excreta) and used to estimate apparent digestibility of dietary N.

Whole body protein turnover was determined by the method of Picou and Roberts-Taylor (1969) from the cumulative excretion of ^{15}N over the 24 h from when Diet 2 was first offered. The declines of enrichments over time (Y_t) in liver and small intestinal N were fitted by single exponential functions of the form $Y_t = A[\exp(-kt)]$, where A is the intercept at the time Diet 2 was first offered, and k is the fractional turnover rate (FTR, %/h) of the N in each tissue.

The procedures employed during the 12-h provision of Diet 2 when birds were 16 days old were repeated when the birds were 23 and 30 days of age.

A two-way (age × sex) analysis of variance was performed using SYSTAT (version 8.0, 1998, SPSS Inc., USA) to discriminate between the main effects of age and sex and their interactions. Multiple comparisons of means were made using the Least Significant Difference procedure (Steel and Torrie, 1980).

III. RESULTS

Live-weight gain increased with age and live weight at 30 days of age was about 80% of that expected from Cobb birds under ideal conditions (Table 1). Organ weights also increased as chickens aged, but the weights of liver and small intestine relative to live weight, and their protein mass relative to live weight decreased with age (Tables 1 and 2).

Table 1 Mean live weight and absolute and relative weights and protein contents in liver and small intestine (SI) of broiler chickens at 16, 23 and 30 days of age

	Age (days)			Sex	
	16	23	30	Female	Male
Mean bird live weight (g)	322^{a}	606^{b}	1009^{c}	627	665
Liver weight (g)	17.5^{a}	29.4^{b}	39.9^{c}	28.9	29.0
SI weight (g)	12.1^{a}	17.1^{b}	19.2^{c}	16.1	16.1
Relative liver weight (g/kg LW)	51.2^{a}	42.7^{b}	36.2^{c}	43.1	43.6
Relative SI weight (g/kg LW)	35.4^{a}	24.7^{b}	17.3^{c}	25.7	25.9
Liver protein mass (g)	2.75^{a}	4.80^{b}	6.25^{c}	4.65	4.55
SI protein (g)	1.77^{a}	2.54^{b}	2.93^{c}	2.43	2.40

$^{a, b, c:}$ Means with different superscripts in any row within age differ significantly ($P<0.05$). There were no significant differences ($P>0.05$) between the two sexes.

Although males tended (P=0.11) to be heavier at 30 days of age, there were no other significant differences between sexes in the measurements reported in Table 1.

Between 16 and 30 days of age, the birds' rate of N intake and N deposition doubled and their excreta N output increased 2.4 times (Table 2). Males had higher N intake and N excretion than females (P<0.05) but N deposition did not differ (P>0.05) between the sexes. Nevertheless, females had higher apparent N digestibility than males (P<0.05), and tended to use absorbed protein more efficiently (N apparently absorbed/N excreted): they also tended (P=0.11) to have a higher overall efficiency of N utilization (N retention/N intake). There was a significant interaction between age and sex for apparent N digestibility (P<0.05).

Whole-body SR and DR increased as birds aged from 16 to 30 days (P<0.05) but when related to metabolic live weight ($LW^{0.75}$), these measures decreased (P<0.05) over the same period (Table 2).

Table 2 N intake, excretion and net deposition and whole body protein synthesis and degradation rates and the percentage of protein synthesised retained in tissues of female and male broilers at 16, 23 and 30 days of age

	Age (days)			Sex	
	16	23	30	Female	Male
N intake (g/bird.d)	2.39^{a}	4.09^{b}	5.09^{c}	3.71^{a}	4.01^{b}
Apparent N digestibility (%)	84.9^{a}	77.5^{b}	80.8^{c}	83.3^{a}	78.8^{b}
N retention (g/bird.d)	1.49^{a}	2.35^{b}	2.91^{c}	2.25	2.25
Protein synthesis rate (g/bird.d)	15.2^{a}	20.9^{b}	27.7^{c}	20.3	22.3
Protein degradation rate (g/bird.d)	5.92^{a}	6.27^{ab}	9.48^{b}	6.23	8.22
Protein synthesis rate (g/kg $LW^{0.75}$.d)	35.7^{a}	30.5^{b}	27.4^{b}	30.8	31.7
Protein degradation rate (g/kg $LW^{0.75}$.d)	13.9^{a}	9.13^{b}	9.28^{b}	10.1	11.4
Fractional synthesis rate (%/bird.d)	26.9^{a}	19.7^{b}	15.6^{c}	20.6	20.8
Fractional degradation rate (%/bird.d)	10.5^{a}	5.88^{b}	5.24^{b}	6.92	7.49
Protein retention/synthesis rate (%)	61.9^{a}	70.4^{b}	66.7^{b}	68.4	64.3

$^{a, b, c:}$ Means bearing different superscripts in the same row within age or sex categories differed significantly (P<0.05).

Whole-body protein fractional SR and DR (%/d) also decreased with age. The ratio of protein retention to protein synthesised increased from 16 to 23 days of age (61.9 *vs* 70.4%; P<0.05), but then decreased from 23 to 30 days of age (70.4% *vs* 66.7%, P<0.05).

Table 3 Fractional synthesis rates (%/bird.d) of whole body protein and of breast muscle, liver and small intestine in young broiler chickens at 16, 23 and 30 days of age

	Age (days)		
	16	23	30
Breast muscle	18.2	15.6	14.9
Liver	28.8	25.9	23.3
Small intestine	51.6	45.4	44.2

Male broilers tended (P=0.12) to have higher rates of whole-body PS (P=0.13) and DR (g/d) than female broilers. There were no significant sex effects or age-sex interactions for tissue weight and protein contents, and thus only the main effects of age are given in Table 2. Fractional PS rates were highest in small intestinal tissue, intermediate in liver (and body tissues as a whole) and lowest in breast muscle tissue, and declined with age (Tables 2 and 3).

IV. DISCUSSION

A tendency for a higher efficiency of amino acid utilization in female than in male birds could be related to a higher efficiency of net absorption or a more efficient utilization of amino acids after absorption. In general, there were only small differences in apparent protein absorption between male and female chickens of different ages. However, the significant age differences and interactions between the age and sex of birds in apparent nitrogen digestibility (see Table 1) may be related to the different requirements for protein and energy with changing tissue composition as birds age, and these factors deserve further study.

The accretion of body protein using the absorbed amino acids in growing broilers is the net effect of higher rates of PS than PD in their various tissues. Absorbed amino acids are the ultimate building monomers for this accretion, but the total rates of PS within the body were 10.2 and 9.5 times their net rates of PD at 16 and 30 days of age respectively. Daily whole-body protein mass turnover at 16 and 30 d on conventional broiler starter/ grower diets was 27% and 16% respectively and daily breast muscle protein turnover was 15-18%. Fractional turnover rates in liver and small intestinal tissues were considerably higher again, viz. 23-52%/d but, because they represented a smaller fraction of the whole-body protein mass, the total amounts of protein turned over in these tissues were less than for muscle. Turnover in the skin and other active tissues were not determined in this study but should also be determined in the future.

Notwithstanding the relatively high estimates of protein turnover in gut and liver tissues, the true values may have been higher because (a) recycling of ^{15}N was assumed to be negligible in making the calculations (which is possibly not correct) and (b) the 'end-product' technique used here would probably not have detected all of the intracellular cycling and re-utilisation of amino acids. Nevertheless, the SR and DR estimates were many times higher than the rates of dietary protein intake and, more particularly, of protein absorption from the gut. As a result, net protein deposition during growth was about 10% of PS rate. Protein turnover is influenced by the age of birds, as shown in this study, but is also regulated in response to plane of nutrition, stress, disease, hormones and level of exercise (Rathmacker, 2000). When the effects of all these factors are better understood, it should be possible through management and dietary manipulation to reduce DR and thereby increase the efficiency of protein deposition in edible meat.

REFERENCES

Rathmacher, J.A. (2000). In: *Farm Animal Metabolism and Nutrition*, pp. 25-48 ed. J.P.F. D'Mello, CABI Publishing, Wallingford, UK.

Summers, J.D., Leeson, S. and Spratt, D. (1988). *Poultry Science*, **68**: 241-248.

Remignon, H., Lefaucheur, L., Blum, J.C. and Ricard, F.H. (1994). *British Poultry Science*, **35**: 65-76.

Tesseraud, S., Peresson, R. and Chagneau, A.M. (1996). *Poultry Science*, **75**: 627-631.

Steel, R.G.D. and Torrie, J.H. (1980). *Principles and Procedures of Statistics* 2nd Ed. McGraw-Hill, Inc, London.

Rathmacher, J.A. (2000). In: *Farm Animal Metabolism and Nutrition*. Ed: D'Mello, J. P. F., CABI Publishing, Wallingford, UK. pp. 25-47.

PERFORMANCE OF BROILERS FED DIETS FORMULATED USING TOTAL OR DIGESTIBLE AMINO ACID VALUES

X. LI[1], K.V. KURKO[2], K.HUANG[1] and W.L. BRYDEN[1,3]

There is increasing interest in the poultry industry to formulate diets based on digestible amino acid values as an indicator of amino acid availability (Ravindran and Bryden, 1999). A number of studies have demonstrated that diets formulated on this basis do improve broiler performance but most studies have only evaluated responses in the starter phase. The object of this study was to evaluate the performance from 0–42 d of broiler chickens fed a diet formulated using total amino acid values (Diet 1) compared to birds fed diets formulated using either published values (Ravindran *et al*., 1998) for digestible amino acids (Diet 2) or using ingredients of known amino acid digestibility (Diet 3) and a diet formulated using an industry feed composition matrix based on amino acid digestibility values (Diet 4).

Diets consisting of sorghum, wheat, canola meal, cottonseed meal, meat and bone meal, soybean meal, minerals and vitamins were formulated to contain 230, 210 and 200g crude protein per kg of diet for starter, grower and finisher, respectively. The apparent metabolisable energy levels were approximately 13 MJ/kg for Diets 1 to 3 and 12.5 MJ/kg for Diet 4. Each diet was fed to 6 pens of 6 male broilers (Cobb); starter from days 1 to 14, grower from days 14 to 28 and finisher from days 28 to 42. Feed consumption and body weight were recorded weekly. On day 42, breast muscle weight and abdominal fat weight measurements were made on 12 birds from each diet following a lethal injection of sodium pentobarbitone. The performance of birds to day 42 is shown in the table.

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P-value
Bodyweight (g)	2050[a]	2426[b]	2437[b]	2420[b]	53.3	0.001
Feed intake (g/bird)	3800[a]	4323[b]	4281[b]	4287[b]	81.6	0.001
Feed conversion (g/g)	1.89[a]	1.83[b]	1.80[b]	1.81[b]	0.018	0.005
Breast muscle (g/kg bodyweight)	121.7[a]	154.3[b]	155.8[b]	172.5[c]	4.29	0.001
Abdominal fat pad (g/kg bodyweight)	23.9[a]	20.9[ab]	19.8[b]	14.8[c]	1.34	0.001

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

Final bodyweight (P<0.001), feed intake (P<0.001) and feed conversion ratio (P<0.005) were significantly improved when diets were formulated on a digestible amino acid basis. These diets also significantly increased (P<0.001) breast muscle weight but decreased P<0.001) abdominal fat pad weight. The change in carcass composition was most apparent with birds fed Diet 4.

The results of this study demonstrate that formulation of diets using digestible amino acid values and fed from hatch to processing can significantly improve bird performance and carcass composition.

Ravindran, V. and Bryden, W.L. (1999). *Aust. J. Agric. Res.* **50**: 889-908.

Ravindran, V., Hew, L.I. and Bryden, W.L. (1998). *Digestible Amino Acids in Feedstuffs for Poultry*, RIRDC, Canberra and Poultry Research Foundation, The University of Sydney, Camden.

[1]Faculty of Veterinary Science, University of Sydney, Camden NSW 2570
[2]Inghams Enterprises Pty Limited, Liverpool NSW 2171
[3]School of Animal Studies, University of Queensland, Gatton Qld 4343

MAXIMUM INCLUSION OF CANOLA MEAL IN BROILER STARTER DIETS FORMULATED ON A DIGESTIBLE OR TOTAL AMINO ACID BASIS

R.A. PEREZ-MALDONADO, K.M. BARRAM and M.F. KEMSLEY

Previous work has have shown that high levels of canola meal (CM) can support satisfactory broiler performance when diets are formulated on a digestible amino acid basis (Perez-Maldonado *et al* 2001). This study investigated the effects of adding 200, 300 and 400 g/kg CM from two processors (Newcastle and Melbourne) to crumbled starter diets formulated on a total or digestible amino acid (AA) basis. There were 13 treatments offered to male broiler (Cobb) chicks (4-25 d) grown in 65 mesh wire cages with 5 replicates per treatment, 8 birds per cage in a completely randomised design. The control diet contained sorghum (45%), wheat (25%), soybean meal (17%), meat and bone and poultry offal meals at 5% each plus vitamins and minerals. Food, water, light and temperature were offered in an environmentally controlled house according to industry practice. Each CM was analysed for proximate and mineral composition, AAs, energy, glucosinolates, total condensed tannins, AME and ileal digestible AA as described by Perez-Maldonado *et al.* 2001.

Treatment		Formulation basis	Feed intake (g/bird)	Growth rate (g/bird)	Feed efficiency (g/g)
Control			1266 ^{ab}	920 ^{abc}	1.382 ^{e}
Newcastle CM	200 g/kg	Total AA	1268 ^{ab}	925 ^{ab}	1.374 ^{cde}
	300 g/kg	Total AA	1223 ^{bcd}	896 ^{abc}	1.374 ^{cde}
	400 g/kg	Total AA	1185 ^{cde}	864 ^{d}	1.388 ^{de}
	200 g/kg	Digestible AA	1235 ^{abc}	921 ^{abc}	1.339 ^{ab}
	300 g/kg	Digestible AA	1188 ^{cde}	896 ^{abc}	1.335 ^{a}
	400 g/kg	Digestible AA	1132 ^{e}	845 ^{d}	1.348 ^{abc}
Melbourne CM	200 g/kg	Total AA	1285 ^{a}	937 ^{a}	1.379 ^{de}
	300 g/kg	Total AA	1208 ^{cd}	878 ^{cd}	1.384 ^{de}
	400 g/kg	Total AA	1222 ^{bcd}	877 ^{cd}	1.400 ^{e}
	200 g/kg	Digestible AA	1218 ^{bcd}	889 ^{bcd}	1.371 ^{cd}
	300 g/kg	Digestible AA	1216 ^{bcd}	892 ^{abc}	1.364 ^{bcd}
	400 g/kg	Digestible AA	1176 ^{de}	855 ^{d}	1.375 ^{de}
LSD (P<0.05)			56.4	45.2	0.0257

$^{a-e}$ Means in a column with different superscripts differ significantly (P<0.05)

Response in 4-25d food intake (FI, g/bird), growth rate (GR, g/bird) and FCR was influenced by CM source, CM level of inclusion and method of formulation. For both CMs, GR and FI were depressed at the highest (400 g/kg) level of inclusion. For Newcastle CM at all three levels of inclusion, formulation on a digestible AA basis resulted in a significantly (P<0.05) better feed efficiency than in the birds given the equivalent diet formulated on a total AA basis. The relative contribution of FI and GR to this response varied with level of inclusion. The results indicate that satisfactory performance can be obtained with broiler starter diets containing up to 300g CM /kg and that growth and feed efficiency on diets containing CM are likely to be improved by formulation on a digestible AA basis.

Perez-Maldonado, R.A., Blight, G.W. and Pos, J. (2001). *Proceedings of the Australian Poultry Science Symposium*, Ed. D. Balnave. **13**: 156-159.

Queensland Poultry Research and Development Centre, PO Box 327, Cleveland, Qld 4163.

MAXIMUM INCLUSION OF CANOLA MEAL IN BROILER FINISHER DIETS FORMULATED ON A DIGESTIBLE OR TOTAL AMINO ACID BASIS

R. A. PEREZ-MALDONADO [1], K.M. BARRAM [1], P. C.TRAPPETT [1] and G. L. KERVEN [2]

The effects of adding 200, 300 and 400 g/kg CM from two processors (Newcastle and Melbourne) to finisher diets formulated on a total or digestible amino acid basis were investigated. A total of 13 treatments (Perez-Maldonado *et al.*, 2002) were offered to male broiler (Cobb) chickens (24-42 d) grown in 65 mesh wire cages with 5 replicates per treatment, 8 birds per cage in a completely randomised design. Food, water, light and temperature were offered in an environmentally controlled house according to industry practices. Growth rates (GR) and feed intakes (FI) were recorded at 42 d of age when two birds per cage from control diet and 200 and 400 g CM/kg treatments were sacrificed and weighed. Digesta from the small intestine (SI) was collected; liver, pancreas and fat pad removed and individually weighed. Analyses are described by Perez-Maldonado *et al.* (2001).

Treatment		Formulation basis	Feed intake (g/bird)	Growth rate (g/bird)	Feed efficiency (g/g)
Control			3074 ^{ab}	1619 ^{ab}	1.901 ^{bcd}
Newcastle CM	200 g/kg	Total AA	2967 ^{bcd}	1605 ^{abc}	1.916 ^{cd}
	300 g/kg	Total AA	2893 ^{cde}	1617 ^{abc}	1.808 ^{a}
	400 g/kg	Total AA	2866 ^{de}	1504 ^{bc}	1.906 ^{bcd}
	200 g/kg	Digestible AA	3016 ^{abc}	1634 ^{ab}	1.855 ^{abc}
	300 g/kg	Digestible AA	2923 ^{cde}	1605 ^{abc}	1.848 ^{abc}
	400 g/kg	Digestible AA	2831 ^{e}	1529 ^{abc}	1.851 ^{abc}
Melbourne CM	200 g/kg	Total AA	3103 ^{a}	1661 ^{a}	1.892 ^{bcd}
	300 g/kg	Total AA	3010 ^{abc}	1598 ^{abc}	1.911 ^{cd}
	400 g/kg	Total AA	2821 ^{e}	1542 ^{abc}	1.843 ^{ab}
	200 g/kg	Digestible AA	2927 ^{cde}	1530 ^{abc}	1.939 ^{d}
	300 g/kg	Digestible AA	2870 ^{de}	1553 ^{abc}	1.865 ^{abc}
	400 g/kg	Digestible AA	2797 ^{e}	1476 ^{c}	1.887 ^{bcd}
LSD (P<0.05)			130.2	141.6	0.0679

$^{a-e}$ Means in a column with different superscripts differ significantly (P<0.05)

For both CMs, inclusion rate affected both FI and GR. There was an overall linear negative relationship between CM inclusion rate and FI and a significant (P<0.05) depression in mean GR on the 400 g/kg CM diets. As a result of this, mean FCR on the 300 g/kg CM diets was lower than on the control or 200 g/kg CM diets. Thus, very satisfactory GR and feed efficiency were obtained on diets containing 300g/kg CM. Contrary to the findings during the starter period (Perez-Maldonado *et al*., 2002) formulating diets on a digestible AA basis did not improve feed efficiency during the finisher period. Inclusion of CM reduced abdominal fat proportion from 14.5 to 9.7 g/kg, SI viscosity from 4.0 to 2.5 cP without effecting liver weight, but relative pancreas weight increased from 1.58 to 1.87 g/kg.

Perez-Maldonado, R.A., Blight, G.W. and Pos, J. (2001). *Proceedings of the Australian Poultry Science Symposium*, Ed. D. Balnave. **13**: 156-159.

Perez-Maldonado, RA., Barram K.M. and Kemsley, M. F. (2002). *Proceedings of the Australian Poultry Science Symposium*, Ed. R.A.E. Pym. **14**: in press.

[1] Queensland Poultry Research and Development Centre, PO Box 327, Cleveland, Qld 4163.
[2] The University of Queensland, School of Land and Food Sciences, St Lucia, Qld 4072.

EVALUATION OF IMMUNOGLOBULIN-FORTIFIED PROTEIN SOURCES IN BROILER DIETS

M.R. KING, P.C.H. MOREL, D.V. THOMAS, B.J. CAMDEN and V. RAVINDRAN

Animal protein sources that contain high levels of immunoglobulins, have been shown to produce performance benefits when included at levels of up to 60 g/kg in pig starter diets (van Dijk *et al*., 2001). No published data is currently available on the use of these protein sources in poultry diets. The present study was designed to evaluate the performance of broiler chickens offered starter diets containing spray-dried bovine colostrum (SBC), spray-dried bovine plasma (SBP) or spray-dried porcine plasma (SPP) as protein sources. The crude protein, lysine and methionine plus cysteine contents (g/kg) of SBC, SBP and SPP were: 816, 777 and 745; 61.3, 66.6 and 59.9; and 28.2, 31.5 and 26.1, respectively. The test proteins were incorporated into a maize-soybean meal diet at a level of 50 g/kg. All diets were balanced to contain similar levels of metabolisable energy, lysine, methionine plus cysteine and dietary electrolytes. Each of the four experimental diets was fed to six replicates of eight male broiler chicks (Ross) from 1 to 14 days of age. On day 14, the experimental diets were removed and replaced with a common grower diet, which was offered until day 35. Data were analysed using the GLM procedure of SAS.

Dietary treatment	Weight gain (g/bird)	Feed intake (g/bird)	Feed/gain
Day 1-14			
Maize-soy control	420	517	1.232[a]
Spray-dried bovine colostrum	435	518	1.193[b]
Spray-dried bovine plasma	427	517	1.210[ab]
Spray-dried porcine plasma	416	501	1.210[ab]
Pooled SEM	7.7	8.5	0.008
Day 1-35			
Maize-soy control	2101	3422	1.647
Spray-dried bovine colostrum	2170	3459	1.619
Spray-dried bovine plasma	2161	3484	1.639
Spray-dried porcine plasma	2121	3442	1.638
Pooled SEM	35.7	66.1	0.012

Dietary treatments had no effect ($P>0.05$) on the weight gain or feed intake of birds during the first 14 days. However, feed/gain of birds fed the SBC-diet was lower ($P<0.05$) than those fed the control diet. Numerical reductions in feed/gain were observed in SBP and SPP diets, but the differences were not significant. Dietary treatments had no effect ($P>0.05$) on later performance 14-35 d (data not shown) or 1-35 d. These results suggest that there may be early performance benefits from supplementing broiler starter diets with specialised globulin proteins.

van Dijk, A.J., Everts, H., Nabuurs, M.J.A., Margry, R.J.C.F. and Beynen, A.C. (2001). *Livest. Prod. Sci.* **68**: 263.

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

MULTI METHOD EFFICACY COMPARISON OF COMMERCIAL ANTIOXIDANTS IN AUSTRALIA AND NEW ZEALAND

T.J. WALSH[1], J. ANDREWS[1], and D. DAMJANOVIC[2]

Summary

The effectiveness of five commercially available antioxidants currently used in Austrailia and New Zealand were evaluated using two test media, poultry fat and tallow. The five antioxidants were Santoquin®, BHT (butylated hydroxytoluene), Endox, Oxistat and Oxicheck. To determine their effectiveness, four stability measurements were run on each antioxidant. These measurements included the Peroxide Value Method (PV), the Oxidative Stability Index (OSI), the Rancimat and the Active Oxygen Method (AOM). Results indicate that a wide variation in efficacy exists between the commercially available antioxidants currently being used in the animal feed industry.

I. INTRODUCTION

The oxidative destruction of dietary nutrients begins during ingredient manufacturing and continues until absorption occurs in the digestive tract of an animal. Therefore an effective antioxidant plays an essential role in any feed quality control program. Preventing oxidative rancidity of fats and oils is important because it allows the metabolizable energy value to be maintained and prevents rancid odors that can lead to a decrease in feed intake by the animal. In addition it protects Vitamins A and E from destruction as well as preventing oxidative loss of the fat-soluble vitamins and pigments even through the digestive process. Several commercial antioxidants are currently available for today's nutritionists, however the relative effectiveness of these antioxidants has not recently been tested. It would be useful to test these antioxidants using several different measurements to see which one gives the best oxidative stability.

The oxidative process begins with free oxygen combining with unstable fat to form peroxy radicals. These radicals can then attack the carbon-hydrogen bonds located next to the double bonds in fat, and create hydroperoxides. Over time, peroxides can decompose into other products such as aldehydes and ketones that are toxic. Once these secondary products are formed, the process cannot be reversed, hence antioxidants need to be added as early as possible to prevent their formation. Factors that affect the rate of oxidation are ingredient type, temperature, surface area exposure to oxygen and time. For example, it is known that storing fats in the summer will increase the rate of oxidation as the rate of oxidation doubles for every 10 ^{o}C rise in temperature.

To measure the effectiveness of an antioxidant several methods are commonly used. These tests measure different products in the oxidation process including peroxides, which are the primary product of oxidation, aldehydes and ketones the secondary products of oxidation, as well as volatile fatty acids, which are tertiary oxidation by-products. The Peroxide Value Method (PV, AOCS, 1993a) measures peroxides by a titration method, in milliquivalents of peroxides per 1000 grams of sample. Generally values below 5 meg/kg are desired. However simply measuring initial PV does not provide a "true" sense of the stability of the ingredient. Therefore it is optimal to also measure PV's at other points along the oxidation curve. This is usually done using the Active Oxygen Method (AOM, AOCS,

[1] 530 Maryville Centre Dr., St Louis, MO 63132 USA
[2] PO Box 157, Drummoyne, NSW 1470

1993b). In this test the fat is heated to 98 ^{o}C and air is bubbled through the sample for 20 h. Peroxides can then be measured by titration at 0, 4 and 20 h. A modified version of the AOM measures the number of hours it takes to reach 20 meq peroxides/kg of fat. For the modified AOM, the greater the number of hours it takes to get to 20 meq/kg of fat, the better the oxidative stability of the fat or the antioxidant being tested. Times above 20 h are desired. Another test is the Oil Stability Index (OSI, AOCS, 1993c)). The OSI test measures the "induction" time or the time it takes until the production of volatile fatty acids, which are tertiary byproducts of oxidation. . The more effective antioxidant will have a longer induction time to the production of volatile fatty acids. The Rancimat test also measures volatile fatty acids, but uses different experimental conditions.

The above methodologies (PV, AOM, modified AOM, OSI and Rancimat) were utilised to test the effectiveness of five antioxidants currently on the Australian and New Zealand market.

II. METHODS

The five antioxidants chosen for comparisons were: Santoquin, BHT (butylated hydroxytoluene), Endox, Oxistat and Oxicheck. Each antioxidant was tested on both poultry fat and tallow at the manufacturers' recommended rates. Results of the relative effectiveness of the five antioxidants are coded and are not reported according to trade name.

Sample Preparation Procedure: According to the recommended usage rates, the calculated amount of each individual antioxidant was weighed into a 250-ml beaker (Table 1). Approximately 100 g of the test fat was added, stirred well and then transferred into a 1 quart bottle. This procedure was repeated three times to ensure complete transfer to the main storage bottle. Additional fat was added to reach a total of 600 g of fat per test fat/antioxidant and mixed well. All test materials were stored at –20^{0}C until used.

Table 1. Calculation of antioxidant inclusion rates to 600 g fat sample.

Antioxidant	Control	BHT*	Santoquin	Endox	Oxistat	Oxicheck
Add'n Level (ppm)	0	200	500	500	500	500
Weight (g)	0	0.120	0.300	0.300	0.300	0.300

* FDA limit of 200 ppm in fat or oil.

Stability Measurement Procedures: The Peroxide Value Method (PV) was measured using a titration procedure (Method #Cd 8-53, AOCS,1993a). The Active Oxygen Method (AOM, Method #Cd 12-57, AOCS, 1993b) was measured by heating the test fat to 98 ^{o}C while air was bubbled through the sample for 20 h. Peroxide levels were measured by titration at 20 h. For the modified AOM test, fat samples were held under the same test conditions until a level of 20 meq peroxide/kg of fat was reached. The Oil Stability Index (OSI) and Rancimat tests were both conducted at 110 ^{o}C. The two tests varied in the quantity of sample used and air flow rate; the OSI test (AOCS Method #Cd 12b-92, AOCS 1993c) uses a 5 g sample and an airflow rate of 2.5 ml/sec while the Rancimat test employs a 2.5 g sample and an airflow rate of 5.6 ml/sec.

The PV, AOM and Rancimat analyses were run at the analytical laboratories of Novus International, Inc. while the Oil Stability Index test was conducted at an outside contract laboratory. Duplicate analyses were run only on the Rancimat data with statistical analysis done using the GLM procedure of SAS.

III. RESULTS

Initial Peroxide Method: Peroxide values were determined on both the tallow and poultry fat to provide a measure of their initial stability value. The initial peroxide value for the poultry fat and tallow were 4.15 and 2.54 meq/kg respectively.

Active Oxygen Method: Table 2 shows the results for the AOM test. The control sample for the tallow was the only treatment to fail the 20-h AOM test. For poultry fat, the control and Products A, D and C failed the 20-h AOM test. Product E was the most effective antioxidant in preventing oxidation under this methodology with Product B performing better than Products A, D and C but less efficacious than Product E. The modified AOM test gave the same ranking of antioxidants as the AOM test for both substrates.

Table 2. Relative effectiveness of the five antioxidants for poultry fat and tallow determined by the AOM and modified AOM methods

Antioxidant	20-h AOM (meq peroxides/kg fat)		Modified AOM (h to 20 meq perodixdes/kg fat)	
	Poultry Fat	Tallow	Poultry Fat	Tallow
A	32.3	3.6	16	53
B	15.3	2.8	22	108
C	208.8	19.3	8	20
D	189.6	6.1	9	33
E	2.3	1.5	165	210
Control	254.7	52.7	8	14

Oil Stability Index: Table 3 shows the results for the Rancimat and OSI data. The two methodologies produced similar results. There was a significant stepwise time effect ($P<.0001$) for the Rancimat induction time of tallow with Product E having the longest induction time (34.7 h) and the control sample having the shortest (3.3 h). For poultry fat, Product E again resulted in significantly longer induction time compared to all other treatments ($P<.0001$). Product C and Product D were not significantly different from one another with induction times of 4.8 h and 5.0 h respectively. Product A and Product B were intermediate, with A performing slightly, although not significantly ($P>0.05$), better with an induction time of 6.5 h.

Table 3. Comparison of the effectiveness of the five antioxidants on poultry fat and tallow using the Rancimat and OSI tests.

Antioxidant	Rancimat Induction Time (h)				OSI Induction time (h)	
	Poultry Fat	SE	Tallow	SE	Poultry Fat	Tallow
A	6.5^{b}	0.11	9.5^{c}	0.18	6.5	14.9
B	5.8^{bc}	0.04	10.9^{b}	0.17	7.4	17.8
C	4.8^{cd}	0.04	7.3^{e}	0.02	4.9	6.6
D	5.0^{bcd}	0.12	7.9^{d}	0.07	5.4	13.4
E	20.8^{a}	1.12	34.7^{a}	0.28	28.7	34.1
Control	4.1^{d}	0.10	3.3^{f}	0.02	4.7	6.0

Means with different superscripts within a column are significantly different ($P<0.05$) (Rancimat test only).

IV. DISCUSSION

All methodologies used to determine the relative effectiveness of the five antioxidants resulted in the same ranking of products for both substrates. This was so despite the different end products determined in the Rancimat and OSI tests than in the PV and AOM tests. Although the Rancimat test data was the only set to be subjected to statistical analysis, given the sensitivity of the statistical test in discriminating between the mean values of this test as indicated by the low standard errors (Table 4), and the similar ranking of products in all tests, the inference is that differences of any magnitude in the other test data shown in Tables 2 and 3, are real

Of the five commercial antioxidants tested, Product E performed the best regardless of the methodology used for comparing their effectiveness. The methodologies used to determine the effectiveness of the commercially available antioxidants confirmed that these products differ in their ability to stabilize different fat sources. However, it must be recognized that these different tests measure different products of oxidation. For example, a low PV does not necessarily indicate that the fat quality is good. A low value could mean that the fat has gone through extensive oxidation, and could have a negative impact on growth and performance. The tests should thus be applied judiciously in establishing an effective quality control program for feed ingredients.

Fat quality and stability during storage is essential for optimum performance of animals. Without proper stabilization during storage, performance measures such as growth rate and feed conversion may suffer. The present results demonstrate that commercially available antioxidants differ significantly in their capacity to provide adequate stability.

REFERENCES

AOCS. (1993a) Peroxide Value: Acetic Acid-Chloroform Method, AOCS Official Method #Cd 8-53. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th Edition, AOCS Press.

AOCS. (1993b). Fat Stability: Active Oxygen Method, AOCS Official Method #Cd 12-57. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th Edition, AOCS Press.

AOCS. (1993c). Oil Stability Index, AOCS Official Method #Cd 12b-92. *Official Methods and Recommended Practices of the American Oil Chemists' Society, 4th Edition*, AOCS Press.

.

EFFECT OF SELENIUM AND VITAMIN E CONTENT OF THE DIET ON LIPID PEROXIDATION IN BREAST MUSCLE TISSUE OF BROILER BREEDER HENS DURING STORAGE

P.F. SURAI[1] and J. E. DVORSKA[2]

Summary

The aim of the present work was to study the effect of organic selenium in combination with vitamin E in the broiler breeder diet on lipid peroxidation in the breast muscle of the breeder hen during storage at -20°C. Supplementing the breeder's diet with 0.2 or 0.4 ppm organic selenium or with 40, 100 or 200 ppm of vitamin E either alone or in combination for 12 weeks increased breast muscle stability during 24 months storage at –20° C. These stabilising effects are considered to be due to increased activity of glutathione peroxidase and increased vitamin E concentration in the muscles.

I. INTRODUCTION

For the last few years consumer demands regarding meat quality have substantially increased. There is consequently a great pressure on the meat industry to enhance the image of meat purchased at the supermarket (Janssens, 1998). The major meat quality characteristics that attract consumer attention include appearance, texture and flavour (Liu *et al*., 1995) as well as tenderness, juiciness and aroma (Janssens, 1998). Among these, appearance has a major impact on the initial decision of the customer to purchase or reject the product (Sheehy *et al*., 1997). Clearly consumers today prefer fresh meat with a minimum loss of water during handling and cooking. Therefore water-holding capacity of the meat (Mahan and Kim, 1999) as well as colour (Froning, 1995) and absence of off-flavours (Sheehy *et al*., 1997) are considered to be the most important meat quality characteristics.

It has been shown that the sensory quality of meat is affected by modern processing technologies (Ouali, 1991). For example, grinding increases oxygen incorporation into muscle, which could increase lipid peroxidation, and cooking releases protein-bound iron into the intracellular pool (Chan and Decker, 1994) which also stimulates lipid peroxidation. In this process free radical production and lipid peroxidation cause membrane structure and quality disruption, which leads ultimately to significant losses in food quality, including off-flavour, off colours and poor texture (Stanley, 1991) as well as to accumulation of toxic products of lipid peroxidation.

Various approaches have been implemented by the meat industry to resolve the problem. One of the most promising solutions could be to enhance oxidative stability of meat by adding antioxidants either to the animals' diet or directly during processing (Decker, 1998). There is an increasing body of evidence which indicates that increased vitamin E supplementation is an effective means of improving meat quality in chickens, turkeys, cattle, pigs and lambs (Sheehy *et al*., 1997; Wulf *et al*., 1995; Buckley *et al*., 1995; Liu *et al*., 1995).

[1]Avian Science Research Centre, Scottish Agricultural College, Auchincruive,Ayr, KA6 5HW, Scotland, UK
[2]Department of Veterinary Medicine, Sumy State Agrarian University, Sumy, Ukraine

Recently we have shown that organic selenium in the breeder diet is deposited in the egg and in embryonic tissues and is effective in enhancing antioxidant defences of newly hatched chicks (Surai, 2000a). The effects of Se were seen during 5-10 days posthatch, when vitamin E concentration in the liver progressively declined and higher emphasis to antioxidant enzymes was observed (Surai, 2000b). However, the benefit to the breeders themselves from organic selenium supplementation has not been studied.

The aim of the present work was to study the effect of organic selenium in combination with vitamin E in the breeder diet on lipid peroxidation in the breast muscle of the hens during storage at -20°C.

II. MATERIALS AND METHODS

Ninety Cobb broiler breeder hens were divided into 9 equal groups and housed in pens at 25 weeks of age. Each hen received one of the nine treatment diets (Table). Selenium was supplemented in the form of Sel-Plex (Alltech, Inc), containing Se mainly as selenomethionine. After 12 weeks of feeding breeders were sacrificed and liver and breast muscle were collected and frozen at – 20°C. After 24 months of storage samples were defrosted and vitamin E, GSH-Px activity and malondialdehyde (MDA, the final product of lipid peroxidation) were determined in muscles from 5 birds in each group. Results of egg and embryo analyses as well as methods used for assay were reported previously (Surai, 2000). Vitamin E was determined by using an HPLC system (Shimadzu Liquid Chromatograph, LC-10AD, Japan Spectroscopic Co Ltd with JASCO Intelligent Spectrofluorometer 821-FP) fitted with a Spherisorb, type S30DS2, 3μ C_{18} reverse phase HPLC column, 15cm × 4.6mm (Phase Separations Limited, UK). Chromatography was performed using a mobile phase of methanol/water (97:3, v/v) at a flow rate of 1.1 ml/min. Fluorescence detection of α-tocopherol involved excitation and emission wavelengths of 295 and 330 nm respectively.

For GSH-Px determination tissue samples were washed in potassium phosphate buffer (10 mM, pH 7.4) at 4°C and homogenised in nine volumes of the same buffer, supplemented with 30 mM KCl. The homogenate was centrifuged (3500 g, for 30 min at 4°C) and the enzyme activities of the supernatant were determined. Se-dependent GSH-Px activity was measured by a coupled reaction with excess glutathione reductase and monitoring the NADPH oxidation at 340 nm using hydrogen peroxide as a substrate. Units of glutathione peroxidase activity were expressed as μmol NADPH oxidized/min/g fresh tissue.

MDA accumulation was determined by HPLC in freshly defrosted tissue after homogenization in phosphate buffer (0.05M) at pH 7.4. Samples of the homogenate were incubated for 1 hour at 37°C either without peroxidation inducers to assess spontaneous lipid peroxidation, or in presence of Fe^{2+}, to determine Fe-stimulated lipid peroxidation.

III. RESULTS

As shown in Table 1, vitamin E concentration in the breast muscle was directly related to dietary levels of vitamin E and, to a lesser extent, to selenium supplementation. Dietary supplementation of organic Se at a level of 0.4 ppm significantly increased vitamin E concentration in the breast muscle. Combinations of vitamin E and organic selenium in the diet were more effective in promoting vitamin E accumulation in breast muscle than vitamin E alone.

Table 1. Effect of selenium and vitamin E on breast muscle quality during storage (n=5)

Dietary supplementation				Breast muscle composition				
Dietary Group	Diet	Vitamin E, mg/kg	Se, mg/kg	Vitamin E, μg/g	Se-GSH-Px, %	MDA, initial level, μg/g	MDA, free peroxidation, μg/g	MDA, Fe-stimulated peroxidation, μg/g
1	Semi-synthetic	-	-	1.34 ± 0.15[a]	63.5[a]	2.46 ± 0.25[a]	3.96 ± 0.44[a]	10.22 ± 0.66[a]
2	Commercial (CD)	-	-	1.66± 0.14[a]	100[b]	1.55 ± 0.16[b]	2.49 ± 0.25[b]	7.66 ± 0.70[b]
3	CD	-	0.2	1.88± 0.39[a]	226.4[c]	0.82 ± 0.03[c]	1.44 ± 0.09[c]	3.28 ± 0.60[c]
4	CD	-	0.4	2.14± 0.13[b]	257.2[cd]	0.56 ± 0.07[df]	0.81 ± 0.11[df]	2.99 ± 0.37[c]
5	CD	40	-	2.55± 0.33[bd]	109.3[b]	0.73 ± 0.03[cd]	1.05 ± 0.13[ed]	2.93 ± 0.24[c]
6	CD	100	-	4.88± 0.61[c]	122.2[b]	0.51 ± 0.07[ef]	0.72 ± 0.12[edf]	2.06 ± 0.43[ce]
7	CD	200	-	5.11± 0.47[c]	119.4[b]	0.33 ± 0.04[g]	0.66 ± 0.10[gf]	1.01 ± 0.15[d]
8	CD	40	0.2	3.22± 0.36[d]	265.4[cd]	0.50 ± 0.05[ef]	0.62 ± 0.10[gf]	1.66 ± 0.30[de]
9	CD	100	0.4	5.88± 0.59[c]	305.9[ed]	0.31 ± 0.05[g]	0.51 ± 0.09[gf]	0.96 ± 0.11[fd]

Values are means ± S.E.M; values in a column which do not have a common superscript are significantly (P<0.05) different

[a]The level of selenium in the semi-synthetic diet was 44 μg/kg and in commercial diet 171 μg/kg. Selenium was supplemented in the form of Sel-Plex. Semi-synthetic and commercial diets contained 4.86 and 10.05 mg/kg α-tocopherol. Both, commercial and semi-synthetic diets were balanced in other nutrients.

The semi-synthetic diet was comparatively low in Se and this caused a significant decrease in Se-GSH-Px activity in the muscle. On the other hand, inclusion of organic selenium in the commercial breeder diet increased Se-GSH-Px activity in muscles more than two-fold. The difference in Se-GSH-Px activity between 0.2 and 0.4 ppm Se supplementation was not dramatic, but higher Se supplementation promoted higher Se-GSH-Px activity in muscles. There was also a trend towards increased Se-GSH-Px activity in muscles associated with vitamin E supplementation of the diet. Combination of organic selenium and vitamin E supplementation were associated with the highest Se-GSH-Px activity in muscles.

As a result of significant differences in dietary antioxidant composition, the initial level of MDA in the breast muscle after 2 year storage at –20 C differed significantly ($P<0.05$) between the various groups. For example, the highest level of the final product of lipid peroxidation was found in muscles from laying hens fed on a semi-synthetic diet and characterised by the lowest vitamin E and Se-GSH-Px activity. The lowest initial level of MDA was found in muscles from birds fed diets supplemented with either 200 ppm vitamin E or 100 ppm vitamin E in combination with 0.4 ppm organic selenium.

Spontaneous and Fe-stimulated lipid peroxidation in the muscles also reflected dietary antioxidant composition and were lowest in the muscles from birds fed the diets supplemented with both vitamin E and organic selenium, or with high levels of either vitamin E (100 or 200 ppm) or Se (0.4 mg/kg) alone.

IV. DISCUSSION

Based on the data presented above and taking into account antioxidant interactions, it is possible to suggest that synergism between Se and vitamin E could work to enhance meat quality. In fact, it has been shown previously that GSH-Px activity in muscles did not change significantly over 8-day storage of beef (Renerre *et al*., 1996). This means that once GSH-Px activity is elevated it is maintained post-mortem. This was also the case in our study where after 24 months of storage Se-GSH-Px activity was significantly higher in the breast muscles from birds supplemented with organic selenium. This suggests a stabilizing effect of dietary Se supplementation on meat quality during meat storage. Our data are in agreement with the findings of DeVore *et al*. (1983). In that study supplementing broiler diets with 0.25 ppm Se substantially increased GSH-Px activity in breast (2.1-fold) and leg (4.1-fold) muscle; and as a result decreased lipid peroxidation was detected (2.5-fold in breast muscle and 3.3-fold in leg muscles) after 4 days storage at 4°C compared to the control group (DeVore *et al*., 1983). These data clearly indicate that GSH-Px significantly contributes to the overall antioxidant defence of muscle, decreasing tissue susceptibility to lipid peroxidation and that increasing oxidative stability of skeletal muscle can be accomplished by organic Se supplementation of the diet.

As clearly seen from the data, the best results in terms of meat stability during frozen storage were achieved with vitamin E supplementation in combination with organic selenium. In support of this, Avanzo *et al*. (2001) have shown that deficiencies of α-tocopherol and Se caused multiple alterations in the antioxidant system and adversely affected the redox state of chicken superficial pectoralis muscle. The evidence suggests that protective effects of Se may be mediated via improvement of other chains of antioxidant defence. For example, Se in combination with vitamin E increased activity of superoxide dismutase in chicken serum (Tras *et al*., 2000). A stabilising effect of Se in combination with other antioxidants on meat quality would be a great advantage in producing so-called ‘designer’ meat. For example, meat enriched with n-3 PUFAs was shown to have increased thiobarbituric acid (TBA) values during storage; and the same meat from antioxidant-

supplemented (Se + vitamins E and C) chickens showed lower TBA values and greater colour stability during storage (Ahn *et al*., 1998).

V. CONCLUSIONS

Our results show that inclusion of organic selenium in the diet of breeders can have beneficial effects not only on egg quality as it was shown previously (Surai, 2000) but breeders themselves can benefit from better antioxidant protection. In our case breast muscle storagability was significantly improved as a result of dietary Se or Se+vitamin E supplementation. Taking into account consumer demand for healthier meat a combination of organic selenium with increased vitamin E supplementation could be an effective means of meeting these demands. However, this approach requires further investigation with broiler chickens.

VI. ACKNOWLEDGEMENTS

We are grateful to the Scottish Executive Rural Affairs Department for financial support for PFS, to the World Poultry Science Association for the Science Award for PFS, to the Houghton Trust, Wellcome Trust and British Poultry Science Ltd for Travel Grants for JED, to Alltech Inc. (USA) for providing Sel-Plex and to F. Hoffmann La Roche Ltd. (Basel, Switzerland) for providing tocol and carotenoids.

REFERENCES

Ahn, C.N., Chae, H.S., Kim, D.W., Yoo, Y.M., Kim, Y.K. and Rhee, Y.C. (1998). *Journal of Livestock Science*, **40**: 96-102.

Avanzo, J.L., De Mendonca, C.X. Jr, Pugine, S.M., and De Cerquwira Cesar, M. (2001). *Comparative Biochemistry and Physiology*, **129C**: 163-173 .

Buckley, D.J., Morrissey, P.A. and Gray, J.I. (1995). *Journal of Animal Science,* **73**: 3122-3130.

Chan, K.M. and Decker, E.A. (1994). *Critical Reviews in Food Science and Nutrition,* **34**: 403-426.

Decker, E.A. (1998). *Trends in Food Science and Technology,* **9**: 241-248.

DeVore, V.R., Colnago G.L., Jensen, L.S. and Greene, B.E. (1983). *Journal of Food Science*, **48**: 300-301.

Froning G.W. (1995). *Poultry and Avian Biology Reviews,* 6: 83-93.

Janssens, G. (1998). *Meat Processing: International Edition*. September/October **42**: 44-46.

Liu, Q., Lanari, M.C. and Schaefer, D.M. (1995). *Journal of Animal Science,* **73**: 3131-3140.

Mahan, D.C. and Kim, Y.Y. (1999). *Asian-Australian Journal of Animal Sciences*, **12**: 287-294.

Ouali, A. (1991). Animal Biotechnology and the Quality of Meat Production (Fiems L.O., Cottyn B.G. and Demeyer D.I., Eds.). Elsevier Science Publishers B.V. Amsterdam, pp. 85-105.

Sheehy, P.J.A., Morrissey,P.A., Buckley, D.J. and Wen, J. (1997). Recent advances in animal nutrition. (Garnsworthy P.C. and Wiseman J., Eds.) Nottingham University Press, Nottingham, pp. 3-27.

Stanley, D.W. (1991). *Critical Reviews in Food Science and Technology*, **30**: 487-553.

Surai, P.F. (2000a). *British Poultry Science,* **41:** 235-243.

Surai, P.F. (2000b). In: *Biotechnology in the Feed industry*. Proc. Alltech's 16th Annual Symposium (Lyons T.P. and Jacques K.A., eds.). Nottingham University Press, Nottingham, UK, pp.205-260.

Tras, B., Inal, F., Bas, A.L., Alyinok, V. and Yazar, E. (2000). *British Poultry Science*, **41**: 664-666.

Wulf, D.M., Morgan, J.B. Sanders, S.K., Tatum, J.D., Smith, G.C. and Williams, S. (1995). *Journal of Animal Science*, **73**: 399-405.

AN AUSTRALIAN MODEL FOR EXPERIMENTAL REPRODUCTION OF CLOSTRIDIAL ENTERITIS IN BROILERS

A. KOCHER[1], G. ANDERSON[2], M. CHOCT[1] and R.R. CARTER[3]

A significant threat to the broiler industry is the sporadic outbreak of necrotic enteritis (NE). Damage to the intestinal mucosa through coccidial infection or a change in the normal intestinal microflora predisposes birds to the proliferation of *Clostridium perfringens* (*CP*) resulting in NE. Presently, the occurrence of NE in poultry is controlled by adding antibacterial growth promotants (AGP) to feed. Although, Australia has no ban on AGP in place there are increased efforts in the poultry industry to find alternatives to AGP. This paper reports the results from two experiments investigating a possible challenge model for the experimental induction of subclinical NE for subsequent evaluation of alternatives to AGP. Day-old male broiler chickens were raised in multi-deck brooders to 13 days of age and fed commercial broiler starter crumbles. From day 13 to the end of the experiment the birds received the experimental diet without AGP and without a coccidiostat. On day 17 the birds were challenged with 7000 each of sporulated oocysts of *E. acervulina* and *E. brunetti* (Cocci challenge, CoC). On days 21, 22 and 23 birds allocated to the challenge groups were inoculated with 1mL *CP* (~10^{8-9}CFU/ml) using a commercially available crop needle. Feed intake and weight gain were measured over the entire experimental period. On day 28 all birds were killed and the intestines were examined for possible damage due to the coccidiosis infection and/or NE.

Treatment	Experiment 1				Experiment 2			
	Live weight g/bird	Weight gain g/bird	Feed intake g/bird	FCR g/g	Live Weight g/bird	Weight gain g/bird	Feed intake g/bird	FCR g/g
Control (C)	1369[a]	922.1[a]	1344.0	1.470[b]	1200[a]	820.6[a]	1507.1	1.837[c]
C+ *CP* 1[1]	---	---	---	---	1151[ab]	785.6[a]	1427.2	1.821[c]
C+ *CP* 2[1]	---	---	---	---	1128[ab]	752.8[ab]	1449.0	1.930[bc]
CoC	1297[ab]	854.7[ab]	1282.9	1.520[ab]	1076[b]	696.4[b]	1494.1	2.142[b]
CoC + *CP* 1[1]	1195[b]	750.8[b]	1226.4	1.644[a]	1081[b]	685.7[b]	1420.8	2.087[b]
CoC + *CP* 2[1]	---	---	---	---	924[c]	535.2[c]	1289.6	2.438[a]
SEM	42.6	43.1	42.0	0.047	46.2	43.9	54.3	0.106
Probability	0.008	0.008	0.121	0.021	0.000	0.000	0.09	0.000

[a,b,c] Values within columns without a similar superscript are significantly different (P<0.05).
[1] CP 1 and CP 2 are two strains of *Clostridium perfringens* isolated from field cases of NE.

The treatments did not result in death or visible gross lesions in the intestine of birds. However, the intestine of birds infected with cocci alone or in combination with *CP* had increased mucosal secretion and redness compared to that of the control birds. Differences in the performance data would suggest that birds were severely affected by the challenge with *Eimera* alone. However, a further reduction in growth and feed efficiency in groups challenged with both *Eimeria* and *CP strain 2*, clearly indicates that a challenge model based on an appropriate combination of coccidia and CP strain can be successful in the experimental induction of clostridial enteritis. Further experiments conducted with a larger number of animals will be necessary to conclusively establish the success of the proposed model.

[1]School of Rural Science and Agriculture, University of New England, Armidale NSW 2351.
[2]DPI, Animal Research Institute, LMB No 4 Moorooka Qld 4105.
[3]Kemin (Aust.) Pty Ltd., 1.02/10 Edgeworth David Avenue, Hornsby NSW 2077.

THE AETIOLOGY AND IMPORTANCE OF SALPINGITIS IN LAYING HENS

R.B. CUMMING

Summary

Acute and chronic bacterial infections of the oviduct (salpingitis) are recorded in most commercial laying flocks and may account for losses of from 1% to 8% over a laying year. Evidence collected over 23 years in laying experiments at the University of New England showed that, out of a total of 12500 hens housed alone in single bird laying cages, none died from salpingitis. Typical commercial losses from vent-peck and salpingitis were recorded in laying hens housed on deep litter (11000 birds) or in three-bird laying cages (48000 birds). The fitting of polypeepers markedly reduced the incidence of vent-peck and salpingitis. From these results it is postulated that salpingitis is an ascending infection of the oviduct following pecking damage to the oviduct by pen or cage mates.

I. INTRODUCTION

Both acute and chronic infections of the oviduct of hens have been recorded from laying flocks ever since mortality records have been kept. The disease appears in nearly all flocks of layers causing losses of from between 2 and 8% over a laying year with a mean incidence of about 4%. The condition has been variously called salpingitis, peritonitis and reproductive breakdown and varies from acute to chronic with characteristic lesions.

In acute cases, post mortem inspection generally confirms that the bird is in good condition, the ovary and oviduct active, and that the bird has obviously very recently been in production. The carcass appears fevered and the breast muscles are darker than normal. The most prominent lesion is marked venous congestion of the oviduct, which may contain small (1 to 4 mm) floccules of yellowish puss. There may be similar floccules of pus in the peritoneal cavity where the blood vessels are also often congested (acute peritonitis).

In the chronic form the bird is usually emaciated with an inactive ovary and the oviduct distended with pus. This may be in fluid form but often occurs as concentric layers of inspissated material. Evidence of a chronic form of peritonitis is often present. The incidence of the different types and degrees of condition varies from flock to flock.

II. METHODS

The laying hens from which the data presented in this paper were derived, were kept at the poultry farm of the University of New England. The objective was to give the birds good practical management as they were part of field demonstrations. These demonstrations, which commenced in 1961, included the production and maintenance of Mycoplasma gallicepticum (MG) free chickens under field conditions, as well as comparing various methods and degrees of feed restriction of the growing pullets between 6 and 18 weeks of age. Some trials included comparisons of breeds and some also compared feeds, feeding regimens and other management practices.

The commercial layer pullets were mostly obtained as day old chicks from various commercial hatcheries in Australia. The layer stock used initially from 1961 to 1970, were principally White Leghorn X Black Australorp crossbreds. Thereafter a greater variety of

"Rockpool", Rockvale Road, Armidale, NSW 2350.

breed and strain crosses were available, including White Leghorn X New Hampshire, New Hampshire X Black Australorp and strain cross White Leghorn. The pullets were reared on the floor on litter in a brooder shed to 6-8 weeks of age. They were then transferred to deep litter rearing sheds until 18 weeks of age, when they were transferred to their laying quarters. These consisted of single bird laying cage, (544 birds) or 20 deep litter sheds each housing 50 birds (total 1000 birds). The pullets were reared under natural day length but received 16 hours of light per day when in the laying quarters. From 1972, a new saw-tooth layer cage shed was available, with commercial three birds cages measuring 300 mm wide by 530 mm deep and 425 mm high. This shed housed 4000 birds and the deep litter pens were no longer used after a couple of years.

The chickens received commercial chicken starter diets to 8 weeks of age and then commercial pullet developer diets until transferred to their laying quarters. Commercial or experimental laying diets were fed from about 18 weeks of age. The pullets in the single bird cages received the same diets and management as the birds on deep litter or in the three bird cages.

All dead birds were autopsied to determine the cause of death where possible. The flocks were serologically free from *Salmonella pullorum* and *Mycoplasma gallisepticum*. When Mareks Disease vaccines became available in the mid seventies, the pullets were vaccinated at the hatcheries before despatch. Infectious bronchitis vaccines were used at three and fourteen weeks of age when they became available. The pullets were beak trimmed at 10 days of age and again at 14 to 16 weeks of age.

In 1969, in an attempt to reduce the losses from vent-peck and salpingitis, polypeepers were fitted to the pullets in half the pens in the deep litter shed. Polypeepers are plastic devices which prevent forward vision of the birds and are attached by a C clip, that locks into the nostrils of the beak. They were fitted to the pullets 10 to 14 days after the birds had been introduced to their laying quarters and had become familiar with their new environment. In 1973 polypeepers were fitted to half the pullets in the three bird laying cages, care being taken to randomise the treatment groups across the shed.

III. RESULTS

(a) 1961 to 1971

This period involved 5440 hens in single bird laying cages and 11000 hens on deep litter. Over this period total annual losses of about 30% were recorded which were essentially similar to those observed in commercial layer flocks. Losses were consistently lower in the birds housed in the single-bird cages than in those on deep litter. This difference was due to the complete absence of any cases of vent-peck or salpingitis in the birds housed singly. Total mortality on deep litter was 27.3%, with vent-peck contributing 6.5% and salpingitis 3.6%. In the single-bird cages total losses were 19%, with no cases of vent-peck or salpingitis.

(b) 1972 – 1984

This period involved 6500 hens in single bird laying cages and 48000 hens in three bird cages. Overall mortality was dramatically reduced when Mareks Disease vaccines were introduced. Again, no cases of vent-peck or salpingitis were recorded in the birds housed singly. Overall mortality was considerably lower in the single-bird cages (11%) than in the three-bird cages (17%) and was essentially due to the difference in combined mortality from vent-peck and salpingitis.

The fitting of polypeepers to the birds on deep litter and in the three bird cages markedly reduced the incidence of vent-peck and salpingitis mortalities. Total losses in birds on deep litter not fitted with polypeepers, were 21%, with vent-peck contributing 11% and salpingitis 1.0%. Fitting polypeepers reduced these losses to 9.5%, 1.1% and 0.2%. Thus on deep litter, polypeepers significantly reduced but did not entirely eliminate mortality due to vent-peck or salpingitis. In the three bird cages, in birds without polypeepers, total losses were 17%, with losses from vent-peck, 8% and salpingitis 1.1%. Fitting polypeepers reduced these losses to 10%, 1.0% and 0.1% respectively.

IV. DISCUSSION

Over the entire twenty three years not a single case of vent-peck or salpingitis was recorded in the 12512 birds housed in the single bird cages. The 59000 pullets housed on deep litter or in the three bird cages, suffered constant and significant losses from vent-peck and salpingitis. The incidence of vent-peck varied from 2 to 8% over the years on deep litter and in the three bird cages.. This variation was possibly in part due to the varying skills of the persons carrying out the beak trimming. However some strains of birds had consistently high mortalities and others consistently low mortalities from vent-peck. The incidence of salpingitis mortality was typically between 10 and 50% of that due to vent-peck. On two occasions, however, when vent-peck losses were low (3.2 and 3.8%) the losses from salpingitis were comparatively high, 2.6 to 2.4% respectively. The overall incidence of salpingitis and vent peck mortality was markedly reduced when polypeepers were fitted

Taking all this evidence into consideration, i.e. the complete absence of salpingitis if vent-peck is eliminated and the reduction in salpingitis if vent-pecking is reduced, it is postulated that salpingitis is an ascending infection of the oviduct following pecking damage to the oviduct by pen mates. The course of the infection that follows varies and is probably due to the degree of damage and contamination of the wounded area. This idea was first proposed by Cumming (1974) and more recently a possible correlation between vent-peck and salpingitis was suggested by Jordan and Pattison (1996).

Losses from vent-peck are one of the major problems in modern laying hens, particularly in the single layer Californian style sheds widely used in Australia which have such a high light intensity. To this loss we can now add the deaths from salpingitis, at roughly one third the number of birds dying from vent-peck. Birds dying from salpingitis may succumb in a few days but, in chronic cases, the birds may survive for up to 150 days (Gross and Siegel 1959). These chronic cases will produce no eggs but will eat food for several weeks and even months thus further increasing the loss to the egg producer.

V. ACKNOWLEDGMENTS

The excellent technical assistance extending over many years of Mr M. Bell, Mr J. Wolfenden, Mrs J. Schaefer and Mrs W. Ball, is gratefully acknowledged.

REFERENCES

Cumming, R.B. (1974). *Proceedings of the 1974 Australasian Poultry Science Convention.* Hobart, Tasmania. p.207.

Gross, W.B. and Siegel, P.B. (1959). *Avian Diseases*. **3**:370-373.

Jordan, F.T.W. and Pattison, M. (1996). *Poultry Diseases*. 4th Ed. W.B. Saunders. pp. 41-42.

THE USE OF STRESS ALLEVIATION COMPOUNDS TO IMPROVE POULTRY PERFORMANCE

D.G. THOMAS and C.J. COOK

Long-term adaptation to physiological stress in poultry, identified by increased circulating concentrations of neurogenic amines and corticosterone, may depress the growth and skeletal development of young birds and lead to weight loss in adults, often despite increased feed consumption (Siegel, 1995). This study investigated the potential of Stress Alleviation Compounds (SAC: a combination of pyridine, amino acid, vitamin and protein compounds) to reduce the physiological response of broilers to cold temperature and enhance performance. The full mode of SAC action is not clear, but they appear to act within the bird by mildly inhibiting corticosterone synthesis in the adrenal gland and serotonin levels in the brain (Frankel *et al*., 1967; Blackburn, 1981). The effects of SAC were compared with existing antibiotic (Surmax®, 0.2g/kg) and mannan oligosaccharide (Bio-Mos®, 1g/kg) products. Day-old male broiler chicks (Ross) were allocated into 24 cages (6 replicates per treatment, 6 birds per cage) and fed a pelleted maize-soy diet without or with the additives. The birds were maintained at 26ºC from 1 to 14 d of age to impose a chronic cold stress. Standard room temperatures were used for the rest of the trial. Body weights and feed intakes were recorded at weekly intervals during the 35-day trial period. Results are tabulated below.

	Day 1-14			Day 1-35			
Treatment	Weight gain (g/bird)	Feed intake (g/bird)	Feed per gain (g/g)	Weight gain (g/bird)	Feed intake (g/bird)	Feed per gain (g/g)	Uniformity[1] (%)
Nil	443[a]	560	1.28[a]	2216[a]	3394	1.55[a]	62.5
SAC	473[b]	582	1.24[b]	2309[b]	3391	1.48[c]	72.7
Antibiotic	444[a]	565	1.27[a]	2254[ab]	3358	1.50[bc]	76.5
Oligosaccharide	468[b]	573	1.23[b]	2185[a]	3343	1.53[ab]	80.0
SED	5.3	11.5	0.014	23.3	57.6	0.017	11.0
Probability	0.003	0.27	0.009	0.02	0.77	0.003	0.42

[a-c] Means in a column without a common superscript are significantly different ($P < 0.05$).
[1] % of birds weighing within $\pm$ 1 standard deviation of the mean body weight of the group.

During the period of cold stress (day 1-14), broilers fed diets containing SAC or mannan oligosaccharide showed significantly ($P<0.05$) improved weight gains and feed efficiency when compared to birds fed the control or antibiotic-supplemented diets. The SAC and oligosaccharide treatments improved weight gains by 6.8 and 5.6% and feed efficiency by 3.1 and 3.9% respectively, when compared to control birds. Over the 35-day trial period, the SAC treatment improved ($P<0.05$) feed efficiency and weight gains by 4.5 and 4.2%, respectively. Antibiotic treatment had no effect ($P>0.05$) on weight gains, but improved ($P<0.05$) feed efficiency by 3.2%. These results indicate that compounds that alleviate different components of the stress response have exciting promise as alternatives to growth promotants.

Blackburn, G.L. (1981) *J. Trauma.* **21**: 707.
Frankel, A.I. (1967). *Endocrinol*. **89**: 1013.
Siegel, H.S. (1995). *Br. Poult. Sci.* **36**: 3.

Bioengineering Technology Group, HortResearch, Hamilton, New Zealand.

SEROTYPE AND STRAIN SPECIFIC MOLECULAR DIFFERENTIATION OF MAREK'S DISEASE VIRUS

R.D. LAYTON and G.W. BURGESS

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by an avian herpesvirus, Marek's disease virus (MDV). There are three recognised serotypes (1, 2 and 3) of which only serotype 1 is pathogenic (Churchill & Biggs, 1967). Vaccination using all three serotypes is currently used as the mainstay for inducing protection against MD. The current method of detecting MDV post vaccination uses cell culture with immunofluorescence. PCRs have been designed using cumbersome phenol/chloroform protocols to extract DNA from feather tips and whole blood (Handberg *et al.,* 2001). There is no current method that can separate different strains of type 1 MDV. The present study uses blood collected on filter paper and PCR to differentiate between serotypes and strains of type 1 following vaccination.

Serotype 1 primers were those described by Zerbes *et al.* (1994) and targeted the gene encoding glycoprotein C. Primers for type 1 strain differentiation, serotype 2 and serotype 3 (HVT) targeted genes within the inverted repeat regions and used sequence data obtained from Lee *et al.* (2000a), Izumiya *et al.* (2001) and Afonso *et al.* (2001) respectively. The strain specific type 1 PCR amplified the entire coding region of the *meq* gene. Clinical samples were collected on treated filter paper (TropBio, Townsville) and extracted at 94°C in TE buffer.

The serotype 3 primers were able to detect viraemia at up to 21 days post vaccination with HVT (Bioproperties Aust. and Intervet). The results compared favourably with those obtained using cell culture isolation on the same sample set. The serotype 1 primers detected viraemia at 21 days post vaccination and were also able to detect challenge with a field strain serotype 1 following vaccination with HVT. The type 1 strain specific PCR amplified two products, of 1200 and 1400 bp in CVI 988 (Rispens). In two Australian isolates, BH 16 (Intervet) and Woodlands (RMIT), only the 1400 bp product was amplified.

The results obtained to date for the serotype specific primers indicate that PCR is a suitable diagnostic tool for MDV. Combined with the simplified inexpensive collection method, it becomes a viable alternative to cell culture given its lower cost and rapid completion time. Differentiation between strains of serotype 1 is not possible using existing cell culture methods. Our PCR has detected differences between imported and local strains of type 1 MDV. The *meq* gene in virulent MDV type1 has been reported at 1019 bp (Lee *et al.,* 2000b). CVI 988 has been reported to undergo detectable changes following attenuation (Lee *et al.,* 2001). Our PCR has confirmed these findings but has also highlighted differences in Australian isolates. This represents possibly exploitable differences in the genome of the Australian isolates. Differences between Australian strains require further investigation.

Afonso, C.L., Tulman, E.R., Lu, Z., Zsak, L., Rock, D.L. and Kutish, G.F. (2001) *J.Virol.* **75:**971-978.
Churchill, A.E. and Biggs, P.M. (1967). *Nature,* **215:**528-530.
Izumiya, Y., Jang, H.K., Ono, M. and Mikami, T. (2001). *Curr.Top.Microbiol.Immunol.* **255:**191-221.
Handberg, K.J., Nielsen, O.L. and Jorgensen, P.H. (2001). *Avian Path.* **30:**243-249
Lee, L.F., Wu, P., Sui, D. X., Ren, D. L., Kamil, J., Kung, H. J. and Witter, R.L. (2000a). *Proc.Nat. Acad.Sci.* **97:** 6091-6096.
Lee, S.I. Takagi, M. Ohashi, K. Sugimoto, C. and Onumma, M. (2000b). *J.Med.Vet.Sci*. **63:**287-292.
Zerbes, M. Tannock, G.A. Jenner, R.J. and Young, P.L. (1994). *Virus Genes* **8:**15-24.

Department of Microbiology and Immunology, James Cook University, Townsville, Qld, 4811.

AUTHOR INDEX

Name	Page(s)	Email Address
Rudkin, C.	158	clare@mailbox.uq.edu.au
Selby, E.	162	
Selle, P.H.	141, 152	sellep@basf-australia.com.au
Sim, J.S.	148	
Song, Y.	54	
Stewart, G.D.	158	gds@sas.uq.edu.au
Suawa, E.	137	
Sulaiman, A.	117	
Sunwoo, H.	148	
Surai, P.F.	187	P.Surai@au.sac.ac.uk
Sutedjo, H.	121	s199331@student.uq.edu.au
Szylo, D.	125	
Thomas, D.G.	197	DThomas@hortresearch.co.nz
Thomas, D.V.	136, 145, 182	D.V.Thomas@massey.ac.nz
Trappett, P.C.	181	
Van Kessel, A.G.	163	
Walsh, T.J.	183	tara.walsh@novusint.com
Wang, Y.	148	
Weir, K.A.	126	Kristy.Weir@flinders.edu.au
Westbury, H.	76	Harvey.Westbury@csiro.au
Whitehead, C.C.	61	colin.whitehead@bbsrc.ac.uk
Wilkinson, S.J.	103, 152	
Wu, Y.B.	127, 145	