AN ANNUAL SYMPOSIUM ORGANISED BY

THE POULTRY RESEARCH FOUNDATION
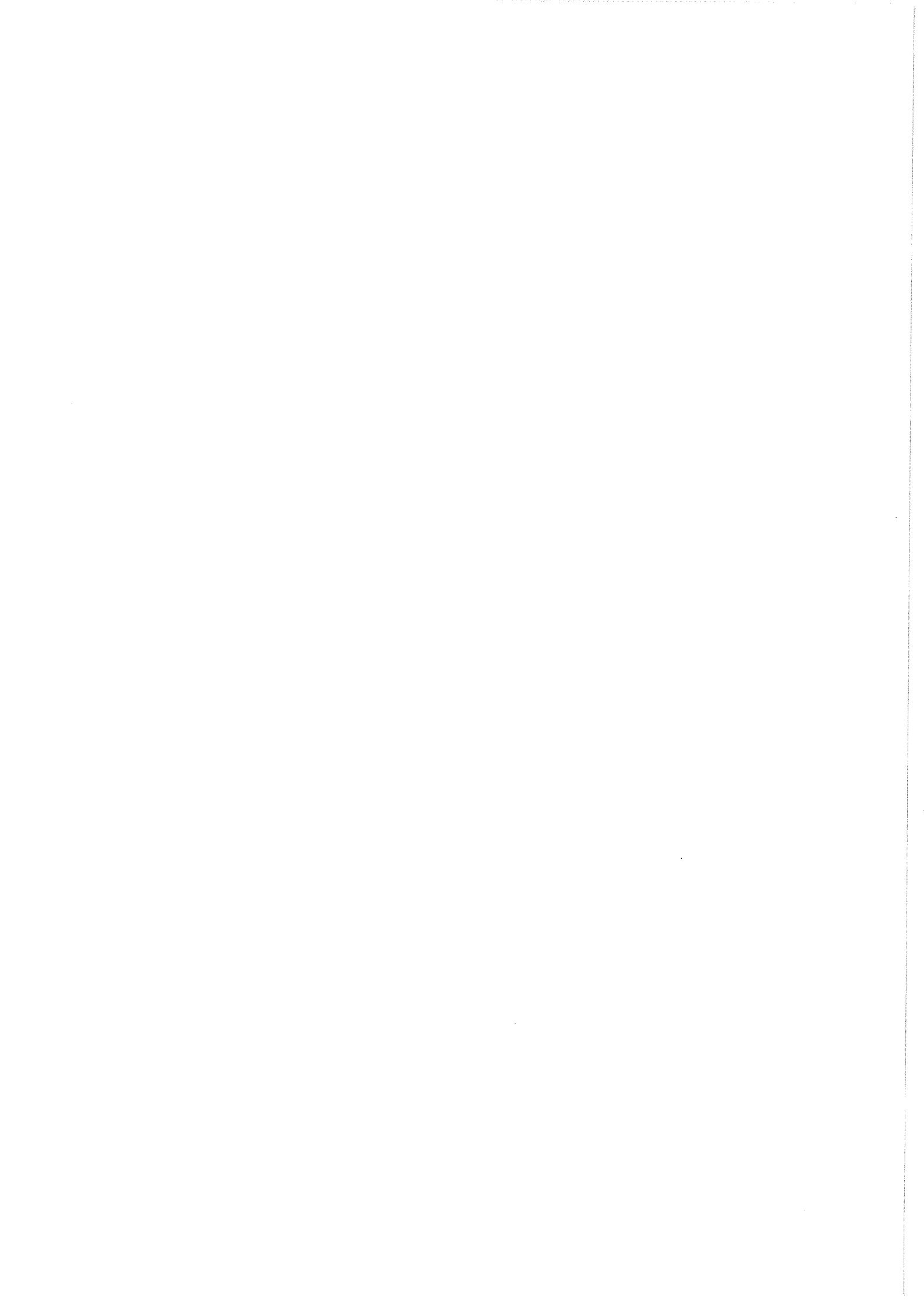
UNIVERSITY OF SYDNEY

AND

THE WORLD'S POULTRY SCIENCE ASSOCIATION

(Australian Branch)

AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

1999

ORGANISING COMMITTEE

Associate Professor D. Balnave (Chair)
Professor E.F. Annison
Associate Professor W.L. Bryden
Associate Professor D.J. Farrell
Professor D.R. Fraser
Dr R.A.E. Pym
Mr R. Roberts
Dr B.L. Sheldon

EDITORIAL COMMITTEE

Associate Professor D.J. Farrell (Chair)
Professor E.F. Annison
Associate Professor D. Balnave
Associate Professor W.L. Bryden
Professor D.R. Fraser
Mr R.J. Hughes
Dr S. Prowse
Dr R.A.E. Pym
Dr B.L. Sheldon

PROCEEDINGS OF THE SYMPOSIUM

Enquiries regarding the Proceedings should be addressed to:

The Director
Poultry Research Foundation
Department of Animal Science
RMC Gunn Building
University of Sydney
Sydney
NSW 2006

SPONSORS OF THE 1999
AUSTRALIAN POULTRY SCIENCE SYMPOSIUM

Speaker Sponsors

Chicken Meat Research and Development Committee of the RIRDC

Egg Industry Research and Development Committee of the RIRDC

Novus International Pty Ltd

Rhône-Poulenc Animal Nutrition Pty Ltd

Gold Sponsors

ADM Australia Pty Ltd

Degussa Australia Pty Ltd

Finnfeeds International Ltd

Pfizer Animal Health

Silver Sponsors

Alltech Biotechnology Pty Ltd

Elanco Animal Health

Fort Dodge Australia Pty Limited

Kemin (Aust.) Pty Limited

Roche Products Pty Limited

Food and Wine Sponsors

Emerald Estate Wineries, South Australia

Millmaster Feeds

AUSTRALIAN POULTRY AWARD

Previous recipients of the award are:

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

SYD WILKINS MEMORIAL PRIZE

Previous recipients of the prize are:

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

CONTENTS

SHORT PAPERS

NUTRITIONAL REQUIREMENTS OF GENETICALLY SELECTED LEAN CHICKENS

B. LECLERCQ

Summary

The main differences between genetically lean (LL) and fat (FL) chickens concern the partition of retained energy between fat and protein, even if LL tend to exhibit a slightly lower energy requirement for maintenance. Lean chickens require diets that are more concentrated in both essential and non-essential amino acids. These particularities of lean chickens may account for the curvilinear responses of commercial flocks to variation in dietary crude protein or amino acid concentration.

I. INTRODUCTION

Several experimental studies on selection for leanness have been performed since 1980. Some of them involved indirect selection for leanness where birds were selected for low feed conversion ratio (FCR) (Pym et al.,1985; Leenstra, 1988) or low plasma very low-density lipoprotein (VLDL) (Whitehead, 1988) for example. Direct selection for leanness has only, for example, been performed in Israel (Cahaner, 1988), in Australia (Pym, 1988) and in France (Leclercq, 1988). The main focus of this paper is on results obtained in experiments performed on lines selected directly for leanness, since in lines selected for low FCR or low VLDL mechanisms other than those related to lipid metabolism may be involved. However reference will be made in places to a comparison of results obtained on lean lines with those coming from lines selected for low FCR and low VLDL. This short review will concern energy and protein metabolism. There are two reasons for studying the feeding of genetically lean broilers. Firstly, commercial broiler breeding companies all now incorporate selection for low FCR in their programmes. Although these programmes have probably modified characteristics other than fatness, they have reduced adiposity in the broiler progeny. Secondly, there is a natural heterogeneity of fatness in commercial flocks, and, if performance responses are different between fat and lean birds due to dietary factors, this heterogeneity could explain curvilinear response curves of commercial crosses to diet changes.

II. CAUSES OF EXCESSIVE FATNESS

Details of the selection programme of our experimental lean line (LL) and fat line (FL) have been given in a previous paper (Leclercq, 1988). Between-line difference in fatness has been extensively studied in our own lines (Leclercq et al., 1989). Both lines were obtained by sib-selection on the abdominal fat (AF) to live weight ratio at 63 days of age. Before this age lean chickens exhibited a slightly lower live weight (LW) than the fat chickens. On the contrary, after 63 days of age, lean chickens exhibited a higher LW (Leclercq et al., 1989). Difference in AF proportion increased from hatching to 63 days of age, when the AF/LW of the fat line was four times that of the lean chickens. Body lipid proportion of the fat line was only twice that of the lean line at 63 days of age. We observed slight differences in plasma T3 and T4, but this difference could not account for a large part of the difference in fatness, since T3 supplementation in the diet did not significantly modify the FL to LL difference (Leclercq et al. ,1988).

Station de Recherches Avicoles, INRA, 37380 Nouzilly, France.

The main causes of the difference are likely of metabolic origin, and hyperphagia is secondary. Although there was a slight difference in feed consumption between lines, we have good evidence that feed intake does not account primarily for the difference in adiposity. There are two arguments for that. Firstly, when FL chickens were pair-fed on LL feed intake they still remained fatter than LL birds (Leclercq and Saadoun, 1982). The second argument is that when broilers were refed after an over-night fast, LL birds consumed their feed more rapidly than the FL. However plasma triglycerides rose more rapidly in the plasma of the FL birds (Leclercq et al.,1990). Thus, there are metabolic factors which cause a partitioning of nutrients between lipid deposition and other utilisation (heat production or protein accretion). Insulin secretion and sensitivity is seen as a critical factor in explaining differences in fattening, since we observed significant differences in glucose to insulin balance (Simon, 1988), insulin sensitivity (Simon and Leclercq, 1985) and recently in insulin receptor or post-receptor phosphorylations (Dupont et al., submitted).

III. ENERGY

We did not observe any difference in metabolisability of dietary energy between fat and lean lines (Leclercq and Saadoun, 1982). This is in contrast to results from lines selected for feed efficiency (Pym, 1985; Leenstra, 1991), which always exhibited higher metabolisability of gross energy compared to control line birds.

Energy requirement for maintenance was not different between lines when it was expressed as kJ per $kg^{0.75}$live weight per day and measured from the carcass analysis method (Leclercq and Saadoun, 1982). In this experiment mean requirements (two experiments) were 406.7 and 453.3 $kJ/kg^{0.75}/d$ for LL and FL birds respectively. This was investigated later by Geraert et al. (1988) on the same lines using respiration chambers. When expressed as $kJ/kg^{0.75}$of lean mass/d, the energy requirement for maintenance of LL birds was significantly lower than in the FL. This difference between lines is probably due to differences in feathering, since we observed that feather proportion was always higher in the LL than in the FL (Leclercq and Guy, 1990 ; Geraert et al., 1993). In the case of lines selected for low FCR, fasting heat production and maintenance energy requirement were lower than in the unselected control line (Pym et al., 1984).

Similarly no significant difference was observed between our lines for dietary induced thermogenesis (DIT) as measured by the difference between total heat production and fasting heat production (Geraert et al., 1988). Lastly, fasting heat production was very similar in both lines, which contrasts with the situation in obese rodents. DIT appeared to be unaffected in FL chickens. It is thus not a problem of partition of energy between heat production and lipid deposition, as has been observed in other genetic comparisons in chickens (Gabarrou et al.,1997). There was no difference in the energy retained to metabolisable energy (ME) intake ratio (Geraert et al., 1988). The only difference concerned the partition of retained energy either as fat or as protein (Geraert et al., 1988). In many nutritional conditions, even under feed restriction, FL chickens tended to store more energy as fat than as protein (Leclercq and Saadoun, 1982; Geraert et al., 1988).

Energetic efficiencies of protein and lipid accretion were estimated in two experiments (Leclercq and Saadoun, 1982). No significant differences were observed between lines. The mean energetic efficiency for protein accretion was 39.8% in both LL and FL birds. The mean energetic efficiency for lipid deposition was 88.5% in LL and 91.7% in FL birds. None of these efficiency coefficients was significantly different between lines. Thus, the main difference induced by sib-selection for abdominal fat concerns the partition of retained energy between lipid and protein.

2

IV. PROTEIN AND AMINO ACIDS

(a) Protein

At a given dietary protein content LL birds always retained more protein as gain than the FL (Leclercq,1984; Geraert *et al.*, 1988; Leclercq and Guy, 1990; Geraert *et al*, 1990). This is a characteristic of all lean lines which was also observed in lines selected for low FCR (Pym and Farrell, 1977) or for low VLDL (Whitehead and Parks, 1988).The question of the sensitivity of lean chickens to low protein diets is more controversial. Lean chickens were found to be more sensitive to low protein diets by Leclercq (1983) and Geraert *et al.* (1990). However, in another experiment, sensitivity to low protein diets as determined by growth rate was found to be very similar in the two lines (Leclercq and Guy, 1990). This was also observed in a line selected for low plasma VLDL. In one experiment (Whitehead, 1990) the low VLDL line was less tolerant of inadequate dietary protein than the high-VLDL line; in another experiment there was no difference (Whitehead and Parks, 1988). In two experiments (Leclercq, 1983; Leclercq and Guy, 1990) slopes of the regression lines between protein gain and protein intake were different between lines; the LL exhibiting a higher slope suggesting that they had a higher dietary protein efficiency (PCE) than the FL. However in another experiment the regression lines were parallel (Geraert *et al.*, 1990). In his own lines, Whitehead (1990) observed that regression lines between PCE and fatness were different, indicating that, at a given degree of adiposity, lean birds were more efficient in converting dietary proteins into body proteins. Whitehead (1990) concluded that genetic selection is a more effective and economic means of producing leaner broilers than by manipulating dietary protein content.

(b) Amino acids

Several experiments were performed recently on comparative responses of LL and FL birds to changes in dietary amino acid concentration. These studies were undertaken following the observations by Larbier and Leclercq (1983) of line differences in plasma free amino acid concentrations which were further confirmed by Pesti *et al.* (1994). In several experiments we observed higher concentrations of sulfur- (SAA) and branched chain-amino acids in the FL than in the LL birds. In contrast levels of non-essential amino acids (NEAA) were always higher in LL than in FL birds and there was always a higher tyrosine to phenylalanine ratio in the LL birds. We firstly investigated the nutritional response of both lines to SAA concentration (Leclercq *et al*, 1993). We found that the lean chickens had a higher proportion of feathers than the FL chickens, although birds of both lines were all homozygous for the sex-linked fast-feathering gene. This was confirmed in another experiment (Geraert *et al.*, 1993). Consequently the LL birds required diets more concentrated in SAA. When weight gain was plotted against SAA intake, both lines appeared to be on the same response curve. However when SAA accretion was plotted against SAA intake, the LL response was higher than that of the FL. Moreover, feather proportion was increased by graded increases in dietary SAA level only in the LL birds. Lastly plasma SAA concentration reached a higher level in FL than in LL birds at lower dietary SAA concentrations, confirming that the SAA requirement was met in the FL at lower dietary SAA levels than in the LL.

Similar observations were made for lysine and arginine (Leclercq *et al.*, 1994). Lean chickens required diets with higher lysine and arginine contents. However when weight gain was plotted against lysine or arginine intake, both lines appeared to be on the same response

3

curves. When body proteins were plotted against lysine or arginine intake, FL gains were slightly lower than those of LL.

We recently compared the FL and LL responses to changes in dietary threonine concentration (Alleman *et al.*, 1999). Again we observed the LL birds to be more sensitive to threonine deficiency than the FL. As seen in Table 1, weight gain in the LL birds reached a plateau at a higher digestible threonine content (5.22 g/kg vs 3.80 g/kg). When weight gain or protein gain were plotted against digestible threonine intake, both lines were on the same response curve, suggesting that threonine is used with similar efficiency in the two lines. When linear regression was calculated on data of the LL at threonine intakes lower than 12 g (linear part of the response curve) during the experimental period (28 to 49 d), the constant of the equation did not differ significantly from zero. So the digestible threonine requirement was estimated as 10.8 mg per g of weight gain during the 28 to 49 day period, or 64.3 mg per g of protein gain.

Table 1.   Responses of lean (LL) and fat (FL) chickens to dietary threonine.

Digestible threonine (g/kg)	3.80	4.28	4.75	5.22	5.70
Weight gain (g/d)					
LL	28.6 a[1]	39.6 b	43.2 bc	46.2 cd	47.7 d
FL	43.7 cd	47.9 d	49.2 d	48.3 d	49.7 d
Feed conversion					
LL	2.87 a	2.45 b	2.27 de	2.19 ef	2.11 f
FL	2.62 b	2.34 cd	2.36 bc	2.36 bcd	2.28 cd

[1]Since variances were not homogenous due to the lowest threonine diet, data were compared at the P=0.05 level using the Mann and Whitney test.

These results agree closely with the recent conclusions from Edwards *et al.*(1997). Dietary threonine content did not influence abdominal fat or breast muscle proportion as we had observed earlier (Leclercq, 1997).

Similarly we compared both lines for their responses to NEAA. Four diets were compared, providing from 130 to 190 g CP/ kg of diet. Essential amino acid contents were maintained in all diets at levels slightly higher than the assumed requirements of the LL birds. Results are given in Table 2. Lean chickens appeared to be less tolerant of low protein diets, even when the requirements for essential amino acids were met. Moreover breast muscle (BM) proportion in both lines responded to graded increases in dietary NEAA, even when maximum daily gain was achieved at lower NEAA contents, as observed in the FL birds.

(c) Appetite for amino acids

We compared both lines for their appetite for protein and amino acids. We firstly observed that when LL and FL chickens had a choice between a low-(140 g CP/kg) and a high-(280 g CP/kg) protein diet, lean birds ate more of the high-protein diet to consume on average 20g CP/kg more than the FL. This trend for LL birds to consume more protein than the FL was significant after one week (Leclercq and Guy, 1990). Similar free-choice

Table 2.   Responses of lean and fat chickens to non essential amino acids.

Dietary Protein (g/kg)	130	150	170	190
Weight gain (g/d)				
LL	45.2 a	46.9 ab	48.5 bc	50.1 c
FL	46.0 ab	46.6 ab	45.8 ab	47.5 abc
RSD=4.58				
Feed conversion				
LL	2.22 a	2.18 ab	2.10 bc	2.06 c
FL	2.41 d	2.37 d	2.41 d	2.35 d
RSD=0.115				
BM/LW (g/kg)				
LL	111.2 bc	115.8 cd	118.0 d	119.9 d
FL	103.3 a	107.2 ab	107.6 ab	115.1 cd
RSD=0.75				

experiments were undertaken for NEAA and lysine. With NEAA, lean chickens were able to consume slightly more of the high NEAA diet than their fat line counterparts (Leclercq and Michel, 1997), as seen in Table 3. In contrast, when birds from both lines were offered free-choice between a low-(4.6 g/kg) or a high- (9.5 g/kg) lysine diet, the mean lysine content of the diet consumed was similar in the two lines. This dietary lysine content was adequate or even in excess for FL birds but was too low to ensure maximum gain in the LL (Leclercq and Michel, 1997), suggesting that the lean chickens were unable to adjust their relative intake of the low- and the high-lysine diets to meet their lysine requirement.

Table 3.   Performance of FL and LL given free-choice of diets.

Diets :	Control	Low Protein[3]	C/LP[1]	L-/L+[2]
Daily gain (g/d)				
LL	49.4 bc	48.7 bc	52.2 c	41.6 a
FL	45.3 ab	46.9 ab	48.4 bc	46.7 a
RSD=5.1				
Mean CP consumed				
LL	193	156	174.4 (5.8)[4]	186.1
FL	193	156	172.5 (5.8)	186.1
Mean lysine content of diet consumed				
LL	9.5	9.5	9.5	8.05 (0.54)
FL	9.5	9.5	9.5	7.97 (0.44)

[1]C/LP= free-choice between control and low-protein diet.
[2]L-/L+= free-choice between low-lysine or high-lysine diets.
[3]Low protein diet was supplemented with EAA.
[4]Standard deviation.

V. CONCLUSION

It is clear that lean chickens require high protein and high amino acid diets notwithstanding their often more efficient use of dietary amino acids. This genetic difference largely accounts for the curvilinear responses of commercial flocks to variation in dietary amino acid concentration. Indeed, in commercial flocks, and even in pure lines, there is a genotypic variability in fatness which explains why all birds do not react similarly to dietary manipulation. This conclusion can be also applied to genetic variability in FCR, since selection for low FCR results in a reduction of food intake at a given daily gain.

The second conclusion is that it should be possible to select indirectly for leanness or FCR by placing half of each family on a low- and the other half on a high-lysine (or protein) diet. Families which exhibit similar performances with the two diets are likely to be fat and less efficient families. In contrast, families which exhibit lower growth performance on the low-lysine (or protein) diet compared to the high-lysine (or protein) diet should be the leanest and more efficient families. This indirect method for selecting efficient birds could be applied to other avian species, like turkeys or ducks, where individual recording of feed consumption or body composition is difficult. Although there are no reports of such selection studies in these species, we know that there is a genetic variability for leanness in all avian species.

These observations suggest also that dietary concentrations of other nutrients (minerals, vitamins) should also be increased in diets of lean chickens and probably in diets of chickens selected for low FCR. Indeed, if the requirements for these nutrients are similar when expressed per g of gain, as the lean or efficient birds consume less feed than the non-selected birds, they require feeds more concentrated in all nutrients. More generally it seems that nutritional recommendations should not be expressed as g per kg of diet but as mg of nutrient per g of gain at a given age. Indeed if genetic selection modifies energy requirement or feed intake it would be more appropriate to adjust dietary concentrations of nutrients to the actual feed intake of the genotype by dividing nutrient requirement by feed intake.

REFERENCES

Alleman, F., Michel, J., Chagneau, A.M., and Leclercq, B. (1999). *British Poultry Science* (in press).

Cahaner, A. (1988). In: Leanness in Domestic Birds. B.Leclercq and C.C.Whitehead, pp71-86 Butterworths, London.

Dupont, J., Chen, J., Derouet, M., Leclercq, B., and Taouis, M. (1999). *American Journal of Physiology*, (in press).

Edwards, H.M., Baker, D.H., Fernandez, S.R. and Parsons, C.M. (1997). *British Journal of Nutrition*, 78: 111-119.

Gabarrou, J.F., Geraert P.A., Picard, M. and Bordas, A. (1997). *Journal of Nutrition*, 127: 2371-2376.

Geraert, P.A., MacLeod, M.G. and Leclercq, B. (1988). *Journal of Nutrition*, 118 :1232-1239.

Geraert, P.A., MacLeod, M.G., Larbier, M. and Leclercq, B. (1990). *Poultry Science*, 69: 1911-1921.

Geraert, P.A., Guillaumin, S. and Leclercq, B. (1993). *British Poultry Science*, 34: 643-653.

Larbier,M., and Leclercq, B. (1983). in: IV *Symposium Inter. Métabolisme et Nutrition* pp 111-115 Ed. *azotés* INRA

Leclercq,B. (1983). *British Poultry Science*, 24: 581-587.

Leclercq, B. (1988). In: Leanness in Domestic Birds, pp25-40Eds B. Leclercq and C.C. Whitehead, Butterworths, London).

Leclercq, B. (1997). *Poultry Science*, **76**: 118-123.

Leclercq, B., and Saadoun, A. (1982). *Poultry Science*, **61**: 1799-1803.

Leclercq, B., Guy, G., and Rudeaux, F. (1988). *Reproduction Nutrition Développement*, **28**: 931-937.

Leclercq, B., Guy, G. and Rudeaux, F. (1989). *Génétique Sélection Evolution*, **21**: 69-80.

Leclercq, B., Hermier, D. and Guy, G. (1990). *Reproduction Nutrition Développement*, **30**: 701-715.

Leclercq, B. and Guy G. (1991). *British Poultry Science*, **32**: 789-798.

Leclercq, B., Chagneau, A.M., Cochard, T. Hamzaoui, S. and Larbier, M. (1993). *British Poultry Science*, **34**: 383-391.

Leclercq, B., Chagneau, A.M., Cochard, T . and Khoury, J. (1994). *British Poultry Science*, **35**: 687-696.

Leclercq, B. and Michel, J. (1997). In: Deuxièmes Journées de la Recherche Avicole pp65-68 ITAVI., Paris.

Leenstra, F. (1988). In: Leanness in domestic birds. Eds. B. Leclercq and C.C. Whitehead edit., Butterworths, London, pp59-70.

Leenstra, F. (1991). *Informacion Tecnica Economica Agraria,* **87A**: 160-165.

Pesti, G.M., Leclercq, B., Chagneau, A.M. and Cochard, T. (1994*). British Poultry Science*, **35**: 697-707.

Pym, R.A.E. (1985). In: Poultry Genetics and Breeding pp97-112. *Eds.* W.G. Will, J.M. Manson and Dr Dewitt, British Poultry Science Ltd, Edinburgh.

Pym, R.A.E., (1988). *Final Report to the Australian Chicken Meat Research Council.*

Pym, R.A.E. and Farrell, D.J. (1977). *British Poultry Science*, **18**: 411-426.

Pym R.A.E. and Nicholls, P.J. (1979). *British Poultry Science,* **20**: 73-86.

Pym, R.A.E., Nicholls, P.J., Thomson, E., Choice, A. and Farrell D.J. (1984). *British Poultry Science*, **25**: 529-539.

Simon, J. (1988). In: Leanness in domestic birds, pp253-268. Eds B.Leclercq and C.C. Whitehead, Butterworths, London.

Simon, J. and Leclercq, B. (1985). *American Journal of Physiology*, **249**: R393-R401.

Whitehead, C.C. (1988). In: Leanness in domestic birds, pp 41-57. Eds B. Leclercq and C.C. Whitehead, Butterworths, London.

Whitehead, C.C. (1990). *British Poultry Science*, **31**: 163-172.

Whitehead, C.C. and Parks, J.R. (1988). *Animal Production*, **46**: 469-478.

OPTIMIZING THE ROLE OF FATS IN DIET FORMULATION

JULIAN WISEMAN

I. INTRODUCTION

Fats and oils are important components of compound poultry diets; they have approximately twice the dietary energy-yielding capacity of carbohydrates, they may contain essential fatty acids and fat soluble vitamins, their physical texture reduces dust in feed mills and they promote diet palatability; their influence on meat quality is also important. They are however extremely variable in terms of chemical composition and nutritional value.

Decisions on whether to use fats and oils will also be based upon other factors including their cost and the presence of appropriate milling technology which often limits the amounts that may be used. Fats and oils are frequently blends of a number of individual ingredients providing a final mixture with a melting point in the region of 40^0 to 50^0C. Storage of blends is usually within this temperature range, requiring specialised facilities (which should be stainless steel or polymer based) and they are usually added in the liquid state. Most compound feeds are pelleted subsequent to mixing and this process is not effective if added fat levels are excessive (beyond approximately 40g fat/kg diet). Addition of further amounts of fat is through liquid fat spraying equipment post-pelleting. To overcome these technological problems, 'dry fat' products, where fats are absorbed on to a solid carrier, and spray-dried products are becoming increasingly available; the use of oil seeds prior to oil extraction (for example soya beans) is a well-established means of adding oil to poultry diets, a process which has the additional advantage of simultaneously providing other nutrients (for example amino acids).

II. UTILISATION OF FATS AND OILS BY POULTRY

The nutritional value of a raw material is governed primarily by its chemical composition (i.e. its ability to provide energy-yielding compounds and specific nutrients) and the degree to which it is digested. Fats and oils are of diverse origin and chemical composition - the animal feed industry is frequently the recipient of by-products from other processes which are often available as blends. A wide range of commodities is thus available including crude vegetable oils, soapstocks, hydrogenated materials, rendered animal tallows, recovered vegetable oils from human food production and even commodities from the refining of oils such as bleaching earths which are sometimes available as 'dry fat' products.

Whilst all these materials (with the exception of the last category) probably have similar total fat contents approaching 1000g/kg, there is considerable variability in chemical composition which has a pronounced influence on their digestibility. A number of reviews have considered both the physiological basis for the digestion and absorption of fats, and the factors which are responsible for the large differences in their subsequent dietary energy values (e.g. Freeman, 1976; Wiseman, 1984).

(a) <u>Influence of chemical structure of fats and oils on digestibility and dietary energy value</u>

There is considerable confusion relating to the apparent metabolizable energy (AME) value of fats primarily because descriptions of the products evaluated are confined to names and origins with no accompanying chemical characterisation of a more precise nature. Systematic studies on the influence of chemical composition of fats and oils on dietary

University of Nottingham Sutton Bonington Campus, Loughborough, Leics LE12 5RD, UK.

energy values for poultry, in terms of the quantitative contribution of the variables involved, are limited. Thus tables of nutritional value of fats and oils are limited to descriptions such as 'beef tallow' and 'vegetable oil'. The need for greater precision in defining source materials in a chemical rather than descriptive manner is of particular importance as fats and oils are rarely fed as a single commodity but rather as blends of a variety of materials. The two chemical variables of most importance are the degree of saturation and content of free fatty acids, with chain length of the constituent fatty acids being of secondary concern.

(i) Degree of saturation and free fatty acid content. Physiologically, the mechanisms of fat digestion and absorption in non-ruminants are well documented (Freeman, 1976). The major site of fat digestion in poultry is the duodenum and this consists of emulsification of dietary fat by conjugated bile salts, followed by hydrolysis of triglycerides by pancreatic lipase into mixtures of 2-monoglycerides and free fatty acids. The subsequent absorption of these products is dependent upon their solubility in bile salt micelles. Polar solutes are more readily incorporated into micelles, and this explains the relatively higher absorption of unsaturated fatty acids compared to saturated fatty acids and the well-established observation that unsaturated fatty acids have a higher digestibility than those that are saturated. Thus oils have a higher AME value than the more saturated fats - this also explains why hydrogenation of oils (even partial) is associated with a reduction in AME value.

However this is an overly qualitative description and, to be of any value to assigning AME values to the wide range of commodities available, a quantitative measurement of the degree of saturation is essential. Whilst reference has been made to the digestion of individual fatty acids, some assessment of the overall commodity is more important. What has been utilised (e.g. Wiseman, 1990) is the ratio of unsaturated (U) to saturated (S) fatty acids (giving U/S). Increasing the degree of unsaturation of a fat through mixing a saturated fat with an unsaturated oil, which is associated with higher U/S ratios, is associated with a non-linear improvement in AME.

The relative superiority of an intact triglyceride compared to hydrolysed fat in terms of AME is also well known (e.g. Young, 1961; Sklan, 1979). Increasing the proportion of FFA would appear to be associated with a linear reduction in fat digestibility (Freeman, 1976).

Systematic studies on the influence of U/S and FFA content of fats and oils on AME and DE values were undertaken by Wiseman and Salvador, 1991 and Wiseman *et al.*, 1991). Materials and their blends of known U/S and FFA content, which covered the range employed in poultry feeding, were evaluated for AME. Chain length of constituent fatty acids was predominantly in the range 16 to 20 carbon atoms. Data generated confirmed that the response of AME to U/S was curvilinear, with the greatest improvement in dietary energy value occurring over the lower range of increase in U/S. The response of dietary energy to FFA was linear.

Evaluation through biological experimentation is a lengthy and costly procedure. Thus there have been many studies attempting to predict AME from chemical composition. Having identified the two major chemical components which have nutritional relevance and generated AME values, a subsequent procedure was regression analysis undertaken to relate AME (dependent variable) to both U/S and FFA content (independent variables).

Equations combining both independent variables are presented in Table 1. Separate functions were derived for both 'young' and 'old' birds as the ability to digest fats improves with age (Fedde *et al.*, 1960; Carew *et al.*, 1972; Wiseman and Salvador, 1989). The influence of both U/S and FFA, together with age, is presented in Figure 1. These functions do represent a considerable improvement, in terms of accuracy of prediction of AME, over those based upon rather more empirical approaches. An example of the latter would be

equations employing iodine value as the independent variable. Values for soya bean oil and rapeseed oil would be different (the former would be higher) whereas AME would be similar.

Table 1.   Prediction equations relating AME, MJ/kg of fats / oils to U/S and FFA (g/kg fat) Age 1 and 2 to 1.5 and 7.5 weeks of age: AME = A + B x FFA + C x $e^{(D \times U/S)}$.

PV is proportion of variance accounted for by regression.

	Age 1			Age 2		
Constant			PV			PV
A	38.112	± 1.418	0.816	39.050	± 0.557	0.925
B	- 0.009	± 0.002		- 0.006	± 0.001	
C	-15.337	± 2.636		- 8.505	± 0.746	
D	- 0.509	± 0.186		- 0.403	± 0.088	

Influence of U/S and FFA on Fat AME
Young and Old Birds
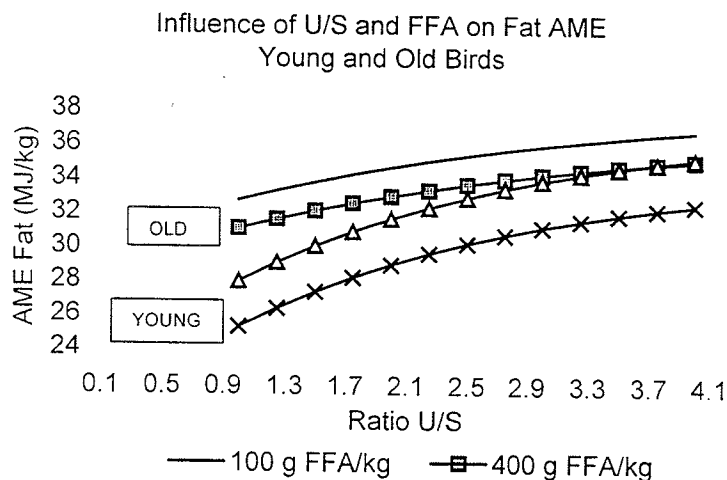
Figure 1.   Influence of degree of saturation (U/S) and free fatty acid (FFA) content on the apparent metabolisable energy value of fats for two ages of broiler.

There are, however, problems with the equations derived as it may not be appropriate to apply them to those fats containing saturated fatty acids of shorter chain length (i.e. below 16 atoms of carbon) (Renner and Hill, 1961). This problem has been studied (Wiseman and Blanch, 1994) where a combination of coconut and palm kernel oils (together with the respective acid oil and with mixtures of the two to give blends of intermediate FFA content) were evaluated. Both combinations consist predominantly of saturated fatty acids, but of chain lengths shorter than 14 atoms of carbon. AME data indicate that content of saturated fatty acids used to calculate the U/S ratio should be based on the sum of myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids but not lauric (C12:0) which behaves like an unsaturated fatty acid.

(ii) Heat damaged fats and oils. It is likely that fats and oils have undergone some form of heat treatment, often in the presence of oxygen. They are relatively unstable (particularly if unsaturated) and are therefore prone to some form of degradation (which explains why

protection of fats and oils through the use of anti-oxidants, added as early as possible in the manufacturing process as oxidative degeneration is irreversible, is crucial). This has prompted numerous studies on the chemical commodities produced during heating and the nutritional implications (e.g. Artman, 1969; Wiseman, 1986).

A large number of modifications to the chemical structure of fats and oils following heating have been identified ranging from simple oxidation products through to dimerization and polymerization (linear and cyclic) of both fatty acids and triglycerides depending upon the substrate in question and the conditions.

The biological effects of feeding these modified structures are also extremely varied both in terms of the actual response in the animal and its severity. It should be noted that even minor adverse biological consequences would have serious effects on overall bird performance. Initially AME will be reduced, and an increasing amount of dietary fat will pass through the gastro-intestinal tract and be excreted. This may have serious implications for litter conditions which may deteriorate through becoming 'greasy', giving rise to a poorer environment and problems of both bird welfare and product quality. It is also possible that the presence of modified fat structures may interfere with the overall digestive process such that general nutrient uptake is impaired. Thus there may be overall nutrient deficiency. Furthermore an actively oxidizing fat or oil will destroy other nutrients present, e.g. vitamins.

Perhaps more concern has been expressed over whether any toxic products are generated following fat and oil heating / oxidation. It does appear however that the majority of these products are only sparingly absorbed and are thus harmless. However, in the case of oxidized fats and oils, defence mechanisms in the gut mucosae to prevent absorption may be stretched such that overall nutrient absorption might be reduced. Death in laboratory animals fed heated fats and oils has been recorded (Andrews et al., 1960) but these are extreme cases.

Because of the potential adverse effects of feeding heat damaged / oxidized fats and oils, there has been considerable interest in developing chemical methods to detect such damage. It is important to note that any method adopted has to be one that measures all products collectively if it is to have any practical application. Peroxide value (PV) has been employed widely for this purpose, but it is an unsound method. In tracing the change in PV over time (Poling et al., 1962)) an increase followed by a reduction was observed. Thus a low PV value may indicate on the one hand a commodity that had not undergone any degradation but, on the other hand, one that had been seriously denatured. The rate of production of oxidized fats may also equal their subsequent degradation. The PV under these circumstances may not change significantly, suggesting a stable situation. Measurement of 'oxidized fat' has been employed but the evaluation is solvent dependent and would not measure those complexes which would not be soluble in polar solvents.

FFA content has been employed frequently to assess damage to oils used in the human food industry but is inappropriate for fats and oils for animal feeding. This is because soapstocks, for example, are perfectly acceptable ingredients for blends (whilst being of lower AME than the original triglyceride) although the FFA content is high. This also explains why assessments of molecular weights or sizes are inappropriate. Thus a fatty acid trimer (zero AME) would generate similar data to a triglyceride (of high value).

One promising technique is based upon estimating the total non-elutable material (NEM) through quantitative gas-liquid chromatography (Waltking et al., 1975; Edmunds 1990). Whilst this method only measures total degraded structures within a fat or oil, it does at least provide guidance as to whether the commodity has been damaged and has proved useful in identifying those commodities (for example some recovered vegetable oils) which are liable to have been excessively heated.

In a trial designed to examine the reduction in dietary energy value likely to result from damage (Wiseman et al., 1992)), a refined sunflower oil was extensively heat damaged. From analysis of the data it was evident that the AME of the NEM fraction, in this material,

was of the order of zero. This indicates the problems identified with heat damaged fats, although no account was taken of associated issues of the presence of the NEM fraction (e.g. reduction in general nutrient uptake).

The experimental program conducted at the University of Nottingham described above had employed the same basal diet to which experimental fats and oils were added. The major raw materials were wheat 300g, maize 275g, soya bean meal 440g and fish meal 125g/kg and, accordingly, the levels of cereal non-starch polysaccharides (NSP) were deliberately kept very low to avoid any possible confounding effects they might have (it had already been established that data generated on AME of fats and oils were no different from those when a synthetic diet was employed). This precaution has, subsequently, proved to have been important.

Recent data have demonstrated that there may be significant interactions between fat digestibility and the nature of the basal diet attributable to high levels of cereal NSP. Thus, both Danicke et al., (1997) and Langhout et al., (1997) have established that basal diets containing high levels of cereal NSP (achieved in the former with 610g rye and in the latter with 500g wheat and 100g rye/kg diet) reduced the digestibility of added fat, the more so with fats that were more saturated. Improved responses were obtained with additions of various exogenous NSPases. The probable reasons for these responses were considered to be linked to the increase in viscosity of intestinal digesta (associated with NSPs) and greater microbial activity which may de-conjugate bile acids, thus reducing their effectiveness in promoting digestion and absorption of fats. It remains to be established whether similar responses would be obtained in the absence of rye (which contains considerably greater levels of NSP than wheat).

(iii) Contaminants within fats and oils. In addition to the products arising from heat damage and oxidation, fats and oils may contain contaminants which are fat soluble. Perhaps the most important group of contaminants are pesticides. Whilst they are potentially damaging to the animal itself, residues within the product are also of concern to the human consumer. Furthermore many of these commodities are rendered even more dangerous following the action of heat (e.g. Metcalfe 1972). Levels within diets should be strictly controlled. Other contaminants found in fats and oils include unsaponifiable matter (plant waxes) which acts as a diluent, water (a diluent but which also has a role in the oxidation process) and polythene (which would compromise fat spraying equipment). These would also cause a minor reduction in total fat content. Finally it is of interest to note that, in any process designed to purify a fat or oil, the materials employed in such purification will themselves become contaminated.

(b) Effect of dietary fats and oils on meat quality

An important feature of poultry production is increasing interest in quality of the product. Carcass fatty acids in poultry can arise from two discrete sources, being de novo synthesis or direct deposition from dietary sources - the latter is the route by which polyunsaturated fatty acids (principally linoleic acid) appear in the carcass.

The degree to which dietary factors influence the fatty acid profile of carcass fat is controlled by a number of factors, including the actual fatty acid profile of the dietary fat and AME (direct deposition of dietary fat is more likely the higher the AME). The basic effects of dietary fat have been recognized for some considerable time. Carcass linoleic acid levels, specifically, have been identified as perhaps the major determinant of the degree of softness of carcass fat. Investigations into the role of linoleic acid have led to recommendations that concentrations above 150g linoleic acid /kg body fat are to be avoided if excessive softness is to be prevented (Whittington et al., 1986), although these studies were with pigs; the lack of a

discrete subcutaneous layer makes it more difficult to comment on softness of carcass fat in broilers.

(i) Effect on physical texture and on keeping / eating quality. The increased use of diets with high AME (particularly as this has been associated with including significant amounts of relatively unsaturated dietary fats) is accompanied by a risk of a negative effect on the keeping quality of the carcass because of the risk of oxidative breakdown of unsaturated fatty acids resulting in development of peroxides and rancidity (Darling *et al.*, 1998). This is because oxidation products, being volatile, give rise to off-odours which will reduce the shelf life of meat. However, the evidence that carcass fatty acid profiles influence the eating quality of meat is less conclusive, although it would appear that the more unstable the fatty acid, the greater the risks. Certainly feeding oils with high levels of long chain polyunsaturated fatty acids (for example fish oil) is not advisable.

Although current recommendations appear to suggest that the polyunsaturated fatty acid content of poultry adipose tissue should be limited (on both textural and eating quality grounds), an opposing viewpoint is occasionally put forward by human nutritionists. A UK Government report (COMA, 1991), in recommending that total dietary fat should represent no more than 33% of total energy intake of the national diet, also proposed that the proportion of this total fat that was saturated should be no more than one third. Recommendations were also established in connection with unsaturated fatty acid intake, which represents an increasing awareness of the importance of different classes of unsaturated fatty acids in human nutrition. The proposed cis mono-unsaturated fatty acid: cis polyunsaturated fatty acid ratio was 2:1 and, furthermore, the ratio of cis ω-linoleic acid (of the n-6 or ω-6 essential fatty acid family) to cis ω-linolenic acid (n-3 or ω-3 essential fatty acid family) was 5:1.

These recommendations apply to the total diet and should not necessarily be used to define optimum fatty acid ratios for individual food items in the human diet. Nevertheless, there is growing interest in modifying fatty acid profiles of animal products, which is comparatively easy in poultry, because of the somewhat negative reputation that these products have of being high in saturated fatty acids which have been implicated in consumer avoidance of these food items. It is possible that broiler meat which was aligned better to the 'optimum' would have a significant impact upon consumer acceptance of them. Nevertheless such a development should not proceed unless there is clear evidence that such modifications to carcass adipose tissue levels are not accompanied by deteriorations in meat quality as defined by technological and organoleptic criteria. In this context the protective function of dietary α-tocopherol acetate has recently been studied (Bartov and Frigg, 1992) where it was considered that, whilst it would not improve performance, it had an important effect on meat stability.

(ii) Rate of change of carcass adipose tissue fatty acids. Problems of unsaturated fatty acids within adipose tissue need to be placed alongside the higher dietary energy values associated with more unsaturated fat blends. Such, seemingly mutually exclusive, objectives might be more easily reconciled if the speed with which carcass fatty acid profiles respond to changes in dietary levels could be established.

Major fatty acids in abdominal fat
Diet change from safflower to tallow
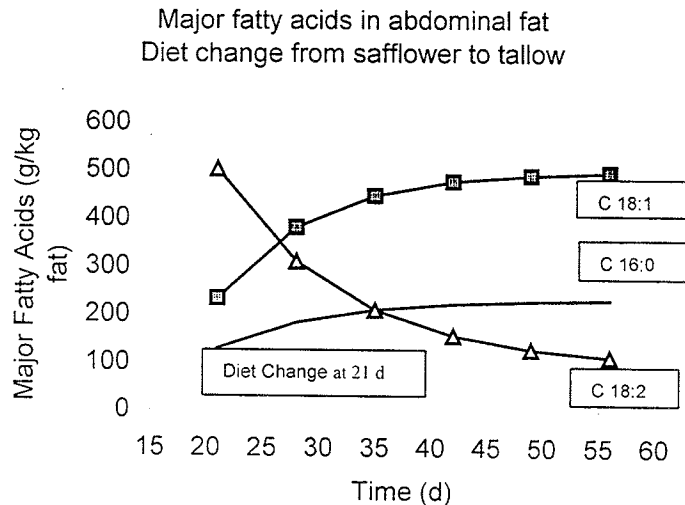
Figure 2   Pattern of change of carcass fatty acids following dietary change

    The more rapid these changes, then the later the introduction of the more saturated lower dietary energy fat. The responsiveness of carcass fat to dietary fat has been studied (University of Nottingham, unpublished) employing diets based upon tallow and safflower oil; data indicate that the rate of change of carcass fatty acid profile is comparatively rapid (Figure 2).

III. CONCLUSIONS

    Fats and oils have an important role in contributing to the AME of compound diets for broilers although they also have other beneficial effects including provision of essential fatty acids, reducing dust in mills and improving diet palatability. It is evident that the chemical composition of the various commodities which are available is extremely variable and this will have a major impact on bird performance and carcass quality. Classification of fats and oils simply in terms of their origin is no longer valid. Quantitative relationships between AME and chemical composition are now available and should contribute significantly to the efficiency of diet formulation.

IV. REFERENCES

Andrews, J.S., Mead, J.F. Griffith, W.H. (1960) *Journal of Nutrition*, **70**: 199.

Artman, N.R. (1969). *Advances in Lipid Research* **7**: 245.

Bartov, I. and Frigg, M. (1992) *British Poultry Science*, **33**: 393.

Carew, L.B., Machemer, R.H. Sharp, R.W. and Foss, D.C. (1972) *Poultry Science*, **52**: 738.

COMA (1991) Dietary reference values for food and nutrients in the United Kingdom. Department of Heath and Social Services, Report on health and social subjects 41, HMSO, London.

Danicke, S., Simon, O., Jeroch, H. and Bedford. M. (1997). *British Poultry Science*, **38**: 546.

Darling, F.M.C., Wiseman, J. and Taylor, A.J. (1998). In: *Progress in Pig Science,* pp429. Eds. J Wiseman, M.A. Varley and J.P. Chadwick, Nottingham University Press, Nottingham.

Edmunds, B.K. (1990). In: *Feedstuff Evaluation.* Eds. J. Wiseman and D.J.A. Cole, Nottingham University Press, Nottingham.

Fedde, M.R., Waibel, P.E. and Burger, R.E. (1960). *Journal of Nutrition*, **70**: 447.

Freeman, C.P. (1976). *Digestion and Absorption in the Fowl*, p117. Eds. K.N. Boorman and B.M. Freeman, British Poultry Science, Edinburgh.

Langhout, D.J., Schutte, J.B., Geerse, C., Kies, A.K., De Jong, J. and Verstegen, M.W.A. (1997). *British Poultry Science*, **38**: 557.

Metcalfe, L.D. (1972) *Journal of the Association of Official Agricultural Chemists*, **55**: 542.

Renner, R. and Hill, F.W. (1961). *Journal of Nutrition*, **74**:259.

Sklan, D. (1979). *Poultry Science*, **58**: 885.

Waltking, A.E., Seery, W.E. and Bleffert, G.W. (1975). *Journal of the American Oil Chemists Society*, **52**: 96.

Whittington, F.M., Prescott, N.J., Wood, J.D.and Enser, M. (1986). *Journal of the Science of Food and Agriculture*, **37**: 753.

Wiseman, J. (1984). In: *Fats in Animal Nutrition*, p277. Ed. J. Wiseman. Butterworths, London.

Wiseman, J. (1990). In: *Feedstuff Evaluation*, p125, Eds. J. Wiseman and D.J.A. Cole. Butterworths, London.

Wiseman, J. (1986). In: *Recent Advances in Animal Nutrition - 1986*, p 47. Eds. W. Haresign and D.J.A. Cole. Butterworths, London.

Wiseman, J. and Blanch, A. (1994). *Animal Feed Science and Technology*, **47**: 225.

Wiseman, J. and Salvador, F. (1989). *British Poultry Science*, **30**: 653.

Wiseman J. and Salvador, F. (1991). *Poultry Science*, **70**: 573.

Wiseman, J., Salvador, F. and Craigon, J. (1991). *Poultry Science*, **70**: 573.

Wiseman, J., Edmunds, B.K. and Shepperson, N. (1992). *Animal Feed Science and Technology*, **36**:41.

Young, R.J. (1961). *Poultry Science*, **40**: 1225 (1961).

NUTRITIONAL RESEARCH ON THREONINE IN BROILERS

M. T. KIDD

Summary

Nutritionists formulating diets for broilers limiting in the most essential amino acids may be reducing profitability as a result of suboptimal performance. Commercial acceptance of methionine and lysine supplements typically results in the total sulfur amino acids and lysine nutrients as being adequate in diets lower in crude protein. However, marginal reductions in crude protein may render the third limiting amino acid (threonine) as deficient, especially in diets primarily composed of sorghum and wheat. It is the objective of this paper to demonstrate the importance of maintaining an adequate threonine minimum in broiler diets. Dose response curves for threonine in growing and finishing broilers are presented.

I. INTRODUCTION

The threonine requirement of broiler chicks has been studied extensively (Thomas *et al.*, 1979; Thomas *et al.*, 1986; Uzu *et al.*, 1986; Thomas *et al.*, 1987; Robbins *et al.*, 1987; Smith *et al.*, 1988; Rangel-Lugo *et al.*, 1994; Holsheimer *et al.*, 1994). However, differences exist in experimental procedures, crude protein levels, dietary energy, dietary lysine, chick age, and environmental conditions which render estimates of threonine requirement for chicks as quite variable. In addition, research reports concerning threonine requirements beyond three weeks of age are scarce.

Uzu (1986) conducted experiments using a variety of threonine deficient basal diets (maize/soybean meal vs. maize/soybean/peanut meal; wheat/soybean meal vs. wheat/soybean/peanut meal). The threonine requirement for feed conversion and growth for 21 to 42 day old broilers was estimated to be 6.8 g/kg diet. Penz *et al.* (1991) also evaluated threonine requirements for broilers from 21 to 42 days of age using a threonine deficient basal diet composed of wheat, maize gluten meal, soybean meal, and meat and bone meal. The threonine requirement for gain and feed conversion was 6.6 g and 6.8 g/kg diet, respectively. Recently, Penz *et al.* (1997) evaluated body weight, feed conversion, and abdominal fat of broilers as affected by dietary threonine and determined that the 21 to 42 day requirement does not exceed 7.09 g/kg diet. Thomas *et al.* (1995) evaluated the 35 to 49 day threonine requirement in a diet containing peanut meal and crystalline amino acids and reported that the threonine requirement did not exceed 0.39 per therm (5.7 g of total threonine/kg diet containing 13.39 MJ AME/kg of diet). Kharlakian *et al.* (1996) evaluated the threonine requirement for 35 to 49 day old broilers by feeding a maize/peanut meal diet. The threonine requirement for gain and feed conversion of males and feed conversion of females was 5.7 g and 5.5 g/kg diet, respectively. However, performance of both male and female broilers in the study of Kharlakian *et al.* (1996) fed the maize/peanut meal basal diets was below that of the maize/soybean meal positive control diet containing 7.0 g dietary threonine/kg. Webel *et al.* (1996) estimated total threonine requirements for broilers of 7.0 g and 6. g/kg diet for the 21 to 42 and 42 to 56 day periods, respectively. In addition, these authors stated that the digestible threonine requirement for 21 to 56 day old chicks should be expressed as 70% of the chicks' respective digestible lysine requirement. Threonine requirement studies for broilers conducted beyond 21 days of age suggest that the NRC (1994) 21 to 42 and 42 to 56 day requirements of 7.4 g and 6.8 g/kg diet, respectively, are too

Nutri-Quest Inc., 1400 Elbridge Payne Road, Suite 110, Chesterfield Mo 63017.

high. This study was conducted to evaluate threonine dose response curves in commercial broilers from 30 to 42 and 42 to 56 days of age on performance and breast meat deposition.

Table 1.   Composition of diets (g/kg, unless otherwise noted).

Ingredients	30 – 42 day experiment		42 – 56 day experiment	
	Control diet	Experimental diet	Control diet	Experimental diet
Maize	650.1	250.6	742.0	665.9
Peanut meal	-	250.0	-	197.0
Sorghum	-	355.8	-	-
Soybean meal	233.5	32.5	109.0	-
Wheat midds	-	-	-	26.0
Poultry meal	50.0	10.0	100.0	-
Poultry oil	37.0	50.0	19.0	57.0
Limestone	12.0	16.5	10.3	12.7
Phosphate	9.5 [1]	13.0 [1]	5.2 [2]	14.1 [2]
Sodium bicarbonate	2.0	4.5	2.6	5.0
L-lysine·HCl	1.1	6.3	1.5	5.7
DL-methionine	0.7	2.4	0.5	1.8
L-isoleucine	-	1.6	-	1.7
L-valine	-	1.1	-	1.2
L-tryptophan	-	0.2	-	0.4
L-threonine	-	-	0.5	-
Sodium chloride	1.5	-	1.9	0.9
Premix	2.1	2.1	7.5	7.5
Variable [3]	0.5	3.4	-	3.1
Calculated composition				
Crude protein	202.4	191.2	178.3	170.5
Threonine	7.6	5.5	6.8	4.5
Lysine	11.0	11.0	9.4	9.4
Methionine	4.9	5.1	3.5	4.1
TSAA	7.9	7.9	6.6	6.6
Isoleucine	8.0	8.0	6.6	6.8
Valine	9.5	9.0	8.3	7.7
Arginine	13.6	15.9	10.6	13.3
Tryptophan	2.4	2.0	1.6	1.8
Glycine + Serine	19.6	16.7	19.0	14.7
Calcium	8.9	10.1	8.5	8.5
Phosphorus, avail.	3.5	3.5	3.5	3.5
DEB, mEq/kg [4]	188	163	150	150
ME MJ/kg	13.39	13.42	13.42	13.42

[1] Dicalcium phosphate; [2] Defluorinated phosphate; [3] Represents graded levels of L-threonine at the expense of washed builders sand in the experimental diets and washed builders sand in the 30-42 day control diet; [4] DEB = dietary electrolyte balance and is defined as sodium + potassium – chloride.

II. MATERIALS AND METHODS

Two experiments were conducted in which broilers received graded levels of total dietary threonine from 30 to 42 days of age or 42 to 56 days of age (Figures 1-6). All broilers in both experiments received common diets prior to the experimental periods. Low protein-threonine deficient diets were formulated with linear programming by using analyzed amino acid values for all protein contributing ingredients (Table 1). Amino acid analysis indicated that threonine treatment additions were in close agreement with calculated levels. The digestible threonine content was calculated in the experimental diets whereby supplemental amino acids were considered to be 100% digestible. Digestible coefficients, as reported by NRC (1994), for maize, soybean meal, and wheat middlings were utilized and average values for peanut meal were calculated by combining data from the NRC (1994) and Zhang and Parsons (1996).

Both experimental facilities consisted of closed-sided houses with positive pressure ventilation. Temperature in the experimental facilities was maintained between 21 and 22° C. Broilers consumed feed and water *ad libitum*. The 30 to 42 day experiment consisted of 120 Ross x Ross male broilers randomized across 56 floor pens (8 treatments with 7 replications/treatment). The 42 to 56 day experiment consisted of 64 Ross x Hubbard male broilers randomized across 64 floor pens (8 treatments with 8 replications/treatment). For each experimental period body weight gain and feed consumption data were collected on a pen basis. Breast meat deposition was measured on eight and five birds per pen at the end of the 30 to 42 and 42 to 56 day periods, respectively.

Data were analyzed by the General Linear Models Procedure of the SAS ® software package (SAS, 1985). Threonine dose response curves were calculated from significant (P<0.05) quadratic regression equations as determined from the General Linear Models Procedure. Pen was the experimental unit for all analysis and percentage data were transformed prior to analysis.

III. RESULTS

In both experiments, birds receiving the threonine deficient diets containing a surfeit of threonine performed as well, or better, than birds receiving the control diet. Mortality was not affected by dietary threonine in either experiment.

(a) 30 to 42 day period

Curvilinear responses (P<0.01; quadratic) to dietary threonine clearly indicate that the experimental diet was deficient in threonine (Figures 1-3). Dietary threonine needs for body weight gain, feed:gain, and breast meat, as calculated by taking 95% of the upper asymptote, were 7.0 g, 7.0 g and 7.8 g/kg diet, respectively. The calculated digestible threonine content needed for performance was 6.3 g/kg diet, but the digestible threonine needed for breast meat was 7.1 g/kg of diet.
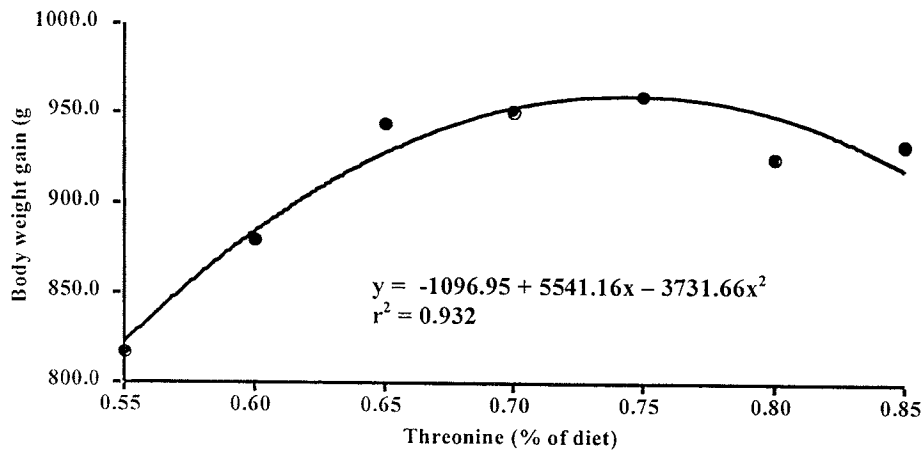
Figure 1.   Curvilinear graph of 30 to 42 day weight gain as affected by graded levels of dietary threonine. 95% of the asymptote is 0.70% threonine.
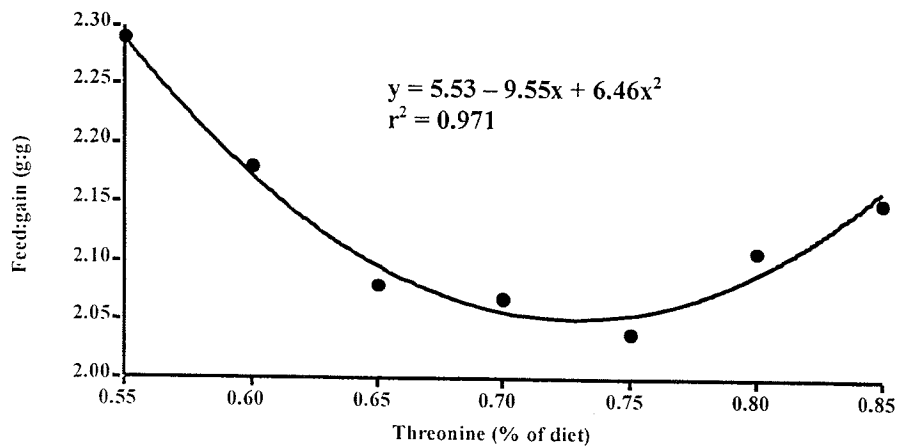
Figure 2.   Curvilinear graph of 30 to 42 day feed:gain as affected by graded levels of dietary threonine. 95% of the asymptote is 0.70% threonine.
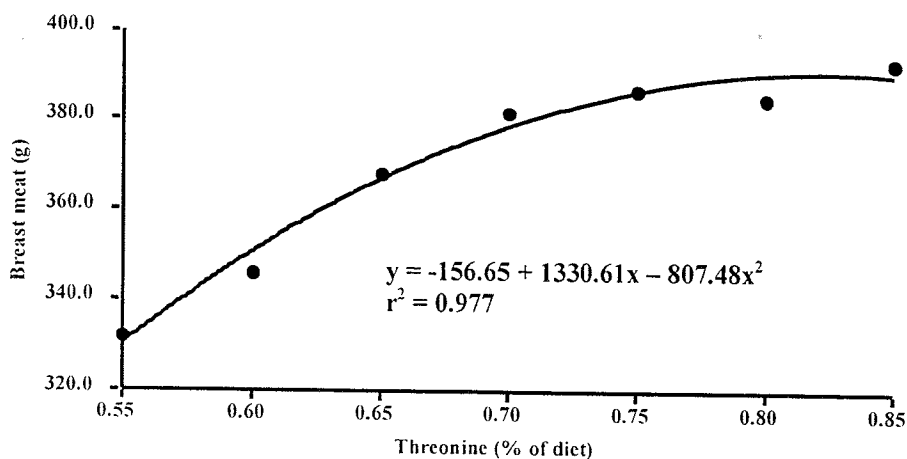
Figure 3.   Curvilinear graph of 30 to 42 day breast meat (skin-less and bone-less *Pectoralis major* and *Pectoralis minor*) as affected by graded levels of dietary threonine. 95% of the asymptote is 0.78% threonine.

(b) 42 to 56 day period

Significant quadratic responses indicated a good dose response to dietary threonine for all parameters measured (Figures 4-6). Regression equations indicated the level of threonine needed for body weight gain and feed:gain was 6.7 g (6.0 g digestible threonine)/kg diet (Figures 4 and 5). The threonine level needed for good breast meat yield (Figure 6) was 6.6 g/kg diet (5.9 g digestible threonine).

$$y = -65.90 + 419.65x - 295.73x^2$$
$$r^2 = 0.961$$
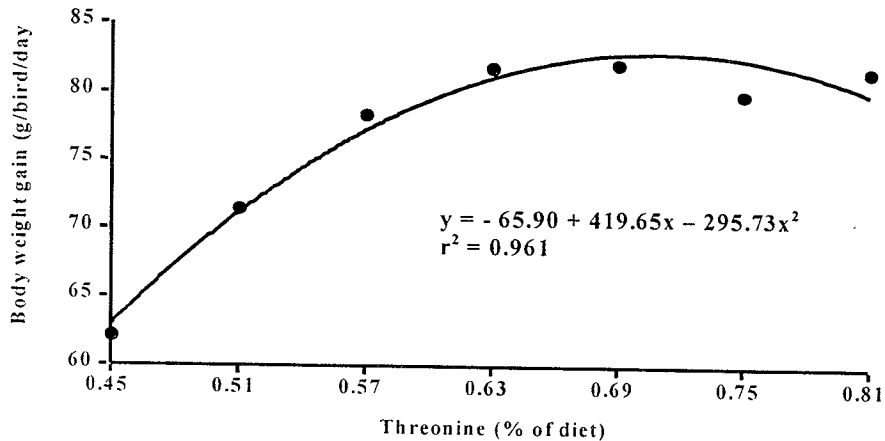
Figure 4. Curvilinear graph of 42 to 56 day body weight gain as affected by graded levels of dietary threonine. 95% of the asymptote is 0.67% threonine.

$$y = 6.986 - 13.014x + 9.196x^2$$
$$r^2 = 0.921$$
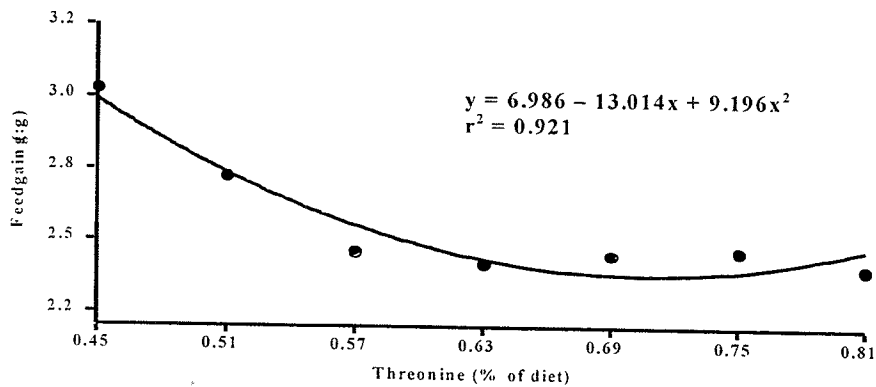
Figure 5. Curvilinear graph of 42 to 56 day feed:gain as affected by graded levels of dietary threonine. 95% of the asymptote is 0.67% threonine.
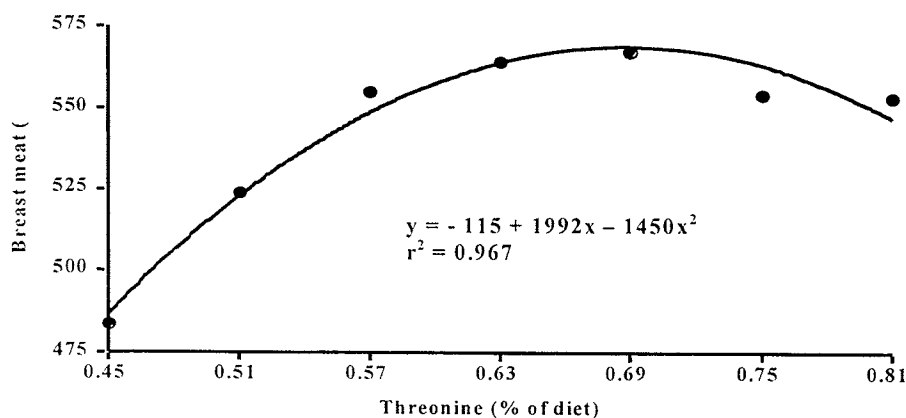
Figure 6.   Curvilinear graph of 56 day breast meat (skin-less and bone-less *Pectoralis major* and *Pectoralis minor*) as affected by graded levels of dietary threonine. 95% of the asymptote is 0.66% threonine.

IV. DISCUSSION

These experiments evaluated both performance and breast meat responses in male broilers as affected by graded levels of dietary threonine. Threonine estimates (per kg of diet) obtained in these experiments suggest that the NRC (1994) threonine requirements from 21 to 42 and 42 to 56 days of age of 7.4 g and 6.8 g per kg of diet, respectively, are safe estimates. In addition, these threonine estimates point to the importance of maintaining a sufficient threonine level in finishing broiler rations so that performance and breast meat deposition are not hindered and are in agreement with the findings of Kidd *et al.* (1997).

REFERENCES

Holsheimer, J.P., Vereijken, P.F.G., and Schutte, J.B. (1994). *British Poultry Science,* **35**: 551-562.

Kharlakian, H.K., Shellem, T.A., Thomas, O.P., and Baer, C.K. (1996). *Proceedings of the Maryland Nutrition Conference* Baltimore, MD, pp. 53-63.

Kidd, M.T., Kerr, B.J., and Anthony, N. B. (1997). *Poultry Science,* **76**: 608-614.

National Research Council (1994). *9th Revised edition.* National Academy Press, Washington, DC.

Penz, A.M., Jr., Colnago, G.L., and Jensen, L.S. (1991). *Poultry Science,* **70** (Suppl. 1): 93.

Penz, A.M., Jr., Colnago, G.L., and Jensen, L.S. (1997). *Journal of Applied Poultry Research,* **6**: 355-361.

Rangel-Lugo, M., Su, C.-L., and Austic, R.E (1994). *Poultry Science,* **73**: 670-681.

Robbins, K.R. (1987). *Poultry Science,* **66**: 1531-1534.

SAS Institute, 1985. *Version 6 Edition.* SAS Institute, Inc., Cary, NC.

Smith, N.K., Jr. and Waldroup, P.W. (1988). *Poultry Science,* **67**: 108-112.

Thomas O.P., Twining, P.V., Jr., Bossard, E.H., Nicholson, J.L., and Rubin, M. (1979). *Proceedings of the Maryland Nutrition Conference,* pp. 44-48.

Thomas O.P., Zuckerman, A.I., Farran, M., and Tamplin, C.B. (1986). *Proceedings of the Maryland Nutrition Conference,* pp. 79-85.

Thomas O.P., Farran, M., Tamplin, C.B., and Zuckerman, A.I. (1987). *Proceedings of the Maryland Nutrition Conference,* pp 38-42.

Thomas O.P., Shellem, T.A., Sprague, M., and Kharlakian, H.G. (1995). *Proceedings of the Maryland Nutrition Conference,* pp. 71-75.

Uzu, G. (1986). Threonine requirement for broilers. *AEC Information Poultry 252.* 03600 Commentry, France.

Webel, D.M., Fernandez, S.R., Parsons, C.M., and Baker, D.H., (1996). *Poultry Science,* **75**: 1253-1257.

Zhang, Y. and Parsons, C.M. (1996). *Poultry Science,* **75**: 514-518.

EMERGENCY DISEASES FROM AN AUSTRALIAN PERSPECTIVE

J.G. FAIRBROTHER

Summary

The Australian Veterinary Emergency Plan was designed to provide a national response plan to combat emergency diseases. The role of the Consultative Committee on Exotic Animal Diseases includes both advice on control and eradication procedures and payment of compensation resulting from an emergency disease outbreak. Endemic emergency diseases are related to minimum farm management standards. The exotic emergency diseases virulent avian influenza and Newcastle disease have occurred in Australia a number of times and every outbreak has been eradicated. Suggestions made in 1995 that the poultry industry should participate in an auditable accredited scheme for the farming sector resurfaced in 1998 as Commonwealth and State governments became more concerned over the increasing compensation payments being made. The industry now faces new major challenges as it reviews its perspective on emergency diseases.

I. INTRODUCTION

"Emergency diseases" is a term that has been introduced into the Australian animal industries' vocabulary in recent times. It covers both exotic animal diseases and endemic animal diseases that from time to time cause severe economic losses in an industry. In the poultry industry context, even as recently as 1996, the term emergency disease related only to the two exotic diseases, Newcastle disease and virulent avian influenza.

II. THE AUSTRALIAN VETERINARY EMERGENCY PLAN

The Australian Veterinary Emergency Plan (AUSVETPLAN) was first published in 1991 although the concept was initiated as early as 1976. It was designed as a coordinated national response plan for the control and eradication of exotic animal diseases. In the introduction to the AUSVETPLAN Summary Document on Poultry (ARMCANZ 1996a) there is the following paragraph:

"Australian agriculture benefits enormously from its freedom from the more devastating epidemic diseases that plague livestock industries in other parts of the world. The introduction of these foreign diseases could cause serious production losses to livestock industries in this country, jeopardise exports of livestock and livestock products and/or have serious public health implications. It is therefore essential that effective contingency plans and well-trained personnel are available to counter any exotic diseases that penetrate our quarantine barriers. Australian policy is to eradicate any introduced exotic animal disease as expeditiously as possible, if this is at all feasible."

It is very difficult to disagree with this statement. However, it is an irony that, of the seven poultry exotic disease outbreaks that have occurred in Australia since 1930, only the first is considered seriously to be the result of a "foreign" disease. There is some doubt about one of the avian influenza outbreaks. There are no conclusions as to the origins of the viruses that caused the remaining five outbreaks.

In any case a stamping-out policy was implemented on each occasion and this involved:

- quarantine of the infected premises and movement controls;
- the slaughter and disposal of the infected and exposed animals;
- decontamination of the infected premises;
- surveillance of susceptible animals; and
- restriction of the activities of the infected and other poultry enterprises.

This policy has proved to be effective in each case. With such a plan in place it is inevitable that legislative backup is required. In Australia, each State and Territory has operational responsibility for the control and eradication of animal diseases, whether endemic or exotic, within its borders. Each State and Territory therefore administers its own exotic disease control legislation. This legislation is further supported by emergency service arrangements. In all cases these provide adequate powers for all essential exotic disease eradication measures. Commonwealth legislation includes powers under the *Quarantine Act 1908* that would be available to support, where appropriate, the States and Territories.

III. CONSULTATIVE COMMITTEE ON EXOTIC ANIMAL DISEASES

In any discussion on emergency diseases it is necessary to mention the Consultative Committee on Exotic Animal Diseases (CCEAD) and the Commonwealth/States Cost Sharing Agreement. The CCEAD is a committee of State/Territory Chief Veterinary Officers (CVOs), the Head of the Australian Animal Health Laboratory (AAHL) and the Chief of the Division of Animal Health of CSIRO. It is chaired by the CVO of Australia (from the Commonwealth Department of Agriculture, Fisheries and Forestry). The consultative committee was originally established in June 1941. Its terms of reference are:

- to consult on exotic and serious epizootic animal disease emergencies;
- to advise on control or eradication methods;
- to make judgments regarding the diagnosis of exotic diseases of livestock for the purpose of invoking the provisions of the Commonwealth/State financial arrangement for combating outbreaks.

Under the Commonwealth/States cost-sharing agreement for the eradication of certain exotic animal diseases, the total cost of eradication is borne by the Commonwealth (50%) and the States/Territories (50%). Each State/Territory pays a proportion fixed according to a formula established for each of the twelve diseases covered. The cost-sharing agreement applies only while CCEAD advises ARMCANZ (Agriculture and Resource Management Council of Australia and New Zealand) that "eradication is considered to be reasonably possible". The cost-sharing agreement is in place for twelve exotic diseases including Newcastle disease (in its classical virulent form) and virulent avian influenza (ARMCANZ 1996b).

IV. ENDEMIC EMERGENCY DISEASES

Emergency diseases of an endemic nature usually caused by a viral infection are invariably controlled by vaccination. Egg Drop Syndrome (EDS '76), very virulent Marek's disease (vvMD) and infectious laryngotracheitis (ILT) are three good examples.

While not a problem in Australia at present, very virulent infectious bursal disease (vvIBD) and *Salmonella enteritidis* could rapidly become emergency diseases if introduced into our poultry flocks. Duck viral hepatitis, duck viral enteritis and turkey rhinotracheitis are other examples. Diseases that are normally controlled by vaccination such as infectious bronchitis (IB) and fowl cholera could become emergency diseases only if there was a breakdown in either

efficacy or supply of good vaccines. Vaccination is not the panacea but only one of the many factors necessary to control an emergency viral disease.

Good management procedures with adequate attention to biosecurity are essential ingredients in any poultry farming operation. It goes without saying that sheds should be proof against wild birds and animals, vermin, pets and unauthorised people. An adequate and safe water supply is an obvious prerequisite to a poultry farm establishment. The logical extension of this concept is that all poultry farms and farm management procedures should adhere to a minimum industry standard. This theme is followed up later in the paper.

V. EXOTIC EMERGENCY DISEASES

The two emergency diseases of an exotic nature, i.e. diseases that do not normally occur in Australia, virulent avian influenza and Newcastle disease, are certainly treated differently.

(a) Virulent avian influenza

The first outbreak of virulent avian influenza in Australia was in Victoria in January 1976. The Victorian isolate was identified at Weybridge UK as Dutch 27 fowl plague virus first isolated in Indonesia in 1927 and not recorded anywhere since. The source of that virus was never established. As I recall, the farm was of such a standard that it was buried along with the birds that died or were slaughtered. Clean up and compensation costs were $220,000.

In May 1985 there was a second outbreak in Victoria, this time in Bendigo. This particular outbreak caused the industry and the State and Commonwealth Governments much concern as the consequences of the devastating 1983-84 outbreaks of AI in Pennsylvania and Virginia in the USA were still very much in people's minds. Clean up and compensation costs were $2.2 million.

Obviously avian influenza was becoming of greater concern to government. In April 1991 the Australian Quarantine Inspection Service (AQIS) discussion paper (AQIS, 1991) on the importation of chicken meat into Australia contained the following commentary:

"AI is found in domestic poultry, seabirds, waterfowl and wild birds. The disease may range from subclinical to one with mild symptoms or may be highly acute and fatal. In countries where outbreaks of the disease have been reported, huge economic losses resulting from mortalities, losses in production, sales and costs of control measures, were incurred.
The virus has a worldwide distribution and several antigenic subtypes are recognised. There is an enormous reservoir of influenza A subtypes in wild (mainly waterfowl) and domestic birds. The migration and congregation of waterfowl in wetlands play an important role in the maintenance of a reservoir for avian influenza viruses." This AQIS comment did not necessarily relate to the Australian situation, but it probably does.

The causative antigenic subtypes isolated in 1976 and 1985 were identical (H7N7). In July 1992, another outbreak of avian influenza occurred in Victoria, in the same area near Bendigo where it occurred in 1985. Specimens were forwarded to the Australian Animal Health Laboratory (AAHL) late on 30th July. A preliminary diagnosis of avian influenza was provided shortly after midnight and this was confirmed later in the morning of 31st July. The property was placed in quarantine and slaughter of the stock commenced on the next day.

Work at AAHL was undertaken to isolate the virus, determine its identity (H7N3) and confirm its pathogenicity by inoculation of chickens and ducks. This was reported by 5th August. The outbreak was confined to one property and eradication was effected rapidly and efficiently, with minimal interruption to interstate trade. The cost of clean-up and compensation was $1.3million.

Avian influenza was diagnosed on a layer property in Lowood, Queensland, in December, 1994. The producer had seen a rapidly increasing mortality rate in one shed. The diagnosis was made quickly using the suite of tests available for avian influenza. The virus was typed as virulent H7N3 strain but was different from the H7N3 responsible for the Bendigo 1992 outbreak. All diagnostic procedures worked well, with the speed of diagnosis allowing disease control programs to be quickly introduced by the Queensland Department of Primary Industries.

The cost of clean up and compensation was $500,000.

In November 1997, another disaster. Avian influenza (H7N4) struck a large well-managed chicken broiler breeder operation in Tamworth NSW. It subsequently spread to a broiler chicken farm and also involved some free range emus and a hatchery in Sydney. A total of 310,565 chickens and 1,232,074 hatching eggs were destroyed during the operation. The total cost involved in the eradication was $4.445 million, which comprised $2.168 million in compensation and $2.277 million in operating costs. Costs were again shared between Commonwealth, State and Territory Governments.

What effect do these repeated outbreaks of avian influenza have on the Australian perspective to emergency diseases?

In February 1988, the Victorian Department of Agriculture and Rural Affairs (DARA) now Department of Natural Resources and Environment (DNRE), issued a Technical Report (Miller and Simpson-White, 1998): *"Guidelines for preventing exotic and serious endemic poultry diseases"*. This publication was developed by a joint DARA/poultry industries working party established to review and upgrade operational procedures to combat exotic and serious endemic poultry diseases. It took two years for 18 people to write the guidelines - admittedly only six made it through the whole process. The extraordinary situation was that at the time of the AI outbreak at Bendigo, some four years later, virtually no reference was made to the Guidelines. Truly an example of having reinvented the wheel. We had not learnt a lot since 1985. The very first points mentioned in the DARA report were:

- sheds are to be secure against entry of all birds ....
- water must be clean and free from contamination by free flying birds ..chlorination and ultra-violet treatment is recommended ....

It went on to mention feed storage, hygiene facilities, visitors' books, dead bird disposal. The perspective hasn't changed, but little has been done by many sections of the industry. In 1989 in NSW, the Agriculture Department issued an AGFACTS sheet on "Poultry health - keeping disease out". Highlighted were:

- surface water can be contaminated by wild birds. Treat any surface water to be used for drinking ...
- bird proof your sheds so that wild birds cannot get into them.

In 1992 at the time of the second Bendigo outbreak, NSW Agriculture issued an information sheet simply headed Avian Influenza (Fowl Plague). Under the heading "Prevention" we read:

.......given that this disease (AI) is now in Australia and the virus is known to be present in wild birds these principles are critical .....:

- bird proof your sheds especially against water fowl ...
- provide a safe water supply.

Again in December 1994 in Lowood, Queensland, An AI outbreak. Siterep 23rd December:

"The most likely source of the outbreak is contamination of creek water by wild water birds .... ."

Water was considered by many people to have been involved in the 1997 AI outbreak in Tamworth. However, Dr George Arzey in Dander (Newsletter of the Australian Veterinary Poultry Association) (Arzey, 1998) in August 1998 expressed his concerns that water (from the Peel river) may not have been involved. He suggested that diseases may be introduced to farms by various routes and that all aspects of biosecurity need to be addressed by producers...... . His article concluded as follows:

"The disease hazards on each farm need to be identified, control measures need to be identified and implemented perhaps in order of cost, ease or consequences. Monitoring for each measure is as important as the implementation. It is not enough to install a footbath in front of the shed. Monitoring compliance and adherence to certain standards is essential."

(b) Newcastle Disease

Newcastle disease (ND) first came to international attention in Newcastle-on-Tyne, England, in 1926. It is a highly contagious viral disease of domestic poultry, cage and aviary birds and wild birds. It is characterised by digestive, respiratory and/or nervous signs. The disease has a number of strains that differ in the severity of their clinical signs, ranging from inapparent infection to a rapidly fatal condition.

There have been three outbreaks of Newcastle disease in Australia. The first two were in 1930 and 1932 in Victoria and as far as can be ascertained they were the same virus from the original source. On that occasion the disease entered Australia on infected poultry carcasses. This happened in Victoria and cooked poultry scraps were, apparently, discarded from a ship in Port Philip bay, picked up by seagulls or other birds and an infection reached the commercial poultry industry. Of course the industry was very small at the time and an eradication program was eventually successful. Nevertheless at the time it was seen as catastrophic for the Victorian industry which was virtually all laying fowls on the ground.

The industry perspective on ND has been based on the premise that it is the major disease threat to the viability of the Australian commercial poultry industry. It is also regarded as a major potential threat to the pet and native bird populations. The significance of the disease has been acknowledged in numerous government publications. Quarantine pamphlets issued by the NSW Department of Agriculture in 1974 and the Commonwealth Department of Health, Quarantine Division in 1984, describe Newcastle disease as "the most feared avian disease in the world".

In the industry's opposition to the importation of chicken meat into Australia much was said about the consequences of an ND outbreak. The cost of attempted eradication of the disease would be very high. Both cost and consequence would depend to a large extent on where the outbreaks occurred. The worst, and most likely scenario would be an outbreak in the Sydney-Newcastle region. The outer Sydney metropolitan area/Hawkesbury/Newcastle-Hunter region supports large numbers of broilers and layers as well as elite breeding flocks, grandparent breeding flocks and multiplication flocks. The cost of quarantine, control and eradication programs to the industry and Commonwealth and State governments are difficult to estimate. However, based roughly on the cost of eradication of three outbreaks of avian influenza in recent years it is suggested that a widespread ND outbreak could cost $30-40 million to eradicate. One of the better known ND outbreaks occurred in California in the early 1970s. That outbreak took two years to eradicate, 11 million birds were destroyed and the total cost was $56m (US). Our Australian estimate is not exaggerated.

If the disease could not be eradicated, very large costs associated with mortality and loss of production due to clinical disease, with purchasing and applying vaccines and loss of trade would be incurred on an ongoing basis.

There is insufficient knowledge of the effects upon and spread of the disease in native bird species and this means that the effect on Australia's native bird population is inestimable. What we do know is that our native bird population is highly susceptible to virulent Newcastle disease and an outbreak would be a disaster.

It is not surprising therefore that the industry was shocked and immensely concerned when ND was confirmed on two farms in western Sydney in September 1998. Subsequently a third farm became infected by chickens it received from one of the Sydney properties. At the time of writing this paper the outbreak appeared to be totally controlled. The three farms had been depopulated and clean up and disinfection were about to commence. The ND virus involved was highly pathogenic. Fortunately transmission of the virus appeared to be by physical contact and quite slow moving. As was the case in the AI outbreaks CCEAD was activated. Also, following a recent change to policy, an Incident Management Group of three government and two industry representatives was established so that deliberations of CCEAD could be quickly disseminated to industry. The anticipated major disaster did not occur on this occasion.

The significant difference between the 1998 Newcastle disease outbreak and all the previous outbreaks was that the costs of clean up and decontamination of the infected properties were to be borne by the property owners.

VI. THE NEED FOR A NEW PERSPECTIVE

In July 1995 I presented a paper at the Queensland Poultry Science symposium on *Avian influenza in review - its significance, cost and control* (Fairbrother, 1995). A year later I spoke at the Queensland Poultry Information Exchange on "*Exotic diseases - the implications of further outbreaks*" (Fairbrother, 1996).

As reported in my 1995 paper, I wrote as follows to the Animal Health Committee of the Standing Committee on Agricultural Resource Management:

"My personal attitude has long been one of dismay and despair at the lack of quarantine precautions and low standard of hygiene at many poultry farms and processing plants in Australia. The lack of bird proofing of sheds and the reluctance of poultry farmers to treat water on their premises and to remove the ability of water fowl to effectively cohabit with their flocks, is appalling.

The accreditation of farms (from a disease viewpoint) would be difficult to implement, although it has some attractions. The problems with accreditation and monitoring of farms to ensure that standards are maintained could become a very costly option. As we move into the ISO 9000 mind-set, however, auditing will become the name of the game and there could be substantial merit in auditing against a Code of Practice.

In any case, cleaning up the operations of many commercial egg farmers and smaller broiler farmers would be a good start. An industry Code of Practice, as was suggested following the 1985 AI outbreak in Bendigo, would also help. Probably such codes already exist, but one wouldn't think so when you have a look around the industry."

The suggestion that following restocking a farm should not be permitted to recommence operations until appropriate bird proofing and water treatment facilities have been installed at the owners expense, is fully supported. There should be no exceptions.

Since I made those outlandish comments in 1995 there have been two more exotic disease outbreaks in 1997 and 1998. We have also had serious problems with vvMD, an emergency with EDS '76 and one or two ILT outbreaks. All were emergencies as far as the industry was concerned.

Times, attitudes, available funds and perspectives are changing - rapidly.

ARMCANZ at its meeting No 12 held on 27 February 1998, in its Resolution No 20, requested, *inter alia*, "AAHC to review with industry involvement procedures for funding and management of AI, in particular the use of compensation, where industry does not meet acceptable management criteria".

In June 1998 the Australian Animal Health Council established a Task Group to review management practices and procedures to reduce avian influenza outbreaks in the poultry industries. The terms of reference of the task group included the following:

1. Describe and critically analyse guidelines for good management practices in the chicken meat and egg laying industries as they relate to the risk of incursion of AI.
2. Review the AUSVETPLAN Enterprise Manual for the poultry industry and compare this with any existing codes or guidelines described in 1 above.
3. Review the industry's adherence to these practices particularly with regard to AI risk prevention and minimisation.
4. Present recommendations on the criteria for payment of compensation for losses following stamping out and cleaning up after an emergency disease situation has passed.

On the 18th September 1998, even before the ink was dry on the terms of reference, Newcastle disease was confirmed on two properties in western Sydney, as already outlined. Along with the disruption to business involving quarantine, movement controls both within and between States, tracing product, logging vehicle movements, waste disposal and surveillance of surrounding farms, there is the impact on overseas trade. Australia has a significant trade in poultry and poultry products, including day-old chickens, particularly to Papua New Guinea and the Pacific islands. The Australian Quarantine and Inspection Service investigated the implications for trade and imposed interim restrictions on the provision of health certificates pending clarification of other countries' certification requirements for poultry, poultry products, ostriches and their products and emu products.

AQIS also contacted individual authorities and Australia's overseas posts as required regarding the continuation of trade based on existing or revised certification and animal health measures applied by NSW Agriculture. The effective and timely introduction of control zones around affected or at risk properties certainly helped minimise any commercial impact. As expected, some countries placed a total ban on exports from Australia while others placed a ban on products from NSW. The length of the ban varied from six months to one year.

Another critical issue that will change the poultry industry's perspective on emergency diseases relates to changes in the rules for compensation payments. In early 1998 the Centre for International Economics completed a report for the Australian Animal Health Council Ltd on *The funding of exotic animal disease management* (Anon, 1998). Subsequently in August 1998 the AAHC conducted a workshop on this matter and in September a small expert working group was established to make recommendations on, among other things:

- the cost sharing proportions between government and industry for specified diseases;
- what costs should be eligible for reimbursement.

It is quite clear that the Commonwealth and States will no longer bear the total costs of compensation resulting from an emergency disease outbreak. Diseases will be categorised and the industry proportion could vary from zero to 80 per cent of the total cost. The final categorisation and industry share of costs should be known by early 1999. How the poultry industry will raise its contribution in the event of an emergency disease outbreak is a moot point.

This raises again the issue of accreditation and auditing of poultry farms in all their forms. Many hatcheries, processing plants, grading floors and stock feed mills already have auditable HACCP plans in place. All these facilities are discussed in the existing AUSVETPLAN Enterprise Manual for the poultry industry (Gilchrist, 1996). This document had

its genesis, at least in part, from the 1988 DARA (Victoria) *Technical Report*. Currently the most critical area is the poultry farming sector.

VII. CONCLUSION

An agreed industry Code of Practice for the operation of poultry farms *from a biosecurity and hygienic practice viewpoint* based on HACCP principles is the first step required. An accreditation system will have to be put in place and be backed with an audit program. The whole program should be driven by industry but it may be necessary to support the program with minimum government regulation if industry cooperation is not forthcoming. Any program must be fair and equitable to both small and large operators. It must be said that a significant percentage of the poultry industry already has suitable programs in place.

Such a program will not necessarily eliminate future emergency disease outbreaks. However, it should result in better biosecurity and a much improved standard of hygiene in the poultry farming sector generally. Only an audited accreditation program will provide the industry generally with confidence that their business will not be jeopardised by a few sub-standard operators.

The Australian perspective has changed over time. It will without doubt become increasingly circumspect about emergency diseases. In the long term everyone will benefit.

REFERENCES

Anon. (1998). The Funding of Emergency Animal Disease Management. Report prepared for the Australian Animal Health Council by the Centre for International Economics, Canberra.

AQIS. (1991). The Importation of Fresh Frozen and Cooked Chicken Meat and Products from USA, Denmark, Thailand and New Zealand, p.10. Department of Primary Industries and Energy, Canberra.

ARMCANZ. (1996a) *AUSVETPLAN*. Summary Document, Poultry. Department of Primary Industries and Energy, Canberra.

ARMCANZ. (1996b) *Summary Document*. Department of Primary Industries and Energy, Canberra.

Arzey, G. (1998). In: *Dander*, p. 10. Ed. T. Faragher, Australian Veterinary Poultry Association, Canterbury, Victoria.

Fairbrother, J.G. (1995). *Proceedings of the Queensland Poultry Science Symposium.* Gatton, Queensland.

Fairbrother, J.G. (1996). *Poultry Information Exchange,* ANA, Gold Coast, pp 9-15. Queensland.

Gilchrist, P.G. (1996). Poultry Industry Enterprise Manual. AUSVETPLAN. Department of Primary Industries and Energy, Canberra.

Miller. L.A. and Simpson-White, P.H. (1988). Guidelines for Preventing Exotic and Serious Endemic Poultry Diseases. Department of Agriculture and Rural Affairs, Victoria. Technical Report. Series No. 151.

LIVING WITH EXOTIC DISEASES

Prof. I. AINI *Idris* *Uni of Malaysia*.

Summary

This paper reviews the early reports of important poultry diseases in Malaysia, the emergence of new diseases and how the country copes in terms of disease control. All the major infectious diseases of chickens reported in other countries, and the new diseases that arise as a result of intensive management systems, seem to find their way into Malaysia.

I. INTRODUCTION

"Exotic" as defined by The Oxford Advanced Learner's Dictionary is "anything that is introduced from another country, not native, or unusual". Longmans Active Study Dictionary of English, defined it as "(as if) from a distant country". For the purpose of this review, the second definition is more appropriate mainly because nobody can be certain of the origin of poultry diseases we are seeing in Malaysia today. Postulates are made based on various evidence and reports. Living with exotic diseases simply means how these diseases are being handled. In this review, the history of major poultry diseases in Malaysia and their control are discussed.

II. POULTRY INDUSTRY

Among the early exotic breeds brought into Malaysia by English sailors in the early 1930s were White Leghorn, Rhode-Island Red, New Hampshire and Barred Plymouth Rock (Mann, 1941). This was the beginning of the expansion of poultry industry in Malaysia. Its development evolved almost on a similar pattern to those in the advanced countries. The introduction of superior breeds (mainly from European countries), vaccines for disease control, high quality feed, advanced technology and favourable government policy, contributed to the transformation of subsistence poultry farming to commercialised and advanced poultry industry with high breeding efficiency and high productivity (Aini, 1993). It is the most developed industry within the livestock sector, though traditional backyard subsistence poultry farming is still important in rural areas.

Among the factors that contribute to the rapid expansion of the poultry industry were: (1) effective control of major diseases through vaccination, (2) liberal policy on importation of breeding stock, (3) structural changes that allow setting up of feedmills, breeder farms and large integrated production units, (4) high domestic demand for poultry meat, (5) introduction of investment incentives by the Government in 1985.

Peninsular Malaysia has been self-sufficient in chicken meat production since 1960 and became a nett exporter in1983. Poultry meat production increased from 21,300 tonnes in 1960 to 678,000 tonnes in 1997. Egg production showed a similar growth pattern, with 12,800 tonnes in 1961 to 360,000 tonnes in 1997 (Watt Poultry Statistical Year Book, 1998).

Other avian species that have been imported into Malaysia include ducks, turkeys, quails, pigeons, pet birds, and in the last three years, ostrich. They also contribute to the collection of exotic avian species in Malaysia.

Faculty of Veterinary Medicine and Animal Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

III. POULTRY DISEASES

Poultry diseases remain a great threat to Malaysian poultry farmers and are responsible for large economic losses to producers. These losses arise from one or more of the following outcomes: high mortality, retarded growth, reduced egg production, low fertility, poor hatchability, reduced product quality, and increased cost of treatment or vaccination. Due at least in part to the globalisation of the poultry industry and the widespread adoption of imported production systems, which give rise to large concentrations of stock, almost all known major poultry diseases have been reported in Malaysia (Aini, 1993). The common husbandry practices in Malaysia also readily facilitate transmission of diseases betweeen flocks and farms. These practices include open type of housing, close proximity of poultry houses and farms, in many areas. Biosecurity measures and routine vaccination programmes are being practised in big commercial farms, especially breeder farms, as a means of controlling important poultry diseases (Aini, 1990). Many breeder farms now practise a closed house system with evaporative cooling.

IV. VIRAL DISEASES

(a) Newcastle disease (ND)

Newcastle disease is still a major threat to the poultry industry in Malaysia, though in general it is under control. The Mukteswar strain of ND virus, a mesogenic strain, was introduced into Malaysia in 1947 for vaccine production. Its introduction, followed by the F strain, was regarded as the greatest single factor that gave fresh impetus to poultry keeping in Malaysia. The velogenic viscerotropic ND, which is the most virulent form of ND, is endemic in Malaysia. It can cause 100% mortality in susceptible birds and primarily affects the trachea, lungs, intestine, proventriculus and caecal tonsils (Lai et al., 1986).

The first case of ND in Malaysia was reported in 1934 (Anon, 1934, cited by Chandrasekaran and Aziz, 1989). This coincides with the introduction of exotic chickens from Europe by the English sailors. Since then, an abundance of reports on ND outbreaks has been documented in local and overseas publications. Outbreaks are usually encountered in small farms where vaccination is not carried out, improperly administered or there is mishandling of vaccine.

Velogenic viscerotropic ND virus was also isolated from an outbreak in quails (Chandrasekaran and Aziz, 1989) and moustache parakeets (Heng and Lim, 1983). Other strains of ND virus have been isolated from wild birds (Mustaffa-Babjee, 1977; Heng and Lim, 1983; Awang et al., 1990).

(b) Infectious bronchitis (IB)

The other important poultry disease is IB. The virus was first isolated in 1964 (Heng et al., 1980). Respiratory signs (Heng et al., 1980), nephrosis - nephritis syndrome (Heng et al., 1980; Azri et al., 1997, Aziz et al., 1996) and mortality reaching 40% (Heng et al., 1980) had been reported. The disease also adversely affected egg production in layer and breeder flocks.

Control of IB by vaccination has helped to reduce its prevalence in this country. However, recurrent outbreaks of IB in vaccinated flocks, especially those associated with kidney lesions, indicated that the vaccine virus does not provide the protection expected against current field strains of IB virus.

(c) Fowlpox (FP)

There is no specific date as to the first report of FP in Malaysia. It is however believed to be present since the 1930s. Dry pox, which is characterised by the formation of an extensive nodular scab-like lesions on the non-feathered parts of the skin, is more common than wet pox, which is characterised by a diphtheritic membrane in the upper respiratory tract of chickens. Pox infection has also been reported in pigeons (Loganathan *et al.*, 1985), turkeys (Aini and Ibrahim, 1986a; Lim *et al.*, 1986a) and ducks (Aziz and Mokhtar, 1989).

(d) Infectious Bursal Disease (IBD)

The presence of the disease was detected serologically in 1985, but later a classical strain of birnavirus was isolated. Outbreaks of clinical IBD with high mortality was first described in Malaysia in 1991 (Loganathan *et al.*, 1992; Hair-Bejo, 1992). Since then, the disease has become one of the most important viral diseases in the country, mainly due to significant economic losses resulting from high mortality, impaired growth and profound immunosuppression. The infection causes necrosis of the lymphoid cells in the bursa of Fabricius, thus impairing its functions. Outbreaks of IBD continue to occur despite the importation of many types of IBD vaccines (Hair-Bejo *et al.*, 1996; Sharifah *et al.*, 1994), probably due to strain variations or improper vaccination regimes (Aini *et al.*, 1996).

(e) Other viral diseases

(i) Marek's disease (MD). The paralytic form of MD in Malaysia was first reported by Wells in 1955 (Loganathan and Harizam, 1987). Subsequently more reports of the disease were published (Omar and Lim, 1968; Omar *et al.*, 1973). To control the disease, layer and breeder chickens were vaccinated at day-old.

(ii) Avian encephalomyelitis (AE) was first confirmed in Malaysia in 1966 (Opitz *et al.*, 1976). Since then sporadic occurrences have been reported (Mustaffa-Babjee, 1985). Avian encephalomyelitis is mainly observed in chicks less than three weeks of age with the signs of depression, head tremors and incoordination, leading to death.

(iii) Infectious laryngotracheitis (ILT) occurs sporadically and is more localised in nature (Mustaffa-Babjee, 1985). Cases have been reported mainly in adult birds.

(iv) Egg drop syndrome (EDS) has been detected serologically and isolated in embryonated duck eggs (Mustaffa-Babjee, 1985). Vaccination is practised by many breeder farms.

(v) Avian reovirus was isolated from tendons of broilers with leg weakness (Sharifah *et al.*, 1989). The broilers, six to seven weeks of age, were observed to be dull, unthrifty, refused to walk and sat on their hocks. The birds had swollen hock joints.

(vi) Avian influenza (AV). The significance of AV in Malaysia is yet to be determined. Aini and Ibrahim (1986b) isolated nine H_3N_6 and five H_4N_6 subtype isolates from ducks. Influenza virus of H_3N_6 subtype caused a mild respiratory distress in experimental chickens (Aini and Ibrahim, 1986b), whereas isolate H_4N_6 was non-pathogenic for young chicks (unpublished data). Two isolates of H_4N_3 subtype have also been isolated from passerine birds (Ibrahim *et al.*, 1990).

(vii) Reticuloendotheliosis/chicken anaemia virus (CAV). Antibodies against these viruses have been detected in Government poultry farms (Chai and Yuasa, 1989). Rozana *et al.* (1995) did a survey in broiler and breeder commercial farms in the southern states of Malaysia and detected chicken anaemia virus serologically. By also using dot-blot method they were able to detect CAV from the liver of chickens showing clinical signs of CAV infection.

(viii) Chick embryo lethal orphan virus. Azri and Mohd-Nor (1990) detected antibodies against this virus in poultry and pet birds, during a routine diagnostic tests on 3693 serum samples from 1985-1989. A variety of clinical signs were reported, including respiratory signs, drop in egg production and some mortality.

(ix) Swollen head syndrome (SHS). An upper respiratory tract infection of chickens was confirmed to be present in Malaysia, (Azri *et al,* 1997; Lim *et al.,* 1994). Recently outbreaks of SHS were reported in a breeder farm, in which the egg production was affected. The syndrome began with sneezing and conjunctivitis, followed by swelling of lachrymal glands, around the eyes, over the head, sub-mandibular region and finally subcutaneous oedema of the head.

(f) Duck diseases

(i) Duck virus hepatitis (DVH). Outbreak of DVH was first reported in 1986 in Peninsular Malaysia. The virus caused enlarged and necrotic livers in ducklings under two to three weeks old (Lim *et al.,* 1986b). The first outbreak occurred in a broiler farm of Muscovy cross (imported from Taiwan) and Pekin ducks. High mortality (80%), opisthotonus and paddling of legs before death were observed in Pekin ducks under 10 days old, while Muscovy cross ducklings of the same age were unaffected.

(ii) Duck virus enteritis (DVE) was first reported in 1992 (Sharifah *et al.,* 1994), in flocks of commercial Pekin ducks of various ages, ranging from 18 days to 18 months old. The infected ducks developed diarrhoea and mortality of the ducks reached as high as 80-100% within 1-5 days. Subsequently vaccines for both diseases were imported into the country.

V. BACTERIAL DISEASES

(a) Pullorum

Pullorum disease was first reported in West Malaysia in 1956 (Shanta, 1958). The disease then became widespread in this country especially among large commercial flocks. The survey carried out between 1965-1968 showed a particularly high incidence of the disease in birds originally imported from the United States (Osman, 1968). The implementation of National Disease Control and Eradication Scheme for *Salmonella pullorum* has brought the prevalence of this disease to a very low incidence or eradicated in many farms.

(b) Fowl Cholera (FC)

The first report of FC outbreak in Malaysia was in 1935 (Whitmore, 1936). The second outbreak was reported in 1949, after a lapse of fourteen years (Lancaster, 1951), with deaths in chicken and ducks. The outbreaks were successfully controlled with the use of sulphamethazine. Sporadic outbreaks occurred after that, until the early 1990s when outbreaks were reported in village chickens, resulting in heavy mortalities. Besides using antibiotics, the farmers resorted to imported vaccines from Taiwan. The Veterinary Research Institute now produces fowl cholera bacterin with a combination of three serotypes (Types A, D and untypeable).

(c) <u>Others</u>

(i)   Infectious coryza (IC). Sporadic outbreaks of IC have been reported mainly in replacement pullets. *H. paragallinarum* was earlier isolated from chickens with respiratory signs (Chong, 1960). This disease is controlled by using antibiotics.

(ii)   Mycoplasmosis is an important chronic respiratory disease, especially in broilers. Mycoplasma was first isolated from a case of avian coryza in 1960 (Thuraisingham, 1963). Since then, *M. gallisepticum, M. gallinarum, M. gallinaceum* and *M. synoviae* have been isolated from commercial chickens.

(iii)   Colibacillosis   is also an important disease, usually occurring together with mycoplasmosis.

VI. PROTOZOAN DISEASES

(i)   Coccidiosis. In spite of advances in nutrition, chemotherapy and management practices, coccidiosis still remains one of the major economic losses to the poultry industry in Malaysia. The disease causes damage to the intestinal tract, interruption in feeding and digestive processes, dehydration, blood loss, and increased susceptibility to other diseases and death. *Eimeria tenella* and *E. necatrix* are the most commonly isolated species which caused the most severe outbreaks.

The anticoccidials used in Malaysia include monensin, salinomycin, halofuginone, lasalocid, sulphaquinoxaline, narasin, totrazuril and amprolium.   Another ionophore, semduramicin, was introduced into Malaysian poultry recently.

(ii)   Leucocytozoon (LU). Kuppusamy (1936) was the first to record the presence of LU in chicken in Peninsular Malaysia. Subsequently, Omar (1968) recognised in 1961 that the disease was caused by *A. caulleryi* with *Culicoides arakawae* as the vector. LU occurs sporadically in areas with marshy ground and is usually associated with stress. Clinically affected birds appear pale and can lead to high mortality. On post-mortem, petechial haemorrhages are usually observed on the thigh and breast muscles, and sometimes the kidneys are haemorrhagic.

VII. FOODBORNE PATHOGENS

Joseph *et al.* (1988) reported that, from 1981-1985, *S. sofia* was commonly isolated and from 1991-1995, *S. Enteritidis (SE)* was the most frequently isolated serotype (Mohktar *et al.,* 1996). Infections caused by SE remains a problem of great concern. The impact of the disease on trade was very much felt by the export farms in particular.

In Malaysia, *Campylobacter,* particularly *C. jejuni,* were found not only in broiler (Joseph, *et al.,* 1983) and village chickens (ranging from 72.6-97.1%) but also in ducks (18.4-75.0%). Puthucheary *et al.* (1994) stated that though published reports in Malaysia gave a low isolation rate in man (about 3%), the true incidence may be 5-10 times greater.

VIII. RECENTLY REPORTED DISEASES

Hypoglycaemia spiking mortality syndrome (Mohd-Haas *et al.,* 1998), Chlamydiosis (Phong *et al.,* 1996; Phong *et al.,* 1997) and *Ornithobacterium rhinotracheitis* (Raymond, 1998), are among the new diseases reported in Malaysia.

IX. DISEASE CONTROL

Most of the major diseases mentioned earlier are under control, through routine vaccination programmes, prophylactic medication, eradication and other control programmes, and most importantly, farmers' education. Strict biosecurity measures are being practised especially in the grandparent and parent stock farms (Aini, 1993).

Vaccination programmes and procedures may differ from one farm to another, depending on the type of vaccines used, source of vaccines, disease situations and risks, and local conditions (Aini, 1993). In the case of coccidiosis, though vaccines are available, the main method of control is by prophylactic medication, through feed or drinking water. Vaccination is not very popular in controlling coccidiosis in Malaysia. Similarly, for mycoplasmosis, chemotherapy is more commonly practised than routine vaccination.

For coccidiosis, more than 30 anticoccidial drugs have been introduced to the broiler industry and their long extensive use in chickens has resulted in the decline in sensitivity of the coccidia to the anticoccidials. More and more anticoccidials are being brought into the country to combat this disease.

Various antibiotics are imported into the country for the control and treatment of most bacterial diseases. The use of antibiotics in intensive farming system has become an integral part of poultry management. Antibiotics are used for prevention as well as for treatment of diseases and also used in feed as growth promoters. The concern by farmers over many clinical diseases has led to indiscriminate use of antimicrobial agents and illegal vaccines. Reports of failures in treatment of respiratory diseases have mounted in recent years.

The Department of Veterinary Services (DVS) Malaysia has approved vaccines for 14 poultry diseases (nine viral, one protozoan and four bacterins) and two for duck diseases. The vaccines are for the following diseases: ND, IB, IBD, MD, ILT, EDS, FB, viral arthritis, AE, IC, FC, colibacillosis, mycoplasmosis, coccidiosis, DVE and DVH. Up to June 1993, a total of 280 vaccines with different trade names have been approved for importation, sale and use in Malaysia subjected to certain rules and regulations as specified by DVS.

Two types of approval are given to imported vaccines, namely provisional approval and final approval. All applications for registration of veterinary vaccines are subjected to "provisional approval" for a one-year period. These provisionally approved vaccines shall be considered for "final approval" when all the requirements stated have been fully and satisfactorily complied by the importer, and the vaccine is found to be justified for use in the country. The justification is based on the potency, efficacy and safety of the vaccine. The final approval is valid for four years and subjected to renewal of the registration at the end of that period (DVS, 1992).

A local vaccine company, Malaysian Vaccines and Pharmaceuticals Sdn. Bhd., was established in 1992. To date the company has produced ND, FP, IB and ND-IB vaccines.

X. CONCLUSION

As the years go by, more and more diseases are being added to the list of poultry diseases reported in Malaysia. The availability of more efficient and reliable tests makes diagnosis of diseases an easier task. Couples with advanced molecular techniques, diseases that were difficult to diagnose before and were therefore not attempted, now can be determined with confidence. Molecular techniques tool enable researchers to differentiate different strains of the organisms and also differentiate vaccine strains from field strains. Genetic variations among various isolates can now be determined using the more sensitive and rapid methods, such as polymerase chain reaction (PCR), followed by restriction fragment length polymorphism (RFLP) and PCR-sequencing. Mankind's ability to

manipulate DNA has opened new doors that will ultimately lead to new changes and opportunities, such as the discovery of new diseases and molecular control of existing diseases. With the advancement of these molecular techniques, perhaps one day we can truly determine exotic pathogens being brought into the country. Meanwhile, we have to satisfy with the efforts in preventing the new diseases and controlling the existing diseases.

Strict regulations, continuous and sustainable surveillance and monitoring programmes will still be carried out throughout the country, especially when the diseases are still present in neighbouring countries or still prevalent in other parts of the world. Malaysia needs more well trained and experienced poultry veterinarians, efficient reporting systems for disease statistics, efficient and reliable laboratory and field services and of course well established control procedures.

XI. ACKNOWLEDGEMENTS

REFERENCES

Aini, I. (1990). *World's Poultry Science Journal.* **46**: 125-132.

Aini, I, (1993). *Proc. Xth International Congress of the World Veterinary Poultry Association,* pp. 41-46. Eds. J. York, S. McAuliffe and V. McWaters.

Aini, I, and Ibrahim, A.L. (1986a). *Kajian Vet.* **18**: 85-87.

Aini, I. and Ibrahim, A.L. (1986b). *Vet. Record* **118**: 130.

Aini, I., Phong, S.F., Hair-Bejo, M., Chulan-Mohsin, U., Jalila, A. and Ibrahim, A.L. (1996). *Proc. 8th Vet. Assoc. Malaysia Scientific Congress,* pp. 241-242. Eds. N. Muniandy, M.K. Vidyadaran, K.T. Lim and A.H. Aziz.

Awang, I.P.R., Ibrahim, H.M., Aini, I. and Ibrahim, A.L. (1990). *Proc. 13th Malaysian Microbiology Symposium.*

Aziz, H.A. and Mokhtar, M.A. (1989). *J. Vet. Malaysia* **1**: 17-20.

Azri, A. and Mohd-Nor, (1990). *J. Vet. Malaysia* **2**(1): 51-53.

Azri, A., Roosevien, R.F.N., Sohayati, A.R., Goh, G.Y., Aminahkadariah, A.L., Chulan, U. (1997). *J. Vet. Malaysia* (In press).

Azri, A., Roslan, M., Zabidah, A. and Rahim, A. (1997). *Proc. 9th Vet. Assoc. Malaysia Scientific Congress,* pp. 52-54. Eds. N. Muniandy, M.Y. Johara and S. Chandrasekaran.

Chai, K.K. and Yuasa, N. (1989). *J. Vet. Malaysia* **1**: 11-16.

Chandrasekaran, S. and Aziz, H.A. (1989). *J. Vet. Malaysia* **1**: 9-15.

Chong, S.K. (1960). *J. Malay. Vet. Med. Assoc.* **2**: 143-161.

Department of Veterinary Services-DVS (1987). In: *Annual Report,* 213 pp. Ministry of Agriculture, Malaysia.

Hair-Bejo, M. (1992). *Proc. 4th Vet. Assoc. Malaysia Congress.* pp. 35-36. Ed. M.K. Vidyadaran.

Hair-Bejo-M., Thu-Zar, T. and Aini, I. (1996). *Proc. 8th Vet. Assoc. Malaysian Scientific Congress,* pp. 224-225. Eds. N. Muniandy, M.K. Vidyadaran, K.T. Lim and A.H. Aziz.

Heng, N.H. and Lim, K.T. (1983). *Kajian Vet.* **15**: 11-16.

Heng, N.H., Lim, K.T.and Lee, C.M. (1980). *Kajian Vet.* **12**: 1-18.

Ibrahim, H.M., Awang, I.P.R., Alexander, D.J., Manrell, R.J., Aini, I. and Ibrahim, A.L. (1990). *Vet. Record* **127**: 528.

Joseph, P.G., Sivanandan, S.P. and Tham, T.Y. (1988a). *Epidem. Inf.* **100**: 351-359.

Joseph, P.G., L.J. Tan, S.P. Sivanandan, Jamnah Omar G.S. Cottew and F. Yeates (1988b). *Trop. Biomed.* **5**: 167-177.

Kuppusamy, A.R. (1936). *Indian Vet. J.* **13**: 25-35.

Lancaster, W.,E. (1951). In: *Federation of Malaya. Report of the Vet. Dept. for 1949*, pp. 19.

Lai, M.C., Ibrahim, A.L. and Aini, I. (1986). *Proc. 35th Western Poultry Dis. Conf.* pp. 102-103.

Lim, K.T., Mahani A.H. and Lim, S.S. (1986a). *Kajian Vet.* **18**: 41-49.

Lim, K.T., Mahani, A.H., Lim, S.S. and Saroja, S. (1986b). *Kajian Vet.* **18**: 129-138.

Loganathan, P. and Haraizam, Y. (1987). *Kajian Vet.* **19**: 55-59.

Loganathan, P., Devi, K.V. and Lo, H.S. (1985). *Kajian Vet.* **17** : 55-61.

Loganathan, P., Sharifah, S.H., Arunasalam, V. and Mahani, A.H. (1992). *J. Vet. Malaysia* 4(2): 103-108.

Mann, G.E. (1941). *Malay Agric. J.* **29**: 107.

Mohd.-Haas, Y., Choo, P.Y., Ng, S.F., Aziz, A.J., Azri, A., Rahmah, S.M.S., Kono, Y. and Ganapathy K. (1998). *J. Vet. Malaysia* (accepted for publication).

Mohktar, A.M., Tham, T.Y. and Siti Hajar (1996). *Proc. 8th Vet. Assoc. Malaysia Scientific Congress*, pp. 71-72. Eds. N. Muniandy, M.K. Vidyadaran, K.T. Lim and A.H. Aziz.

Mustaffa-Babjee, A. (1977). *Bull Off. Inst. Epiz.* **89**(9-10) : 835-839.

Omar, A.R., Lo, H.S. and Teoh, K.C. (1973). *Australian Vet. J.* **49**: 319-320.

Omar, A.R. and Lim, S.Y. (1968). *Kajian Vet. Malaysia-Singapore* **1**: 224-235.

Omar, A.R. (1968). *Kajian Vet.* **1**: 109-124.

Opitz, H.M., Maamor, A. and Lim, K.T. (1976). *Kajian Vet.* **8**: 13-17.

Osman, D. (1968). *Paper Presented at Vet. Off. Conf., Kuala Lumpur.*

Phong, S.F., Aini, I., Al-Ajeeli, K.S. and Lee, P.C. (1997). *Proc. 9th Vet. Assoc. Malaysia Scientific Congress*, pp. 268-269. Eds. N. Muniandy, M.Y. Johara, S. Chandrasekaran.

Phong, S.F., Aini, I., Jalila, A., Al-Ajeeli, K.S. and N. Salim (1996). *Proc. 8th Vet. Assoc. Malaysia Scientific Congress*, pp. 243-244. Eds - N. Muniandy, M.K. Vidyadaran, K.T. Lim, A.H. Aziz.

Puthucheary, S.D., Parasakthi, N. Liem, S.T. and Chee, Y.W. (1994). *Singapore Med. J.* **36**: 453-456.

Raymond, C. (1998). *Personal Communication.*

Rozana, A.S., Aini, I., Al-Ajeeli, K.S., Jalila, A. and Salim, N.B. (1995). *J. Vet. Malaysia.* 7(2): 77-79.

Shanta, C.S. (1958). *J. Vet. Med. Assoc.* **2**: 80.

Sharifah, S.H., Mahani, A.H., Loganathan, P. and Lim, K.T. (1989). *J. Vet. Malaysia* **1**: 17-27.

Sharifah, S.H., Mahani, A.H., Taniguchi, T. and Salmah, M. (1994). *J. Vet. Malaysia* 6(1): 17-20.

Sharifah, S.H., Ong, G.H., Mahani, A.H., Wan-Kamil, W.M., Aini, I. and Ibrahim, A.L. (1994). *J. Vet. Malaysia* 6(2): 65-69.

Thuraisingam, S. (1963). Federation of Malaya Report on the Veterinary Division, Ministry of Agricultural and Cooperatives for year 1959 and 1960, Kuala Lumpur pp. 44.

Watt Poultry Statistical Yearbook (1998). *Poultry International* **37**(9): 12-29.

Whitmore, S.H. (1936). In: *Annual Report of the Veterinary Department, Straits Settlements for 1935*, pp 52.

FATTY ACID MODIFIERS OF BIOCHEMICAL AND MOLECULAR ACTIONS IN BONE

B.A. WATKINS

Summary

New research indicates that dietary lipids influence bone formation rates in animals and collagen synthesis in chondrocyte cultures. Feed sources of fatty acids are hypothesized to modulate the local biosynthesis of eicosanoids in bone to alter formation rates, but may also affect the production of reactive oxygen species in epiphyseal cartilage. For example, bone modeling was optimal in chicks when (n-3) fatty acids were supplied in the diet to moderate the effects of (n-6) fatty acids. Feed sources of (n-3) fatty acids elevated the concentrations of 20:5(n-3) and 22:6(n-3) in epiphyseal and articular cartilage, and in cortical and trabecular bone in chicks. Moreover, (n-3) fatty acids reduced the concentration of 20:4(n-6) in bone polar lipids, decreased *ex vivo* prostaglandin E_2 (PGE_2) production in bone organ culture, increased bone formation rate, and improved mechanical properties of bone. These observations are believed to be the result of long-chain (n-3) fatty acids affecting osteoblastic activity by modifying autocrine and paracrine signals that govern bone modeling. Since PGE_2 exhibits biphasic effects on bone formation, stimulating bone formation at a low concentration but inhibiting it at higher concentrations, (n-3) fatty acids may directly enhance osteoblastic bone formation. Another potential effect of (n-3) fatty acids may be up-regulation of insulin-like growth factor-1 (IGF-1) anabolic action on bone. The recent discovery of the Cbfa1 gene, which controls differentiation of osteoblasts in animals, provides an opportunity to explore nutrient gene regulation of bone formation. The Cbfa1 gene controls other genes that influence osteoblastic bone formation. A lack of its expression results in boneless mice which have only cartilage (Ducy *et al.*, 1997). The relationship between nutrients and gene expression is relatively new, and recent research demonstrated that dietary fatty acids regulate hepatic gene transcription. This paper presents research that describes how fats and antioxidants support bone formation and benefit cartilage function to optimize bone modeling in poultry.

I. BONE CELLS AND BONE METABOLISM

Bone is a multifunctional organ that consists of a structural framework of mineralized matrix and contains heterogeneous populations of chondrocytes, osteoblasts, osteocytes, osteoclasts, endothelial cells, monocytes, macrophages, lymphocytes, and hemopoietic cells. This milieu of cells produces a variety of biological regulators that control local bone metabolism. Systemic calcitropic hormones [parathyroid hormone (PTH), estrogen, and $1,25(OH)_2vitaminD_3$] and autocrine and paracrine factors, including prostaglandins, cytokines, and growth factors orchestrate the cellular activities of bone modeling to increase the length, diameter, and shape of long bones in animals. Bone growth includes the activities of bone matrix formation, matrix mineralisation, and bone resorption. Bone matrix is produced and mineralised through the activity of osteoblasts while bone matrix resorption is accomplished by specialized multinucleated cells called osteoclasts. The combined and cooperative activities of osteoblasts and osteoclasts result in a bone architecture that provides mechanical support and maintains normal serum concentrations of calcium and phosphorus. The effects of feed fats on altering the amount of local factors produced in bone will be the focus of this paper.

Lipid Chemistry and Molecular Biology Laboratory, Department of Food Science, Purdue University, West Lafayette, IN, 47907-1160, USA.

II. BONE GROWTH

Bone growth and modeling are regulated by complex interactions between an individual's genetic potential, environmental influences, and nutrition. These interactions produce a bone architecture that balances functionally appropriate morphology with the skeleton's role in calcium and phosphorus homeostasis. Long bones of poultry increase in length and diameter by a process called modeling. Bone modeling represents an adaptive process of generalized and continuous growth and reshaping of bone governed by the activities of osteoblasts and osteoclasts until adult bone structure is attained in poultry. This growth requires that bone cells function normally. Bone modeling is distinct from bone remodeling which describes the local, coupled process of bone resorption and formation that maintains skeletal mass and morphology in the mature hen.

Bone is a dynamic connective tissue consisting of living cells embedded within or lining surfaces of a mineralized organic matrix. Bone provides mechanical support for the body, and through attachment of muscles, allows for locomotive movement through space. Furthermore, skeletal tissue protects vital organs and serves as a metabolic reservoir of calcium and phosphate for the body. Anatomically, the bones of the skeleton can be classified according to their individual shapes: flat (bones forming the roof of the skull, scapula, and ilium), short (tarsal bones), irregular (vertebrae), and long (humerus, radius, ulna, femur, and tibia).

All bone is derived from mesenchymal tissue, however, two different histogenetic processes exist for producing bone: one direct and another indirect through a temporary cartilage model. Intramembranous ossification occurs within presumptive flat bones by direct differentiation of mesenchymal cells into osteogenic cells. Osteoblasts deposit organic matrix within their embryonic connective tissue membrane which becomes mineralized. Long bones are formed by endochondral ossification, a process where embryonic mesenchymal cells differentiate into chondroblasts which secrete hyaline cartilage matrix and produce a cartilage model of the future bone. Diaphyseal and, later, epiphyseal centers of ossification develop following local cartilage mineralisation and invasion by the vasculature. Cartilage matrix is removed and replaced with bone by newly arrived osteogenic cells. The location of a plate of cartilage interposed between epiphyseal and metaphyseal regions of a bone provides the means for bones to grow in length. In this process, chondrocyte proliferation, matrix production, mineralization, and vascular invasion is balanced with removal of mineralized trabeculae from the metaphyseal side of the growth plate through osteoclastic activity. The diameters of bones increase via intramembranous ossification through apposition of bone matrix by osteoblasts located within the periosteum. Cortical bone serves primarily mechanical and protective functions.

III. REGULATION OF BONE METABOLISM

Bone formation and bone resorption are regulated by systemic hormones and factors produced locally primarily by osteoblasts (Watkins, 1992). Systemic hormones involved in stimulating bone formation include, insulin, growth hormone, and estrogen; while those involved in stimulating bone resorption include, $1,25\text{-}(OH)_2\text{vitaminD}_3$, PTH, and thyroid hormone. In addition, calcitonin and glucocorticoids inhibit bone resorption.

IGF-1, also called somatomedins, are described as paracrine or autocrine regulatory polypeptides of cells. These compounds stimulate growth and synthesis of DNA, RNA, and proteins in cells. IGF are mitogenic and stimulate differentiation in a variety of cell types. Pituitary growth hormone (GH) controls tissue biosynthesis and secretion of IGF-1 (or somatomedin C) postnatally and it is through IGF-1 that the tissue effects of GH are mediated.

Serum concentration of IGF-I is maintained by liver synthesis under the influence of GH. Much of the circulating IGF is bound to plasma IGF binding proteins (IGFBP). The amount of IGF-1 and IGF-2 produced by bone cells is species dependent. In the human, neonatal mouse, and chicken more IGF-2 than IGF-1 is produced in the skeletal tissues (Baustista et al., 1991). While IGF-2 is generally more abundant than IGF-1, IGF-1 appears to be under greater regulatory control in bone (Canalis et al., 1991). For example, prostaglandin E_2 (0.01-1 μM) elevated IGF-1 mRNA and polypeptide levels by 1.9- to 4.7-fold; however, prostaglandin E_2 did not increase IGF-2 mRNA or polypeptide levels in bone organ cultures (McCarthy et al., 1991).

In addition to the cytokines and growth factors which act as local modifiers of bone metabolism, certain eicosanoids [e.g., prostaglandins (PG), leukotrienes (LT)] also exert stimulatory effects on bone formation and resorption (Table 1). In 1970, Klein and Raisz (1970) reported that PGE_1, PGE_2, PGA_1, and $PGF_{1\alpha}$ increased the release of ^{45}Ca into the media from cultured fetal rat bone. Since then, numerous studies have demonstrated that PGEs stimulate bone formation as well as bone resorption (Raisz, 1993; Marks and Miller, 1993). Raisz (1993) reported that infusion of PGE_2 at a high concentration depressed osteogenesis in fetal rat calvariae. PGE_2 stimulates bone formation at low concentrations but it may be inhibitory at high concentrations.

Similar to the PG, the LT also play an important role in bone metabolism. Ren and Dziak (1991) demonstrated that LTB_4 inhibited cell proliferation in cultured osteoblasts isolated from rat calvaria in a dose-dependent manner, but LTB_4 may interact with PG to regulate osteoblast activity. Other reports indicate that LTC_4, LTD_4, and 5-HETE stimulated isolated avian osteoclasts to resorb bone.

IV. DIETARY LIPIDS MODIFY THE FATTY ACID COMPOSITION OF BONE

Although the importance of lipids in cartilage mineralisation and bone biology has been well documented, research describing the relationships between dietary lipids and chondrocyte function and prostanoid effects on bone formation has, until recently, received little attention (Watkins and Seifert, 1996). Analysis of epiphyseal cartilage in animals revealed a low concentration of (n-6) fatty acids and 3-4% Mead acid [20:3(n-9)] (Adkisson et al., 1991). [Mead acid accumulates in animal tissues during a deficiency of the essential fatty acid linoleic acid.] Mead acid was not reduced in cartilage of chicks given a rich dietary source of 18:2(n-6); however, consumption of (n-3) fatty acids [20:5(n-3) and 22:6(n-3)] elevated their concentration in cartilage (Xu et al., 1994). These findings indicate that epiphyseal cartilage, which is responsible for longitudinal bone growth, may selectively incorporate certain dietary fatty acids.

Table 1.   Reported responses of autocrine and paracrine factors in bone[1]

Responses observed in bone	Cytokine, eicosanoid, or peptide growth factor[2]
Bone formation or matrix production	FGF, IGF, PGE, TGF-ß
Bone resorption	EGF, IL, LT, PDGF, TGF-α, TNF-α
Collagen synthesis	FGF, IGF, TGF-ß

[1]Adapted from Watkins (2). [2]Epidermal growth factor = EGF; Fibroblast growth factor = FGF; Interleukin = IL; Insulin-like growth factor = IGF; Leukotriene = LT; Platelet-derived growth factor=PDGF; Prostaglandin E = PGE_2; Transforming growth factor = TGF-α, TGF-ß; Tumor necrosis factor = TNF-α.

Recent investigations suggest that vitamin E benefits bone growth and cartilage activity. Chicks given supplemental vitamin E demonstrated higher bone formation rates (Xu et al., 1995). In cartilage, the ability to protect against lipid peroxidation may be restricted to nonmineralized regions of cartilage. Research indicated that the mineralized area of growth plate cartilage has limited enzymatic capacity for handling oxidized lipid species because superoxide dismutase and catalase activities are low in this region. The enrichment of chicken epiphyseal chondrocytes with 18:2(n-6) resulted in cellular injury [elevated lactate dehydrogenase (LDH) activity] and depressed collagen synthesis when compared to cells supplemented with oleic acid and no fatty acids (Watkins et al., 1996). Consistent with the effect of linoleic acid on chondrocyte injury (elevated LDH activity in culture media), enrichment with 18:2(n-6) lowered chondrocyte collagen synthesis; however, vitamin E restored collagen synthesis in these cells. Accompanied with the reduction in collagen synthesis in primary cultures of epiphyseal chondrocytes is an elevated production of PGE_2 (Watkins and Chen, 1997). The decrease in collagen synthesis observed with (n-6) fatty acid enrichment appears to be related to membrane damage and impaired cell function, for which vitamin E is protective. Since the onset of peroxidative reactions within biological membranes can impair cell behavior and function, vitamin E and antioxidant systems designed to protect chondrocytes may be minimal in epiphyseal cartilage. Collectively, these data suggest that diets marginal in vitamin E or which lead to oxidative stress (linoleic acid) and tissue depletion of vitamin E, impair normal bone and cartilage function.

Studies with chicks demonstrated that dietary lipids modify the fatty acid composition of cortical and trabecular bone. Chicks given diets containing trans-18:1 or (n-3) fatty acids had increased concentrations of these fatty acids in bone. The conjugated linoleic acid (CLA) isomers in anhydrous butter oil were also found in bone tissues of animals given CLA (Li and Watkins, 1998). Watkins et al. (1996, 1997) reported that chicks given a blend of menhaden oil + safflower oil in a semi-purified diet had a lower concentration of 20:4(n-6) but higher concentrations of 20:5(n-3) and 22:6(n-3) in cortical bone polar lipids compared to those given soybean oil. As the concentration of 20:4(n-6) decreased in tibial bone of chicks given 20- and 22-carbon (n-3) fatty acids so did the ex vivo PGE_2 production in bone organ culture (Watkins et al., 1996, 1997). Since diets that moderate ex vivo PGE_2 production in bone organ culture were associated with higher rates of bone formation in vivo, it is presumed that dietary lipids [(n-3) fatty acids and CLA] impact bone formation and resorption activities by modulating PGE_2 biosynthesis.

V. DIETARY FATTY ACIDS ALTER BONE FORMATION

Determining the consequence of altering the fatty acid composition of cartilage and bone with dietary lipids has been one aim of our research. Our investigations indicate that dietary lipids influence bone formation and chondrocyte cell function. For example, kinetic analyses of bone modeling revealed that total fractional labeled trabecular surfaces and bone formation rate (BFR) were significantly greater in chicks given menhaden oil + safflower oil compared to those given soybean oil (Watkins et al., 1996). A rather intriguing observation was that the increased BFR in chicks given 20- and 22-carbon (n-3) fatty acids was associated with a 3.5-fold decrease in ex vivo PGE_2 production in tibia. Under this dietary condition 20:5(n-3) predominates over arachidonic acid as an eicosanoid precursor since its concentration was 10-fold higher, while arachidonic acid concentration was about 50% lower in tibia. Thus, principle metabolites from 20:5(n-3) might include PGE_3 or leukotrienes (LTB_5, LTC_5, LTD_5, LTE_5). Providing aspirin in the diet of these animals abolished ex vivo PGE_2 (20 to 36-fold decrease) production in bone but BFR was sustained. The effects of dietary fatty acids and related factors

on the osteoblast and osteoclast are summarized in Figure 1.
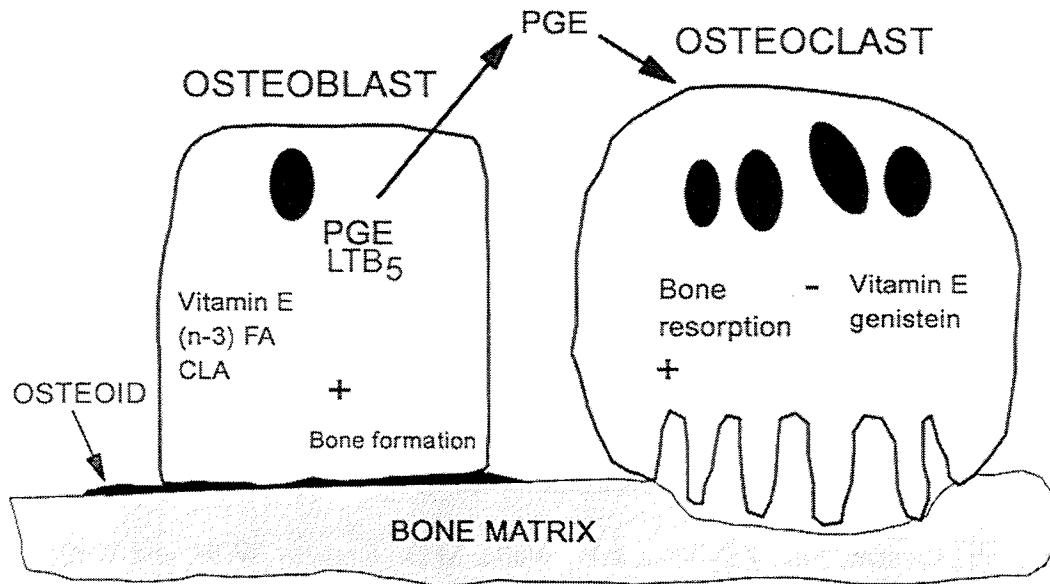
Fig. 1. Observed and potential effects of dietary fatty acids and related compounds on osteoblastic and osteoclastic activity in bone. Excessive biosynthesis of PGE_2 may depress bone formation and lead to increased bone resorption. Altering the production of eicosanoids (PGE and LTB) appears to optimize bone formation by osteoblasts and moderate bone resorption by osteoclasts. Vitamin E may benefit bone formation and reduce excessive bone resorption by decreasing free radicals.

PGE_2 exhibits biphasic effects on bone formation, stimulating bone formation at a low concentration but inhibiting it at higher concentrations, and excess production of PGE_2 is perhaps associated with bone pathology. The higher amount of bone PGE_2 in chicks given soybean oil could have stimulated an increase in bone resorptive activity that reduced bone volume and trabecular number. It appears that dietary 20- and 22-carbon (n-3) fatty acids aid in moderating PGE_2 production in bone to optimize bone formation and perhaps prevent excessive bone resorption.

VI. CONCLUSIONS

Recent data demonstrating dietary lipid effects on bone metabolism indicate that dietary 20- and 22-carbon (n-3) fatty acids reduce skeletal production of PGE_2 to enhance bone formation and optimize bone modeling. Improved bone modeling may reduce the incidence of abnormal bone modeling in rapidly growing chickens. Furthermore, antioxidant nutrients may enhance bone formation and reduce the production of free radicals which contribute to bone resorption (Watkins *et al.* 1997b). Because fat constitutes a significant amount of the energy in poultry rations, the type of fat consumed can significantly influence the metabolic and physiological processes controlling bone modeling in animals. Likewise, the levels of antioxidant nutrients and flavonoids may contribute to better bone growth by reducing the formation of free radicals and lipid peroxides.

The production of PGE$_2$ in bone was significantly decreased in chicks consuming menhaden oil compared to the amount in those given soybean oil. Decreasing bone resorption and stimulating bone formation with dietary (n-3) fatty acids may afford a means to maximize bone mineral accretion in growing animals and minimize mineral mass loss in the laying hen. Future research on fats and antioxidant compounds in bone biology will benefit poultry and other livestock. This work can now be directed at nutrient gene regulation of bone formation because of the recent discovery of the Cbfa1 "master gene" which influences osteoblastic activity. Research on the Cbfa1 gene might provide some insight on how (n-3) fatty acids increase bone formation in the chicken.

ACKNOWLEDGMENTS

REFERENCES

Adkisson, H.D., Risener, F.S., Zarrinkar, P.P., Walla, M.D., Christie, W.W. and Wuthier, R.E. (1991). *The FASEB Journal,* **5**: 344-353.

Bautista, C., Baylink, D.J. and Mohan, S. (1991). *Biochemistry Biophysics Research Communications,* **176**: 756-763.

Canalis, E., Centrella, M. and McCarthy, T.L. (1991). *Endocrinology,* **129**: 2457-2462.

Ducy, P., Zhang, R., Geoffroy, V., Ridall, A.L. and Karsenty, G. (1997). *Cell,* **89**: 747-754.

Klein, D.C. and Raisz, L.G. (1970). *Endocrinology,* **86**: 1436-1440.

Li, Y. and Watkins, B.A. (1998). *Lipids,* **33**: 417-425.

Marks, S.C. and Miller, S.C. (1993). *Endocrine Journal,* **1**: 337-344.

McCarthy, T.L., Centrella, M., Raisz, L.G. and Canalis, E. (1991). *Endocrinology,* **128**: 2895-2900.

Raisz, L.G. (1993). *Journal of Bone and Mineral Research,* **8**: S457-S465.

Ren, W. and Dziak, R. (1991). *Calcified Tissue International,* **49**: 197-201.

Watkins, B.A. (1992). Factors involved in the local regulation of bone growth, In: *Bone biology and skeletal disorders in poultry,* pp. 67-86. Whitehead, C.C. (ed), Carfax Publishing Co., Abingdon, England.

Watkins, B.A. and Seifert, M.F. (1996). Food Lipids and Bone Health, In: *Food Lipids and Health,* pp. 71-116. McDonald, R.E. and Min, B.D. (eds), Marcel Dekker Inc., NY, NY.

Watkins, B.A., Xu, H. and Turek, J.J. (1996). *Proceedings of the Society for Experimental Biology and Medicine,* **212**: 153-159.

Watkins, B.A., Shen, C-L., Allen, K.G.D. and Seifert, M.F. (1996). *Journal of Bone and Mineral Research,* **11**: 1321-1332.

Watkins, B.A., Shen C-L., McMurtry, J.P., Xu, H., Bain, S.D., Allen, K.G.D. and Seifert, M.F. (1997). *The Journal of Nutrition,* **127**: 1084-1091.

Watkins, B.A. and Chen, Y. (1997). *Journal of Bone and Mineral Research* **12**: S297.

Watkins, B.A., Seifert, M.F. and Allen, K.G.D. (1997). *World Review of Nutrition and Dietetics,* **82**: 250-259.

Xu, H., Watkins, B.A. and Adkisson, H.D. (1994). *Lipids,* **29**: 619-625.

Xu, H., Watkins, B.A. and Seifert, M.F. (1995). *Calcified Tissue International,* **57**: 293-300.

THE CHALLENGE OF INTESTINAL IMMUNITY AND VACCINATION

WENDY I. MUIR

Summary

Current knowledge of the mechanisms of the avian intestinal immune system is reviewed. With improved understanding of these mechanisms, re-evaluation of vaccination techniques for stimulation of the mucosal immune system is likely to be fruitful. Advances in the design and delivery of mucosal vaccines in mammals justify their evaluation in chickens. Additionally, the regulatory influence of T cells, and their cytokine profiles, on the production of IgA antibody in mammalian species is intently debated. Advances in this area of avian immunology are hindered by the limited number of sequenced and cloned avian cytokines. It is likely that cytokine manipulation will facilitate significant advances in mucosal vaccines, particularly if coupled with the delivery of antigen in appropriate adjuvant/vehicle formulations.

I. INTRODUCTION

The immune system responds to the constant challenge of environmentally derived antigens through the combined actions of innate (non-specific) and acquired (specific) immunity. Antigen-specific activation of the intestinal immune system is important for protection from pathogens present in the gastrointestinal tract (GIT). Optimisation of the intestinal immune response requires delineation of the molecular events following antigen challenge. This information is essential for the design and delivery of vaccines. The majority of new strategies for intestinal vaccination have been developed in mammals, and the challenge and opportunity of applying them to birds is discussed.

II. MUCOSAL IMMUNITY: AN OVERVIEW

The intestine is one of the many mucosal surfaces in the body that respond to antigens in a similar manner. The immune response to an antigen located at the mucosal surface involves stimulation of lymphocytes and the local secretion of immunoglobulin A (IgA). Antigen present at mucosal sites is typically taken into the mucosa-associated lymphoid tissue across the epithelial surface. In the intestine of mammalian species Peyer's patches (PP) are the follicular inductive sites and the epithelium overlying PP is enriched with modified epithelial cells, microfold cells (M cells), which sample lumenal antigen, transporting them from the intestinal lumen to the dendritic and lymphoid cells below. The PP consist of a B cell area, characterised by germinal centres in lymphoid follicles, and an interfollicular T cell zone, comprised of diffuse lymphoid tissue and high endothelial venules.

Following antigen presentation, B and T cell lymphoblasts leave the PP via the efferent lymphatics, passing through the mesenteric lymph nodes entering the systemic circulation via the thoracic duct, as shown on Figure 1. Circulating B cells are retained at distant mucosal effector sites through contact with their specific homing receptors in high endothelial venules. Here they undergo clonal expansion and mature into IgA secreting plasma cells.

Department of Veterinary Anatomy and Pathology, Faculty of Veterinary Science, University of Sydney, Sydney, NSW, 2006, Australia.

Intestinal lumen

Affector site

Effector site

B and T blasts

M cell

antigen

plasma cell

T cell

IgA antibody

PP   Peyer's patch

GC   Germinal centre

lymph node

thoracic duct
lymph

blood circulation

PP

liver

bile ducts

to other mucosal sites e.g. lungs, urogenital tract.
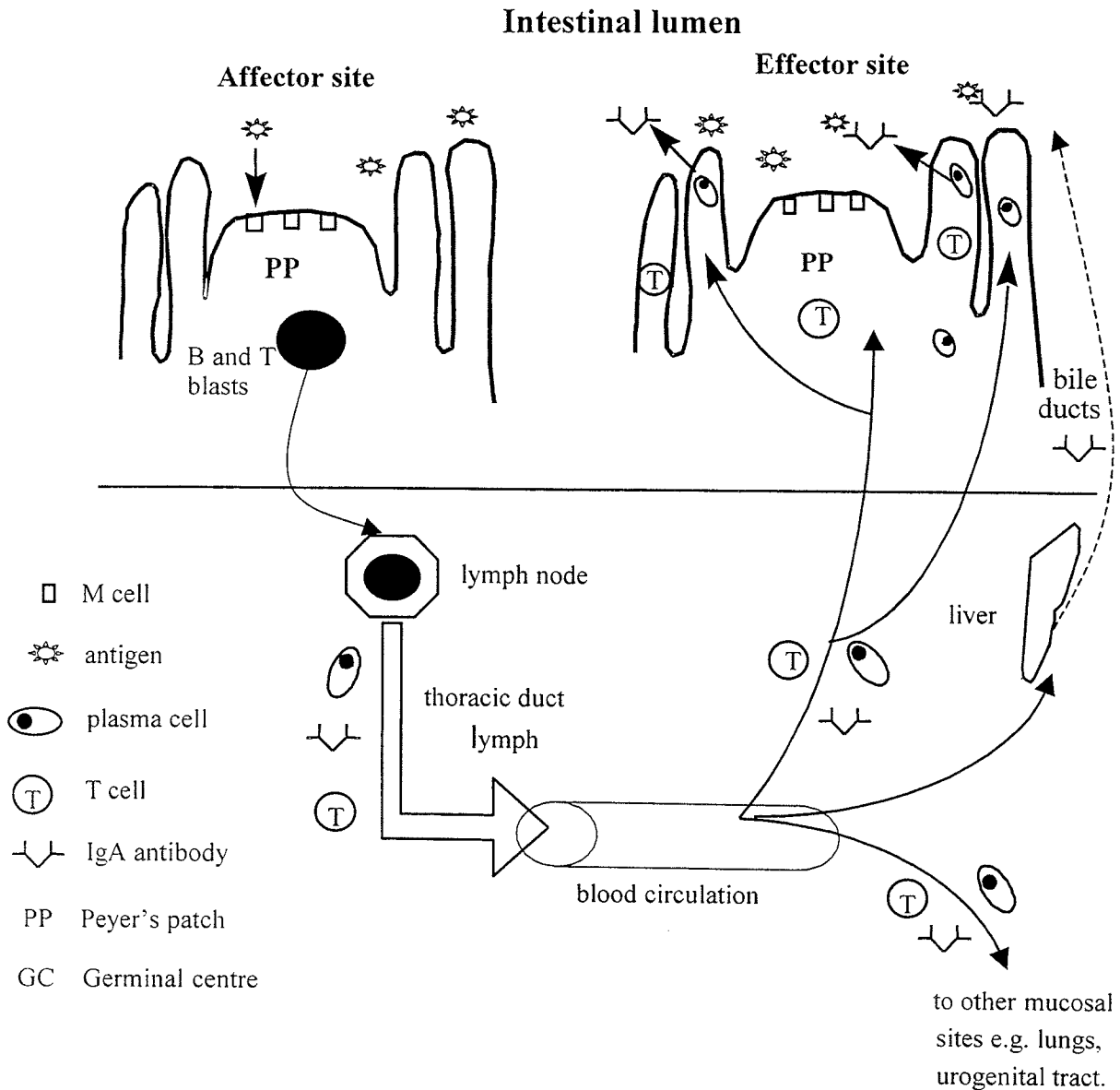
Figure 1.   The migration of lymphocytes following presentation of antigen to the gut associated lymphoid tissue.

Regulation of the mucosal immune response is complex. T cell help is required for the induction of a humoral immune response and it involves direct cell-cell contact and signals from soluble T cell derived cytokines. Mosmann and Coffman (1989) identified two distinct patterns of cytokine expression by T helper (Th) cells in mice: Th1 cells, promoting cell mediated immunity through the production of interleukin (IL)-2, interferon (IFN) and tumour necrosis factor (TNF) and, Th2 cells which promote antibody responses, and typically produce IL-4, IL-5, IL-6 and IL-10. While this polarisation of responses is not rigid, cytokine profiles at the mucosal surface do favour Th2 responses and IgA production. For the initiation of an IgA B cell response, signals from T cell membrane contact and secretions of IL-4 and transforming growth

factor (TGF)-β are crucial for promoting isotype switching to IgA. Subsequently, T cell contact is minimal, with terminal differentiation and IgA antibody secretion being largely dependent on IL-5, IL-6 and IL-10 secretion.

The circulation or trafficking of lymphocytes in chickens has received limited research attention; however, it is assumed that the response to an antigen presented to the gut associated lymphoid tissues (GALT) of chickens follows a similar pattern to that of mammals (Figure 1). Muir (1998) has reviewed the avian GALT.

III. MUCOSAL IMMUNITY AND VACCINATION

As the initial encounter with infectious agents or food antigens frequently occurs at mucosal surfaces, it is expected that the induction of local immune responses at these sites would be most effective for local protection. Vaccines directed at enhancing the intestinal immune response are often administered orally. However, when using inactivated vaccines large, multiple doses of antigen are required to induce an IgA antibody response which is typically quite variable and of short duration (Newby, 1984). This is partly the outcome of antigen degradation, denaturation and its elimination from the GIT, permitting only small quantities of fully active antigens to be presented to the GALT. Further, presentation of antigen across the intestinal epithelial cells activates suppressive T cells which dampen an antigen-specific response (Bland and Warren, 1986).

The difficulties of oral vaccination with non-replicating antigens has necessitated the search for vaccine technology capable of optimising mucosal immune responses. The main facets of vaccinology open to manipulation are the antigen delivery system, the adjuvant and the route of immunisation.

(a) Mucosal antigen delivery systems

(i) Biodegradable microspheres contain antigen dispersed throughout a sustain release polymer which protects the antigen from the environment, controlling its rate of release and aiding in its uptake by PP (Eldridge *et al.*, 1990). Antigen is released either via dispersion through the matrix pores or by degradation of the matrix. A microparticle system using the copolymers of lactic and glycolic acids to form biocompatable and biodegradable poly(DL-lactide-co-glycolide) (DL-PLG) microspheres is suitable for both systemic (Eldridge *et al.*, 1991) and mucosal (Allaoui-Attarki *et al.*, 1997; Muir *et al.*,1994) vaccination, and is able to codeliver antigen and cytokine (IL-5 and IL-6) (Rafferty *et al.*, 1996).

Despite the promising results obtained following oral immunisations using microspheres in mammals, there are very few reports of their use in chickens. Widders *et al.* (1996) delivered *Campylobacter jejuni* flagellin in microspheres, inducing a minimal increase in gut IgG. Unfortunately, gut IgA antibody titres were not determined. Multiple oral delivery of DL-PLG microspheres to chickens demonstrated no benefits compared to oral delivery of the unencapsulated antigen (Muir, 1996). However, *in-ovo* delivery of DL-PLG microspheres did invoke higher antigen-specific serum IgG and serum, biliary and intestinal IgA titres compared to native antigen (Noor, 1995).

(ii) Liposomes consist of concentric lipid bilayers encompassing an aqueous compartment, which facilitate direct contact between antigen, lymphoid tissues and cells. The success of liposomes for oral delivery of antigen is equivocal (Michalek *et al.*, 1994; Walker, 1994). Liposomes must be delivered orally to efficiently induce a mucosal immune response, but their formulation and surface charge may affect their stability and immunogenicity. Significant antigen interaction with

the immunological properties of surface charged liposomes requires that each antigen/liposome formulation be considered separately (Gregoriadis and Panagiotidi, 1989).

Published investigations of the use of liposomes in chickens are scarce. Jing-sheng and Yi-zhu (1993) compared the antibody response of liposomes to an oil emulsion containing inactivated EDS-76 virus. The liposomally induced immune response followed a similar pattern to that of the oil emulsion preparation. Fatunmbi *et al.* (1992) delivered avian influenza antigen in avridine containing liposomes, concluding that the positively charged liposomes generated substantial antibody titres.

(iii) Immune-stimulating complexes (ISCOMs) are negatively charged cage-like structures that are formed naturally when mixing cholesterol and the saponin Quil A, which can also act as an adjuvant. ISCOMs deliver intact immunogens proteins to the mucosal immune system; however a larger dose of ISCOM associated antigen is required for oral than parenteral priming. Used parenterally, ISCOMs incite humoral and cell-mediated immunity.

Rehmani and Spadbrow (1995) did not identify any benefits following the oral delivery of a live strain of Newcastle disease virus in ISCOMs to chickens. However, Sundquist *et al.* (1996) found a positive dose-dependent antibody response in chickens which received intramuscular immunisations of *Mycoplasma gallisepticum* ISCOMs.

The immunomodulatory capacity of Quil A in chickens remains unclear. Reid and Blackall (1987) and Rehmani and Spradbrow (1995) reported no improvement in antibody responses when incorporating Quil A with *Haemophilus paragallinarum* or Newcastle disease virus. However, when Quil A was included in a vegetable oil based adjuvant with antigen, which was delivered intraperitoneally (IP), Muir *et al.* (1995) found notable increases in the number of jejunal antigen-specific IgA secreting plasma cells, and antigen-specific IgA antibody titres in serum and intestinal scrapings.

Immunisation with non-replicating plasmid constructs, Naked DNA, containing antigen-encoding DNA enables the foreign gene product to be expressed in the immunised animal for periods up to 60 days (Wolff *et al.*, 1990). Fynan *et al.* (1993) used purified plasmid DNA expressing an influenza A virus hemagglutinin glycoprotein for protection from a subsequent challenge. In both murine and avian models DNA inoculation via intramuscular, intravenous and mucosal routes provided partial protection from the challenge. Interestingly, epidermal injection of beads coated in the plasmid (representing only 4 μg DNA) yielded a 95% survival rate in challenged mice. Using a similar vector system Robinson *et al.* (1993) reported 50% survival in DNA-immunised chickens following challenge compared to less than 2% survival with the control birds.

(b) Mucosal adjuvants

Adjuvants enhance positive amplification and dampen suppressive signals of the immune system, thereby altering the kinetics, quantity and class of immunoglobulin produced.

Simultaneous mucosal administration of cholera toxin (CT) and certain antigens induces systemic IgG and mucosal IgA. CT improves penetration of the intestinal epithelium by the antigen and enhances antigen-specific IgA responses via activation of CD4+ Th-2 cells (Yamamoto *et al.*, 1997).

Oral immunisation of CT with either tetanus toxoid (Meinersmann and Porter, 1993) or inactivated infectious bursal disease virus (Hoshi *et al.*, 1995) in chickens did not stimulate mucosal immunoglobulin production. In the former, CT suppressed the antigen-specific response. However, Takada and Kida (1996) found that intranasal administration of inactivated

Newcastle disease virus with CT-B subunit enhanced IgA and IgM antibody titres in nasal washings, with significant reductions in the recovery of challenge virus.

Lipopolysaccharide (LPS), from the cell wall of *E. coli*, acts as an adjuvant when given by a different route at a different site to the antigen. Monophosphoryl lipid A is a modified, nontoxic form of LPS which specifically deactivates CD8+ suppressor cell function (Baker *et al.*, 1988) and upregulates CD4+ Th cells (Schneerson *et al.*, 1991). The mechanisms of mucosal adjuvanticity of Lipid A derivatives are complex, being an intricate balancing act between stimulating oral tolerance and immunity.

There is a paucity of reports on the application of LPS as mucosal adjuvants in chickens.

(c) The route of administration of antigen to the mucosa

(i) Parenteral immunisation. The poor immunogenicity of native non-replicating antigens presented to the intestinal mucosal surface can be reversed when appropriate parenteral priming is followed with a local oral booster. Following IP immunisation, antigen is presented to the PP across the serosal surface. Intraperitoneal immunisation induces substantial mucosal immunity in sheep and pigs, with antigen emulsified in either Freund's complete adjuvant, or a vegetable oil based Auspharm adjuvant (patent pending, Husband, 1993).

Intraperitoneal immunisation of chickens with antigen in either Auspharm adjuvant (Muir *et al.*, 1995) or Montanide ISA 50 (Widders *et al.*, 1996) induced significant mucosal IgA antibody titres. Interestingly, only in the former report did an oral booster immunisation further enhance intestinal IgA antibody titres. Following IP immunisation with *Campylobacter jeuni* antigens in Montanide ISA 50 (Widders *et al.*, 1996), no significant increase in serum or intestinal specific IgA was detected. However, Muir *et al.* (1998) have shown IP immunisation with whole killed *Salmonella typhimurium* in Auspharm adjuvant reduced organ infection following challenge with homologous bacteria.

(ii) In-ovo delivery. Embryonic exposure to antigen increases the interval between vaccination and exposure to environmental antigens, facilitating precocious priming of the immune system. *In-ovo* immunisations for Marek's disease virus and Newcastle disease virus (Johnston *et al.*, 1997; Sarma *et al.*, 1995; Sharma and Ahmad, 1994) have demonstrated improved protection from a challenge, compared to conventionally immunised chickens. *In-ovo* delivery of antigen into the amniotic fluid also stimulates precocious development of mucosal immune function in a non-specific manner (Noor *et al.*, 1995), invoking significant increases in total IgA antibody in serum, bile and intestinal scrapings. Delivery of vitamin E into the embryonic amnion stimulated humoral immunity (total antibody, IgG and IgM titres), and cellular immunity (macrophage phagocytic activity) in a non-specific manner (Gore and Qureshi, 1997).

IV. CHARACTERISATION OF THE DOMINANT IMMUNE RESPONSE

While attempting to optimise a desired mucosal IgA response, consideration of the prevalent T cell response is also essential. This is broadly delineated by the predominant CD4+ Th cell subset and its cytokine profile. However, following *in-vivo* studies and the use of cytokine gene knockout mice, there is ongoing debate of the requirements for particular cytokines for the induction of mucosal IgA antibody responses.

It is widely speculated that IL-4 is required for differentiation of the B cell to an IgA secreting plasma cell. Husband *et al.* (1994) found IL-4 knockout mice almost entirely devoid of IgA plasma cells in mucosal tissues and with no detectable IgA response following peroral

immunisation of antigen and CT (Vajdy *et al.*, 1995). However, Okahashi *et al.* (1996) maintain that IL-4 knockout mice have normal mucosal IgA levels.

Similar discrepancies exist with respect to the exact role of IL-6 in the terminal maturation of B cells into plasma cells. Ramsay *et al.* (1994) found very few IgA plasma cells in the intestine of IL-6 deficient mice. Following an intestinal challenge, no local antigen-specific IgA response was induced. To the contrary, Bromander *et al.* (1996) found normal total serum IgA levels and IgA secreting plasma cells at the intestinal site, and no differences in antigen-specific responses following immunisation of IL-6 knockout mice with several antigens.

Further, the innate Th cell profiles can be manipulated by delivering exogenous cytokines. In 1993, Ramsay and Kohonen-Corish showed that the coexpression of murine IL-5 and antigen in a recombinant vaccinia virus vector system notably upregulated the antigen-specific IgA response in the lungs after intranasal inoculation. Ramsay *et al.* (1994) and Baqar *et al.* (1993) found delivery of recombinant IL-6 upregulated IgA responses, and in the latter this correlated with improved protection from *Campylobacter jejuni*. In this way the appropriate microenvironment which is known to favour a specific type of immune response can be presented at either the afferent or efferent sites of the immune system by the supply of an exogenous source of cytokine using recombinant vector systems. Cytokine therapy can be utilised to either restore defective immune responses and/or to enhance standard responses.

Immune responses in chickens do follow the Th1 and Th2 paradigm (Kasier, 1996), but the absence of cloned cytokines and their antibodies make identification of the most pertinent cytokines for regulation of a specific intestinal immune response difficult. However, delivery of lymphokine containing conditioned media generated from T lymphocytes of *Salmonella enteritidis* immune chickens to day old chicks induced rapid infiltration of polymorphonuclear cells, in particular heterophils, with improved adherence, chemotaxis and phagocytosis (Kogut *et al.*, 1995) into the intestinal lamina propria. This correlated with improved protection from *S. enteritidis* and facilitated cross protection between serotypes (Kogut *et al.*, 1996). Protection of neonates has been observed following deposition of the lymphokine preparation into the amnion *in-ovo* (McGruder *et al.*, 1995). These results indicate the potential for cytokine gene therapy to manipulate avian mucosal immune responses and increased resistance to pathogens; however, it is only with further characterisation and cloning of avian cytokines that these techniques can be used to benefit the poultry industry.

V. CONCLUSIONS

Ongoing research will enable further characterisation of the avian intestinal immune system. Concurrent consideration of the regulatory mechanisms of the intestinal immune response, vaccine delivery and mucosal adjuvants is required to improve local immune responses and protection from pathogens. Mucosal adjuvants and technologies for vaccine and cytokine delivery have enabled enhanced mucosal immune responses in mammals; however, the application of these in the chickens remains unclear. It is only through avian-specific investigations that these technologies will be optimised for use in the poultry industry.

VI. ACKNOWLEDGEMENTS

REFERENCES

Allaoui-Attarki, K., Pecquet, S., Fattal, E., Trollé, S., Chachaty, E., Couvreur, P. and Andremont, A. (1997). *Infection and Immunity*, **65**: 853-857.

Baker, P., Hiernaux, J., Fauntleroy, M., Prescott, B., Cantrell, J. and Rudbach, J. (1988). *Infection and Immunity*, **56**: 1076-1083.

Baqar, S., Pacheco, N. D. and Rollwagen, F. M. (1993*). Antimicrobial Agents and Chemotherapy* **37**: 2688-2692.

Bland, P. W. and Warren, L. G. (1986). *Immunology*, **58**: 9-14.

Bromander, A. K., Ekman, L., Kopf, M., Nedrud, J. G. and Lycke, N. Y. (1996). *Journal of Immunology,* **156**: 4290-4297.

Eldridge, J. H., Hammond, C. J., Meulbroek, J. A., Staas, J. K., Gilley, R. M. and Tice, T. R.(1990). *Journal of Controlled Release*, **11**: 205-214.

Eldridge, J. H., Staas, J. K., Meulbroek, J. A., Tice, T. R. and Gilley, R. M. (1991). *Infection and Immunity,* **59**: 2978-2986.

Fatunmbi, O. O., Newman, J. A., Sivanandan, V. and Halvorson, D. A. (1992). *Vaccine*, **10**: 623-626-626.

Fynan, E. F., Webster, R. G., Fuller, D. H., Haynes, J. R., Santoro, J. C. and Robinson, H. L. (1993). *Proceedings of the National Academy of Science, USA*, **90**: 11478-11482.

Gore, A. B. and Qureshi, M. A. (1997). *Poultry Science*, **76**: 984-991.

Gregoriadis, G. and Panagiotidi, C. (1989). *Immunology Letters*, **20**: 237-240.

Hoshi, S., Nakamura, T., Nunoya, T. and Ueda, S. (1995). *Vaccine*, **13**: 245-252.

Husband, A. J. (1993). *Vaccine*, **11**: 107-112.

Husband, A, J., Bao, S., Muir, W. I., Ramsay, A. J. and Ramshaw, I. A. (1994*). Reproduction and Fertility Development*, **6**: 381-388.

Jing-sheng, L. and Yi-zhu, W. (1993). *Proceedings of the Tenth International Congress of the World Veterinary Poultry Association*, p.188. Ed. J. York. Sydney. Australia,.

Johnston, P., Liu , H., O'Connell, T., Phelps, P., Bland, M., Tyczkowski, J., Kemper, A., Harding, T., Avakian, A., Haddad, E., Whitfill,C., Gildersleeve, R. and Ricks, C.A. (1997). *Poultry Science*, **76**: 165-178.

Kaiser, P. (1996). Poultry Immunology, p. 8-114. Ed. T.F. Davison, T.R. Morris and L.N. Payne. Carfax Publishing Co. Oxford..

Kogut, M. H., McGruder, E. D., Hargis, B. M., Corrier, D. E. and DeLoach, J. R. (1995). *Journal of Leukocyte Biology*, **57**: 56-62.

Kogut, M. H., Tellez, G., McGruder, E. D., Wong, R. A., Isibasi, A., Ortiz, V. N., Hargis, B. M.and DeLoach, J. R. (1996). *Avian Pathology*, **25**: 737-749.

McGruder, E. D., Ramirez, G. A., Kogut, M. H., Moore, R. W. Corrier, D. E., DeLoach, J. R.and Hargis, B.M. (1995). Poultry Science, 74: 18-25.

Meinersmann, R. J. and Porter, R. E. (1993). *Avian Diseases*, **37**: 427-432.

Michalek, S. M., Eldridge, J. H., Curtiss, R. and Rosenthal, K. L. (1994). *Handbook on Mucosal Immunology*, pp 73-390. Academic Press, Inc. San Diego..

Mossman, T. R. and Coffman, R. L. (1989). *Annual Reviews of Immunology*, 7: 145-173.

Muir, W. I. (1996). PhD Thesis, University of Sydney, Sydney, Australia.

Muir, W.I. (1998). *Poultry and Avian Biology Reviews*, (In press).

Muir, W. I., Husband, A. J., Gipps, E. M. and Bradley, M. P. (1994*). Immunology Letters*, **42**: 203-207.

Muir, W. I., Bryden, W. L. and Husband, A. J. (1995). *Avian Pathology*, **24**: 679-692.

Muir, W. I, Bryden, W. L. and Husband, A. J. (1998). *Poultry Science*, (in press).

Newby, T. J. (1984). Local Immune Responses of the Gut, pp 143-198. Ed. T. J. Newby and C. R. Stokes, CRC Press Inc., Boca Raton

Noor, S.M., (1995). MVetSci. Thesis. University of Sydney, Sydney, Australia.

Okahashi, N., Yamamoto, M., Vancott, J. L., Chatfield, S. N., Roberts, M., Bluethmann, H., Hiroi, T., Kiyono, H. and McGhee, J. R. (1996). *Infection and Immunity*, **64**: 1516-1525.

Rafferty, D. E., Elfaki, M. G. and Montgomery, P. C. (1996). *Vaccine*, **14**: 532-538

Ramsay, A. J., Husband, A. J., Ramshaw, I. A., Bao, S., Matthaei, K. I., Koehler, G. and Knopf, M. (1994). *Science*, **264**: 561-563.

Ramsay, A. J. and Kohonen-Corish, M. (1993). *European Journal of Immunology*, **23**: 3141-3145.

Rehmani, S. F. and Spadbrow, P. B. (1995). *Veterinary Microbiology*, **46**: 63-68.

Reid, G. G. and Blackall, P. J.(1987). *Avian Diseases*, **31**: 59-63.

Robinson, H. L., Hunt, L. A. and Webster, R. G. (1993). *Vaccine*, **11**: 957-960.

Sundquist, B. G., Czifra, G. and Stipkovits, L. (1996). *Vaccine*, **14**: 892-897.

Sarma, G., Greer,W., Gildersleeve, R. P., Murray, D. L. and Miles, A. M. (1995). *Avian Diseases*, **39**: 211-217.

Schneerson, R., Fattom, A., Szu, S. C., Bryla, D., Ulrich, J. T., Rudbach, J. A., Schiffman, G. and Robbins, J. B. (1991). *Journal of Immunology*, **147**: 2136-2140.

Sharma, J. M. and Ahmad, J. (1994). Advances in Avian Immunology Research, pp 273-277. Ed. T.F. Davison, N. Bumstead and P. Kaiser Carfax Publishing, Abingdon, England..

Takada, A. and Kida, H. (1996). *Veterinary Microbiology*, **50**: 17-25.

Vajdy, M., Kosco-Vilbois, M. H., Kopf, M., Kohler, G. and Lycke, N. (1995). *Journal of Experimental Medicine*, **181**: 41-53.

Walker, R. I. (1994). *Vaccine*, **12**: 387-400.

Widders, P. R., Perry, R., Muir, W. I., Husband, A. J. and Long, K. A. (1996). *British Poultry Science*, **37**: 765-778.

Wolff, J.A., Malone, R.W., Williams, P., Chong, W., Ascadi, G., Jani, A. and Flegner, P.L (1990). *Science*, **247**: 1465-1468.

Yamamoto, S., Kiyono, H., Yamamoto, M., Imaoka, K., Yamamoto, M., Fujihashi, K., Van Ginkel, F. W., Noda, M., Takeda, Y. and McGhee, J. R. (1997). *Proceedings of the National Academy of Sciences, USA.*, **94**: 5267-5272.

METABOLIC AND ORGAN MASS RESPONSES TO SELECTION IN CHICKENS

I. R. WALLIS[1], M. KONARZEWSKI[2], R. MCDEVITT[1] and A. GAVIN[1]

Summary

How has genetic selection changed broilers? We studied this by comparing metabolic rates (MR) and organ sizes of broilers and layers up to 14 d of age. Although broilers grow six-fold faster than layers in the first days after hatching, the strains have similar resting metabolic rates (RMR). Compared to layers, broilers attain higher PeakMR, have a higher relative mass of leg and pectoral muscles and have slower maturing muscles. There was a positive correlation between RMR and the masses of leg muscles, intestine and liver. For birds weighing less than 100g, strains did not differ in relative mass of heart, liver and small intestine. In birds weighing over 100g, broilers had heavier intestines but not a larger heart or liver, suggesting an imbalance between metabolic demands and morphology. Broilers always had the smallest brains, this is an energetically expensive organ.

I. INTRODUCTION

The rate of development varies ten-fold among avian species of similar mass (Starck and Ricklefs, 1998). Three main factors explain this variation. First, food abundance and, second, gut function may both limit growth rate (GR). Thirdly, GR may depend on the ratio of immature muscles able to grow, to those maturing at the expense of growth (Ricklefs, 1979). Researchers often use broad interspecific comparisons to study links between variation in growth and ecological factors (Starck and Ricklefs 1998). They now realise that manipulation studies will help their understanding of variation in life-history. The existing poultry strains, selected for diverse traits, are a key resource for studying divergent selection within a species.

Despite better husbandry, metabolic diseases afflict fast-growing broilers. The recent onset of these diseases suggests limits to GRs are near. Thus, studying how poultry change with selection may aid our understanding of evolution and help solve a serious problem.

II. MATERIALS AND METHODS

(a) Measurements of metabolic rates and organ sizes

We measured RMR in individual Ross 308 broilers (RF) of both sexes and in males of the Euribrid Hisex layer strain (L). We used birds aged 1-14d to study the period when GRs diverge most between strains (Ricklefs, 1985). All measurements, made with a typical open circuit system fitted with mass flow controllers, began with a 30 min equilibration followed by a 2 h measure of RMR. Depending on the size of bird, we used chambers of 1.1 or 2.3 L, flow rates of 400-900 mL/min and temperatures of 30-34°C (±0.2°C). After measuring RMR, we subjected birds of 1-6 d to cold by reducing the chamber temperature at 0.5°C/min to as low as -7.0°C. We assumed the bird reached PeakMR when O_2 use declined with decreasing temperature. To estimate RMR we took the lowest 4-min value that did not change by more than 0.01% in O_2 concentration. We defined PeakMR as the highest O_2 use averaged over 2 min. Directly after a metabolic trial, the birds were killed, weighed and sexed. We weighed (±0.1g) the yolk residue, empty gut, heart, liver, brain, and the pectoral and leg muscles.

[1]Department of Biochemistry and Nutrition, SAC, Auchincruive, Ayr, Scotland, KA6 5HW.
[2]Institute of Biology, University of Warsaw, Branch in Bialystok, Poland.

(b) Data analysis and statistics

To remove interactions between strains, BM and age we statistically divided the birds into two groups (<100g; >100g) and analysed each group separately. We tested for strain contrasts by ANCOVA, with sex as a main effect and lean body mass (LBM) and age as covariates. For organ masses, we included the dissector as a main effect and subtracted organ mass from LBM before analysis. To study the link between RMR and PeakMR we correlated residuals from stepwise, multiple regression of RMR and PeakMR on age, sex and LBM. The residuals are measures of RMR and PeakMR free of the significant effects of LBM, age and sex. We used similar analyses to examine the relationship between MR and organ masses.

III. RESULTS

(a) Growth rate and metabolic rate (Table 1)

RF chickens grew six-fold faster to 100g (P<0.001) and four-times faster from 100 g (P<0.001) than did L-birds. Despite these differences in GR, RMR did not differ between strains. In contrast, PeakMRs normalised with LBM and age as covariates, are higher in RF-than L-chickens. A positive correlation (r=0.42; P<0.001) between residuals of RMR and PeakMR over both strains show that chickens with a high RMR often have a high PeakMR.

Table 1. Adjusted means (SD) (ie with LBM differences removed) for GR, MR, muscle and organ mass for RF and L birds. Different letters in a mass class indicate significance (P<0.05).

	Body mass less than 100 g		Body mass more than 100 g	
	RF	L	RF	L
Growth rate (g/d)	12.8 (1.12) [a]	2.01 (0.97) [b]	27.9 (2.1) [a]	6.6 (2.0) [b]
RMR (mL O2/h)	115 (4.6) [a]	116 (3.8) [a]	295 (22.9) [a]	272 (20.3) [a]
Peak MR (mL O2/h)	267 (6.9) [a]	220 (6.6) [b]		
Leg muscle mass (g)	6.1 (0.10) [a]	5.1 (0.09) [b]	14.2 (0.83) [a]	11.3 (0.69) [b]
Leg muscle water (g)	4.5 (0.09) [a]	4.1 (0.07) [b]	10.9 (0.26) [a]	9.9(0.25) [b]
Intestine (g)	3.8 (0.12) [a]	3.5 (0.15) [a]	8.6 (0.71) [a]	6.0 (0.23) [b]
Brain mass (g)	1.07 (0.021) [a]	1.16 (0.018) [b]	1.39 (0.018) [a]	1.51 (0.019) [b]

[1] Values with different superscripts are significantly different (P<0.05)

(b) Muscle and organ growth (Table 1)

In both weight classes, RF birds had the heaviest leg muscles (P<0.001), partly as these muscles contained more water than those of the L birds (P<0.001). Pectoral muscles did not differ in mass between strains but those of RF birds always had more water (P<0.01).

After removing the effect of lean body mass, dissecting person, and age, there was no difference in intestinal mass of RF and L chickens weighing less than 100g. In birds weighing over 100g, RF chickens had heavier intestines both absolutely (P<0.001, fig. 1) and relatively (P<0.001, Table 1). The LBM- and age-corrected masses of liver and heart did not differ between strains for either size category. When corrected for LBM, RF chickens in both weight classes had lighter brains than did L chickens (P<0.05, P<0.001).

(c) Correlations between organ mass and metabolic rates (Table 2)

For RF and L birds weighing less than 100g, we found positive correlations between RMR residuals and those for the masses of leg muscles, intestines and liver, but not for the masses of heart and pectoral muscles. These correlations were often unclear within strains.
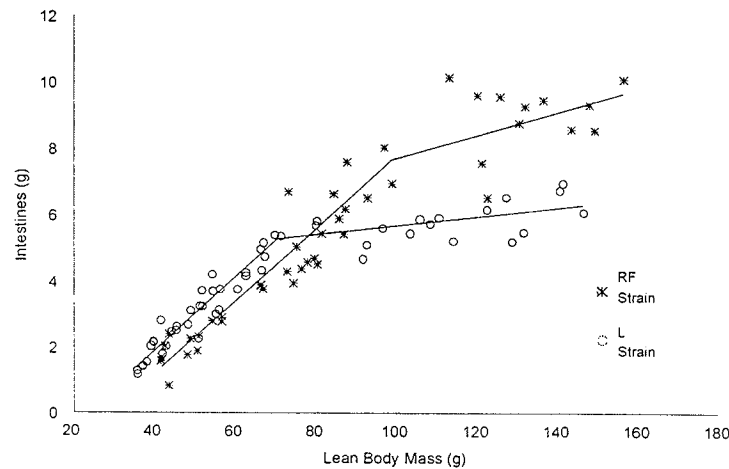
Figure 1.   The relationship between LBM and the mass of intestines in RF and L chickens.

There were significant correlations of residuals for RMR with those for the masses of the leg and pectoral muscles and the liver. Similar correlations do not exist for the heart and intestines. Repeating these analyses for chickens weighing more than 100g showed that only the correlation between RMR and heart mass was significant (r=0.41, P=0.01,both strains; r=0.58, P=0.03, within RF). Similar analyses with PeakMR showed a correlation with leg- (r=0.31, P=0.01, both strains; r=0.34, P=0.06, within RF) and pectoral muscle mass (r=0.39, P=0.002, both strains; r=0.43, P=0.02, within RF), but not with the mass of the heart.

Table 2.   Correlations coefficients and P values (in brackets) between RMR and organ masses using residuals from multiple regression in birds weighing less than 100 g.

	Pooled data	RF strain	L strain
Leg muscle	0.34 (0.004)	0.52 (0.003)	0.26 (0.11)
Pectoral muscle	0.18 (0.14)	0.37 (0.04)	-0.17 (0.30)
Heart	0.26 (0.03)	0.16 (0.42)	0.29 (0.08)
Intestines	0.27 (0.02)	-0.05 (0.74)	0.28 (0.08)
Liver	0.35 (0.004)	0.23 (0.22)	0.47 (0.004)

IV. DISCUSSION

A chick's energy budget can be divided into the energy used for growth and that for respiration (RMR). Theoretically, there are three possible relationships between the increased growth rates of chicks and RMR (Konarzewski, 1995): (1) a negative relationship stemming from the trade-off between the higher demands for growth that can be covered only by reducing RMR; (2) no relationship between growth and RMR suggesting that birds do not need larger metabolic machinery for faster growth, or that they can run larger metabolic machinery without increasing RMR; and (3) a positive relationship resulting from the high respiratory costs of the larger metabolic machinery needed to support high growth rates.

Despite large differences in GRs between strains, RMR did not differ. This agrees with Visser's (1991) failure to find a link between GRs and RMR in published poultry data. The independence of RMR and GR suggests that a chick's total energy budget is not limited by a physiological ceiling on nutrient acquisition. Many researchers (eg, Jackson and Diamond, 1996) believe that gut capacity limits growth (and thus the total energy budget) because selection for high GR often increases the relative gut size. The evidence, though, is

not unanimous. Ricklefs and Marks (1985) did not find any differences in gut size between fast- and slow-growing Japanese quail and Mitchell and Smith (1991) reported relatively smaller viscera in fast-growing strains of chicken. How do RF birds grow six times faster to 100g than do L birds, when both have the same relative mass of intestines? It seems that an increase in the mass-specific absorptive surface (Uni *et al.*, 1995) allows this fast growth, rather than a mere increase in intestinal mass. After about 5 d, increases in intestine size in RF birds suggest that birds later responded to a limiting gut size.

When birds are cold they produce heat mainly by isometric contraction of pectoral and leg muscles. Earlier studies showed that fast growing birds have a lower PeakMR, possibly due to slow maturation of muscle (Ricklefs *et al.*, 1995). In contrast, this study showed that RF birds had higher PeakMR than L-strain birds, possibly because a higher relative muscle mass offset delayed muscle maturation. Furthermore, the maintenance cost of a larger muscle mass was reflected in variation of RMR, which in turn was positively correlated with Peak MR.

No correlation exists among heart mass and MR. Moreover, RF birds achieved higher PeakMR than L birds even though their hearts were no bigger. Thus, our data do not support the hypothesis that selection for fast GRs has impaired oxygen delivery in young broilers. Instead, the results imply that the heart of the modern broiler is working harder than it once did and is perhaps a weakness that appears in some birds as the degenerative disease, ascites.

This study showed that even just after hatching, when differences in GR are highest, layers and broilers have similar sized internal organs and maintenance costs. This suggests that a simple re-scaling of metabolic machinery does not explain the changes in broilers. Also, our study does not support hypotheses linking variation in avian GRs to changed maintenance costs and it gives only partial support to the existence of gut capacity limiting GR. The higher water content of muscles in RF birds did not impair their metabolic capacity. Thus, this study gives limited support only to an inverse relationship between GR and tissue maturation (Ricklefs, 1979). Instead, our results suggest that muscle maturation and digestive capacity may both limit GRs at different periods of development.

V. ACKNOWLEDGMENTS

REFERENCES

Jackson S. and J. Diamond (1996). *Evolution,* **50**:1 638-1650.
Mitchell M and M.W. Smith (1991). *Comparative Biochemistry and Physiology,* **99A**: 251-58.
Ricklefs R.E. (1979). *Biological Reviews,* **54**: 269-290.
Ricklefs R.E. (1985). *Poultry Science,* **64**:1 563-1576.
Ricklefs R.E. and H.L. Marks (1985). *The Auk,* **102**: 323-333.
Ricklefs R.E., R.E. Shea and I-O. Choi (1994). *Evolution,* **48**: 1080-1088.
Starck J.M. and R.E. Ricklefs (1998). In: Growth and Development. Evolution within the Avian Altricial-precocial Spectrum, pp. 247-266. Eds. J.M. Starck and R.E. Ricklefs. Oxford University Press, New York.
Uni Z., Y. Noy and D. Sklan (1995). *British Poultry Science,* **36** :63-71.
Visser G.H. (1991). Ph.D. dissertation, University of Utrecht.

PERFORMANCE AND GUT CHARACTERISTICS OF GRIT-FED BROILERS

G.P.D. JONES and R.D. TAYLOR

Summary

Three experiments examined the performance and gut characteristics of broilers fed either soluble or insoluble grit as it has been shown in layers that the inclusion of grit in the diet leads to an improvement in apparent metabolisable energy. The results obtained in the experiments were inconsistent and inconclusive, although it was shown that bird growth and performance may be improved by the inclusion of grit in the diet. The type of grit used may influence any result obtained.

I. INTRODUCTION

In earlier research (Taylor, 1998), responses in apparent metabolisable energy (AME) have been found in layers fed particulate, as opposed to ground, limestone although substantial quantities of the particulate limestone pass through the digestive tract relatively unchanged (Taylor and Jones, 1998).

The improvement in AME was not associated with an increase in gizzard musculature which is commonly found when feeding whole grains. The provision of particulate limestone had no influence on α-amylase activity and ileal viscosity was not influenced by feeding particulate limestone. It is possible that the improvement in AME is through an increased mixing of the digesta with the presence of large particles in the digestive tract thereby disturbing the viscous contents of the intestinal segments and providing greater interaction between the digestive enzymes and the digesta contents. However, the effects of solubilised calcium may also alter AME, possibly through changes in digesta pH.

The improvement in AME in layers through particulate calcium feeding may also be evidenced in broilers. Poultry have immature digestive tracts for a substantial part of their early productive life (Ritz *et al.* 1995). Recently, this immaturity has been overcome to some extent, and with mixed success, with the application of exogenous enzymes to the feed.

The experiments presented here examine the effects of two particulate grits on the performance and gut characteristics of broiler chickens.

II. METHODS

Soluble (limestone) and insoluble (granite) grit was provided *ad libitum* to broiler chickens from 1 - 24 days of age. The grit was mixed with a previously crumbled, wheat/soyabean based broiler starter diet (12.56 MJ AME and 220 g CP/kg). The birds were housed in electrically heated brooders for the duration of each of two experiments and were maintained under 24 hr fluorescent lighting. Feed and water were provided *ad libitum*.

In experiment 1, four replicates of 6 birds were fed either the unadulterated diet or the diet with inclusion of either of the two grits. In experiments 2 and 3, six replicates of 6 birds were fed the control diet with or without insoluble (granite) grit and with or without the inclusion of the exogenous feed enzyme, Avizyme 1302 (0.5 g/kg).

At 24 days of age, the birds were killed by cervical dislocation and the gizzard and proventriculus removed and weighed both full and empty. The contents were then washed and separated. The intestine was separated into the duodenum, jejunum and ileum and the

University of New England, Armidale, NSW 2351.

length of each recorded. In experiment 3, the viscosity of the contents of each segment was additionally determined. The proventriculus was scored for the presence or absence of distension or dilatation in experiments 2 and 3.

The results were analysed by analysis of variance with the general linear models procedure of SAS (SAS Institute, 1989). Where appropriate, significant least squares (LS) means were separated using paired sample t-tests and are presented with appropriate standard errors (SE). The binary data (proventricular dilatation score) were analysed as a general linear model using Splus (MathSoft Inc., 1997). The significance of treatment differences was tested by comparing the change in deviance due to each treatment contrast with the critical region of the X^2_1 distribution. In the tables, unlike letters (a,b or c) within a column are significantly different at $P < 0.05$.

III. RESULTS

Bird performance to 24 days in experiment 1, as well as the measures of gastrointestinal weight and length, were unaffected by grit provision in experiment 1 (Table 1). As expected, gizzard grit content was greater $(P < 0.05)$ in the grit-fed birds, with those birds fed the insoluble (granite) grit retaining more in the gizzard than those fed the limestone grit. Gizzard grit content was not related to proventriculus or gizzard weight although gizzard weight was poorly correlated to the length of the duodenum and ileum. All intestinal segment lengths were interrelated.

Table 1.   Body weight and gut measurements from broilers fed a control diet or the control diet supplemented with insoluble grit or limestone grit *ad libitum*.

Diet	Body weight (g)	Proventriculus weight (g/kg body wt)	Gizzard weight (g/kg body wt)	Gizzard grit (g)	Duodenum length (cm/kg body wt)	Jejunum length (cm/kg body wt)	Ileum length (cm/kg body wt)
Control	1050	5.9	22.3	0^c	24.6	66.0	53.6
Control + insoluble grit	1013	4.6	22.0	7.8^a	24.4	66.1	56.7
Control + lime grit	993	5.1	21.2	3.4^b	24.4	68.0	55.5
SE	33.7	0.38	0.77	0.52	1.10	2.24	2.09

In experiment 2, the treatments had no effect on bird bodyweight (Table 2). Proventriculus weight was decreased $(P < 0.05)$ by both grit and enzyme addition although gizzard weight was not influenced. The decrease in proventriculus weight was associated with an decrease in dilatation score (Table 3).

Table 2.   Body weight and gut measurements from broilers fed a control diet or the control diet supplemented with insoluble grit *ad libitum*, enzyme or the grit plus enzyme.

Diet	Body weight (g)	Proventriculus weight (g/kg bwt)	Gizzard weight (g/kg bwt)	Gizzard grit (g)	Duodenum length (cm/kg bwt)	Jejunum length (cm/kg bwt)	Ileum length (cm/kg bwt)
Control	939	8.5^a	20.7	0^a	26.1^a	68.8	58.3^{ab}
Control/grit	954	5.2^{bc}	21.8	8.5^b	23.5^b	74.8	60.7^a
Control/enzyme	977	6.7^b	19.6	0^b	24.2^{ab}	69.8	54.5^b
Control/grit/enzyme	981	5.1^c	21.7	8.3^a	23.6^b	67.5	54.8^b
SE	21.6	0.57	0.83	0.45	0.89	1.95	1.38

Broilers provided enzyme or grit plus enzyme in experiment 3 were heavier (P < 0.05) than those fed without either (Table 4). Proventriculus weight was greater (P < 0.05) in those birds fed grit plus enzyme as was gizzard weight. The duodenum was longest in birds provided the control (unsupplemented) diet as were the jejunum and ileum.

Table 3. Analysis of deviance of proventricular dilatation counted as binary data (present = 1, absent = 0).

Diet	Dilatation present no.	SE no. present	Probability	SE (probability)
Control	8[b]	2.11	0.44	0.025
Control/grit	1[a]	0.97	0.06	0.011
Control/enzyme	2[a]	1.33	0.11	0.016
Control/grit/enzyme	0.18[a]	0.42	0.01	0.005

Table 4. Body weight and gut measurements from broilers fed a control diet or the control diet supplemented with insoluble grit *ad libitum*, enzyme or the grit plus enzyme.

Diet	Body weight (g)	Provent. weight (g/kg bw)	Gizzard weight (g/kg bw)	Provent. grit (g/kg bw)	Gizzard grit (g/kg bw)	Duodenum length (cm/kg bw)	Jejunum length (cm/kg bw)	Ileum length (cm/kg bw)
Control	1137[b]	6.9[b]	16.2[b]	0[b]	0[b]	23.5[a]	56.3[a]	53.2
Control/grit	1182[ab]	5.7[ab]	18.0[ab]	0.53[a]	6.5[a]	21.2[b]	54.5[ab]	48.5
Control/enzyme	1203[a]	6.0[ab]	17.4[b]	0[b]	0[b]	21.3[b]	52.4[b]	49.0
Control/grit/enzyme	1230[a]	4.6[a]	19.9[a]	0.08[b]	7.6[a]	21.4[b]	50.8[b]	49.5
SE	22.3	0.50	0.79	0.145	0.41	0.63	1.33	1.54

Intestinal viscosity (Table 5) of the duodenal contents was not (P > 0.05) influenced by treatment, however, in the jejunal and ileal segments, digesta viscosity was reduced by enzyme addition. Grit consumption did not (P >0.05) reduce digesta viscosity.

Table 5. Intestinal segment viscosity (cP ± SE) from broilers fed a control diet or the control diet supplemented with insoluble grit *ad libitum*, enzyme (Avizyme 1302) or the grit plus enzyme.

Diet	Duodenum		Jejunum		Ileum	
Control	3.5	0.65	8.8[b]	0.58	16.7[b]	0.82
Control/grit	4.9	0.48	8.5[b]	0.56	18.0[b]	0.85
Control/enzyme	3.9	0.44	3.9[a]	0.58	6.7[a]	0.82
Control/grit/enzyme	3.7	0.51	3.5[a]	0.60	6.5[a]	0.80

Proventricular dilatation (Table 6) was significantly reduced (P < 0.05) only when grit and enzyme were added to the diet. Providing grit alone or including enzyme in the diet did not reduce the incidence of dilatation.

Table 6. Analysis of deviance of proventricular dilatation counted as binary data (present = 1, absent = 0).

Diet	N	Dilatation present no.	SE no. present	probability	SE (probability)
Control	19	6[b]	2.03	0.32	0.025
Control/grit	20	3[b]	1.60	0.15	0.018
Control/enzyme	20	5[b]	1.94	0.25	0.022
Control/grit/enzyme	19	1[a]	0.97	0.05	0.012

IV. DISCUSSION

The three experiments presented represent a preliminary investigation into the phenomenon of increased AME with the feeding of grits to layers and when applied to broiler chicken stock.

Although no differences were apparent in experiments 1 and 2, the third experiment demonstrated a non-significant improvement in bodyweight when grit was provided. This increase was associated with a small grit retention and a small increase in gizzard musculature; however these did not account for the full weight difference and although not measured, it is unlikely that any retained grit in the lower digestive tract was of sufficient quantity to account for the difference.

The cage system used precluded any direct measure of AME or food conversion ratio (FCR); however the relationship between digesta viscosity and FCR (Bedford, 1996) indicates that FCR was not improved by grit feeding in experiment 3 although the decrease in size of the duodenum, jejunum and ileum (experiments 2 and 3) indicate a more rapid and efficient digestion of nutrients (Marquardt, 1996).

It is postulated that the presence of grit in the gizzard leads to the improved grinding of the grain fraction of the diet thereby disrupting the starch granule cell walls and increasing the surface area of the starch.

In the first experiment, the decreased level of soluble (limestone) grit in the gizzard indicates that the birds may be eating to a calcium appetite rather than a grit appetite *per se.*

The conflicting results obtained in these experiments indicate that the source of grit may be important to the results obtained. Similarly, the decrease in proventricular dilatation observed in Experiment 2 when grit was provided to the birds was not noted in the third trial. The presence of this dilatation has implications for the broiler predisposed to the ascites syndrome (Jones and Cumming, 1993).

From the results of the three experiments presented it is apparent that the phenomenon requires further investigation to elucidate the effects measured to date.

V. ACKNOWLEDGEMENTS

REFERENCES

Bedford, M.R. (1996). *Proceedings 1st Chinese Symposium on Feed Enzymes*, pp 19-28.

Jones, G.P.D. and Cumming, R.B. (1993). *Australian Poultry Science Symposium*, **5**: 88.

Marquardt, R.R. (1996). *Proceedings 1st Chinese Symposium on Feed Enzymes*, pp 5-17.

Ritz, C.W., Hulet, R.M., Self, B.B. and Denbow, D.M. (1991). *Poultry Science*, **74**: 1317-1322.

Taylor, R.D. (1998). *Proceedings of the Australian Poultry Science Symposium*, **10**: 103-106.

Taylor, R.D. and Jones, G.P.D. (1998). *Proceedings of the Queensland Poultry Science Symposium,* **7**: 8-1-8.8.

AMINO ACID REQUIREMENT OF MALE BROILER CHICKENS FROM 20 TO 40 DAYS OF AGE IN RELATION TO THE DIETARY CRUDE PROTEIN LEVEL

S. MACK[1], J. B. SCHUTTE[2], J. DE JONG[2] and S. VAN CAUWENBERGHE[3]

Summary

The aim of this experiment was to determine the effect of reducing the crude protein (CP) content of a diet optimized for the ideal ratio of seven essential amino acids (AA) on the performance of broiler chickens from 20 to 40 days of age. Three isoenergetic basal diets (13.2 MJ AMEn/kg) consisting mainly of corn and soybean meal were formulated to contain 197, 178, and 160 g/kg of CP, respectively. Each basal diet contained 11.5 g/kg true faecal digestible lysine and the essential amino acids methionine + cystine, threonine, tryptophan, arginine, valine and isoleucine relative to lysine at the previously found ideal ratio of 75%, 63%, 19%, 112%, 81%, and 71%, respectively (Mack *et al.*, 1998). The basal diets were supplemented with or without essential and/or non-essential amino acids to form six treatments. Reducing the CP content from 197 to 178 g/kg led to similar bird performance when the dietary glycine + serine content was kept constant at 18 g/kg. Further reducing the CP content to 160 g/kg required the additional supplementation of leucine, phenylalanine, tyrosine and histidine to obtain their ideal ratio to lysine of 109 % (leu), 105 % (phe+tyr), and 32 % (his) (Baker, 1996).

I. INTRODUCTION

Applying the ideal amino acid profile in formulating broiler diets provides distinct benefits. Once an ideal ratio of essential amino acids to lysine is established for a certain age period one can focus on determining accurately the lysine requirement under a variety of conditions. Based on this the dietary specifications for all other indispensable AA can simply be set by applying their ideal ratio to lysine instead of looking into the full range of essential amino acids individually. Moreover, formulating diets according to the ideal protein concept allows for the most efficient and economical use of dietary protein by maximizing nitrogen utilization and minimizing nitrogen excretion. The latter is already or will become a pressure point in a number of countries world-wide due to legislative restrictions.

In a previous study on the ideal protein concept the optimum dietary ratio of methionine + cystine, threonine, tryptophan, arginine, valine and isoleucine relative to lysine was determined to be 75%, 63%, 19%, 112%, 81%, and 71%, respectively, in broiler chickens of 20 to 40 days of age (Mack *et al.*, 1997). Throughout the experiments an identical corn-soybean meal based diet containing 190 g crude protein and 13.2 MJ AMEn per kg was used. In two experiments within the aforementioned study the optimum true faecal digestible (TFD) lysine content was determined to be 11.5 g/kg diet. The present experiment was planned as a follow-up study with the previously indicated ideal amino acid profile, energy content and the optimum dietary lysine concentrations implemented in corn-soybean meal based diets of varying crude protein contents fed to broiler chickens from 20 to 40 days of age. The aim was to elucidate the relationship between the level of dietary protein and amino acids and to determine at which protein level other essential and non-essential amino acids are limiting.

[1]Degussa AG, Feed Additives Division, Applied Technology, P. O. Box 1345, 63403 Hanau, Germany;
[2]TNO-ILOB, P.O. Box 15, 6700 AA Wageningen, The Netherlands;
[3]Eurolysine, 153 rue de Courcelles, 75817 Paris Cedex 17, France;

II. METHODS

A total of 1800 day-old male broiler chicks of a commercial cross (Ross) were fed a commercial starter diet adequate in nutrients and energy until 20 days of age. At 20 days of age the birds were distributed to 36 pens following a randomized block design. Pens were randomly assigned to six dietary treatments (Table 1) each consisting of six replicate floor pens with 50 birds/pen. The floor pens were littered with wood shavings and were stocked with 12.5 birds per square meter net floor space. The birds were housed in an insulated and ventilated broiler unit under a lighting regime of 23 h light and 1 h dark. Temperature in the broiler unit was gradually reduced from 28°C during the first week to 20°C during the last days of the experiment. Pelleted experimental diets and water were offered *ad libitum*. Three isoenergetic basal diets with different CP content were formulated consisting mainly of corn and soybean meal (Table 2). Their content of true fecal digestible Lys, Met+Cys, Thr, Trp, Arg, Ile and Val was constant and matched the ideal ratio for these essential amino acids and the lysine concentration which in a previous study was found to optimize performance (Mack *et al.* 1997). The basal diets were supplemented with or without essential (EAA) and/or non-essential (NEAA) amino acids to form the experimental diets. Added amino acids were assumed to be 100% digestible. The basal diets were formulated according to the analyzed AA content of the individual feedstuff batches (Llames and Fontaine, 1994) combined with table values for true faecal AA digestibility, except for NEAA for which table values for true faecal digestiblity were not available and therefore table values for apparent faecal digestibility were used. At 29 and 40 days of age feed intake (FI) and body weight gain (WG) were recorded. Feed conversion rate (FCR) was calculated as kg feed per kg gain corrected for mortality. At 41 days of age after feed deprivation for 10 h carcass quality was determined in 48 birds per treatment according to the directives set up by the Working Group V of the WPSA European branch. The recorded data were analyzed by the GLM procedure for the effects of CP level, supplemented NEAA and supplemented EAA on performance criteria. Differences between selected treatment means were tested for significance by a linear contrast procedure (SAS Institute Inc., 1987). All statements of significance are based on $P<0.05$.

Table 1.   Experimental diets.

Experimental diet	Total CP content (g/kg)	Addition of NEAA[1]	Addition of EAA (Leu, Phe+Tyr and His[2])
A	197	-	-
B	178	-	-
C	178	+	-
D	160	-	-
E	160	+	-
F	160	+	+

[1]Addition of NEAA up to the apparent faecal digestible level in diet A. Glycine (Gly) and serine (Ser) were added as Gly. Proline, alanine, asparagine and glutamine were added in the form of glutamic acid. Apparent faecal digestible amino acid contents are based on table values for the feed ingredients (Dutch Bureau of Livestock Feeding, 1996).

[2]Addition of Leu, Phe+Tyr and His up to the true digestible levels required to match their ideal ratio to lysine of 109%, 105% and 32%, respectively, (Baker, 1996). (*I. e.* 12.5 g Leu, 12.1 g Phe+Tyr and 3.7 g His per kg diet). True facal digestible amino acid contents are based on table values for the feed ingredients (Degussa, 1993, Rhone-Poulenc, 1993, Heartland Lysine Inc., 1995).

Table 2.   Calculated true faecal digestible nutrient content of the basal diets (g/kg as fed).

Calculated nutrient content (g/kg)	Diet A	Diet B	Diet D
CP[1]	197	178	160
AMEn[2]	13.2	13.2	13.2
Lysine (Lys)	11.5	11.5	11.5
Methionine (Met)	5.8	6.2	6.6
Methionine + cystine (Met+Cys)	8.6	8.6	8.6
Threonine (Thr)	7.1	7.1	7.1
Arginine (Arg)	12.9	12.9	12.9
Tryptophan (Trp)	2.0	2.0	2.0
Isoleucine (Ile)	8.2	8.2	8.2
Leucine(Leu)	14.7	12.5	10.5
Valine (Val)	9.1	9.1	9.1
Phenylalanine (Phe)	8.4	7.0	5.6
Tyrosine (Tyr)	6.1	5.1	4.1
Histidine (His)	4.0	3.3	2.6
Aspartic acid (Asp)[3]	16.0	12.9	9.8
Glutamic acid (Glu)[3]	30.5	25.0	19.5
Proline (Pro)[3]	10.2	8.9	7.5
Alanine (Ala)[3]	7.8	6.7	5.6
Glycine (Gly)[3]	6.5	5.5	4.4
Serine (Ser)[3]	8.7	7.4	6.0

[1]Calculated as total content
[2]Calculated as MJ/kg diet
[2]Stated as apparent faecal digestible content

III. RESULTS AND DISCUSSION

General performance level of the chickens was high: only 0.8% of the birds died during the experimental period. Tables 3 summarizes the effects of the experimental treatments and probabilities for WG, FCR, FI and carcass quality. The dietary protein content affected all listed performance criteria significantly. The results suggest that in addition to Lys, Met, Thr, Trp, Arg, Val and Ile other amino acids become limiting at dietary CP levels of 178 g/kg or lower (diet A vs diet B). Adding NEAA to the diet containing 178 g/kg CP (diet C) resulted in improved WG, FCR and breast meat yield which was not significantly different from dietary treatment A. Previous research conducted by Schutte et al. (1997) in broiler chicks from 0 to 21 days of age indicated that adding glycine but not glutamic acid to a low protein diet adequate in EAA improved performance significantly. Hence, the improved performance achieved in the present experiment with diet C compared to diet B suggests that the synthesis of Gly in the metabolism of older chickens might not be sufficient and that there is still a considerable amount to be supplied via the feed. The numerical differences in performance observed between treatments A and C might be due to the differences in the content of true faecal digestible Leu, Phe+Tyr and His with the His to Lys ratio in diet C being lower than what was found to be ideal in an earlier study (Baker, 1996). Supplementing basal diet D (160 g CP/kg) with NEAA and EAA (Diet F) allowed a performance level equal to the high protein diet A.

Table 3.   Effects of experimental treatments on bird performance from 20 to 40 days of age.

Exptl diet	CP content (g/kg)	Addition of NEAA	EAA	WG (g)	FCR (g/g)	FI (g/bird)	Breast meat (g/kg)[1]	Abdominal fat (g/kg)[1]
A	197	-	-	1813	1.738	3150	178	26.3
B	178	-	-	1746	1.799	3140	171	27.2
C	178	+	-	1792	1.755	3144	175	25.0
D	160	-	-	1600	1.862	2979	165	29.1
E	160	+	-	1650	1.814	2993	168	24.6
F	160	+	+	1780	1.746	3108	180	25.4
SEM	-	-	-	14	0.011	20	2	0.7
Source of variation						Probability (Pr > F)		
CP				0.0001	0.0001	0.0001	0.0009	0.0298
NEAA				0.0096	0.0012	0.8717	0.3088	0.0001
EAA				0.0001	0.0002	0.0003	0.0004	0.4655
Contrast								
A vs B				0.0028	0.0005	0.7282	0.0407	0.3688
A vs C				0.3119	0.2786	0.8438	0.3491	0.2100
A vs F				0.1168	0.5777	0.1542	0.4803	0.3511
B vs C				0.0337	0.0092	0.8802	0.2440	0.0362
E vs F				0.0001	0.0002	0.0003	0.0004	0.4655

[1] As g/kg live weight at 41 days of age

IV. CONCLUSIONS

The present experiment indicates that the CP content of corn-soybean meal based diets which are optimized for the ideal ratio of Met+Cys, Thr, Trp, Arg, Ile and Val to Lys can be reduced from 198 g/kg to 178 g/kg during 20 to 40 days of age without decreasing bird performance when dietary apparent faecal digestible Gly+Ser is maintained at 15.2 g/kg. When further reducing the CP content to 160 g/kg, Leu, Phe+Tyr and His have to be added according to their ideal ratio to lysine (Baker, 1996) in order to obtain optimal performance.

REFERENCES

Baker, D. H. (1996). *Zootecnica International*, **11**: 32-35.

Degussa (1993). Digestible Amino Acids in Feedstuffs for Poultry (Table). Degussa AG, Feed Additives Division, Applied Technology, P. O. Box 1345, 63403 Hanau, Germany.

Dutch Bureau of Livestock Feeding (1996). *Composition, Digestibility and Energy Value of Feedstuffs* (CVB, Lelystad, The Netherlands).

Heartland Lysine (1995). True Digestibility of Essential Amino Acids for Poultry (Table). Heartland Lysine Inc. 8430 W. Bryn Mawr Ave. Chicago, I, USA.

Llames, C. and Fontaine, J. (1994). *Journal of the Association of Analytical Chemists*, **77**: 1362-1402.

Mack, S., Bercovici, D., De Groote, G., Leclercq, B., Lippens, M., Pack, M., Schutte J. B., and Van Cauwenberghe, S. (1998). *Proceedings Australian Poultry Science Symposium*, **10**: 192-196. Ed. R. A. E. Pym.

Rhone-Poulenc (1993). Rhodimet Nutrition Guide (Table), 2nd edition, Rhone-Poulenc Animal Nutrition, 42 Av. Aristide Briand, 92164 Antony, France.

SAS Institute (1987). SAS User's Guide: Statistics, Version 6 edition, Cary, NC, USA.

Schutte, J. B., Smink, W. and Pack, M. (1997). *Archiv für Geflügelkunde*, **61** (1): 43-47.

WELFARE AND PRODUCTIVITY OF HENS IN A BARN SYSTEM AND CAGES

JOHN L. BARNETT

Summary

This experiment compared hens housed in cages and a barn in 3 compartments of a commercial shed. There were 4 sampling periods commencing at 3 weeks after introduction when the birds were 20, 29, 40 and 64 weeks of age, respectively. The barn hens had a lower body weight, egg production and egg yolk colour score. Egg microbial quality was poorer in the floor eggs. Feather condition and cover was better in the barn treatment until 29 weeks of age but poorer in older hens. Corticosterone concentrations and bone strength were higher and there were fewer broken bones in the barn treatment. Notwithstanding the above findings, cautious interpretation is required as the experiment had a number of constraints.

I. INTRODUCTION

While there is a lack of scientific knowledge of alternative systems as a whole, there is considerable industry experience which is based on some traditional elements of poultry husbandry that were in use prior to the introduction of the battery cage. This experiment compared the welfare and production of hens in a barn system and conventional cages in a commercial environment. While it was originally intended to conduct 6 replicates of the experiment using 2 flocks over 2+ years, this did not eventuate; thus, the following data refer to 3 replicates only and considerable caution is required when discussing the findings of this experiment in relation to barn systems in general.

II. METHODS

A barn shed divided into 4 compartments, at a commercial farm with Hisex hens, was used for this experiment. There were 2 treatments: i) Cage - hens housed in conventional cages (2 birds/cage with a space allowance of 1504 cm^2/cage). Twenty four cages were installed in each of 3 compartments and fenced off from the remainder of the compartment. These birds had manual feeding and egg collection. ii) Barn - hens in 3 compartments in a barn system (900 hens/compartment and 7 birds/m^2) with litter (wood-shavings) on the floor and nest boxes; the birds could perch on the feeder lines and on entry platforms to the nest boxes.

The birds were reared in cages at the farm until placed into the barn shed at 16 weeks and 3 days of age; the cage birds were randomly selected from the floor and placed into treatment 4 days later. All birds had been beak-trimmed. During week 1 in the barn shed, a coccidiostat was supplied via the drinking water. Both treatments were given the same feed. Further management details are provided in Barnett (1998).

The experiment commenced in September (spring) and there were 4 sampling periods involving different birds, commencing at 3 weeks after introduction to treatment when the birds were 20, 29, 40 and 64 weeks of age, respectively. At each sampling period from each compartment, the following measurements were taken from 6 birds from cages and 10 birds from the barn system. (i) Corticosterone concentrations on the basis of a blood sample taken from the brachial vein within 60 s of being caught; the corticosterone assay method is described

Animal Welfare Centre, Victorian Institute of Animal Science, Private Bag 7, Sneydes Road, Werribee, Victoria. 3030.

in Barnett *et al.* (1994); (ii) feather condition and cover and foot, digit and clawfold condition were subjectively scored on a 4 point system where a score of 4 indicates a good condition (Tauson, 1986); (iii) claw length, measured on the middle toe of both feet (subsequently averaged) with vernier callipers; (iv) body weight using a portable set of scales. In addition: six eggs were collected at 29 weeks of age from the floor, egg belts and/or cages of each compartment and examined for microbial contamination using standard methods (Anon. 1992); physical egg quality measurements (egg weight, equatorial egg shell thickness, albumin quality and yolk colour) were made on eggs collected at the 4 sampling periods; egg production was measured once per month for each compartment for a 24 h period by research staff and for the shed by farm staff who made twice-daily collections of eggs and daily collections for dead birds; 30 hens at the start of the experiment and 48 hens at the end of the experiment were euthanased for bone strength determinations; the assay method is described in Barnett *et al.* (1997). The data from within each barn or cage compartment were averaged and treatment and age effects determined by analysis of variance, using the compartment mean as the unit of measurement. For bone strength, individual bird data were used for the analyses. In addition, sick/culled hens on the day of fortnightly visits to the farm by research staff were taken for post mortem and faecal samples were collected at each sampling period for parasitological examination; these data are not included in this paper.

III. RESULTS AND DISCUSSION

The egg production data, collected monthly by research staff, did not account for mortalities and while they are likely to be an inaccurate measure of daily egg production, the relatively lower production in the Barn treatment is likely to be real (Fig. 1). The producer's records for hen day egg production for the shed, taking mortalities into account, also indicate lower production in the Barn treatment. One reason for a lower egg production in the Barn treatment is the lower body weights of birds in this treatment (P<0.001; Table 1) which would be expected to result in lower overall production. Possible reasons for the poor body weight and egg performance include inadequate dietary composition (as there may be an increased energy requirement in barn housed hens), inadequate feeder places and/or a change in social behaviour around the feeders resulting in displacement and/or food wastage. While floor eggs were a significant problem at this farm (Fig. 1), overseas experience (and local experience with other shed designs) suggests that a low incidence of floor eggs can be achieved; in a survey of 29 aviary systems the mean percentage of floor eggs was 5.2 % and ranged from 0.9-13.9 % (van Horne, 1997). Features that may reduce the incidence of floor eggs are: introducing birds to the barn system at a younger age (so that they can learn the location of nest boxes), rearing birds on the floor rather than in cages with perches, partially slatted raised floors incorporating the feeding and drinking systems and adjacent to the nest boxes (to get birds off the floor and encourage nest box use) and providing limited access to the nests (by closing them off at night to encourage an association between nest box availability and egg laying). Total mortalities for the Barn and Cage treatments were 10.8 and 5.6 %, respectively, for combined compartments (P<0.05; Chi-square test).

Egg yolk colour score was lower (P<0.01) in the Barn than the Cage treatment (Table 1). There was a significant interaction between housing and age for egg yolk colour score (P<0.05); egg yolk colour score was better in the Cage treatment at all ages (P < 0.05); in the first sampling period the mean values were 3.9 vs. 6.7 (P<0.001), except for the final sampling period where the colour scores were similar (13.2 vs. 13.0 for the Barn and Cage treatments, respectively). Poor egg yolk colour in the first sampling period may have been due to either variable food intake, typical of barn hens (Parkinson, personal communication) or a coccidial

infection (Barnett, 1998) that was evident at this time. There were no overall differences in equatorial egg shell thickness, although there were some age effects (P<0.001; Table 1). While the comparisons of microbial egg quality were constrained by having a manual system for cages compared to an automated system for the eggs laid in nest boxes in the barn system, the floor eggs from the Barn treatment (compared to the cage or belt eggs) had a poorer microbial quality, based on the increased counts (mean log values of cfu/egg are presented) on the shell surface of *E. coli* (P>0.05; 1.2 vs. 0.7) coliform bacteria (P<0.05; 1.7 vs. 0.7) and total viable counts (P<0.05; 1.75 vs 0.7). Reducing floor eggs will reduce this problem. There was no evidence of *Salmonella* contamination in any samples.
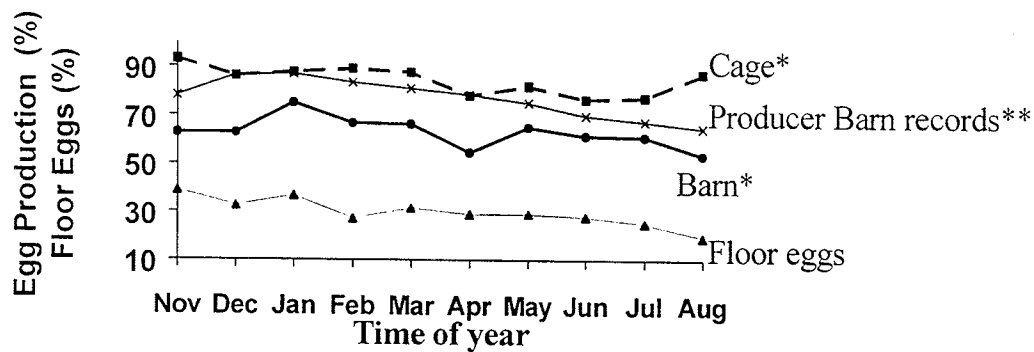
Figure 1.   Mean egg production.

*Production per hen housed (includes floor eggs in the Barn) based on monthly records collected by research staff; **shed hen day egg production (weekly data averaged over 4 week periods).

Table 1.   Effect of housing and age on body weight, claw length, corticosterone concentrations, feather condition and cover and egg quality.

Parameter	Housing		Age (weeks)				LSD[1]
	Barn	Cage	20	29	40	64	
Body weight (kg)	2.01^x	2.25^y	1.98^y	2.10^{yp}	2.12^{yq}	2.32^z	0.013
Claw length (mm)	18.5	18.7	16.0^a	16.2^a	20.1^{ab}	22.0^b	5.69
Corticosterone (nmol/L)	1.54^y	0.46^x	1.65^z	1.26^{bxz}	0.70^a	0.39^{ax}	0.483
Total plumage score	3.43	3.43	3.94^z	3.64^y	3.24^x	2.91^v	0.012
Back plumage score	3.67^a	3.92^b	4.00	4.00	3.83^q	3.33^p	0.177
Tail plumage score	3.18^y	2.90^x	3.59^z	3.07^y	2.94^x	2.55^v	0.010
Base of tail score	3.40^x	3.70^y	4.00^q	3.85^{zp}	3.37^y	2.98^x	0.096
Egg weight (g)	62.1	63.4	51.8^{ap}	61.8^b	66.4^{abcq}	71.1^c	7.91
Haugh units	70.0	71.7	91.3	68.8	66.2	57.1	76.02
Roche colour score	10.1^p	11.2^q	5.31^x	12.03^{py}	12.06^{py}	13.08^{qy}	0.695
Shell thickness (mm)	0.42	0.40	0.40^x	0.43^z	0.42^y	0.40^x	0.0087

[1]Least Significant Difference at P=0.05; abc, pq, vxyz, different letters denote a significant difference at P<0.05, P<0.01 and P<0.001, respectively.

Corticosterone concentrations were higher in the Barn than the Cage treatment (P<0.001; Table 1) and provide *prima facie* evidence for a chronic stress response in the Barn treatment. Supporting evidence includes the lower body weights, egg production (i.e. impaired reproductive performance) and higher parasite burdens (Barnett, 1998).

While there were no treatment effects on claw length (P>0.05), claws grew longer with age; claws at 64 weeks of age were longer than those at 20 and 29 weeks of age (P<0.05; Table 1). There were no treatment or age effects on the condition score of the footpads, clawfolds or digits, all having mean scores very close to 4 at all times. There were no treatment effects on overall feather condition and cover (P>0.05; Table 1). There was a significant interaction term (P<0.01) for total plumage condition and cover score; at 20 and 29 weeks of age the score was higher in the Barn treatment (mean values for the Barn and Cage treatments were 3.98 vs 3.89 and 3.73 vs. 3.54 for 20 and 29 weeks of age, respectively; P<0.05), while it was higher in the Cage treatment for the older ages (mean values for Barn and Cage treatments were 3.20 vs 3.27 and 2.82 vs. 3.01 for 40 and 64 weeks of age, respectively; P<0.05). Feather condition and cover scores declined as the birds became older.

Reduced bone strength in the humeri of caged hens (P<0.001) was an important observation here; there was also a trend for reduced femur strength in the Cage treatment (P=0.052). At the start of the experiment, the mean breaking strength (±SE) of the humerus and femur were 152.7± 11.16 and 110.8± 10.69 Newtons (N), respectively; at 64 weeks old bone strength was 182.1± 30.36 vs. 115.0± 10.68 N for the femur and 146.2± 16.88 vs. 81.8± 8.09 N for the humerus, in the Barn and Cage treatments, respectively. Three birds (6.5 %) from cages had broken bones compared to 0 from the barn system (P<0.01; Chi-square test), and this finding of weaker bones in caged birds is a relatively consistent one in the literature.

IV. CONCLUSIONS

In conclusion, the barn hens had a lower body weight, better feather condition and cover until 29 weeks of age, poorer feather condition and cover from 40 weeks of age, higher levels of stress, poorer egg microbial quality (but only from the floor eggs), higher bone strength and fewer broken bones, lower egg production and a lower egg yolk colour score. Although limited, these results indicate areas to target to improve the efficiency and perhaps welfare in barn hen systems; these include growth rate, egg production and weight and social behaviour and rearing effects that may affect nesting, feeding and injurious pecking behaviours. With greater industry experience and further scientific inputs, a number of the problems in non-cage systems for laying hens can be overcome.

REFERENCES

Anonymous. (1992). Compendium for the Microbiological Examination of Foods, 3rd Edition, Eds. Vanderzand, C. and Splittstoesser, D.F., American Public Health Association, Washington DC.

Barnett, J.L. (1998). The welfare and productivity of hens in a barn system and cages. Final report presented to Rural Industries Research and Development Corporation, project DAV 112A.

Barnett, J.L., Glatz, P.C., Newman, E.A. and Cronin, G.M. (1997). *Australian Journal of Experimental Agriculture*, **37**: 11-18.

Barnett, J.L., Hemsworth, P.H., Hennessy, D.P., McCallum, T.H. and Newman, E.A. (1994). *Applied Animal Behaviour Science*, **41**: 87-100.

Horne, P.L.M. van. (1997). In: *Proceedings of the 5th European Symposium on Poultry Welfare*, pp. 125-126. Eds. P. Koene and H.J. Blokhuis. World's Poultry Science Association.

Tauson, R. (1986). Ph.D. Thesis, Swedish University of Agricultural Sciences: Uppsala, Sweden.

THE GENETIC BASIS OF THE INTRODUCTION OF THE BLUE EGG GENE INTO THE CHINESE SILKIE

D.W. TERRY

Summary

An Australian strain of Naked Neck, known locally as a 'Turken', has a pea comb and lays a blue or green shelled egg. The pea comb and blue egg genes are derived from the Araucana breed and the naked neck gene from the Transylvanian Naked Neck breed. A breeding program based on a knowledge of the genetics of the distinctive traits displayed by the 'Turken' has successfully introduced these traits into the Chinese Silkie to produce two new varieties - the blue egg laying Chinese Silkie and the naked neck blue egg laying Chinese Silkie. An investigation of the genetic basis of a delay in comb development observed in blue egg laying Silkie hens found that the delay was due to blue egg layers having a true walnut comb and that Australian show Silkies are rose combed and not walnut combed as required by the British Standard.

I. INTRODUCTION

The Chinese Silkie breed is characterised by a number of unique features including silkie feathering, purple-black skin, turquoise blue ear lobes and a blue coloured comb. The Araucana breed's distinctive feature is its ability to lay blue or green shelled eggs and the Transylvanian Naked Neck breed has reduced body feathering, particularly in the neck region.

Poultry chromosome maps indicate that the genes for pea comb (P), blue egg shell colour (O) and naked neck (Na) are all linked on chromosome 1 (Crawford, 1990). The blue egg gene (O) is dominant to all other egg shell colours (o), the pea comb gene (P) is incompletely dominant to the single comb gene (p) and the naked neck gene (Na) is incompletely dominant to the full feathered gene (na) (Crawford, 1976). The blue egg shell gene (O) interacts with other shell pigments to produce a wide range of shell colours ranging from a pale blue to an olive green. The pea comb gene (P) produces a three bladed comb, with the blades side by side and the central blade a little higher than the lateral blades. This configuration resembles an opened pea pod and hence the name for this comb type. The naked neck gene (Na) removes the feathering from the neck region and reduces the number of feather tracts on other parts of the body (Hutt, 1949). Heterozygous individuals Na/na have a tuft of feathering on the neck whereas homozygous individuals Na/Na lack this feathering and have less feathering of the body in general (Crawford, 1976).

'Turkens' possess at least one copy of chromosome number 1 carrying the blue egg (O), pea comb (P) and naked neck genes (Na) (Bitgood et al, 1983). The original 'Turken' stock consisted of two males and five females. An inbred cross between one rooster and two hens produced offspring with silkie feathering (p=0.3) indicating that the strain is also carrying the autosomally recessive silkie gene (h). Chromosome maps locate the silkie gene on the same chromosome as the pea comb (P), blue egg shell (O) and naked neck genes (Na). The blue egg shell and pea comb genes are located on the p arm of the chromosome and the naked and silkie genes are on the q arm. 'Turkens' which produce offspring with silkie feathering possess one copy of chromosome number 1 carrying all four linked genes (O P Na h).

6 Bula Place, Bensville, N.S.W. 2251, Australia.

The aim of the breeding program was to introduce both the naked neck and blue egg traits into the Chinese Silkie breed by outcrossing Silkie with Turken and then backcrossing with Silkie for a number of generations to fix the Silkie traits.

II. METHODS

A 'Turken' hen that had produced silkie offspring when crossed with one of the 'Turken' roosters was crossed with a Chinese Silkie rooster. The Silkie breed has a rose comb R/R p/p, lays white or brown shelled eggs o/o, is fully feathered na/na and has silkie feathering h/h. The interaction of the rose comb loci (R) and the pea comb locus (P) produces the rose phenotype (R/R p/p). These offspring were then backcrossed to Chinese Silkie for 10 generations.

The 'Turken' hen was also crossed with an Ancona rooster to determine her genotype at the four gene loci of interest. The Ancona breed has a single comb r/r p/p, lays a white shelled egg o/o, is fully feathered na/na and has non-silkie feathering H/H.

III. RESULTS AND DISCUSSION

Nineteen chickens, all with walnut combs, were produced from the 'Turken' and Silkie cross.

Table 1.   Offspring from the Turken hen and Silkie rooster cross[1].

Sex	Normal feathering walnut comb	Normal feathering naked neck walnut comb	Silkie feathering walnut comb	Silkie feathering naked neck walnut comb
Male	2	2	4	4
Female	3	2	2	0

[1] The sex ratio of the hatch was 12 males to 7 females. All 7 hens laid blue eggs.

Twenty one chickens, all with pea combs, were produced from the 'Turken' and Ancona cross.

Table 2.   Offspring from the Turken hen and Ancona rooster cross[1].

Phenotype	Number of males	Number of females
Pea combed and full feathering	7	5
Pea combed and naked neck	5	4

[1] The sex ratio of the hatch was 12 males to 9 females. All 9 hens laid blue eggs.

The results from both crosses recorded in Tables 1 and 2 establish that the 'Turken' hen was homozygous at both the blue egg locus O/O and at the pea comb locus P/P, as all female offspring produced (n=16) laid blue eggs. These results also establish that the 'Turken' hen was heterozygous at both the naked neck locus Na/na and the Silkie locus H/h. Her genotype at the four gene loci of interest was therefore O/O P/P Na/na H/h. She had one chromosome carrying the blue egg shell gene (O), the pea comb gene (P), the naked neck gene (Na), and the silkie gene (h) which was critical for the success of the breeding program.

The four chickens from the 'Turken' hen and Silkie rooster cross that had naked necks and silkie feathering were retained for breeding. They were all males so it was not possible to

determine whether they were also carrying the blue egg gene. These roosters were back crossed to Silkie hens the following breeding season. The results are shown in Table 3 below.

Table 3.   Offspring from the four naked neck Silkie roosters and normal Silkie hens.

Sex	Naked Neck Walnut Comb	Naked Neck Rose Comb	Normal Silkie Walnut Comb	Normal Silkie Rose Comb
Female	9	7	6	15
Male	13	11	9	9

The naked neck Silkie hens (n=9) and normal Silkie hens (n=6) referred to in table 3 had walnut combs and laid blue eggs. Crosses between naked neck blue egg laying silkie hens and normal silkie roosters also produced normal feathered silkie hens laying blue eggs (n=6). These arose out of single cross over events between the pea comb gene (P) and the naked neck gene (Na) taking place during ovum formation.

A cross between blue egg laying naked neck Silkie hens O/o P/p Na/na h/h and normal Silkie roosters o/o p/p na/na h/h was not useful in obtaining linkage distances between the four genes of interest for several reasons. The blue egg shell gene (O) is sex limited and all male data are to be discarded, as it is not possible to determine whether males are carrying the blue egg gene, even if they have a walnut comb, as cross overs do produce walnut combed birds that do not carry the blue egg gene. The blue egg gene (O) and pea comb gene (P) are so closely linked (about 4 map units) that no cross overs in this region were detected in the female offspring produced. Cross overs in this region produce two phenotypes: hens that are rose combed, fully feathered and lay blue eggs and hens that are walnut combed, naked necked and lay white eggs. The pea comb gene (P) and naked neck gene (Na) are so far apart on different arms of chromosome 1 that they segregate independently of each other. The arrangement of genes on the chromosome of the naked neck blue egg laying Silkie is such that it does not permit the detection of cross overs between the naked neck gene (Na) and the silkie gene (h). There is, however, extensive published data on the map distances between these four genes (Crawford, 1990).

All silkie hens that lay a blue or green shelled egg show what appears to be a delay in the expression of their comb genes. This was evident from the time of hatching, when these chickens had smooth skin in the comb region, until adulthood when their combs were significantly smaller and more flattened than the brown egg laying Silkies. The data in Table 4 was collected from one year's breeding program and was recorded to test the relationship between the rates of comb development and the blue egg laying trait.

Table 4.   Table showing phenotypes and numbers of hens showing each phenotype.

	Blue egg laying delay in comb development	Blue egg laying normal comb development	Brown egg laying delay in comb development	Brown egg laying normal comb development
Naked neck	12	0	0	13
Normal feathered	9	0	0	14
$X^2 = 49.5$				

The delay in comb development was not related to the presence or absence of the naked neck gene (Na) as both naked neck and fully feathered Silkie hens laying blue eggs displayed the delayed expression. An investigation into the difference in rates of comb

development between blue and brown egg laying Silkies found that the two groups carry different genes for comb type. Silkies laying blue eggs have a true walnut comb R/R P/p whereas those laying brown eggs have a rose comb R/R p/p (Crawford, 1998; personal communication). Not only do the blue egg laying Silkies inherit a rose gene (R) from each parent but they also inherit a pea comb gene (P) linked to the blue egg gene from their blue egg laying mothers and a single comb gene from their Silkie fathers (p) and hence develop a walnut comb R/R P/p. The presence of both the rose gene and the pea gene results in the walnut phenotype.

Evidence to support this explanation is provided by the presence of a ridge of thickened skin running lengthwise over the keel of the breastbone of blue egg laying Silkies and the absence of this ridge in the brown egg laying Silkies. The presence or absence of a breast ridge has been shown to be a useful marker in identifying comb phenotypes (Crawford, 1961). Not only does the pea gene produce a ridge of thickened skin over the keel but it also reduces the size of wattles in both sexes. Homozygous P/P individuals have very small wattles; heterozygous individuals P/p birds have a significant reduction in wattle size and homozygous p/p birds show normal wattle development (Crawford, 1990). All blue egg laying Silkies examined (n=21) have significantly smaller wattles than those possessed by brown egg laying Silkies (n=52). All show Chinese Silkies examined have wattles that are normal in size confirming the view that they have a rose comb and not a walnut comb and therefore do not carry the pea comb gene.

IV. CONCLUSIONS

The Chinese silkie is an ancient breed which was mentioned in a famous medicinal book written in the Ming dynasty. In the article 'The Mystical Taihe Black-Boned Chicken' published in the *People's Daily* (February 1987) the author lists ten unique features possessed by the Chinese Silkie: blue comb, green earlobes, five toes, bearded, feathered shanks, white silkie feathering, crest, black bones, black muscles and black skin. Two novel features have been added to this list: a naked neck and blue/green shelled eggs.

This breeding project has shown that the Australian show Silkie and possibly those in other parts of the world (Crawford,1998; personal communication) does not conform genetically to the British Standard of a walnut comb but indeed possesses a rose comb. A plausible explanation would be that the British Standard, when they used the term 'walnut' to define the comb of the Silkie, meant that the comb and its surface should resemble the surface of a walnut and be approximately the size of a walnut. This phenotype can be readily achieved with a rose genotype.

Not only has this breeding program established two new varieties of Silkies but it has produced a comb type that is genetically true to the standard.

REFERENCES

Bitgood, J.J., Otis, J.S. and Shoffner, R.N. (1983) *Poultry Science,* **62**: 235-238.
Crawford, R.D. (1961) *Poultry Science,* **60**, No. 1
Crawford, R.D. (1976) *Poultry Science,* **55**: 820-822.
Crawford, R.D. (1986) *Poultry Science,* **65**: 1859-1862.
Crawford, R.D. editor (1990) Poultry Breeding and Genetics Elsevier Science Publishers, Amsterdam.
Hutt, F.B. (1949) Genetics of the Fowl. McGraw-Hill Book Co. Inc., New York.

THE EFFECT OF DL-METHIONINE AND BETAINE SUPPLEMENTATION ON GROWTH PERFORMANCE AND CARCASS COMPOSITION IN MALE BROILERS

R.M. MCDEVITT[1], S. MACK[2] and I.R. WALLIS[3]

Summary

We measured growth performance and carcass characteristics of male broiler chickens fed a series of diets containing betaine or DL-methionine (met) or a combination of both to see whether betaine could replace part of the methionine in broiler rations.

Supplementation with DL-met increased feed intake, weight gain and decreased FCR compared to birds fed control diets; however, the addition of betaine to these diets had no effect. Birds fed DL-met supplemented diets had relatively more breast muscle than birds fed diets with no DL-met. Addition of betaine to the DL-met diets improved the relative breast muscle yield. The addition of DL-met or betaine to the diet decreased relative abdominal fat pad mass. Birds fed 0.12% DL-met had lighter relative viscera masses, with or without added betaine.

Our data do not support the hypothesis that betaine can successfully substitute for methionine in broilers fed diets that are marginally deficient in methionine plus cystine.

I. INTRODUCTION

Methionine has three crucially important roles in vertebrate metabolism. Firstly it is an essential amino acid, secondly it is a precursor of cysteine and thirdly, methionine plays a key role as a methyl group donor. When methionine acts as a methyl donor it produces the compound homocysteine that together with serine ultimately produces cystine. In order for homocysteine to then be subsequently converted back to methionine, another methyl donor, such as betaine or choline, is required (Baker *et al.*, 1983; Finklestein and Martin, 1984).

The methyl donor betaine is a naturally occurring methylated derivative of glycine and is found in most organisms. In poultry, the methylation properties of betaine may also be important during lipid metabolism. In this role, betaine may reduce and redistribute carcass fat (Saunderson and MacKinlay, 1990). Carcasses with less fat could also result if betaine spared methionine, leaving more of the available essential amino acid for protein synthesis. This means that better use of dietary nutrients would leave fewer amino acids for deamination and eventual synthesis into adipose tissue.

There is some evidence that betaine has a sparing effect in broiler (Virtanen and Rossi, 1995) and in layer (Lowry *et al.*, 1987) diets; however, there is also evidence to the contrary (Rostagno and Pack, 1996; Schutte *et al.*, 1997). The objective of this study was to determine whether betaine could either spare methionine or enhance the effect of methionine in terms of growth and carcass composition of male broiler chickens. We therefore measured several indices of growth performance; live weight gain, feed intake and feed conversion efficiency (FCR), and the yields of breast meat and abdominal fat depots in male broiler chickens fed diets supplemented with either DL-methionine (DL-met) or betaine or a combination of both.

[1]Department of Biochemistry and Nutrition, S.A.C. Ayr, KA6 5HW, Scotland, U.K.
[2]Degussa AG, Feed Additives Division, P.O. Box 1345, D-63403 Hanau, Germany.
[3]Division of Botany and Zoology, A.N.U., Canberrra 0200, ACT, Australia.

II. METHODS

Male Cobb 500 day-old chicks (n=432) were group brooded at 31°C for one week, with gradual reduction to 21°C at 21 days. The lighting pattern throughout the experiment was 21.5 h per day. At 7 days of age the birds were individually weighed, wing-tagged and assigned to treatments using a stratified randomisation procedure. Birds were kept 12 to a pen (1.0 m X 2.0 m) in deep litter and there were 6 replicates per treatment. Each pen had a tube feeder and a bell drinker. The birds were vaccinated as per standard broiler commercial practice in Britain. The diets used in the feeding programme during the experiment were; day 0-7 (commercial starter) day 7-21 (experimental grower) and day 21-42 (experimental finisher). Both of the experimental diets consisted mainly of wheat, peas and soya bean meal, and were adequate in energy and all nutrients except methionine. There were 6 experimental treatments (Table 1).

Table 1.   Levels of methionine and cystine in the basal diet and the supplemented levels of DL-methionine and betaine per dietary treatment.

	Treatment[1]					
	A	B	C	D	E	F
DL-methionine (g/kg)	0.0	0.0	0.6	0.66	1.2	1.2
Betaine (g/kg)	0.0	0.5	0.0	0.5	0.0	0.5

[1]Basal diet contained 6.3 g methionine + 5.7 g methionine/kg

Body weight was recorded on days 7, 21 and 42, and feed intake (per pen) was measured on a weekly basis. At day 42, the birds were killed by stunning and allowed to bleed out and the weights of the following carcass composition parameters were recorded; plucked and bled carcass, eviscerated carcass, breast muscle (BRM) and abdominal fat pad (AFP).

Multifactorial general linear model analysis of variance (ANOVA) and where appropriate (to correct for differences in body mass), analysis of covariance (ANCOVA) were used to analyse the data. To control the alpha level for multiple comparisons between means, and keep the error probability at alpha (5%), the Bonferroni correction was used (Bolton, 1997). Data are means and the SEM per measurement is given. The data presented here are for the overall growth period (7-42 d).

III. RESULTS

(a) Growth performance

Dietary treatment had a significant effect on live weight gain over the whole growth period (P≤0.05). Weight gain (Table 2) increased significantly with increasing levels of DL-met (0.0 to 1.2 g/kg) supplementation. The addition of betaine to the DL-met supplemented diets did not cause an improvement in weight gain. The birds with the greatest weight gain (2347 g) were those supplemented with 1.2 g DL-met alone, and this was 600 g heavier than the birds fed the control diet and those supplemented with only 0.5g/kg betaine

Addition of DL-met, at both levels of inclusion, to the control diet caused a significant increase in feed intake over the whole growth period compared to birds fed the control diet and the control diet supplemented with 0.5 g betaine (P≤0.05). The further addition of betaine to any of the diets had no additional effect on feed intake (Table 2). Over the whole

35 d measurement period, birds fed DL-met supplemented diets had similar feed conversion ratios (FCR) at both the 0.6 and the 1.2 g/kg inclusion level. The FCR in birds fed diets unsupplemented with DL-met was significantly higher compared to those whose diets were supplemented with DL-met (P≤0.05). The addition of betaine to the diets did not significantly improve FCR.

Table 2.   Performance in male broilers fed varying levels of DL-met and betaine (g/kg). Viscera, abdominal fat pad (AFP) and breast muscle (BRM) masses are corrected for the effect of body mass (g, C)

Diet (DL-met, betaine)	Weight gain (g)	Feed intake(g)	FCR (g/g)	Viscera (g, C)	AFP (g, C)	BRM (g, C)
A (0.0, 0.0)	1748^{cl}	3770^{b}	2.15^{b}	518^{a}	39.4^{a}	258^{a}
B (0.0, 0.5)	1673^{c}	3651^{b}	2.18^{b}	517^{a}	38.4^{a}	260^{a}
C (0.6, 0.0)	2191^{b}	4256^{a}	1.94^{ab}	521^{a}	37.7^{a}	289^{b}
D (0.6, 0.5)	2225^{b}	4328^{a}	1.95^{a}	511^{a}	32.1^{b}	324^{c}
E (1.2, 0.0)	2347^{a}	4509^{a}	1.92^{a}	493^{b}	30.6^{b}	333^{c}
F (1.2, 0.5)	2277^{ab}	4450^{a}	1.96^{a}	493^{ab}	29.8^{b}	354^{d}
Pooled SEM	27.6	64.4	0.033	7.52	1.53	7.25

[1]Means within columns without a common letter are significantly different (at P≤ 0.05).

(b) Carcass composition

The masses of the plucked and bled carcass and the eviscerated carcass were affected by dietary treatment in the same way. The carcasses from the birds fed the control diet and the betaine (0.5 g) diet were significantly lighter than those of the birds on the other treatments (P≤0.05). The carcasses of those birds fed the highest level of DL-met (1.2 g) were significantly heavier than those fed diets with less DL-met (0.6 g). The addition of betaine to any of the diets did not result in a significant increase in carcass mass. Birds fed diets containing DL-met also had significantly heavier viscera (P≤0.001)than birds fed diets without DL-met. However, after correcting for treatment differences in carcass mass, the birds fed the two diets with 1.2 g DL-met had lighter viscera than the birds fed all the other diets (P ≤ 0.01) (Table 2).

Dietary treatment had a significant effect on both breast muscle (BRM) and abdominal fat pad (AFP) masses. The addition of DL-met to the diet had a significant positive effect on absolute BRM mass (P≤0.001) and there was evidence of a positive interaction between DL-met and betaine (P≤0.05). At 1.2 g DL-met, the addition of betaine to the diet had no significant effect on BRM, but, at the 0.6 g DL-met/kg, betaine did have a significant effect, resulting in a mean increase in BRM of 40 g. However, when BRM was corrected for the effect of body mass (Table 2) both DL-met and betaine did have a significant effect on BRM (P≤0.01). As the dietary concentration of DL-met increased so did corrected breast mass; likewise the addition of betaine to the diet in the presence of DL-met promoted synthesis of breast muscle. The fact that betaine had no effect, in the absence of DL-met, but a positive effect, in the presence of DL-met, suggested an interaction between the two additives (P = 0.065).

Supplementary DL-met increased the absolute mass of the AFP (P<0.001). Adding 0.6 g DL-met to the basal ration led to carcasses with heavier AFPs, but a further increase in DL-met from 0.6 g to 1.2 g kg had no additional effect. Correcting for treatment differences in body mass (Table 2) showed that adding either DL-met (P<0.05) or betaine (P<0.05)

makes a smaller relative AFP. Birds fed diets with 1.2 g DL-met or 0.6 g DL-met and 0.5 g betaine had smaller AFPs than those fed diets with no DL-met or with 0.6 g DL-met but no betaine.

IV. DISCUSSION

The results of this experiment suggest that betaine cannot substitute for DL-met in diets that are marginally deficient in methionine plus cystine. Adding betaine alone to the basal ration, which contained 2.9 g and 2.6 g DL-met in the grower and finisher phases, respectively, did not significantly improve body mass, feed intake, FCR, or body composition of male broiler chickens. In contrast, adding DL-met to the basal ration stimulated food intake, increased weight gain and improved FCR compared to control or betaine only diets.

The improved balance of dietary amino acids changed the carcass composition too, by increasing both the absolute and relative amounts of breast meat and by decreasing the deposition of abdominal fat. However, there were clear indications of a synergistic relationship between DL-met and betaine. As expected from the requirement for methionine (3.8 - 5.0 g/kg; NRC, 1994), adding 1.2 g DL-met to the basal diet generally resulted in a larger response than adding 0.6 g DL-met/kg.

From a wider energetics perspective, an interesting biological finding in this study was the reduction in relative visceral mass in birds fed diets with 1.2 g DL-met. Considering that relative food intake, after removing treatment differences in body mass, was similar across treatments, one would expect all birds to have similar masses of metabolically active tissue. Instead, it seems that birds fed diets that were deficient in methionine were attempting to compensate by maintaining more viscera in an attempt to maximise nutrient intake (Konarzewski *et al.*, 1990). However, when supplied with sufficient methionine, visceral mass no longer needed to compensate in the same way.

V. CONCLUSION

Our data demonstrate that betaine cannot replace DL-met with regard to improving growth performance in diets that are marginally deficient in methionine. However, betaine may have a role in poultry feeding with regard to its ability to modify carcass composition.

REFERENCES

Baker, D.H., Halpin, K.M., Czarnecki, G.L. and Parsons, C.M. (1983). *Poultry Science,* **62**: 133-137.

Bolton, S. (1997). In: Pharmaceutical Statistics, 3rd edn. Marcel and Decker, New York.

Finklestein, J.D. and Martin, J.J. (1984). *Journal of Biological Chemistry,* **259**: 9508-9513.

Konarzewski, M., Lilja, C., Kozowski, J. and Lewoñczuk, B. (1990). *Journal of Zoology (London),* **222**: 89-101.

Lowry, K.R., Izquierdo, O.A. and Baker, D.H. (1987). *Poultry Science,* **66**: 135.

National Research Council (1994). In: Nutrient Requirements of Poultry. National Academy Press, Washington.

Rostagno, H.S. and Pack, M. (1996). *Journal of Applied Poultry Research,* **5**: 150-154.

Saunderson, C.L. and MacKinlay, J. (1990). *British Journal of Nutrition,* **63**: 339-349.

Schutte, J.B., DeJong, J., Smink, W. and Pack, M. (1997). *Poultry Science,* **76**: 321-325.

Virtanen, E. and Rossi, L. (1995). *Proceedings. of the 8th Australian Poultry Science Symposium.* Ed. D. Balnave. pp. 88-92.

SYNTHETIC METHIONINE SOURCES INCREASE BREAST MEAT YIELD AND REDUCE ABDOMINAL FAT IN GROWING BROILER CHICKENS

I.R. WALLIS

Summary

Feeding broilers increasing amounts of DL-methionine (DL-met) and methionine hydroxy analogue (MHA) enabled the testing of three hypotheses: 1) birds fed diets balanced in amino acids (AA) yield more breast meat and less fat; 2) diets balanced in AA reduce variation in body mass (BM) and carcass parts; 3) DL-met is more potent than MHA. Birds ate more, grew faster and had lower FCR with increasing amounts of DL-met or MHA. However, variation in food intake did not explain all the variation in BM. Independent of BM, DL-met and MHA both increase breast meat yield and reduce abdominal fat (AFP). However, on an equimolar basis, MHA was only 78, 68 and 71% as potent as DL-met for growth, FCR and breast meat yield, respectively. Increments (0.5g/kg) of DL-met (but not MHA) above the amount of Met in the basal diet (ca. 3 g/kg) reduced variation in BM, breast and AFP mass.

I. INTRODUCTION

Methionine (Met) plays three key roles in vertebrate metabolism: as an essential AA, a precursor of cysteine and in methyl group transfer. These competing roles make Met ideal for studying the effect of AA balance on growth and carcass composition. This is because feeding a marginally deficient Met diet might have wide-ranging detrimental effects on the bird.

Much research links diet and carcass composition in broilers (see Summers and Leeson 1979). However, few reports study the effect of a single essential AA (EAA) and fewer still at dietary amounts from below to above the requirement. I could find no reports where researchers studied the response to one EAA and adjusted carcass data allometrically for treatment differences in BM. This surprises me because: 1) EAAs are costly; 2) the ideal balance of EAA presumably enhances growth and meat yields, reduces body fat and aids the selection of breeding progeny; and 3) balancing an animal's AA intake conserves resources.

Nutritionists add Met to broiler diets as either DL-met or MHA. These compounds differ in absorption processes (Maenz and Engele-Schaan, 1996), metabolism (Gordon and Sizer, 1965) and potency (Baker, 1994). Thus, either compound may alter dietary AA balance. If so, the sizes of a demand tissue, eg breast meat, and of an energy sink, eg AFP, may differ between birds fed DL-met or MHA. However, detecting these responses needs careful analysis. While regression equations can describe growth responses, many scientists try to remove the confounding effects of BM by analysing carcass parts as ratios of BM. This assumes that the mass of the part varies isometrically with BM. However, body parts often scale allometrically and should be compared using ANCOVA (Packard and Boardman, 1988).

In a 31d trial, I studied the broilers' responses to added DL-met or MHA, from below to above the Met requirements, to test three hypotheses: 1) supplementary sulphur AA increase breast meat yield and reduce the size of the AFP; 2) AA supplements reduce variation in BM and in the sizes of carcass parts; 3) DL-met is more potent than MHA.

Department of Biochemistry and Nutrition, SAC, Auchincruive, Ayr, Scotland KA6 5HW.
Present address: Division of Botany and Zoology, ANU, Canberra ACT 0200.

II. MATERIALS AND METHODS

Ross 1 broiler cockerels were fed a common starter diet with DL-met providing half the added Met (2.5g/kg) and MHA, the rest. On day 7 they were allocated to treatments (6 replicates of 50 birds in deep litter pens) in a completely randomised design. The nine treatments were, a basal diet deficient in Met and the basal supplemented with about 0.5, 1.0, 1.5 or 0.2 molar g/kg of active DL-met (Degussa AG) or MHA (Alimet®, Novus). The birds had free access to grower pellets. The composition of the basal diet (g/kg/DM) was 3.1 Met, 3.4 Cys, 36 nitrogen, 15.3 AME MJ/kg and fed between days 7 and 21 d, and the finisher pellets (2.7 Met, 2.8 Cys, 34 nitrogen, 15.8 MJ AME/kg from day 21 to 38. Birds were weighed individually on day 38, and 17 were randomly chosen from each pen, killed and their breast meat and AFP dissected and weighed.

(a) Statistical analysis

For the growth and food intake data I used ANOVA or ANCOVA followed by a least significant difference test to separate means. Linear regression showed that breast and AFP masses vary allometrically with BM minus breast mass (BMbrs) and BM minus AFP mass (BMafp), respectively. I first analysed breast and AFP mass by ANCOVA using the respective covariates BMbrs and BMafp (Packard and Boardman 1988). Secondly, I fitted an exponential model to measure the birds' responses to DL-met and MHA as follows:

$$Y = A + B[1 - e^{-C1(X1 + C2.X2)}] \qquad \textit{Equation 1}$$

where Y = average BM gain, FCR or breast meat yield; A = the intercept; B = the maximum response to either AA; $C1$ = the slope of the response curve for DL-met; $C2$ = the ratio of the MHA response to the DL-met response; and $X1$ and $X2$ = the intake of DL-met and MHA (BM gain and breast meat) or determined increment of DL-met or MHA above basal (FCR).

The coefficients of variation (CV) for the individual BM data and the masses of breast tissue and AFP were compared using an F-test (Lewontin, 1966). For this test I used data for individual birds (n = 117) but regarded CV's as significantly different when $P < 0.01$.

III. RESULTS

(a) Mortality, food intake, body mass and food conversion ratio

Two of 2900 birds died in the adaptation period. Average mortality during the trial was 2.3% (s.e. 0.34%), ranging from 0% (0.5g/kg DL-met) to 4.7% (1.0g/kg MHA) (P<0.05). The mass of dead birds did not differ between treatments (mean 1.1, SE 0.18 kg).

Table 1. Means and SD for food intake, BM, adjusted BM (for food intake) and FCR at 38d.

Treatment	Food intake (g/bird)		BM (g)		Adj BM (g)	BM CV %	FCR 7-38 d	
Basal	3102[a]	26	1492[a1]	25	1578[a]	16.6[e]	2.25 [f]	0.033
+0.5g/kg met	3273[b]	25	1680[b]	21	1721[b]	17.1[e]	2.09 [e]	0.020
+1.0g/kg met	3285[b]	14	1756[c]	6	1772[c]	13.7[cd]	1.99 [d]	0.045
+1.5g/kg met	3428[cd]	23	1979[e]	18	1948[e]	13.1[bc]	1.84 [ab]	0.032
+2.0g/kg met	3577[e]	34	2077[f]	25	2022[f]	11.5[a]	1.80 [a]	0.015
+0.5g/kg MHA	3354[bc]	20	1705[bc]	18	1720[b]	14.9[d]	2.10 [e]	0.015
+1.0g/kg MHA	3462[d]	47	1772[c]	20	1754[bc]	12.8[bc]	2.04 [de]	0.028
+1.5g/kg MHA	3524[de]	44	1888[d]	26	1849[d]	12.4[ab]	1.96 [cd]	0.013
+2.0g/kg MHA	3451[cd]	28	1919[de]	22	1904[e]	12.9[bc]	1.89 [b]	0.017

[1]Values with different superscripts are significantly different (P<0.05).

There was a stepwise increase in food intake within the DL-met diet series that was less distinct in birds fed diets with MHA. Birds fed 1.0g/kg MHA ate more than did those fed 1.0g/kg DL-met, but the opposite was true at the highest amounts of AA (Table 1). On day 7, birds weighed less than the breeder's guidelines (113 versus 180g). Each increment of DL-met produced a heavier bird and tended to reduce variation in BM (CV, Table 1). The response to MHA differed. Birds fed the two lower amounts of MHA had similar BM as did those fed the two upper amounts. The means of BM, normalised for food intake, show that each increment of DL-met or MHA increased BM. This confirms the differences in FCR (Table 1).

(b) <u>Breast meat and abdominal fat pad sizes (Table 2)</u>

ANCOVA showed that increasing amounts of dietary DL-met or MHA produced birds with more breast meat (both absolute and relative) and relatively less fat. Within the DL-met treatments, sequential increases (P<0.05) in relative breast meat occurred at the 0.5, 1.0 and 1.5g/kg DL-met, but adding 2.0g/kg DL-met led to no further increase. In contrast, each increment of MHA elicited a significant response in breast size (P < 0.05). Birds fed diets with 1.0 or 1.5g/kg DL-met had significantly heavier breasts than did birds fed 1.0 or 1.5g/kg MHA. The CVs for the carcass, breast meat and AFP masses all tended to decrease in stepwise fashion with each increment of DL-met but not with MHA.

Table 2.   Masses (mean, SD) of the breast and AFP (g) and CVs for carcass components in birds aged 38 days. The adjusted means are corrected to a mean BM with ANCOVA.

Treatment	Breast mass		Adj breast mass	CV Breast mass	AFP mass		Adj AFP mass	CV AFP mass
Basal	170^a	21	181^a	24^a	32.8^a	5.9	40.9^b	41^e
+0.5g/kg met	206^b	14	213^b	20^{ab}	39.1^{ab}	4.4	42.7^{ab}	35de
+1.0g/kg met	238^c	11	242^c	18^{bc}	39.4^{ab}	2.1	40.7^b	32^{cd}
+1.5g/kg met	279^{ef}	7	273^{ef}	15^{cd}	37.9^b	4.1	33.2^c	27^{ab}
+2.0g/kg met	294^f	12	283^f	14^d	39.9^b	3.1	32.9^c	23^a
+0.5g/kg MHA	210^b	18	212^b	21^{ab}	36.2^{ab}	4.2	38.9^b	31^{bcd}
+1.0g/kg MHA	230^c	10	232^c	17^{ab}	44.2^c	4.2	44.9^a	27^b
+1.5g/kg MHA	250^d	11	247^{cd}	16^{ab}	39.5^b	1.7	38.0^b	26^{ab}
+2.0g/kg MHA	265^e	16	259de	17^{ab}	36.2^{ab}	1.3	33.0^c	29^{bc}

(c) <u>Growth, FCR and breast meat response curves and confidence intervals (CI)</u>

Table 3.   Responses of broilers from 7 to 38 days to intake of DL-met and MHA (gain, breast mass) and to determined increments of DL-met or MHA above basal amounts (FCR).

Body mass gain = 1352 + 773*[1-e$^{-0.19*(met+0.78*MHA)}$]; r^2=0.90   *Equation 2*
Asymptote 95% CI: A = 1297-1404; B = 610-936; C1 = -0.097-0.29; C2 = 0.67-0.90

FCR = 2.26 - 0.61*[1-e$^{-6.20*(met+0.68*MHA)}$]; r^2=0.83   *Equation 3*
Asymptote 95% CI: A = 2.21-2.30; B = 0.37-0.85; C1 = 1.5-10.9; C2 = 0.54-0.82

Breast mass = 158 + 161*[1-e$^{-0.244*(met + 0.71*MHA)}$]; r^2=0.89   *Equation 4*
Asymptote 95% CI: A = 144-172; B = 134-187; C1 = 0.24-0.36; C2 = 0.59-0.83

Exponential equations (2-4) show that, for growth and FCR from 7-38 d, MHA is 78% and 68% as effective as DL-met. The model describing the change in the mass of breast meat with increasing intake of DL-met or MHA estimated MHA to be 71% as effective as DL-met.

IV. DISCUSSION

There is no obvious explanation for the poor growth of chicks in the rearing phase. There was evidence, though, of compensatory growth: by day 38 the heaviest broilers weighed more than the top quartile of birds in the Ross Breeders guideline.

The experiment proved the first hypothesis that feeding increasing amounts of dietary methionine will produce a bird with relatively more breast meat and relatively less fat. I chose breast meat as the response tissue because it accounts for up to 30% of the edible meat and as much as 50% of the edible protein on the carcass (Summers et al., 1988). Presumably, the carcass differences partially explain the lower FCRs at the highest amounts of dietary Met. Depositing protein uses less energy than depositing fat, mainly because protein deposition is accompanied by a large deposition of water. Clearly, apart from economic considerations, any improvements in animal efficiency reduces the flow of wastes to the environment.

The experiment also proved the second hypothesis that balancing available AA reduces variation in BM and carcass traits. This has implications for animal breeding in that feeding a diet with the optimal AA balance will reduce variability and increase the precision of selection. This confirms the finding of Marks and Britton (1978) that broiler strains fed diets with 18% protein responded to selection better than those fed diets with 14% protein. Reducing variation between birds has other advantages, eg. in processing. Although Met is often a limiting AA in poultry rations, it is only one of ten essential AAs. Presumably, optimal dietary amounts of all available AAs (ideal protein), will enhance growth and minimize variation between birds.

Regardless of whether the data is analysed with ANOVA or an exponential model I draw the same conclusion: that MHA is a less potent source of Met than is DL-met. This adds to the debate over efficacy that has lasted since the 1950s (see review by Baker 1994). The relative growth rates, food intakes and FCRs of birds fed either DL-met or MHA in this experiment agree closely with those of recent experiments of similar design (eg., Huyghebaert 1993). All show that DL-met is more effective than MHA. Based on equivalent amounts of active ingredient, MHA averages 75% of the potency of DL-met. However, commercial MHA is only 88% pure so based on weight it is 66% as effective as DL-met.

REFERENCES

Baker, D.H. (1994). In: Amino Acids in Farm Animal Nutrition. pp. 37-62. Ed. J.P.F. D'Mello. CAB International, Wallingford, UK.

Gordon, R.S. and Sizer, I.W. (1965). *Poultry Science,* **44**: 673-78.

Huyghebaert, G. (1993). *British Poultry Science,* **34**: 351-9.

Lewontin, R.C. (1966). *Systematic Zoology,* **15**: 141-2.

Maenz, D. and Engele-Schaan, C.M. (1996a). *Journal of Nutrition,* **126**: 529-36.

Marks, H.L. and Britton, W.M. (1978). *Poultry Science,* **57**: 10-16.

Packard, G.C. and Boardman, T.J. (1988). *Physiological Zoology,* **61**: 1-9.

Summers, J.D. and Leeson, S. (1979). *Poultry Science,* **58**: 536-42.

Summers, J.D., Leeson, S. and Spratt, D. (1988). *Poultry Science,* **68**: 241-48.

DIET SELECTIONS OF GROWING BIRDS OFFERED PAIRED-CHOICES OF ISO-ENERGETIC DIETS DIFFERING ONLY IN METHIONINE CONCENTRATIONS

G.N. HINCH, J.V. NOLAN, J.J. LYNCH and E.S. THOMSON

Summary

Forty-eight cockerels were offered choices between pairs of feeds formulated to differ only in methionine concentration, i.e. between high-low, high-medium or medium-low. The choices were offered after an initial exposure to the diets from four weeks of age, followed by a one week rearing period on a low methionine feed. The diets contained 1.7, 2.7 and 6.6 g methionine /kg for low, medium and high respectively. The birds on the high-low choice and medium-low choice showed an immediate preference for the higher methionine feed while the birds given the high-medium choice did not appear to exhibit a preference for either alternative.

I. INTRODUCTION

Hens given a choice between different feeds appear to be able to balance their diets. Forbes and Shariatmadari (1994) and Karunajeewa and Than (1984) have shown that birds can select a well-balanced diet from several imbalanced ones. Hens are also able to select from diets with different protein concentrations in order to maintain adequate protein intake (Kaufmann et al., 1978 and Steinruck et al., 1990). The latter workers suggested that the appropriate choice is made within 7 h of the ingestion of a particular feed.

It seems unlikely that animals would have specific ways of detecting 'protein' in its many and varied forms in the diet, but it is more likely that they might be able to detect specifically some individual amino acids. This has been demonstrated by Newman and Sands (1983) and Murphy and King (1989) who reported that birds are able to distinguish between diets that differed only in the content of specific essential amino acids. The present experiment was designed to determine whether layer bird strains can distinguish between feeds differing only in methionine content and, secondly, how that choice was affected by the levels of methionine in the feeds offered.

II. MATERIALS AND METHODS

Forty-eight Isa Brown cockerels were raised in a group on a chick starter crumble (Ridley's AgriProducts, Tamworth) from day-old until they were 4 weeks of age. The birds were then allocated at random to individual cages and maintained at temperatures between 24-27 °C. Each cage had two identical feed bins at the front and water was freely available from a trough at the back of each cage.

Three feeds with identical apparent metabolisable energy (AME) and nutrient concentrations were formulated to chick starter specifications (11.0 MJ/kg, 171 g/kg crude protein) except that their methionine contents were varied by including different amounts of synthetic DL-methionine in place of cornflower. The diets were designated either 'low', 'medium' or 'high' and contained respectively 1.7, 2.7, or 6.6 g methionine /kg, and were coloured yellow, red and green, respectively, using food dyes.

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

Three treatment groups were each assigned a dietary pair ('high-low', 'medium-low' or 'high-medium') representing the choice birds would be offered during a test period after they had been given a period of familiarisation on both diets. This period commenced when the birds were about 650g live-weight, and the 16 birds in each treatment group were allowed to become familiar with their two feeds during a period of 4 days of exposure to each. From Day 9 to Day 15, all birds were then offered a low-methionine chick starter diet with no added dye from both feeders (deficiency-feeding period). From Day 16 to Day 29, each treatment group was offered its pair of feeds as a choice from the two feeders.

Feed in both containers was weighed and topped up daily, and the intake for the previous 24h was calculated. Live weight of birds was recorded on 4 occasions, viz. at the start of the experiment, and after 15, 21 and 29 days.

Paired t tests were used to determine whether feed intakes had changed between the start and finish of the familiarisation period, deficiency period and choice period of the experiment. The proportion of the higher methionine feed selected in each treatment was determined and compared to random selection on each day of the choice period. A linear regression model was used to determine the relationship between methionine intake and liveweight gain during the choice phase of the experiment.

III. RESULTS AND DISCUSSION

Feed intake increased from Day 1 (26 ± se 3.06 g/d) to Day 8 (81 ± se 2.84 g/d) (P<0.001) and then declined between Day 9 (81 ± se 1.84 g/d) and Day 15 (73 ± se 1.90 g/d) (P<0.01), when all birds were given only the low methionine feed. Total intake subsequently increased for all treatments during the choice phase of the experiment from day 16 (74 ± se 3.02 g/d) to day 29 (79 ± se 2.99 g/d) (P<0.05) when the birds had access to a second feed of higher methionine concentration.

The proportion of the higher methionine feed selected from pairs within each choice treatment is shown in Figure 1. Birds offered a high-low combination ingested predominantly from the high methionine option (from the first day). Birds offered a medium-low combination also exhibited a preference for the higher methionine diet from the first day that choices were offered, ingesting around 80% of their intake from the medium diet. For the group with the choice between the high and medium-methionine feeds, intakes of the two feeds did not differ (P>0.05) from a random choice.

The combinations of the two feeds of different methionine content in each treatment produced a different total methionine intake 0.49 ± se 0.03 g, 0.40 ± se 0.04 g and 0.18 ± se 0.01 g methionine/bird per day for the high-low, high-medium and medium-low treatments, respectively. The mean intakes of methionine within treatments increased only slightly throughout the choice-feeding period and the gain in weight by the birds during the choice-feeding period did not differ between treatments. Weight gain was linearly related to total feed intake (r^2 = 0.64) and the mean feed conversion efficiency (0.42) did not differ between treatments.

In the deficiency-feeding period before the choice of feeds was offered, all birds received the same low-methionine diet and the decline in intake during this period suggests that the diet was imbalanced, and that methionine intake (0.12g/bird/day) was lower than required. Likewise during the period of choice-feeding, the medium-low group had the lowest methionine intake (mean dietary methionine concentration 2.4 g/kg; mean intake about 0.18 g/bird per day). This intake of methionine was only about 53% of the concentration (4.5 g/kg) recommended, yet intake and growth rate did not differ significantly from that of the other two treatments. We therefore conclude that the actual requirement for methionine for

this strain of birds, at this stage of growth, was about 0.15-0.18 g /bird per day, under the conditions present in our experiment.
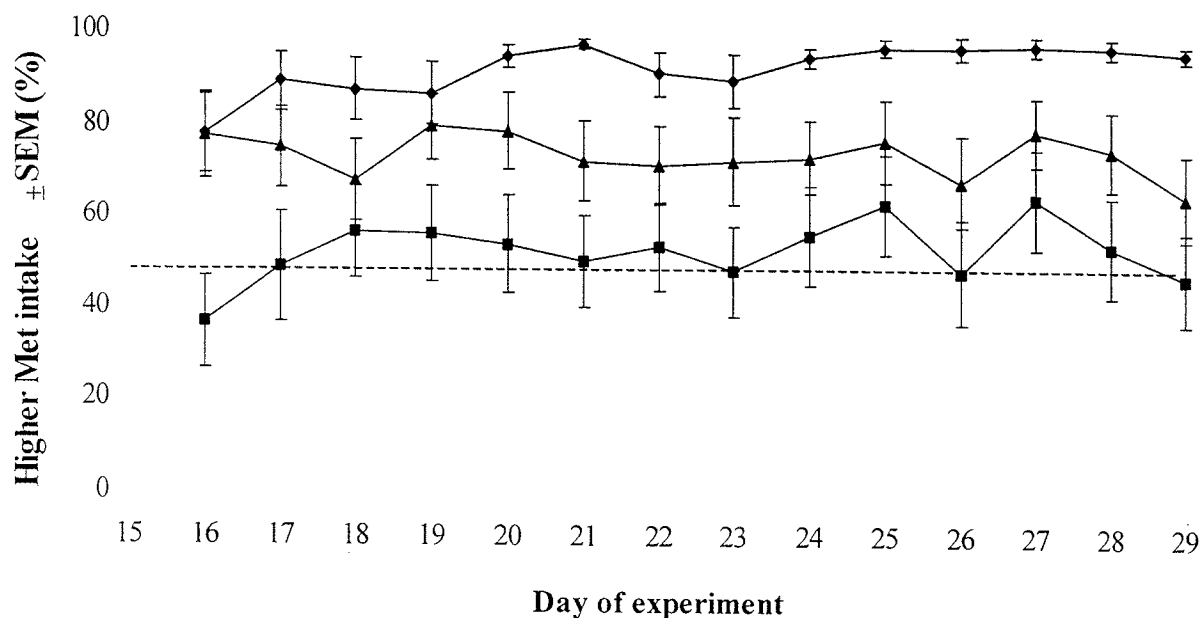
Figure 1.   The mean (± se) daily proportion of the higher methionine feed selected during the experimental period when the birds were given a choice between high and low methionine diets (♦), medium and low methionine diets (▲) and high and medium methionine diets (■). The dashed line indicates random selection.

The results of the choice-feeding tests suggest that the birds avoided the low-methionine feed (possibly because of an aversion formed during the familiarisation period and implied by the decreased food intake during the deficiency-feeding period). Both groups with the low-methionine feed as one of the alternatives during the choice feeding period avoided this feed or favoured the alternative familiar feed. An alternative explanation to aversion is that the birds were exhibiting, during the first day, sensory specific satiety (Provenza, 1996) which is the phenomenon occurring when animals fed exclusively on a feed will show a preference for an alternative familiar feed when given a choice. However the deficient feed was not coloured during the deficiency-feeding period and therefore the change in cues should have induced a similar response in all groups on the first day of choice. Given that choices were not similar for all treatments it seems likely that the initial rejection of the low-methionine feed, when the choice-feeding period commenced, was due to memory of the negative nature of this feed experienced during the familiarisation period.

However, the extent of difference in feed choices on day one between treatments when the low methionine diet was offered appeared to be dependent upon the degree of difference in methionine concentration between feeds, with birds offered the high-low choice selecting more strongly than those offered the medium-low combination. The birds given the high-low contrast showed a clear preference for the high methionine feed and the between-bird consistency by the end of the experiment was notable. In contrast the medium-low group, where the difference between feeds was less, showed a lesser preference for the medium feed. Moreover the between-bird variation in selection did not decrease over the period of the experiment, suggesting that a proportion of the birds may not show a preference for the

medium methionine diet. Four of the 16 birds consistently chose the low methionine diet but appeared to adapt to this diet by increasing intake (88 vs. 72g/day).

The high-medium group showed no preference for either feed possibly because neither of these feeds was deficient in methionine and therefore there would have been no basis for the birds to form any kind of negative or positive association with these feeds during the period of familiarisation.

There was no evidence of any adverse effects of higher intakes of methionine in this experiment as growth rates were directly related to feed intake and efficiency of feed conversion was similar in all groups. This is in keeping with other studies, where methionine toxicity has only been reported at intakes of 2 g or more per day (Benevenga, *et al.*, 1979).

It could be argued that these results fit a hypothesis that birds exhibit a 'specific appetite' for methionine. However, Booth (1985) argues that most preferences for specific nutrients are expressed because the animal has developed a specific aversion to the deficient diet included as the other option in the dietary choice and the present experiment does not refute such a observation.

IV. CONCLUSIONS

Chickens made clear choices between two virtually identical diets differing only in their colour and in methionine concentration. The feed avoidance appeared to be based on the requirements of individual birds and to be facilitated by large contrasts between the feeds. Further studies are needed to confirm the rapid rate of choice (within one day) is due to recognition of previous effects of the foods or is associated with an ability to detect differences in methionine directly.

REFERENCES

Benevenga, N.J., Smolin, L.A., Engstrom, M.A., Steele, R.D., Benes, I., Cifka, J., Ng, L.T., Pascaud, M., Griminger, P., Fisher, H., Khalil, A.A., Thomas, O.P., Combs, G.F., Hebert, J.A., Teekell, R.A., Watts, A.B., Hafez, Y.S., Chavez, E., Vohra, P.and Kratzer, F.H. (1979). *Journal of Nutrition*, 9: 1661-1663.

Booth, D.A. (1985). *Annals of New York Academy of Science*, **443**: 22-41.

Forbes, J.M. and Shariatmadari, F. (1994). *Worlds Poultry Science Journal*, **50**: 7-24

Karunajeewa, H. and Tham,S.H. (1984). *British Poultry Science*, **25**: 99-109.

Kaufmann, L.W., Collier, G. and Squibb, R.L. (1978). *Physiology and Behaviour*, **20**: 339-344.

Murphy, M.E. and King, J.R. (1989). *Physiology and Behaviour*, **45**: 423-430.

Newman, R.K. and Sands, D.C. (1983). *Physiology and Behaviour*, **31**: 13-20.

Provenza, F.D. (1996). *Journal of Animal Science*, **74**: 2010-2020.

Steinruck, U., Roth, F.X. and Kirchgessner, M. (1990). *Archivs fur Geflugelkunde*, **54**: 173-183.

THE INFLUENCE OF MULTI-COMPONENT PECTINASE ENZYMES ON ENERGY AND AMINO ACID AVAILABILITY IN VEGETABLE PROTEINS

W. D. COWAN[1], D. R. PETTERSSON[1] and P. B. RASMUSSEN[2]

Summary

The influence of a multi-component pectinase enzyme upon nutrient availability has been studied in a balance trial and then a performance trial in broiler chickens. Energy and amino acid availability was increased in a range of vegetable proteins through enzyme addition. In a subsequent feeding study, diets were formulated with reduced levels of lysine and sulphur amino acids and it was observed that enzyme supplementation could partly compensate for this reduction.

I. INTRODUCTION

The cell wall composition of vegetable proteins differs from that of wheat and barley, both in chemical and organisational terms. The main cell wall polymers of plant cell walls were described by Bacic et al. (1988) as being cellulose, xylan, mannan, xyloglucan, mixed link beta glucan, pectic polysaccharides and lignin. For the primary cell wall, cellulose is found together with a matrix of other polysaccharides. In mono-cotyledenous plants, e.g. wheat and barley, this is typically xylan or beta glucan and in dictoyledenous plants, Pectic compounds and xyloglucan are more prevalent (Düsterhöff, 1993). Enzymes which should degrade such plant walls will then need to contain enzyme activities appropriate to the substrates which will be encountered.

This paper reports results from balance trials in which a multi-component pectinase enzyme was used to influence the availability of energy and amino acids in different vegetable protein sources. A feeding study was then used to investigate whether or not the ability of the enzyme preparation to improve amino acid availability could be used in diet formulations.

II. MATERIALS AND METHODS

(a) Balance trials

The availability of amino acids and energy in several vegetable protein sources was determined using a modification of the method of Bourdillon et al. (1990). In this method, digestibility of nutrients derived from the differences in these parameters obtained with a basal diet and with a diet in which one of the test proteins has replaced a portion of the basal diet. In the present study, the test materials were included in the diet at 250 g/soya meal or 250 g/kg sunflower or rape seed. Diet composition is shown in Table 1. Each treatment group consisted of four cages of eight female broiler chicks.

At 14 days of age birds were assigned to each of 40 cages and the appropriate test diet introduced. After a 10 day acclimatisation period, all excreta were collected over 96 hours. Birds were fed *ad libitum* except for a six hour fasting period before the start and the end of

[1]Novo Nordisk S.A. 282 Chartridge Lane, Chesham, Bucks HP5 2SG, UK.
[2]Novo Nordisk A/S, DK 2880 Bagsvaerd, Denmark.

the collection period. Excreta samples were pooled, stored at -20 °C and analysis performed on the freeze dried pooled excreta.

Table 1   Composition of basal diet for balance trials.

Component	Content (g/kg)	Component	Content (g/kg)
Maize	500.0	L-lysine HCl	1.10
Soyabean flour (500 g CP)	190.0	DL-methionine	2.50
Full fat soyabean	100.0	Limestone	10.2
Fish meal	30.0	Mono-calcium P	9.00
Tapioca	77.5	Salt	3.00
Animal fat	20.0	Vit/mineral mix	10.00
Soyabean oil	30.0	Diamol[1]	16.70

[1]Diamol is a proprietary insoluble inorganic marker used in balance studies

(b) Feeding study

The ability of the enzyme to improve amino acid digestibility was also studied in a broiler experiment. A total of 2070 day old broilers were sexed and divided into pens, each containing 32 males and 37 females. Broiler diets were formulated to be iso-caloric (13 MJ/kg) but to contain a low (9.8 g), medium (11.6 g) or high level of lysine (14.3 g /kg. Methionine content was also modified in a similar manner. For each diet one group of five pens received the standard diet: a second group of five pens the same diet plus 500 mg/kg pectinase. To limit potential inaccuracies in diet composition resulting from too many diet variations, a single diet was used throughout the feeding period for each group. Diet compositions are shown in Table 2.

Table 2.   Diet compositions for feeding experiment (g/kg diet).

Nutrient	Low Lysine	Normal Lysine	High Lysine
Sorghum	415.0	415.0	415.0
Wheat	100.0	100.0	100.0
Maize	100.0	100.0	100.0
Soyabean meal	221.6	220.4	204.0
Rapeseed (00)	50.0	50.0	50.0
Fish meal	32.0	37.0	50.0
Animal fat	40.0	30.0	30.0
Soya oil	6.0	10.0	10.0
Di-calcium phosphate	16.6	16.0	14.8
Limestone	4.5	4.6	4.4
Salt	2.9	2.9	2.9
DL-Methionine	1.4	2.5	3.9
L-Lysine Hl	-	1.5	4.0
Vitamins, premix, growth promoter	10.24	10.24	10.24
Energy (MJ AME/kg)	12.98	12.93	13.04
Crude protein	190.2	193.2	195.0
Lysine	9.9	12.0	14.0
Methionine	4.5	5.7	7.2
Calcium	7.9	7.9	7.9
Phosphorus (av)	3.5	3.5	3.5

All parameters were subjected to a 3-factorial analysis of variance using Statgraphics 6.0. An additional analysis of means of using LSD test at P<.005 was also made.

III. RESULTS

The results of dietary amino acid digestibility for all amino acids and for energy level following enzyme supplementation are shown in Table 3. Enzyme supplementation increased dietary amino acid and energy availability on all raw materials tested. When the effect on the individual amino acids was examined, it could be seen that the effect on digestibility was uniform and no specific amino acid was increased above the others.

The results of the growth experiment are shown in Tables 4 and 5. Reduction in lysine levels reduced growth and resulted in a poorer FCR, with the highest efficiency seen at the high lysine addition level. Supplementation with pectinase improved performance for the low and normal lysine level diets, but it had no effect on diets containing the highest lysine level. Mortality tended to be higher in the groups receiving the highest lysine levels but this was not affected by Pectinase addition.

Table 3.   Effect of enzyme on diet amino acid digestibility for diets containing different protein sources and derived raw material AMEn.

		Basal - enz	Basal + enz	Soya - enz	Soya + enz
Amino digestibility	acid	83.7 %± 0.6	84.1 %± 0.5	80.4 %±0.5	83.4 %± 1.8
AMEn (MJ/kg)		-	-	8.1 ± 0.4	8.8 ± 0.6
		Sunflower - enz	Sunflower + enz	Rape seed - enz	Rape seed + enz
Amino digestibility	acid	82.7 %± 1.0	84.5 %± 0.9	81.2 %± 0.8	83.1 %± 0.6
AMEn (MJ/kg)		5.7 ± 0.7	6.3 ± 0.8	12.3 ± 0.7	13.4 ± 0.7

Table 4.   Probability values from ANOVA for body weight, feed conversion, daily growth and feed intake (analysis at 6 weeks of age).

Source of variation	Body weight	Feed conversion	Daily growth	Daily feed intake
Ration (R)	***[1]	***	***	***
Pectinase (E)	0.23	0.02	0.23	0.48
Sex (S)	***	0.02	0.23	0.48
R x E	0.39	0.028	0.39	0.27
R x S	***	0.18	***	0.004
E x S	0.09	0.82	0.09	0.26
R x E x S	0.9	0.76	0.9	0.89

[1]*** : p<0.0001

Table 5.   Effect of lysine and pectinase on broiler growth in iso caloric diets containing different levels of amino acids.

	Low lysine	Low lysine + pectinase	Normal lysine	Normal lysine + pectinase	High lysine	High lysine + pectinase
FCR	1.956 [a]	1.900 [a]	1.855 [b]	1.833 [b]	1.810 [c]	1.819 [b]
Final wt (kg)	1.883	1.897	2.051	2.083	2.088	2.081
Mortality	7.4 %	9.9 %	11.9 %	10.5 %	17.2 %	14.5 %

Means without a common superscript are significantly different at $P<0.05$.

IV. CONCLUSIONS

Addition of multi-component pectinase enzymes can improve nutrient availability in diets containing vegetable proteins. By selecting enzymes with activity against the polysaccharides found in these raw materials improved nutrient availability can be achieved. The magnitude of the energy improvement is related to the protein source used and can be expected to show some variation according to the quality of the particular raw material.

This improvement in digestibility will not only improve diet quality but can reduce pollution by increasing retention of nitrogen and other organic materials. Adjustment of dietary nutrient levels will also result in an improved overall utilisation of raw materials but care should be taken to formulate diets correctly to take advantage of this observation.

REFERENCES

Bacic, A., Harris, P.J. and Stone, B. A. (1988). In: *The Biochemistry of Plants. A Comprehensive Treatise.* **Vol. 14.** pp 297-371. Ed J. Preiss. Academic Press, San Diego.

Bourdillon, A., Carré, B., Conan, L., Duperray, J., Huyghebaert, G., Leclercq, B., Lessire, M., Mcnab, J. and J. Wiseman, (1990). *British Poultry Science*, **31**: 557-565.

Düsterhöft, E. M. (1993). Characterisation and Enzymatic Degradation of Non-Starch Polysaccharides in Lignocellulosic By-Products. Ph.D. Thesis University of Wageningen, The Netherlands

EFFECTS OF COMMERCIAL ENZYMES ON WET DROPPINGS IN FOUR STRAINS OF LAYERS FED A BARLEY-BASED DIET

M. CHOCT

Summary

An experiment was conducted to examine the effect of four commercial enzyme products on wet droppings and egg production in four strains of birds (Isa, Hyline CB, Tegel SB2, Tegel HiSex) fed a barley-based diet. Average total egg production was 89.8% and was not affected by any of the treatments. The enzymes markedly ($P<0.01$) reduced feed consumption. The CB birds consumed significantly ($P<0.01$) less feed than all the others. The percentage of non-saleable egg production was 8.9% of the total and was affected ($P<0.01$) by diet, but not by breed. The percentage of dirty eggs averaged 2.4% and was not affected by diet or breed. But broken eggs made up 6.5% of the total production and diet significantly ($P<0.01$) influenced it and breed of birds tended to have an effect ($P=0.053$) as well. There was also a significant ($P<0.01$) diet x breed interaction with responses of enzymes in broken egg percentage differing between breeds of birds. The excreta moisture content differed widely ($P<0.01$) due to both diet and breed. Thus, birds fed the control diet and HiSex birds in general had the wettest excreta among the five diets and four strains tested. The excreta from birds fed this diet also appeared very moist and runny. There was a significant ($P<0.01$) diet x breed interaction on this parameter as well with some breeds of birds responding differently depending on the enzyme source.

I. INTRODUCTION

Watery and sticky droppings in layers have been a problem in the egg industry for years. The major loss to the industry is through increased percentage of dirty eggs, management and hygiene problems such as increased gases and odour in the shed. In tiered cage systems fitted with deflectors or scrapers, wet droppings can be particularly problematic. The wet dropping problem in layers is multifaceted. Factors including nutrition, diuresis, strains, kidney damage from infectious bronchitis or mycotoxins, contaminants in the water and leakage in water troughs all contribute to wet droppings in layer sheds. It is well understood in broilers that increased dietary non-starch polysaccharides (NSP) cause watery and sticky droppings because these polymers increase gut viscosity and hold a large amount for water. Nowadays, NSP-degrading enzymes are routinely used in broiler diets to improve the nutritive value of rations and to alleviate watery and sticky dropping problems (Bedford and Classen, 1992; Choct *et al.*, 1995). The use of enzymes in layer diets is not as widespread as in broilers since increase in egg production due to enzyme supplementation is not often achieved. However, many layer farmers are starting to use enzymes to address wet dropping problems, especially where a tiered cage system is used and the birds are fed diets based on viscous grains (wheat, barley and triticale).

The current experiment was conducted to examine the efficacy of commercially available feed enzymes in reduction of excreta moisture levels and in enhancing production in four strains of laying hens fed a barley-based diet.

School of Rural Science and Natural Resources – Animal Science, University of New England, Armidale, NSW 2351, Australia.

II. MATERIALS AND METHODS

A 4 x 5 factorial experiment was conducted to examine the effect of four commercial enzyme products (basal, enzymes 1 to 4) and four strains of birds (IsaBrown, Hyline CB, Tegel SB2, Tegel HiSex) on wet droppings and egg production. There were thirty replicates of two birds per cage for each dietary treatment. The experiment lasted 6 weeks with a one-week adaptation period and a five-week experimental period. Weekly excreta moisture was determined by collecting droppings twice during a week and drying them at 80°C in a force-air oven. Collectable, broken and dirty eggs were separately recorded daily for total egg production. Weekly feed intake was determined by measuring feed residues in the feeders once a week. Egg quality parameters were also determined by collecting eggs over two days during the last week of the trial (Roberts et al., 1999). The enzymes were supplied by four different commercial companies and were recommended to be used in barley-based layer diets. The diets were formulated with the enzymes added "over the top" at the manufacturers' recommended dosage levels and manufactured by Ridley AgriProducts, Tamworth (See Table 1 for diet composition). The experiment was approved by the Animal Care and Ethics Committee of the University of New England and was conducted at the Laureldale Poultry Research Station of the University of New England.

Table 1.   Composition of experimental basal diets (g/kg).

Barley (100 g CP)	633.4	Meat meal (500 g CP)	110.0
Tallow	20.0	Canola meal (360 g CP)	16.7
Cottonseed meal (410 g CP)	40.0	Soybean meal (480 g CP)	40.0
Rice pollard (130 g CP)	75.0	Limestone	61.7
Salt	1.2	Choline chloride (750 g CP)	0.3
Alimet	0.5	Premix	1.2

Calculated AME and key nutrients

AME (MJ/kg)	11.2	Protein	170.0
Calcium	36.0	Available P	6.9

III. RESULTS

This trial was conducted when the birds were between 37-42 weeks of age. Average total egg production was 89.8% and was not affected by any of the treatments. Daily feed intake was markedly influenced by both diet (P<0.01) and breed (P<0.05). Thus, birds fed the control diet consumed a larger amount of feed than those fed the enzyme-containing diets. Hyline CB consumed less feed than all the other strains. The percentage of dirty eggs was 2.4% and was not influenced by diet and breed. The percentage of broken eggs was 6.5% of the total and was markedly affected by diet (P<0.01) and tended to be affected by breed (P =0.053). There was also a significant (P<0.01) diet x breed interaction with responses of enzymes differing between breeds of birds. The excreta moisture content differed widely (P<0.01) due to both diet and breed. Enzymes significantly (P<0.01) reduced it. There was also a significant (P<0.01) diet x breed interaction on this parameter as well with some breeds of birds responding differently depending on the enzyme source. Data are shown in Table 2.

Table 2.   Total, broken and dirty egg production, feed intake (g/bird/d) and excreta moisture (EM) of four strains of birds fed a barley diet with four commercial enzyme products.

		Total lay (%)	Broken (%)	Dirty (%)	Intake	EM (%)
Diet						
	Control	89.7[a]	6.5[b]	2.4[a]	110.8[a]	72.1[a]
	Enzyme 1	90.7[a]	5.1[b]	2.1[a]	106.2[b]	70.8[c]
	Enzyme 2	89.9[a]	8.4[a]	2.6[a]	106.0[b]	71.7[ab]
	Enzyme 3	89.9[a]	6.7[ab]	2.5[a]	108.4[b]	71.6[ab]
	Enzyme 4	88.3[a]	5.7[b]	2.4[a]	105.8[b]	71.2[bc]
	Pool SE	0.95	0.63	0.31	1.09	0.25
Breed						
	IsaBrown	89.4[a]	7.5[a]	2.2[a]	107.5[a]	71.4[b]
	Hyline CB	89.5[a]	5.3[b]	2.4[a]	104.9[b]	71.2[b]
	Tegel SB2	90.0[a]	6.9[a]	2.5[a]	108.8[a]	71.0[b]
	Tegel HiSex	88.8[a]	6.4[ab]	2.4[a]	109.2[a]	72.2[a]
	Pool SE	0.85	0.56	0.28	0.98	0.22
Diet x Breed						
Control	IsaBrown	89.5	9.2	2.4	113.9	72.2
	Hyline CB	90.8	3.8	2.0	106.6	71.8
	Tegel SB2	89.5	7.9	2.3	108.0	71.1
	Tegel HiSex	88.8	5.3	2.8	114.5	73.1
Enzyme 1	IsaBrown	91.0	4.0	1.1	103.6	70.5
	Hyline CB	91.4	5.5	2.0	105.4	69.9
	Tegel SB2	89.1	6.5	3.2	108.0	70.5
	Tegel HiSex	91.3	4.7	2.0	107.8	72.4
Enzyme 2	IsaBrown	90.0	8.0	1.5	103.2	70.6
	Hyline CB	91.6	10.5	2.8	105.3	71.6
	Tegel SB2	88.3	8.4	3.5	109.7	72.8
	Tegel HiSex	89.7	7.0	2.4	105.8	71.7
Enzyme 3	IsaBrown	89.6	7.9	3.0	107.3	71.5
	Hyline CB	90.4	3.1	1.9	104.1	72.1
	Tegel SB2	90.5	7.2	2.6	108.2	70.6
	Tegel HiSex	89.0	8.7	2.4	113.9	72.1
Enzyme 4	IsaBrown	89.3	8.4	3.0	109.3	72.4
	Hyline CB	89.7	3.9	3.2	103.2	70.5
	Tegel SB2	90.5	4.6	1.1	106.4	70.1
	Tegel HiSex	83.7	6.2	2.6	104.1	71.7
Probability Values						
Diet		0.488	0.006	0.854	0.001	0.003
Breed		0.385	0.053	0.830	0.014	0.009
Diet x breed		0.611	0.012	0.101	0.125	0.002

[ab] Means within a column for diet or breed with the same superscript do not differ at $P<0.05$.

IV. DISCUSSION

Surprisingly, a diet containing 63% barley supported excellent egg production; this approached 90% and the percentage of dirty eggs was low (2.4% vs 5.1% reported by Hadorn and Gloor, 1995). None of the four enzymes used in the current trial had any effect on the total egg production or proportion of dirty eggs. This is somewhat unexpected since other studies have demonstrated significant increases in egg production (Kumar et al., 1997) and reduction in dirty eggs (Hadorn and Gloor, 1995). It is, however, worth noting that the total egg production in the current trial was high. The number of broken eggs differed widely (P<0.01) due to diet and breed of birds. For example, for the Hyline CB, the number of broken eggs made up 3.8% of the total egg production for birds fed the control diet, but it was 10.5% for birds fed Enzyme 2. The reason for this interaction may be because of the short experimental period of only 6 weeks for the enzymes to have a clear effect. The increased number of broken eggs did not appear to be due to poor eggshell quality (Roberts et al., 1999). Also some enzyme suppliers recommend an energy uplift from enzyme addition to the diet. Whether the current "over the top" application caused an energy : protein imbalance is not known. Whatever the reason, any conclusion to be drawn as to the real significance of this finding may have to await further, perhaps, longer term studies.

The feed intake of the birds were not high, but the enzymes still markedly (P<0.01) reduced it from 110.8 g/d to 106.6 g/d giving a small saving in feed cost. The Hyline CB had the lowest intake of all the strains compared. The moisture level of the excreta from birds given the control diet was generally lower than expected in a high-barley diet, but enzyme supplementation further reduced (P<0.01) it. The droppings from HiSex birds were wetter (P<0.01) than all the other strains. There was also a significant (P<0.01) diet x breed interaction for excreta moisture. The interaction between enzymes and bird strains is highly complex which makes it difficult to draw a clear conclusion.

V. ACKNOWLEDGEMENTS

REFERENCES

Bedford, M. and Classen, H. (1992). Journal of Nutrition, **122**: 560-569.

Choct, M., Hughes, R. J., Trimble, R. P., Angkanaporn, K. and Annison, G. (1995). Journal of Nutrition, **125**: 485-92.

Hadorn, R. and Gloor, A. (1995). In: Proceedings of the 2nd European Symposium on Feed Enzymes, p.289. Eds. W. van Hartingsveldt, J.P. van der Lugt, and W.A.C. Somers. Zeist, The Netherlands.

Kumar, A. Dingle, J. and Creswell, D. (1997). In: Recent Advances in Animal Nutrition in Australia, p. 226. Eds: J.L. Corbett, M. Choct and J.B. Rowe. Armidale, NSW.

Roberts, J.R., Choct, M. and Ball, W. (1999). Australian Poultry Science Symposium, **11**: (in press).

THE INFLUENCE OF CARBOHYDRASE AND PROTEASE SUPPLEMENTATION ON AMINO ACID DIGESTIBILITY OF LUPIN-BASED DIETS FOR BROILER CHICKS

A NAVEED[1], T ACAMOVIC[1] and M R BEDFORD[2]

Summary

An experiment was conducted to evaluate the effectiveness of xylanase, protease and cellulase on the nutritive value of lupin based diets for young broiler chicks. The lupins used were *Lupinus albus* which were cultivated in the UK and incorporated as a ground mash at 400g/kg of diet. Apparent ileal digestibility coefficients for amino acids in the diet were about 0.85 for the untreated and protease treated diets but about 0.7 for the carbohydrase treated diets. The effects of enzymes on digestibility coefficients of amino acids did not reflect animal performance.

I. INTRODUCTION

Europe is not self-sufficient in protein for inclusion in animal diets and currently there is considerable consumer demand around the world, especially in the UK and Europe, to produce diets for animals that are free from animal proteins. The major leguminous component that contributes protein in poultry diets is soya bean meal. Other protein sources which are potential alternatives to soya bean meal include the seeds from legumes such as peas, beans and lupins which grow well in temperate and Mediterranean climates (Carre, 1997). They are also considered as environmentally friendly crops, fixing nitrogen. Of the three legume seeds mentioned, lupins tend to have the highest content of protein at 300-450 g/kg dry matter. They are thus of considerable interest to arable farmers in Europe as an alternative crop and also to nutritionists as a source of protein for inclusion in monogastric diets.

Of the cultivars that are grown commercially, *Lupinus angustifolius* is produced in greatest quantity and primarily in Australia. *Lupinus albus* tends to have a higher protein content and yield than *L. angustifolius* and has been grown successfully in France and the UK. Low alkaloid cultivars of lupins have relatively few compounds that exhibit toxic or antinutritional characteristics. A limitation of legume seeds in general and lupins in particular, however, is their relatively high content (300-400g/kg) of a mixture of complex non-starch polysaccharides (NSPs). These compounds tend to have adverse effects when ingested by poultry (Carre, 1997). The major NSPs in lupins are α-galactosides and these vary in composition between lupin cultivars. They are attributed with adverse effects on intake of diets and digestibility of nutrients (Carre *et al.*, 1985; Evans *et al.*, 1993; Carre, 1997; Naveed *et al.*, 1998; Ferraz de Oliveira, 1998). Other effects of increased dietary NSPs include increasing the viscosity of intestinal contents, increased litter moisture content and alteration of the profile of intestinal and litter microflora (Ferraz de Oliveira *et al.*, 1994; Carre, 1997; Ferraz de Oliveira, 1998; Bedford and Schulze, 1998; Rubio *et al.*, 1998).

Although the content of antinutritional and toxic proteinaceous compounds in lupins is very low the proteinaceous components of lupins tend to be somewhat refractory and thus

[1]Department of Biochemistry and Nutrition, National Centre for Poultry Studies, SAC, Ayr, Scotland, KA6 5HW, UK.
[2]Finnfeeds International Ltd, Market House, High St., Marlborough, Wiltshire, SN8 1AA.

tend to have relatively low digestibility coefficients (Cerletti *et al.*, 1983; Ferraz de Oliveira and Acamovic, 1995; Carre, 1997; Ferraz de Oliveira, 1998).

The use of supplementary dietary enzymes in poultry diets to reduce the adverse dietary effects of NSPs and proteins in poultry diets is used widely with varying degrees of success (Bedford and Schulze, 1998). The success of such enzyme supplementation of lupin-based diets for poultry has had varying degrees of success in improving animal performance and nutrient utilisation and appears to be dependent on the type and quantity of lupins used in the diets as well as the enzymes used (Bryden *et al.*, 1994; Ferraz de Oliveira and Acamovic, 1995; Annison *et al.*, 1996; Ferraz de Oliveira, 1998; Naveed *et al.*, 1998).

Since *Lupinus albus* has been produced in high yields in the UK, an experiment was conducted to investigate the efficacy of the use of carbohydrases and proteases in lupin-rich diets for broilers.

II. MATERIALS AND METHODS

The animal experiments were approved by the Animal Ethics Committee of SAC. Diets were formulated to meet the requirements of young broilers (NRC, 1994) and were designed to be marginally adequate in nitrogen and energy while being isonitrogenous and isoenergetic. The diets were formulated to contain 400g lupins/kg diet.

The lupins were grown in the UK and obtained from Dr. Ian Shield, IACR, Rothamstead, Herts. The enzymes used were Protease- P_3X (from a selected strain of Bacillus), Xylanase-GC, Cellulase-BG (from controlled fermentation of selected Trichoderma species) and a mixture of the above three enzymes. The activity per gram of the product as determined was 100,000 protease units, 40,000 xylanase units and 4500 cellulase units respectively. The enzymes were added as supplements to the diets at 0.2, 0.05 and 0.5 g/kg respectively. One hundred and twenty 11-day-old, male broilers were randomly distributed among five treatments, using four birds per cage and six cages per treatment in such a way that the weight range was minimised within each tier.

Birds were fed the diets for 21-days (from 11 to 32 days of age) after which time the birds were euthanased by an overdose of pentobarbitone and a 30cm section (immediately anterior to the ileocaecal junction) of the ileum removed. The contents were carefully washed out of the intestines with distilled water into a petri dish, frozen, lyophilised and ground prior to analyses for Cr by atomic absorption of the oxidised Cr, and amino acids after hydrolyses, by HPLC. Digestibility coefficients of the amino acids were calculated and subjected to ANOVA according to the GLM procedure (Minitab 10.5).

III. RESULTS AND DISCUSSION

All birds survived the experimental period in good health. Apparent ileal amino acid digestibility coefficients are presented in Table 1.

The apparent digestibility coefficients of lysine cystine and the dispensable, indispensable and total amino acids are relatively low but similar to those published elsewhere (Ferraz de Oliveira and Acamovic, 1995; Carre, 1997; Ferraz de Oliveira, 1998). This is likely to strongly reflect the digestibility coefficients of the amino acids in lupins since lupin protein contributed about 76% of the protein to the diets. The digestibility coefficients for methionine tended to be somewhat higher than the others, but this may reflect the fact that the diets were supplemented with synthetic methionine. Diet 2, where protease was added, had a significantly ($p<0.05$) higher digestibility coefficient for methionine than that for the

Table 1. Apparent ileal digestibility coefficients of some individual, dispensable (Disp) and indispensible (Indisp.)amino acids in lupin-based diets with and without treatment with protease, xylanase and cellulase.

Treatment[1]	Lysine	Methionine	Cystine	Disp.	Indisp.	Total
1	0.785	0.967	0.789	0.849	0.830	0.841
2	0.847	0.975	0.806	0.868	0.845	0.856
3	0.809	0.922	0.673	0.783	0.737	0.762
4	0.628	0.909	0.567	0.741	0.703	0.724
5	0.603	0.862	0.546	0.718	0.655	0.686
LSD[2]	0.159	0.039	0.074	0.102	0.147	0.125
P=	0.007	<0.001	<0.001	0.016	0.046	0.032

[1]Treatments: diets are maize/soyabean/lupin (400g/kg) mash. 1, lupin; 2, lupin supplemented with protease; 3, lupin supplemented with xylanase; 4, lupin supplemented with cellulase; 5, lupin supplemented with protease, xylanase and cellulase.
[2]Least significant difference (P<0.05).

carbohydrase supplemented diets. Supplementation of the diets with the cellulase reduced the digestibility coefficients of the amino acids compared to the unsupplemented diet or the diet supplemented with protease alone.

The apparent ileal digestibility coefficients of the amino acids presented here do not reflect the growth data where growth, intake and efficiency of feed conversion were all much higher for the diets which were supplemented with xylanase and cellulase compared to the unsupplemented diet and that supplemented with protease (Naveed *et al.*, 1998). It thus appears that the xylanase and cellulase may have improved the palatability of the diets by reducing the viscosity and water holding capacity of the diets, thus allowing increased intakes. The increased intake of diet would thus result in a large increase in the intake of digestible amino acids thereby more adequately meeting the requirements of the birds for amino acids, and energy. This increased intake, and thus throughput of diet, may encourage the proliferation of microbes in the lower gastrointestinal tract which may contribute to endogenous losses of amino acids thus reducing the apparent digestibility coefficients. Further work should assist in elucidating the apparently anomalous data.

IV CONCLUSIONS

The nutritional value of *Lupinus albus* for poultry grown in the UK is poor but can be enhanced by enzyme treatment. Treatment of lupin-based diets with proteases has a beneficial effect on the digestibility coefficients of amino acids, whereas supplementation with xylanase and cellulase appears to have a detrimental effect on the apparent ileal digestibility coefficients of amino acids, despite improving animal performance. The detailed mechanisms whereby these effects on lupin-based diets are exhibited require elucidation.

REFERENCES

Annison, G., Hughes, R.J. and Choct, M. (1996). *British Poultry Science,* **37**: 157-162.
Bedford, M.R. and Schulze, H. (1998). *Nutrition Research Review,* **11**: 91-114.
Bryden, W.L., Gill, R.J. and Balnave, D. (1994). *Proceedings Australian Poultry Science Symposium,* **6**: 115.

Carre, B. (1997). *Proceedings Australian Poultry Science Symposium,* **9**: 46-53.

Carre, B., Brillouet, J.M. and Thibault, J.F. (1985). *Journal of Agriculture and Food Chemistry,* **33**: 285-292.

Cerletti, P. Restani, P., Duranti, M., Marriana, A and Semino, A. (1983). In: *Perspectives for Peas and Lupins as Protein Crops.* pp. 308-321. Eds. R. Thomson and R. Casey. Martinus Nijhof, London.

Evans, A.J., Cheung, P.C.K. and Cheetham, N.W.H. (1993). *Carbohydrate Polymers,* **22**: 37-47.

Naveed, A., Acamovic, T. and Bedford, M.R. (1998). *British Poultry Science,* **39**: (in press).

Ferraz de Oliveira, M.I. (1998). Enzyme Treated *Lupinus spp.* Seeds as an Alternative Source of Protein for Broilers. PhD thesis, Aberdeen University, Aberdeen, Scotland, UK.

Ferraz de Oliveira, MI, Hillman, K. and Acamovic, T. (1994). In: Plant Associated Toxins. pp. 195-200. Eds. S M Colegate and P R Dorling. CAB International, Wallingford, UK.

Ferraz de Oliveira, M.I. and Acamovic, T. (1996). In: Protein Metabolism and Nutrition, pp. 185-187. Eds. A.F. Nunes, A.V. Portugal, J.P. Costa and J.R. Ribeiro, EAAP Publication 81. Santarem, Portugal.

Rubio, L.A., Brenes, A., Setien, I., Asuncion, G. de la, Duran, N. and Cutuli, M.T. (1998). *British Poultry Science,* **39**: 354-359.

AN EVALUATION OF MICROBIAL PHYTASE IN SORGHUM-BASED BROILER DIETS.

P. H. SELLE[1], V. RAVINDRAN[1], P.H.PITTOLO[2] and W. L. BRYDEN[1]

Summary

The effects of microbial phytase on performance, toe ash content, nitrogen retention and apparent metabolisable energy were assessed in standard and modified sorghum-based broiler diets. Protein, amino acid, energy and phosphorus specifications, and ingredient costs were reduced in the modified diet. On this diet the feed enzyme significantly (P <0.05) increased weight gain, feed conversion efficiency, nitrogen retention and apparent metabolisable energy. The performance responses of birds on the modified plus phytase diet, relative to the standard diet, suggest that microbial phytase has the potential to reduce dietary specifications and feeding costs.

I. INTRODUCTION

From 1991 microbial phytase has been included in Dutch pig and poultry rations as a feed enzyme that released phytate-bound phosphorus (P) and lowered P excretion in the manure. The usefulness of microbial phytases in improving the bioavailability of phytate-P in broiler diets is now well documented (Kornegay, 1966). However, locally generated data demonstrate that, in addition, phytase increases the apparent ileal digestibility of amino acids in feed ingredients and diets and apparent metabolisable energy (AME) of diets (Ravindran *et al.*, 1998; Cabahug *et al.*, 1999).

To assess the broader potential of microbial phytase, a sorghum-based standard grower diet was formulated and then modified by lowering the specifications (% reductions) for crude protein (7.5), AME (1.9) and total phosphorus (8.7). The objective of the present study was to examine the effects of a microbial phytase feed enzyme (Natuphos®5000 Granulate; BASF AG, Germany) on the performance and nutrient utilisation of broilers offered standard and modified diets.

II. MATERIALS AND METHODS

Day-old male broiler chicks (Cobb) were raised in battery brooders housed in an environmentally controlled room and were provided with a commercial broiler starter diet from day 1 to 6. On day 7, birds were weighed and assigned to 24 pens (10 birds/pen) on the basis of body weight.

A sorghum-based standard grower diet was formulated (Table 1) and modified on a least-cost basis by reducing the dietary specifications (g/kg) of protein by 17.10, available P by 1.16, calcium by 1.27 and AME by 0.24 MJ/kg. For the following critical amino acids calculated dietary specifications (mg/kg) were reduced for available lysine (778), methionine (135), cystine (77), threonine (544), tryptophan (132) and isoleucine (770). Microbial phytase, in granular form, was added to both treatment diets at 600 FTU/kg (120g/t). The diets were then cold-pelleted (65°C) and crumbled.

[1]Department of Animal Science, The University of Sydney, Camden, NSW 2570.
[2]Millmaster Feeds, Merrylands, NSW 2160.

Table 1. Ingredient composition and specifications (g/kg) of the standard and modified broiler diets.

	Standard diet	Modified diet
Sorghum (126 g CP)	695.00	670.80
Soyabean meal (474 g CP)	145.00	85.00
Meat and bone meal (546 g CP)	75.00	50.00
Canola meal (345 g CP)	60.00	70.00
Cottonseed meal (453 g CP)	5.00	40.00
Millrun (133 g CP)	-	60.00
Limestone	2.50	5.50
Vitamin/trace mineral premix	5.00	5.00
Sodium bicarbonate	3.85	4.35
Salt	0.60	0.50
Choline chloride	0.25	0.25
Lysine-HCl	4.15	4.80
DL methionine	3.65	3.70
Threonine	-	0.10
Diet specifications		
AME (MJ/kg)	12.38	12.14
Crude protein	227.15	210.05
Calcium	9.19	7.92
Total P	8.15	7.44
Available P	5.02	3.86
Crude fibre	35.61	41.79

The birds received constant fluorescent illumination and were allowed free access to the diets and water. Each diet was fed to six replicate pens from 7 to 25 days of age. Body weights and feed intake were recorded at weekly intervals.

The AME and nitrogen retention values were determined using a classical total excreta collection method. During the last four days of the feeding trial (day 22-25), feed intake was monitored, and the excreta were collected daily at 09.00 hrs, dried for 24 h at 80°C in a forced-air oven and pooled within a pen. On day 25, all surviving birds were euthanased by intracardial injection of sodium pentobarbitone, and the toes were removed for the determination of ash content. Representative samples of diets and excreta were analysed for gross energy and nitrogen. The gross energy of diet and excreta samples were determined using an adiabatic bomb calorimeter (Gallenkamp) standardised with benzoic acid. Nitrogen content was determined by the method of Sweeney (1989) using a FP-428 nitrogen determinator (LECO Corporation, St Joseph, Michigan). Two-way analysis of variance of the General Linear Models procedure was used to determine main effects and interaction of diet type and enzyme supplementation. Differences were considered significant at P < 0.05, although probability values up to P < 0.10 are shown if the data suggest a trend. Means with a significant F ratio were separated by a test of Least Significant Difference.

III. RESULTS

Only one death was recorded during the trial (on the standard diet). Main effects of diet type and phytase were observed for the performance parameters (Table 2). Weight gains (P <0.001) were higher and the feed intake (P <0.10) and feed conversion ratio (FCR; P

<0.001) were lower in the birds fed on the standard diet than those on the modified diet. Phytase improved weight gain of birds fed on both diets, but the magnitude of response was greater in the modified diet resulting in a diet type x phytase interaction (P <0.10). Phytase increased feed intake of birds in both diets, whereas FCR was improved only in the modified diet as indicated by a diet type x phytase interaction (P <0.01).

Table 2.   Weight gain (WG, g/bird), feed intake (FI, g/bird), feed conversion ratio (FCR, g/g), toe ash (ASH, %) apparent nitrogen retention (NR, %) and apparent metabolisable energy (AME, MJ/kg as is) of broilers fed two diet types with microbial phytase from 7 to 25 days of age[1].

Treatment	WG	FI	FCR	ASH	NR	AME
Standard diet	895 [b2]	1360 [a]	1.52 [c]	12.76	58.2 [d]	12.55 [a]
Standard diet + phytase	918 [b]	1409 [b]	1.53 [c]	12.75	57.7 [c]	12.88 [b]
Modified diet	824 [a]	1392 [ab]	1.69 [a]	12.53	51.2 [a]	12.36 [a]
Modified diet + phytase	887 [b]	1427 [b]	1.61 [b]	12.92	56.1 [b]	12.85 [b]
Pooled SEM	11.3	14.0	0.013	0.143	0.013	0.088
Probabilities						
Diet type	0.001	0.094	0.001	NS	0.004	NS
Phytase	0.002	0.009	0.017	NS	NS	0.001
Diet type x phytase	0.103	NS	0.002	NS	0.053	NS

[1]Mean of six replicates (10 birds/pen).
[2]Means in a column with the same superscript (a-d) are not significantly different (P >0.05).

Toe ash contents were unaffected by diet type or phytase addition (Table 2). Nitrogen retention in birds fed on the modified diet (0.537) was lower (P <0.001) than in those fed on the standard diet (0.580). The diet type x phytase interaction (P <0.06) indicated a response in nitrogen retention to phytase addition in birds given the modified diet. Diet type had no effect on AME values, but phytase improved (P <0.001) the AME values in both diets.

IV. DISCUSSION

The results confirm the value of microbial phytase as a tool to reduce nutrient specifications in broiler feed formulations. As expected, the weight gains and FCR were lowered as a result of modification of the diet. Addition of phytase to the modified diet increased (P <0.05) weight gain and FCR by 7.6 and 4.7%, respectively. Birds receiving the modified diet had similar rates of gain but the FCR was 5.9% lower (P <0.05).

Diet modifications or phytase did not affect toe ash contents, and this was unexpected. The diets were analysed for total P and plant feedstuffs for phytate-P from which estimates of dietary nonphytate-P were calculated. The standard diets contained (g/kg) 6.95 total P, 2.80 phytate-P and 4.15 nonphytate-P whereas the corresponding values for the modified diets were 6.55, 3.40 and 3.15 respectively. The NRC (1994) recommendations for nonphytate-P requirement for broilers to 3 weeks is 4.5 g/kg and 3.5 from 3-6 weeks of age. Since toe ash is a sensitive indicator of P status in birds, the results suggest that a level of 3.15 nonphytate-P g/kg was adequate for bone mineralisation of broilers in this study. It follows that the enzyme effects on bird performance in modified diets cannot be explained on the basis of improved P availability.

On the modified diets phytase improved N retention (P <0.05) by 9.6% from 0.512 to 0.561. This is consistent with the findings of Cabahug *et al.* (1999) who recorded increases in the apparent ileal digestibility of amino acids following phytase addition to the five plant feed ingredients used in this study. Kies and Selle (1998) reviewed the negative effects of phytic acid on protein utilisation by broilers which is the rationale for such responses to added phytase. Overall, phytase increased (P <0.001) AME values by 3.3% from 12.46 to 12.87 MJ/kg. The reasons for the positive effect of phytase on energy are unclear but Farrell *et al.* (1992) found that the addition of phytase to sorghum-based broiler diets resulted in small but significant (P <0.01) increases in nitrogen-corrected metabolisable energy.

Relative to the standard diet the addition of phytase to the modified diet compensated for gain (895 versus 887 g/bird) and partially compensated for feed conversion (1.52 versus 1.61:1). However, it is noteworthy that, based on a relevant survey of prices (NSW Agriculture; January 1998) modification of the diet resulted in a reduction in ingredient costs of approximately $24.25 per tonne. This finding indicates that the addition of microbial phytase to sorghum-based broiler diets, coupled with modifications to the formulations, has the potential to reduce the cost of liveweight gain depending on relative feed ingredients prices.

REFERENCES

Cabahug, S., Ravindran, V., Ravindran, G., and Bryden W.L. (1999). *Proceedings of the Australian Poultry Science Symposium.* Ed. D.J. Farrell. **11**: (in this volume).

Farrell, D.J., du Preez, J. J., Bongarts, M., Betts, M., Sudaman, A., Thomson, E. and Ball, W. (1992). *Proceedings of the Australian Poultry Science Symposium.* Ed. R. J. Johnson. **4**: 1116-1119.

Kies, A. K. and Selle, P. H. (1998). *Proceedings of the Australian Poultry Science Symposium.* Ed. D. Balnave. **10**: 28-131.

Kornegay, E. T. (1966). In: Phytase in Animal Nutrition and Waste Management, pp 275-288. Eds. M. B. Coelho and E. T. Kornegay. (BASF Corporation: Mount Olive , N. J.)

Ravindran, V., Cabahug, S., Bryden, W.L. and Selle, P.H. (1998). *Proceedings Maryland Nutrition Conference for Feed Manufacturers,* pp. 156-165.University of Maryland, College Park.

NRC. (1994). Nutrient Requirements of Poultry. National Research Council. (National Academy Press: Washington, DC.)

Sweeney, R. A. (1989). *Journal of the Association of Official Analytical Chemists,* **72**:770.

INFLUENCE OF DIETARY INCLUSION RATE OF WHEAT ON AME, DIGESTA VISCOSITY AND ENZYME RESPONSE

R.J. HUGHES and P. ZVIEDRANS

Summary

Three energy balance experiments examined the effects of dietary inclusion rate of wheat and response to enzyme dose rate on apparent metabolisable energy (AME), digesta viscosity and growth performance of broiler chickens 24-31 days of age. Semi-purified basal diets containing sorghum, casein, minerals and vitamins were used in each experiment. The AME of wheat was significantly ($P<0.05$) reduced (13.3 versus 14.8 MJ/kg DM) when inclusion rate of wheat was increased from 400 to 800 g/kg in experiment 1 which was conducted about 10 weeks after harvest. Viscosity of ileal digesta increased logarithmically with linear increase in wheat inclusion rate in experiments 2 and 3; however there was a lack of evidence that inclusion rate of wheat and its AME value were related after storage of grain for 8 months. Feed enzymes were very effective in reducing ileal viscosity and improving the AME of wheat, with the results pointing to the need to match dose rate of enzyme to dietary inclusion rate of wheat.

I. INTRODUCTION

A fundamental mathematical assumption that must be made for least cost feed formulation by linear programming methods is that nutritive values of feed ingredients are additive. For example, the value assigned to apparent metabolisable energy (AME) for one ingredient is assumed to be independent of the dietary inclusion rate of that ingredient and is not influenced by the presence of any other ingredient or component of the diet. There is a rapidly growing body of evidence that this assumption does not always hold true in commercial practice.

A prime example is the effect of soluble non-starch polysaccharides (NSPs) on digesta viscosity, starch digestibility and AME (Annison, 1993) and the ameliorating effect of exogenous glycanases (Choct et al., 1996) which in the last 5-10 years have become a common component in broiler feeds (Bedford and Morgan, 1996). Small increments in dietary concentration of NSPs such as arabinoxylan from wheat or β-glucan from barley can result in non-linear increases in viscosity of digesta with a resulting loss of performance, presumably by interference with digestion and absorption of nutrients (Annison, 1993). On the other hand, one of the modes of action of feed enzymes is understood to be the reduction of digesta viscosity by depolymerisation of NSPs through cleavage of glycosidic links between sugar molecules in main and side chains (Smits and Annison, 1996).

Until recently, it was often the practice to include a feed enzyme product in the diet at a fixed rate advised by the manufacturer, irrespective of the likely dietary level of the substrate the enzyme was intended to degrade. Nowadays the usage of feed enzymes in commercial practice is becoming more sophisticated with attempts to match the dose rate with the anticipated substrate level in order to reduce the cost of enzyme supplementation. It is quite possible that additional benefits from the careful matching of enzyme dose to substrate level will become evident with increased understanding of the modes of action of enzymes and impact on other digestive processes.

SARDI, Pig and Poultry Production Institute, Nutrition Research Laboratory, University of Adelaide, Roseworthy, SA 5371.

This paper describes three experiments to examine (1) the effects of increasing the dietary inclusion rate of wheat on AME, digesta viscosity and performance of broiler chickens 24-31 days of age, and (2) the responses to enzymes added at fixed or variable rates.

II. MATERIALS AND METHODS

Day-old broiler chickens of mixed sex were raised in floor pens on a commercial starter crumble to 21 days and then on finisher pellets. At 24 days of age, birds were weighed in groups of five and transferred to metabolism cages in controlled temperature rooms for classical AME studies involving quantitative measurements of feed intake and excreta output. Semi-purified basal diets contained (per kg) 780 or 800 g sorghum (depending on whether the diet contained 20 g Celite digestibility marker, 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%). Wheat replaced sorghum in the basal diets as required. The diets were fed for seven days (chickens 24-31 days of age). In each study, dietary treatments were replicated at least four times. The first three days enabled the chickens to adapt to the cages and the feeds. During the following four days, all excreta were collected and dried. Feed intake was measured during the adaptation and collection phases of the study. Birds were weighed in groups at the end of the seven day period. Two birds per cage were killed to provide ileal digesta. Digesta were stored in ice until centrifuged at 12,000 g for 12 minutes then frozen. Viscosity was determined on thawed supernatant using a Brookfield DVIII viscometer at 25°C with a shear rate 5-500 s^{-1}. 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*)*. Data were analysed using an analysis of variance, and Duncan's multiple range test for separating mean values.

III. RESULTS AND DISCUSSION

The results of Experiment 1 are summarised in Table 1. Dietary inclusion rate of wheat had significant (P<0.05) effects on calculated AME of wheat, dry matter digestibility, feed conversion and growth. Results for sorghum B are included in the table for comparative purposes as this sorghum and the wheat were used in subsequent experiments. The post-harvest storage period for the wheat was about 10 weeks when Experiment 1 was conducted.

Table 1.   Effects of dietary inclusion rate (IR, g/kg) of wheat on feed intake (FI, g/bird 24-31 days), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of diet and grain, and dry matter digestibility (DMD, g retained/g eaten). Means (n=4) having a common postscript letter are not significantly different (P<0.05).

Grain	IR	FI	GR	FCR	AME diet	AME grain	DMD
Sorghum A	800	102 a[1]	350 ab	2.05 bc	15.2 a	15.2 a	0.77 a
Sorghum B	800	102 a	314 b	2.28 a	15.1 a	15.2 a	0.77 a
Wheat	800	98 a	325 b	2.14 ab	13.3 b	12.9 b	0.66 b
Sorghum A/wheat	400/400	106 a	384 a	1.93 c	14.8 a	14.4 a	0.74 a
Pooled SEM		2	13	0.06	0.2	0.4	0.01

[1]Values with the same letter are not significantly different (P>0.05).

The observed reduction in AME of wheat at the higher inclusion rate (Table 1) is consistent with the hypothesis that as the dietary concentration of soluble NSPs increases with wheat in the diet, the resulting elevation of digesta viscosity will adversely affect digestion and absorption of nutrients. Hence the AME value assigned to wheat for feed formulation purposes should be adjusted for anticipated inclusion rate of wheat, particularly if used at high levels.

The results of Experiment 2 are summarised in Table 2. Linear increase in dietary inclusion rate of wheat resulted in logarithmic increase in digesta viscosity, a linear decrease in dry matter digestibility, and no significant (P>0.05) effect on calculated AME of wheat or on feed intake. Growth rate indicated a parabolic response to inclusion rate, with maximum growth rate at 390 g/kg (wheat in equal proportion to sorghum in the diet). A similar pattern was observed in feed conversion ratio with the best response occurring at 390 g/kg. The lack of effect of inclusion rate on calculated AME is inconsistent with the apparent depression of AME of wheat at the higher inclusion level observed in experiment 1. It is possible that any anti-nutritive factor in the wheat was reduced by post-harvest storage (8 months) as was reported by Choct and Hughes (1997).

Table 2. Effects of dietary inclusion rate (IR, g/kg) of wheat and Enzyme A (g/tonne) on feed intake (FI, g/bird 24-31 days), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of diet and wheat, ileal viscosity (IV, cP) and dry matter digestibility (DMD, g retained/g eaten). Means (n=6) having a common postscript letter are not significantly different (P<0.05).

IR	Enzyme A	FI	GR	FCR	AME diet	AME wheat	IV[1]	DMD
0	0	112 a[2]	374 d	2.10 a	14.9 ab	-	2.6 e	0.76 a
195	0	117 a	420 ab	1.95 c	14.4 bcd	13.0 bc	6.2 cd	0.73 ab
390	0	116 a	436 a	1.87 cd	14.2 cd	13.5 abc	9.7 c	0.71 bc
585	0	113 a	414 abc	1.91 cd	13.6 e	13.2 abc	16.5 b	0.68 d
780	0	110 a	391 bcd	1.97 bc	12.9 f	12.8 c	39.3 a	0.63 e
0	500	113 a	385 cd	2.06 ab	15.0 a	-	2.9 e	0.76 a
390	500	114 a	421 ab	1.90 cd	14.5 abc	14.4 a	4.4 de	0.73 ab
780	500	110 a	428 a	1.81 d	13.9 de	14.1 ab	6.0 cd	0.69 cd
Pooled SEM		2	10	0.03	0.2	0.4	3.8	0.01

[1]Viscosity data were log transformed for analysis of variance.
[2]Values with the same superscript are not signficantly different (P>0.05).

Digesta viscosity was significantly (P<0.05) reduced by addition of Enzyme A to the diets containing wheat at 390 and 780 g/kg, however viscosity remained significantly elevated and dry matter was depressed on the highest level of wheat compared with the sorghum diet (wheat 0 g/kg). Addition of Enzyme A to the diet containing wheat at 780 g/kg significantly (P<0.05) improved growth rate, feed conversion, AME of wheat and dry matter digestibility, whereas there were no corresponding improvements (P>0.05) for wheat at 390 g/kg. These results tend to suggest that the dose rate (500 g/tonne) was sufficient for a diet based entirely on wheat (780g/kg) and possibly in excess for a lower inclusion rate of wheat where there was little or no need to use enzyme to reduce ileal viscosity.

Logarithmic increases in ileal viscosity were also observed in experiment 3 (Table 3). Enzyme B at 200 g/tonne significantly (P<0.05) reduced ileal viscosity and improved the AME of wheat at 490 g/kg, along with feed conversion and dry matter digestibility. Increasing the dose rate to 400 g/tonne produced no further benefit. For wheat at 780 g/kg, Enzyme B at 200 g/tonne significantly (P<0.05) reduced viscosity but there were no

responses in AME, growth or conversion. However, increasing the dose rate of Enzyme B to 320 g/tonne for the higher level of wheat resulted in significant (P<0.05) improvement in dietary AME and dry matter digestibility. Reduction of ileal viscosity and improvements in AME of wheat and feed conversion approached significance. The very low value of 10.6 MJ/kg DM for wheat at 490 g/kg shown in Table 3 was unexpected. We cannot offer an explanation for this puzzling result other than to say that excreta output and energy content were elevated for all replicates.

Table 3. Effects of dietary inclusion rate (IR, g/kg) of wheat and dose rate of Enzyme B (g/tonne) on feed intake (FI, g/bird 24-31 days), growth rate (GR, g/bird), feed conversion ratio (FCR, g feed/g gain), AME (MJ/kg dry matter) of diet and wheat, and dry matter digestibility (DMD, g retained/g eaten). Means (n=6) having a common postscript letter are not significantly different (P<0.05).

IR	Enzyme B	FI	GR	FCR	AME diet	AME wheat	IV[1]	DMD
0	0	108 a	329 c	2.30 a	14.9 a	-	3.4 cd	0.75 a
490	0	113 a	361 ab	2.20 ab	12.5 cd	10.6 c	15.4 b	0.64 bc
780	0	112 a	372 a	2. 12 bc	12.3 cd	12.0 b	49.0 a	0.63 bc
0	200	109 a	338 bc	2.26 a	14.8 a	-	3.2 d	0.75 a
490	200	109 a	382 a	1.99 c	14.1 b	13.7 a	4.6 cd	0.73 a
780	200	110 a	365 ab	2.11 bc	12.1 d	11.8 b	5.9 c	0.61 c
490	400	106 a	369 ab	2.02 c	14.0 b	13.6 a	4.2 cd	0.72 a
780	320	110 a	387 a	2.00 c	12.8 c	12.7 b	5.0 cd	0.65 b
SEM		2	10	0.04	0.2	0.3	6.5	0.01

[1]Viscosity data were log transformed for analysis of variance.

IV. CONCLUSIONS

The results of three energy balance studies point to the existence of an inverse relationship between dietary inclusion rate and AME value for wheat soon after harvest but not in grain stored for several months. Further studies are warranted in this area. There was clear evidence that enzyme dose rates need to be matched with the inclusion rate of wheat.

V. ACKNOWLEDGMENTS

REFERENCES

Annison, G. (1993). *Australian Journal of Agricultural Research*, **44**: 405-422.
Annison, G., Choct, M. and Hughes, R.J. (1994). *Proceedings Australian Poultry Science Symposium*, Ed. D. Balnave **6**: 92-96.
Bedford, M.R. and Morgan, A.J. (1996). *World's Poultry Science Journal*, **52**: 61-68.
Choct, M. and Hughes, R.J. (1997). In: Recent Advances in Animal Nutrition in Australia 1997, pp146-150. Eds. J.J. Corbett, M. Choct, J.V. Nolan, J.B. Rowe. The University of New England; Armidale.
Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. and Annison, G. (1996). *British Poultry Science*, **37**: 609-621.
Smits. C.H.M. and Annison, G. (1996). *World's Poultry Science Journal*, **52**: 203-221.

SPRAYING ENZYME BEFORE OR AFTER FAT COATING : IN VITRO RECOVERIES AND IN VIVO EFFICACIES

A.M. PEREZ-VENDRELL[1], J. BRUFAU[1], G. UZU [2] and P.A. GERAERT[2]

Summary

Several trials were performed to determine the different possibilities for spraying a liquid NSP-enzyme. The spraying procedure, i.e. spraying before, immediately after or some time after fat coating, and the effect of fat temperature and amount were investigated. *In vitro* recoveries were measured either by colorimetric or viscometric methods in two laboratories. *In vivo* evaluation of efficacies through AME determination and growth trials were also performed on wheat and barley-based diets untreated or treated with ROVABIO™ EXCEL LC enzyme according to the different spraying procedures.

It appears that neither the spraying procedure, the fat quantity and the fat temperature had an effect on the enzyme activity recovery or efficacy. Indeed, recoveries ranged between 90 and 118 % of the administered dose. One-21 day feed conversion ratio was decreased by 9 % by the enzyme supplementation of the barley-based diet, irrespective of the spraying procedure. However, the recommendation is to spray the liquid enzyme after sieving and prior to fat coating.

I. INTRODUCTION

Non starch polysaccharide (NSP)-enzymes or *endo*-hydrolases are proteins of high molecular weight which catalyse depolymerisation of NSP. Their tridimensional structure houses a catalytic site but also makes the enzymes sensitive to physico-chemical conditions. Indeed, enzymes are particularly sensitive to feed processing treatments which often employ temperature, moisture, pressure as in pelletisation, expansion, anaerobic pasteurisation and extrusion. In such conditions, irrespective of the commercial products, studies have demonstrated that more than 60 to 80 % of the feed enzymes incorporated prior to pelleting were destroyed at a pelleting temperature above 80°C (Spring and Gadient, 1996). The use of liquid enzymes sprayed on to the final feed after feed processing has thus been largely recommended when processing conditions may be too determined for the enzymes. With liquid application, losses less than 10 % and accuracy better than 10 % can be expected (Spring and Gadient, 1996). However, when trying to fit a liquid post-pelleting enzyme application system into a feed manufacturing plant, questions are raised about the best possible location and procedure. Due to the fact that enzymes are normally diluted in water, it has often been recommended to spray the enzyme before the fat coating. However, the temperature of the pellets at the outlet of the press may be too high for the addition of the enzyme. The quantity of fat and its temperature may also have an effect on enzyme activity.

A series of experiments was designed to investigate the effect of different application procedures and processing conditions on enzyme activity and efficacy.

II. MATERIALS AND METHODS

Three experiments were designed to study the effect of spraying the enzyme either before or after fat coating. The diet was wheat or barley-based with a minimum of 500 g of

---

[1]IRTA, Centre de Mas Bové, Apartat 415, 43280 Reus, Spain.
[2]Rhône-Poulenc Animal Nutrition, 42 Av A. Briand BP 100 92164 ANTONY Cedex, France.

barley/kg. Rovabio™ Excel LC contains xylanase and β-glucanase activities resulting from the fermentation process of *Penicillium funiculosum*. The product was sprayed on to the pellets (at 0.2 l/t) prior to fat coating, immediately after or thirty minutes after fat coating to ensure sufficient fat cooling.

The effect of fat temperature and fat quantity at the time of application were also investigated. *In vitro* recoveries were measured by colorimetric (azo-arabinoxylan) or viscometric methods (Sabatier and Fish, 1996). *In vivo* efficacies were determined by digestibility and growth tests. Apparent metabolisable energy (AME) was measured in growing broilers with ad libitum intake and total excreta collection (European reference method, Bourdillon *et al.*, 1990). Growth performance (weight gain and feed efficiency) was measured on birds in floor pens and were grown from 1 to 41 days of age.

III. RESULTS AND DISCUSSION

Table 1 reports the *in vitro* recoveries measured on a barley-based diet using the colorimetric (azo-arabinoxylan) method. Similar results were obtained on a wheat-based diet (not shown here). The values were within the expected range of variation.

Table 1.   Relative enzyme recoveries (%) after fat coating.

Treatment[1] Diet	Before	Immediately after	After 30 min
Starter	100	90	99
Grower	100	104	92

[1]ROVABIO™ EXCEL LC was sprayed at 0.2 l/ton feed, before, immediately after or 30 minutes after fat coating.

In vivo efficacies were also measured using male broilers (Table 2). There were no differences between the three procedures : spraying before, after or 30 minutes after fat coating. The overall effect of the enzyme used reached a 9 % decrease in feed conversion ratio with EXCEL addition between 1 and 21 days of age. There was a 4.6 % reduction in feed conversion between 21 and 41 days of age.

The enzyme supplementation also improved feed digestibility as measured by the increase in the energy value of the feed by 661, 883 and 736 kJ/kg, or 5.7, 7.6 and 6.3 % for the spraying before, after, or 30 min after the fat coating. For the barley-based diet, a small non significant numerical advantage of spraying immediately after fat application was observed. When repeating the same experiment on a wheat-based diet, no difference was seen between the three application procedures. In the case of the ROVABIO™ product tested the two activities, xylanase and β-glucanase, had the same origin and thus similar recoveries could be found irrespective of the enzyme activity. However, when products are based on mixing different activities from different micro-organisms, the stability of the different enzyme activities might be worth further investigation.

Table 2. Effect of liquid enzyme spraying procedure on *in-vivo* efficacies : digestibility (AME_N, 12 replicates x 2 chicks) and growth performance (6 replicates x 80 chicks) of male broilers fed a barley-based diet.[1]

	Feed conversion ratio (g feed : g gain)		AME_N (MJ/kg)
	1-21 days	21-41 days	
Control	1.939[a]	2.138[a]	11.77[a]
Before	1.759[b]	2.048[b]	12.33[b]
After	1.764[b]	2.023[b]	12.55[b]
After 30 min	1.775[b]	2.047[b]	12.80[b]
S E	0.012	0.014	79.9
Treatment effect	< 0.001	< 0.001	< 0.001

[1]ROVABIO™ EXCEL LC was sprayed at 0.2 l/ton onto the pellets, before, immediately after, or 30 minutes after fat coating.

Added fat might also be sprayed on to the pellets at different temperatures depending on the desired technological parameters. The amount of fat added (up to 30 g/kg) or the temperature of the fat (up to 88°C) at the time of spraying did not have a significant effect on enzyme activity recoveries.

Table 3. Effect of fat temperature and fat quantity on enzyme recovery (%).

Quantity (g/kg)	15	30
Temperature of fat (°C)		
64	114	118
80	110	117
88	105	117

Fat was immediately sprayed after ROVABIO™ EXCEL LC pulverisation

Post-pelleting spraying of enzymes might thus be done either before or at any time after fat coating. However, in order to ensure maximum recovery and efficacy, any liquid enzyme should be sprayed after sieving of the pellets. Indeed, fines have a larger surface area : weight ratio than pellets. Thus, higher enzyme concentrations will be obtained in the fines than in the pellets. Birds, which often refuse to feed on fine particles, may not ingest enough enzyme. Furthermore, if fines are recycled to the pellet die, denaturation of enzymes would occur. Consequently, post-pelleting enzyme spraying should be done after sieving.

REFERENCES

Bourdillon, A., Carré, B., Conan, L., Duperray, J., Huyghebaert, G., Leclercq, B., Lessire, M., McNab, J. and Wiseman, J. (1990). *British Poultry Science,* **31**: 557-565.
Günther, C., Beudeker, R.F., and Vahl, J.L. (1997). *Feed Mix,* **5**: 24-27.
Sabatier, A.M., Fish, N.M. (1996). *Journal of Applied Poultry Research,* **6**: 61-68.
Spring, W.G., and Gadient, M. (1996). *South European Feed Manufacturers Conference,* May 9-10th 1996, Reus, Spain, 6 pp.

ROLE OF ENZYMES IN REDUCING VARIABILITY IN NUTRITIVE VALUE OF MAIZE USING THE ILEAL DIGESTIBILITY METHOD

C.L. WYATT, M.R. BEDFORD AND L.A. WALDRON

Summary

The energy content of maize and sorghum is considered to be relatively constant. However, there is little data to support such an assumption, and in fact that which is published indicates there may be considerably more variation than is currently accepted. Thus, a study was conducted to evaluate the variability in ileal energy values in 28 day old broilers between eight different commercial maize samples and to evaluate the impact of enzyme addition on this variation. In the absence of enzyme the standard deviation of ileal digestible energy values between maize samples was quite high (± 0.34 MJ/kg; 2.8%). Addition of a specific enzyme product had two significant effects: (1) average ileal energy content was increased by 0.41 MJ/kg (3.3%), and (2) a clear reduction in variation between samples of 50% (± 0.17 MJ/kg). Ileal starch and fat digestibility values were improved by more than 1% with enzyme supplementation. Addition of enzymes resulted in a greater benefit with poorer quality maize compared with better quality maizes. These results would indicate a more consistent nutritional quality of maize and bird performance in diets containing an appropriate enzyme supplementation.

I. INTRODUCTION

Maize has traditionally been the preferred cereal for domestic animals, with its dietary energy value being one of the highest of the feed grains. The energy content of this cereal is assumed by many to be relatively constant. The degree to which each cereal grain differs from batch to batch is dependent not only on the variety of the grain and the conditions under which it is grown, but also the conditions to which it is subjected during the feed manufacturing process. Attempts to determine the variation in feeding value of each cereal has been addressed recently for barley (Scott and Boldaji, 1997; Kocher *et al.*, 1997), wheat (Classen *et al.*, 1995; Hughes *et al.*, 1996) and maize (Leeson *et al.*, 1993). It is evident that each study detected considerable variation from sample to sample regardless of cereal type. It is also interesting to note that the variability and range (i.e. difference between best and worst sample) is actually not dissimilar between the three grains. Thus, it is important to understand that cereal source can be a major contributor to variation in performance of diets of identical formulation but different raw material origin.

It is now well established that in wheat and barley (likewise in triticale and rye), the viscous non-starch polysaccharides account for as much as 70-80% of the variation in feeding value (Barrier-Guillot *et al.*, 1997; Bedford, 1997; Choct *et al.*, 1996; Smits and Annison, 1996). In maize and sorghum, however, this is clearly not the case; instead it appears to be starch structure that plays a large role in the digestibility of these grains in the chick. Starch structure has a strong bearing on its rate of digestion, and may therefore be of great importance in determining its feeding value. The rate of digestion of maize starch is not intrinsically as rapid as that for wheat and barley and is controlled by several factors including starch crystallinity, drying and processing, and the subsequent formation of retrograde starch (Brown, 1996). However, most nutritionists do not consider maize or sorghum digestion as being poor, in fact most would argue that starch is better than 98% digested. In the case of

Finnfeeds, Market House, High Street, Marlborough Wiltshire SN8 1AA UK.

faecal digestibility, this is certainly the case. However, recent evidence presented by Noy and Sklan (1995) suggests that at the ileal level, starch digestibility rarely exceeds 85% between 4 and 21 days of age, despite ever increasing amounts of amylase output with increasing age. Likewise, Choct et al. (1996) reported an ileal digestibility of only 90% for the starch in a sorghum diet for broilers. Such a phenomenon will result in more starch escaping small intestinal digestion, which provides a fermentation source for large intestinal resident microbes. Overall, it appears that maize starch (and fat/protein) digestion may not be as complete in the chick as previously thought. Recent advances in enzyme technology have allowed for the development of specifically designed enzyme products, which targets cereal starch and the vegetable proteins. Thus, a study was conducted to evaluate the variability in ileal energy values between eight different commercial maize samples and the impact of enzyme addition in 28 day-old broilers.

II. METHODS

Eight different samples of commercially-grown maize were collected from various countries (France, USA, South Africa, Thailand), and then tested under uniform conditions at the Roslin Institute, Scotland. Each maize sample was used at 530 g/kg in an otherwise unchanged diet containing 380 g soybean meal, 36 g animal fat/kg and amino acid and mineral supplements. The ileal digestibility values were measured in broilers at day 28 with each of the eight experimental diets being tested with and without enzymes (Avizyme 1500) added at 1kg/tonne. This enzyme contains xylanase, amylase and protease.

Four cages of four male Cobb broilers per treatment received the diets over a period of 14 days prior to sampling of ileal digesta. Diets contained titanium oxide as an indigestible marker and were fed as mash ad libitum. Feed and digesta samples were analysed for their energy, starch, fat and protein content to determine ileal digestibility coefficients for each parameter. Data were analysed using ANOVA according to the GLM procedures of SAS.

III. RESULTS AND DISCUSSION

The ileal digestibility assay was shown to be a very accurate and sensitive method to assess differences between maize samples and the subsequent impact of supplemental enzymes. The average dietary energy value across all maizes fed without enzymes was 12.24 MJ/kg with a range from 11.71 to 12.56 MJ/kg (Figure 1). Supplementing enzymes significantly (P= 0.006) increased ileal digestible energy values across all diets by 3.2% with an average value of 12.63 MJ/kg. The main effect of enzyme supplementation was to significantly increase ileal starch and fat digestibility values by 1.0% (95.4 vs 96.3%) and 3.5% (74.3 vs 77.1%), respectively. The increase in starch and fat digestion in the small intestine will increase energy availability to the bird by reducing the loss of nutrients to microbes in the caeca. Ileal protein digestibility was increased by 1.3% (79 vs 80%) with enzyme supplementation (P= 0.1), which could be the result of lower endogenous nitrogen losses.

Enzyme addition had two effects: the average energy content was enhanced by 0.405 MJ/kg (3.3%, P<0.001), and at the same time there was a clear reduction in the variability to only ± 0.167 MJ/kg. A larger enzyme effect on the poorer quality maizes and a smaller effect on already good quality batches of maize brought about the latter effect. The correlation between initial maize quality and response to enzyme was significant and is shown in Figure 2. For the two poorest batches of maize tested in this study, which were from Thailand and

Avizyme effect +3.2% (P=0.006)
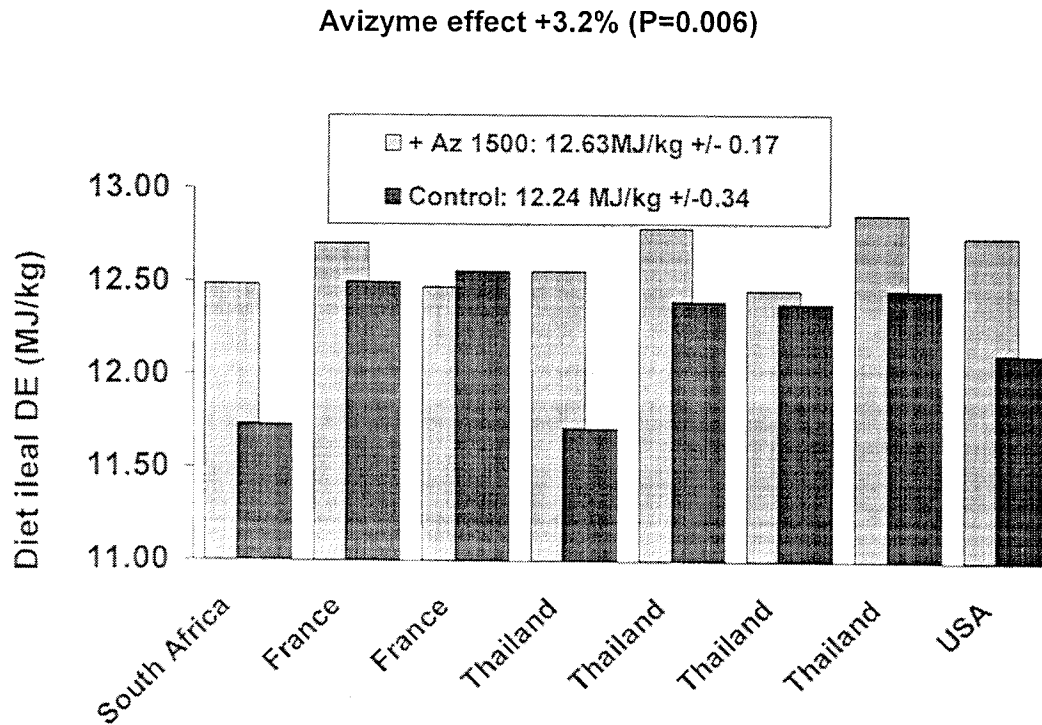
Figure 1.   Effects of enzymes on ileal energy digestibility of maize-based diets in broiler chicks at 28 days of age across 8 different maize batches. (Roslin Institute, Edinburgh, unpublished.)

South Africa, the energy response to enzyme supplementation was 0.85 MJ and 0.76 MJ/kg, respectively (Figure 1). This increase in energy corresponds to more than 1.26 MJ if entirely allocated to the maize fraction of the diet. The findings reported so far may indicate that feeding specific enzymes in the diet may reduce variability in the nutritional value of individual batches of maize. In conclusion, more consistent nutritional quality and bird performance may be expected in diets containing an appropriate enzyme supplement.
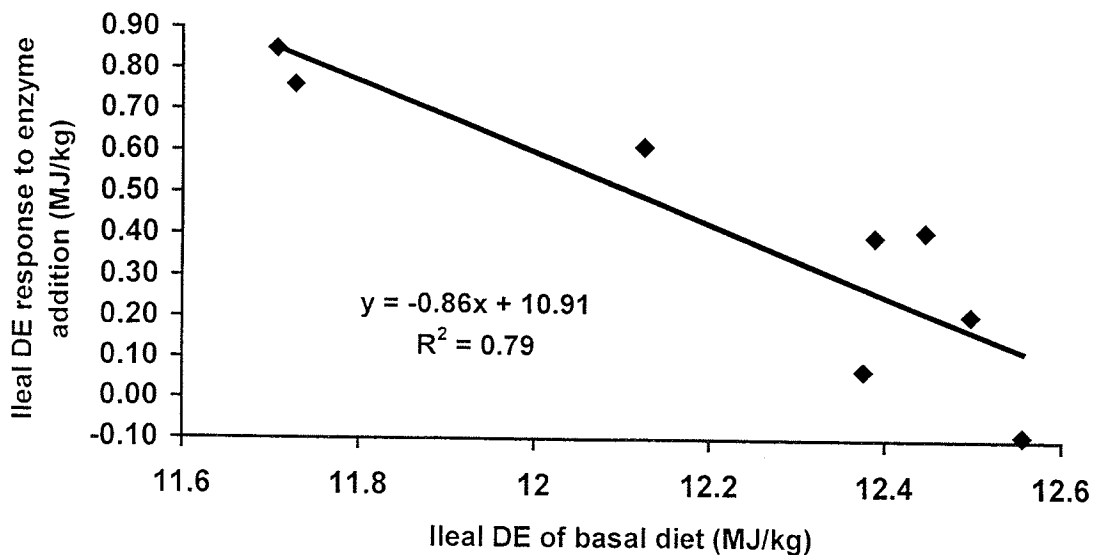
Figure 2.   Effect of the energy content of the enzyme-unsupplemented diet on the energy response to enzyme addition. (Roslin Institute, Edinburgh, unpublished.)

IV. CONCLUSIONS

Results of growth and digestibility studies show an important potential for improvement in energy availability in maize-based diets. The current results demonstrate that the use of the appropriate enzymes can have a direct effect on ileal energy availability from different maize-based broiler diets. Enzyme addition resulted in a larger benefit with poorer compared with better quality maizes. As a result, a more consistent nutritional quality and bird performance may be expected in maize diets containing an appropriate enzyme supplementation.

REFERENCES

Barrier-Guillot, B., Bedford, M.R., Metayer, J.P. and Gatel, F. (1997). *Deuxiemes Journees de la Recherche Avicole,* April 8-10.

Bedford, M.R. (1997). *Poultry International*, June, pp. 56-59.

Brown, I. (1996). *Nutrition Reviews,* **54**: S115-S119.

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

Classen, H.L., Scott, T.A., Irish, G.G., Hucl, P., Swift, M. and Bedford, M.R. (1995) *Proceedings of the 2nd European Symposium on Feed Enzymes*, Noordwijkerhout, NL, pp 65-77.

Hughes, R.J., Kocher, A., Acone, L., Langston, P. and Bird, J.N. (1996) *Proceedings 10th Australian Poultry and Feed Convention*, Melbourne, pp. 232-235.

Kocher, A., Hughes, R.J. and Barr, A.R. (1997). *Proceedings of the Australian Poultry Science Symposium* Ed. D. Balnave, **10**: 142-145.

Leeson, S., Yersin, A. and Volker, L. (1993). *Journal of Applied Poultry Research,* **2**: 208-213.

Noy, Y. and Sklan, D. (1995). *Poultry Science,* **74**: 366-373.

Scott, T.A. and Boldaji, F. (1997). *Poultry Science,* **76**: 594-598.

Smits, C.H.M. and Annison, G. (1996). *World's Poultry Science Journal,* **52**: 203-221.

INVESTIGATIONS INTO THE EFFECT OF XYLANASES AND PECTINASES ON BROILER PERFORMANCE IN SORGHUM BASED DIETS WITH LOW LEVELS OF WHEAT

W. D COWAN[1], D. R. PETTERSSON[2] and G. M. ROSS[3]

Summary

The influence of xylanase and pectinase based enzyme products on broiler performance was examined in diets based predominantly upon sorghum as the main cereal. Addition of a multi- component pectinase enzyme resulted in an increase in final weight and reduction in feed conversion ratio (FCR), that was dose related. Xylanase addition also improved performance. Combinations of the two enzymes improved performance at the lower enzyme dose but, at the higher pectinase dose rate, was not as effective as the high dose of pectinase alone.

I. INTRODUCTION

Xylanase containing enzyme preparations are widely used today to enhance nutrient availability in wheat based broiler diets. The mode of action has been extensively studied and the beneficial effects of NSP-hydrolysing enzymes on performance parameters are generally explained by softening of a so called 'cage effect' on the one hand and by decreasing digesta viscosity on the other hand. The 'cage effect' results in a form of encapsulation of nutrients that prevents access by endogenous digestive enzymes. Viscosity seems to mediate not only an impaired nutrient digestibility but, in addition, modifications in morphology and histology of the intestine, protein and energy metabolism and microbial population in the gastro intestinal tract.

Other cereals that may be used in broiler diets include corn and sorghum and these are not generally associated with high intestinal viscosity levels. The non-starch polysaccharide (NSP) content of their fibre is also significantly different from that found in wheat and barley (Cowan, 1994). Huyghebaert et al. (1995) reported that in balance studies, xylanases and a multi-component pectinase produced only a limited increase in the AMEn value but that larger increases could be observed with soya bean meal.

Huyghebaert (1997) also reported that the AMEn value of wheat was also a function of inclusion level within the test diet. At low inclusion rates, wheat has an apparently higher AMEn than at higher levels due to interaction with other dietary components. As a result of this observation it is possible that, at low inclusion levels, wheat addition will not depress dietary AMEn. Studies of the effect of enzyme addition on the AMEn of sorghum had not resulted in any significant increase in energy availability (Hughes et al., 1998) However, in all of these low wheat circumstances the vegetable protein content of the diet might be more important and hence the differential effect of the two enzymes types needed to be investigated.

This study was made in order to examine the effect of a mono-component xylanase enzyme and a multi-component pectinase, either singly or in combination, on the performance of broilers fed a diet containing sorghum as the main cereal and with a limited inclusion of wheat.

[1]Novo Nordisk S.A. 282 Chartridge Lane, Chesham, Bucks HP5 2SG, UK.
[2]Novo Nordisk A/S, DK 2880 Bagsvaerd, Denmark.
[3]Novo Nordisk Bioindustrial PTY, 22 Loyalty Road, NSW 2151, Australia.

II. MATERIALS AND METHODS

A feeding experiment was carried out in August-September, 1998 at the Pig and Poultry Production Institute in South Australia, using 4080 broiler chickens divided up into 48 groups each consisting of 85 male or female birds. The diet was formulated to reflect current practice within the broiler industry and contained a low level of wheat. A three-phase feeding programme was used and the diet compositions are shown in Table 1.

Table 1.   Composition (g/kg feed) of diets used in performance trial.

Component	Starter diet	Grower diet	Finisher diet
Wheat	150.00	150.00	150.00
Sorghum	444.95	496.50	552.85
Meat meal	65.00	55.00	65.00
Blood meal	10.00	7.50	-
Soyabean meal	185.00	145.00	120.00
Cotton meal	40.00	40.00	25.00
Canola	55.00	60.00	60.00
Tallow	20.00	20.00	10.00
Vegetable oil	10.00	5.00	2.50
Limestone	5.00	5.00	2.50
Salt	0.55	1.70	1.35
Sodium bicarbonate	3.85	2.35	2.40
L-lysine	2.05	1.75	1.55
DL-methionine	2.60	2.70	2.35
Choline chloride	1.00	-	-
Premix	5.00	7.50	4.50
Crude protein	228	209	193
AME (MJ/kg)	12.17	12.18	12.15
Lysine	13.0	11.3	9.90

To each diet the following enzyme additions were made; xylanase (100 FXU/kg feed), pectinase (1000 PSU/kg feed or 3500 PSU/kg feed), xylanase plus pectinase (100 FXU plus 1000 PSU/kg feed or 100 FXU plus 3500 PSU/kg feed). Performance figures were recorded at 16, 30 and 42 days of age. Statistical analysis of the results was performed using the base SAS statistical analysis programme.

To compare performance of broilers receiving the different treatments, FCR values were normalised to a liveweight of 2.3 kg using the procedure of Pesti and Rogers (1997).

III. RESULTS

The performance results for weight gain, FCR and FCR adjusted to a liveweight of 2.3 kg, are shown in Table 2. Addition of xylanase or pectinase resulted in an increase in final weight and a reduction in FCR, with the highest dose of pectinase giving the largest increase in weight. The live-weight gain was statistically ($P<0.05$) higher in the case of the higher dosage of pectinase and for xylanase plus the lower dosage of pectinase. Addition of both enzyme types did not result in a further increase in weight or a reduction in FCR.

Table 2.   Performance results for broilers fed a low sorghum diet supplemented with xylanase and/or pectinase.

Treatment	Live weight gain (kg)	FCR	Adjusted FCR[1]
Control	2.303 [c2] ± 0.197	1.72 ± 0.04	1.713
100 FXU xylanase	2.318 [bc] ± 0.169	1.69 ± 0.04	1.686
1000 PSU pectinase	2.318 [bc] ± 0.192	1.70 ± 0.04	1.690
3500 PSU pectinase	2.364 [a] ± 0.195	1.72 ± 0.05	1.705
100 FXU xylanase + 1000 PSU pectinase	2.359 [ab] ± 0.182	1.70 ± 0.04	1.690
100 FXU xylanase + 3500 PSU pectinase	2.331 [abc] ± 0.207	1.71 ± 0.04	1.705

[1]FCR adjusted to a final weight of 2.3 kg (Pesti and Rogers, 1997).
[2]Means with a different superscript (a-c) are significantly different (P>0.05).

IV. CONCLUSIONS

Addition of a xylanase or pectinase to diets containing low levels of wheat resulted in an improvement in weight gain and a reduction in FCR. Addition of both enzymes gave a final weight that was not statistically different from that obtained with the higher dosage of pectinase. Addition of xylanase together with the higher dosage of xylanase tended to reduce the final weight slightly compared to the combination with the lower pectinase dosage. The result for live weight gain was statistically significant in the case of the higher dosage of pectinase and for xylanase plus the lower dosage of pectinase. The lowest adjusted FCR values were obtained with xylanase, the low dose pectinase and the low dose pectinase plus xylanase. The effect of enzymes upon these low wheat diets was anticipated to be a result of the hydrolysis of non- starch polysaccharides contained within the vegetable protein portion of the diet, rather than upon the sorghum content. Huyghebaert et al. (1995) demonstrated that xylanase as well as pectinase can affect nutrient availability in vegetable proteins. This somewhat surprising observation can be explained by an examination of the fibre make up (Slominski and Campbell, 1990, Düsterhöft, 1993) where it can be seen that xylo-glucans form a significant part of the composition and these may well be partly degraded by xylanases (Düsterhöft, 1993).

The apparent additive effect of xylanase and pectinase compared to a high dose of pectinase implies that there is a maximum release of nutrients obtainable by enzyme addition under these circumstances. The relative role of the two enzyme sources merits further study to determine the optimum balance of enzyme activities to improve nutrient availability and performance of low wheatand sorghum based broiler diets.

REFERENCES

Cowan, W. D. (1994). In: Proceedings of the Second International Round Table on Animal Feed Biotechnology, Eds. S. K. Ho, D.A. Leger and E. E. Lister. Ottawa, Canada.

Düsterhöft, E.M. (1993). In Characterisation and Enzymatic Degradation of Non-Starch Polysaccharides in Lignocellulosic By-Products. Ph.D.   Thesis University of Wageningen, The Netherlands.

Hughes, R. J., Pettersson, D. R. Cowan, W. D, and Ross, G. M. (1998). Unpublished results.

Huyghebaert, G (1997). Animal Feed Science and Technology, 68: 55-66.

Huyghebaert, G., Hastrup, T., Cowan, W. D. and Rasmussen, P.B.(1995). Australian Poultry Science Symposium, 7: 130-134.

Pesti, G. M. and Rogers, S. R. (1997). *Journal of Applied Poultry Research,* **6**, 4, 368-372.

SAS (1988). SAS User's Guide: Statistics, SAS Institute Inc., Cary, NC.

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

APPARENT METABOLISABLE ENERGY VALUES, XYLAN CONTENTS AND DIGESTA VISCOSITY IN RELATION TO BROILER PERFORMANCE ON WHEAT DIETS

C. LIANG and Y. G. LIU

Summary

Wheat when used as the main energy source for broiler chickens is associated with various nutritional problems, including considerable variation in growth rate, feed conversion ratio and wet droppings. This paper reviews relationships in wheat between apparent metabolisable energy (AME), arabinoxylan content and digesta viscosity in relation to broiler performance. Published data showed contradictory results in predicting wheat AME based on its arabinoxylan content. High wheat inclusion in diets often leads to increased gut viscosity, but to a much lower extent compared to those from rye and barley. Enzymes may play a role in improving bird performance on wheat-based diets, which are often associated with a reduction in gut digesta viscosity. However, evidence appears to be mixed and insufficient to support a performance prediction simply on gut digesta viscosity. Overall enzyme effects appear to be more attributable to a wide spectrum of activities rather than to a single feed enzyme. More studies are required to improve our knowledge on the exact mode of action of exogenous enzymes to alleviate the negative influence of low AME wheats on broiler performance.

I. INTRODUCTION

Wheat is a major feed grain in many countries. Its use has tended to increase in many regions where maize and soybean meal have been the traditional ingredients. This has been due mainly to the unstable maize supply and the currency devaluation in some countries. Non-conventional feed ingredients such as wheat, barley and rye are all attracting more attention. During the last ten years significant research into using wheat for broilers has been published. In this paper, research findings on wheat AME values, xylan contents and digesta viscosity in relation to broiler performance, are reviewed.

II. WHEAT AME AND XYLAN CONTENT

Compared with maize as an energy source, broilers fed wheat-based diets showed considerable variation in performance. Wiseman and Inborr (1990) published figures from the UK for 1988 and 1989 showing that feed conversion ratio (FCR) averaged 2.1 but ranged from 1.85 to 2.38. Such variation in FCR may cause serious management problems and economic loss for broiler producers. The variation in performance was due mainly to a considerable variation in apparent metabolisable energy (AME) values of different wheats for broiler; these ranged from 10.35 to 15.94 MJ/kg based on 202 wheat samples collected since 1960. Molar *et al.* (1983) reported AME values of 13 wheat varieties grown in four different locations in Australia; these indicated that the AME values were both variety and location dependent. It was later concluded that the low AME of some wheat was the cause of poor bird performance.

As AME determination requires special techniques, skills and time, efforts were made to develop a rapid prediction of wheat AME values. For example, there is a need to define a

Kemin Industries (Asia) Pte Ltd, 12 Senoko Drive, Singapore 758200.

relationship between wheat AME and the contents of wheat cell-wall material commonly containing anti-nutritional factors, because of their resistance to digestion by the animal's enzymes. These constituents include lignin, cellulose and non-cellulosic polysaccharides (Theander et al., 1989). Arabinoxylan (or pentosan) is the most abundant in wheat compared with oats and barley. Therefore, attempts were made to correlate the contents of wheat arabinoxylan with wheat AME, or performance of poultry fed wheat-based diets.

Two major studies were conducted to determine relationships between the contents of pentosan (or arabinoxylan) and AME values in various wheat varieties grown in Australia (Rogel et al., 1987; Annison, 1991). Surprisingly, the correlation was positive instead of negative (r = 0.5142). Investigations by Annison (1991) found a negative correlation between AME values and the total NSP (r = 0.91), but AME was not correlated with the alkaline-extracted NSP or total arabinoxylan.

III. USING DIGESTA VISCOSITY TO PREDICT BROILER PERFORMANCE

Viscosity is a measurement of a fluid's resistance to flow, typically assayed by using a viscometer with cone and plate. The unit for viscosity is either mPa's (SI) or cP (CGS), with the same measurement values. For digesta viscosity, broilers were killed and dissected between 7 and 35 days of age. Their jejunal and ileal segments were quickly ligated to prevent post-morten digesta movement. Total intestinal contents were collected from the entrance of the bile duct down to the jejunum (for foregut viscosity) and from the jejunum to the ileum for distal-gut viscosity. About 2g of fresh and homogenised digesta were immediately centrifuged at 12,000 g for 5 min and the supernatant was taken for viscosity measurements (Bedford et al., 1991). The same measurement can be tested on wheat extract.

From a number of trials a reduced viscosity was observed following enzyme treatment (e.g. Teitge et al., 1991), especially when barley and rye were used as major energy sources (Bedford and Classen, 1992). They also developed a prediction method for broiler FCR based on logarithm viscosity from 1 to 1,000 cP. For wheat diets, Bedford (1995) published a short abstract claiming a correlation coefficient between broiler FCR and digesta viscosity (r^2 = 0.94), and this was extended to relate enzyme dosage of feed with viscosity of wheat (Bedford, 1997). A careful review of literature on this subject revealed that such correlations were based mostly on diets with exceptionally high viscosity (i.e. 100 to 1000cP) as discussed below.

In order to determine the effect of wheat non-starch polysaccharides (NSP) on broiler performance, Choct and Annison (1992) added alkaline-extracts of wheat milling byproducts into a sorghum-based diet. This alkaline-extract was prepared in a number of steps, including alkaline extraction and hydrolyses by alpha-amylase and proteinase. The final preparation was indeed extremely viscous when dissolved in water. The viscosity values of a 1% and 2% solution were as high as 84 and 2500 cP, respectively. When adding the alkaline-extract to diets at 25 g and 40 g/kg, the viscosity reached 3,000 and 5,000 cP. These diets indeed exerted negative impacts on the digestibility of starch, protein and lipids. However, the viscosity values of these diets would not be encountered under practical conditions. Studies have reported the range of viscosity values for wheat extracts between 1.3 and 5.9 cP (Veldman and Vahl, 1994; Dusel et al., 1998), which would lead to a digesta viscosity value lower than 50 cP.

Table 1 summarises the results of digesta viscosity of broilers fed barley, rye and wheat-based diets and shows that the actual range of digesta viscosity varies enormously. Values for broilers fed a barley diet, from 13 to 93.2 cP; a rye-based diet from 3.2 to 114 cP, and a wheat diet from 2.2 to 21.1 cP. These data suggest that both rye and barley may cause

digesta viscosity of up to 100 cP at high inclusions. For broilers fed wheat-based diets, the fore-gut viscosity ranged from 1.5 to 21.1 cP and the distal viscosity from 2.7 to 39.2 (Allen et al., 1996; Bedford et al.,1991; Bedford, 1997; Teitge et al., 1991; Veldman and Vahl, 1994). A correlation coefficient between the viscosity values and their respective FCR was calculated to be R = -0.3869. Since digesta viscosity values higher than 40 cP for wheat based diets are not commonly encountered under practical circumstances, any extrapolation of the relationship beyond this value must be done with caution. Using Bedford's (1997) data set but transforming it from a log scale into a numerical scale (with the actual viscosity values now from 1 to 100 cP), no meaningful correlation between broiler performance and digesta viscosity can be seen.

Table 1.   Typical gut digesta viscosity values of broiler fed rye, barley and wheat as their energy main source.

Diet type	Viscosity in fore-gut (cP)	Reference
Barley	17.2-93.2	Philip et al. (1995)
	13-29	Almirall et al. (1995)
Rye	18.4-44.4	Bedford and Classen (1993)
	3.2-114	Bedford et al. (1990)
Wheat	2.9-5.6	Veldman and Vahl (1994)
	7.5-35.6	Allen et al. (1996)
	2.2-21.1	Dusel et al. (1998)

Veldmann and Vahl (1994) observed no bird performance differences between two varieties of wheat differing in extract viscosity and pentosan content. In another well-designed study, Dusel et al. (1998) found no clear relationship between determined extract viscosity and FCR of wheat-based diets. Supplementation of xylanase had a positive effect on broiler performance, but the benefit in broiler performance can be explained neither by extract viscosity nor by arabinozylan content. Also, differences in extract viscosity of wheat were not reflected in broiler digesta viscosity. It has also been found that at low viscosities (<10cP) further reductions in viscosity did not result in further improvement in performance. It is therefore concluded that absolute digesta viscosity is not a reliable indicator for predicting performance although, in many cases, the positive effect of enzymes on performance was combined with a decreased digesta viscosity (Dusel et al., 1998).

IV. SINGLE VS MULTI-ENZYMES IN WHEAT DIETS FOR BROILER'S

Although the method for predicting wheat AME and the exact mode of action of using digestive enzyme additives in broiler wheat diets are not yet available, current research findings have shown the effectiveness of exogenous enzymes on broiler performance (Teitge et al., 1991; Veldman and Vahl, 1994; Allen et al., 1996; Dusel et al., 1998). In countries where wheat is used in broiler diets, exogenous enzymes are commonly added to alleviate possible negative effects of low AME wheats on broiler performance. Xylanase is generally believed to be the principal enzyme for wheat diets for broilers. However, a comprehensive study conducted in the UK did not support such hypothesis. The trial was designed to compare the effectiveness of eight different commercial enzyme products on broiler performance in wheat-based diets (van Beek, pers. comm.). The two major parameters used in the study were FCR and xylanase activity. Results demonstrated that the broiler FCR was largely improved by products with multi enzyme activity, but there was no correlation between FCR and xylanase activity. This observation does not rule out the importance of

xylanase in wheat-based diets for broiler, but rather implies that the overall effect may be attributable to a wide spectrum of enzyme activities as well as their synergistic combination.

In conclusion, the results here suggest that there is no clear negative correlation between arabinoxylan content and the wheat AME. Digesta viscosity varies widely in the small intestines of birds and does not necessarily reflect wheat extract viscosity. Additional evidence does not support a viable prediction of broiler performance based simply on viscosity data. More studies are needed in order to understand the exact mode of action of these exogenous enzymes in alleviating the negative effects of low AME wheat on broiler performance.

REFERENCES

Allen, C. M., Bedford, M.R. and McCracken, K.J. (1996). *British Poultry Science*, **37**: S44-S45.

Almirall, M., Francesth, M., Perez-Vendrell, A., Brufau, J. and Esteve-Garcia. E. (1995). *Journal of Nutrition*, **125**: 947-955

Annison, G. (1991). *Journal of Agricultural Food Chemistry*, **39**:1252-1256.

Bedford, M. R. (1995). *Poultry Science*, **74**, Suppl. 1, p18.

Bedford, M. R. (1997). In Recent Advances in Animal Nutrition in Australia 1997, pp.1-7. Eds. J.L. Corbett, M. Choct, J.V. Nolan and J.B. Rowe, The University of New England, Armidale.

Bedford, M. R. and Classen, H. L. (1993). *Poultry Science*, **72**: 137-143.

Bedford, M. R., Classen, H.L and Campbell, G. L. (1991). *Poultry Science*, **70**: 1571-1577.

Bedford, M. R. and Classen, H. L. (1992). *Journal of Nutrition*, **122**:5 60-569.

Beek, van E. (1996). Personal communication.

Choct, M. and Annison, G. (1992). *British Journal of Nutrition*, **67**: 123-132.

Dusel, G., Kluge, H. and Jeroch, H. (1998). *Journal of Applied Poultry Research*, **7**: 119-131.

Mollah, Y., Bryden, W. L., Wallis, I. R., Balnave, D. and Annison, E. F. (1983). *British Poultry Science*, **24**: 81-89

Philip, J. S., Gilbert, H. J. and Smithard, R. R. (1995). *British Poultry Science*, **36**: 599-603.

Rogel, A. M, Annison, E. F., Bryden W. L. and Balnave, D. (1987). *Australian Journal of Agricultural Research*, **38**: 639-649.

Teitge, D. A., Campbell, G. L., Classen H. L. and Thacker, P. A. (1991). *Canadian Journal of Animal Science*, **71**: 507-513.

Theander, O., Westerlund, E. Aman, P. and Graham, H. (1989). *Animal Feed Science Technology*, **23**: 205-225

Veldman, A. and Vahl, H.A. (1994). *British Poultry Science*, **35**: 537-550.

Wiseman, J. and Inborr, J. (1990). In Recent Advances in Animal Nutrition, pp. 79-102. Eds. W. Haresign and D.J.A. Cole. Butterworths, London.

LUPIN OLIGOSACCHARIDES: NUTRIENTS OR ANTI-NUTRIENTS?

A. KOCHER[1,3], R.J. HUGHES[1] and M. CHOCT[2]

Summary

Two AME bioassay studies were conducted to evaluate the nutritive value of ethanol extracted lupin meal. In experiment 1, groups of four birds per cage were fed four semi-purified diets containing untreated and oligosaccharide-reduced, ethanol extracted lupin meal from two cultivars of *L. angustifolius* (cv Gungurru and cv Danja). No significant differences in feed conversion and excreta moisture content were observed among the diets. AME was significantly reduced in diets containing ethanol extracted lupin kernels. Ileal digesta viscosity was significantly increased by removal of oligosaccharides. The second experiment was conducted as a single bird AME bioassay involving four dietary treatments: untreated and ethanol extracted meals of two lupin species (*L. angustifolius* cv Gungurru and *L. albus* cv Kiev mutant). AME and dry matter digestibility of *L. angustifolius* was significantly reduced by removal of oligosaccharides. No such differences were found in diets containing *L. albus*.

I. INTRODUCTION

The lupin species grown in Australia contain low levels of alkaloids making them more suitable for inclusion in pig and poultry diets. High levels of oligosaccharides (OS), in particular α-galactosides, limit their inclusion rate in these diets. Due to a lack of endogenous α-galactosidase monogastric animals cannot digest α-galactosides. 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). However, the nutritive value of legume α-galactosides in broiler diets remains unclear. Ethanol removal of OS from soyabean increased nitrogen corrected true metabolisable energy (TME_N) (Coon *et al*, 1990) whereas ethanol extraction of canola meal resulted in a decrease of TME_N (Slominski *et al.*, 1994). There is little information available on the effects of lupin oligosaccharides on energy availability in broiler diets. The inclusion of 30 g/kg dried α-galactosides from *L. albus* had no effect on bird performance or nutrient digestibility on the jejunum and ileum (Brenes *et al.*, 1989).

This paper reports the results of two studies measuring the effect of oligosaccharides from lupins on performance, apparent metabolisable energy and gut viscosity in broilers.

II. MATERIALS AND METHODS

(a) Preparation of oligosaccharide-free lupin meal

The first experiment included meal from two cultivars of *L. angustifolius*. These were Gungurru and Danja. In experiment 2, kernel of the two lupin species *L. angustifolius* cv Gungurru and *L. albus* cv Kiev mutant were used. In both experiments lupin kernels were

[1]SARDI, Pig and Poultry Production Institute, Nutrition Research Laboratory, University of Adelaide, Roseworthy SA 5371.
[2]University of New England, Division of Animal Science, Armidale NSW 2351.
[3]Current address:University of New England, Division of Animal Science, Armidale 2351.

finely ground and oligosaccharides were extracted in 80% ethanol using a modified procedure described by Coon *et al.*, (1990).

(b) Experiment 1

(i) Diet formulation. Lupin kernel (untreated or ethanol-extracted) was included at 300 g/kg in a semi-purified diet based on sorghum and casein (sorghum 543 g/kg, casein 91 g/kg) and vitamin and minerals 66 g/kg. The basal diet without lupin was included as a control. Each diet was cold pelleted and replicated six times in a randomised block design.

(ii) *AME trial.* The AME study was conducted at the Parafield Poultry Research Centre, Adelaide. Day old mixed sex broiler chickens (Ingham IM98) were raised to 24 days of age in floor pens on commercial starter crumbles. At the start of the AME study birds were weighed in groups of four (two males and two females) and transferred to metabolism cages. A three day period during which feed intake was measured enabled the birds to adapt to their new environment. During the following four days feed intake was measured and all excreta was collected. Excreta moisture content of excreta voided during the second day of the collection period was measured. At the end of the 7 d experimental period all birds were weighed. Two birds from each cage (one male, one female) were killed by intravenous injection of pentobarbitone and ileal digesta were pooled and kept on ice prior to centrifugation then measurement of viscosity.

(c) Experiment 2

(i) Diet formulation. Four semi-purified diets based on sorghum (550 g/kg) and starch (29 g/kg) were prepared with 350 g/kg untreated and ethanol extracted lupin meal. The diets had originally been used in a study to investigate the effects of lupin oligosaccharides on energy digestion in growing pigs (van Barneveld *et al.*, 1996). In the current study additional methionine and lysine were added to meet the nutritional requirements of broilers. Each diet was cold pelleted and replicated twelve times in a randomised block design.

(ii) *AME trial.* The AME study was conducted at the Pig and Poultry Production Institute, Roseworthy. Day old mixed sex broiler chickens (Ingham IM98) were raised to 20 days of age in floor pens on commercial starter crumbles. The birds were transferred at 20 days of age two per cage to individual metabolism cages and given two days to adapt to the new environment. At 22 days of age, one bird was removed from each cage. The remaining chickens were weighed individually. Other procedures were the same as for Experiment 1. At the end of the 7-day experimental period, all birds were weighed individually.

(d) Viscosity measurement and analysis of feed and excreta

Ileal samples were centrifuged at 10,000 *g* for 10 minutes. Viscosity of supernatant was measured in the CSIRO Glenthorne laboratories, Division of Human Nutrition, Adelaide, with a Brookfield DVIII rheometer at 25°C with a CP40 cone and shear rate of $5 - 500$ s^{-1}. Gross energy of excreta and milled feed was measured using a Parr isoperibol bomb calorimeter. Dry matter content of each sample of lupin, and pelleted and milled feed was determined by drying at 105°C.

III. RESULTS

(a) Experiment 1

The removal of oligosaccharides by ethanol extraction significantly ($P<0.05$) reduced AME of the diet and increased ileal viscosity regardless of the lupin cultivar (Table 1). No significant effects were measured for the feed conversion ration (FCR) and the excreta moisture content.

Table 1.   Feed conversion (FCR, g feed/g gain), dietary apparent metabolisable energy (AME, MJ/kg dry matter), excreta moisture content (EM, g/kg)) and ileal viscosity (IV, cP) of untreated and ethanol-extracted lupin kernel meal (n=6; means ± SD).

Lupin cultivar	Ethanol extraction	FCR	AME MJ/kg DM	IV cP	EM g/kg
Gungurru	No	2.01±0.08	13.8±0.3[b]	6.8±4.0[c]	730±20
Gungurru	Yes	2.15±0.25	13.0±0.3[c]	15.2±10.2[ab]	740±30
Danja	No	2.04±0.06	13.4±0.3[b]	8.4±3.0[bc]	740±30
Danja	Yes	2.13±0.19	12.6±0.5[c]	17.6±7.0[a]	740±30
Sorghum control		2.12±0.09	15.4±0.2[a]	3.0±0.6[c]	570±40

[abc] Means with a common superscript are not significantly different ($P<0.05$).

(b) Experiment 2

AME of diet and dry matter digestibility in the diet containing ethanol-extracted *L. angustifolius* was significantly ($P<0.05$) lower than in the diet with untreated lupins (Table 2). No such differences were found between diets containing ethanol-extracted or untreated *L. albus*. Chickens fed *L. albus* had a significantly better FCR than chickens receiving *L. angustifolius*.

Table 2.   Feed conversion (FCR, g feed/g gain), dietary apparent metabolisable energy (AME), excreta moisture content (EM) and dry matter digestibility (DMd, g/g) of untreated and ethanol extracted lupin kernel meal.(n=12; means ± SD).

Lupin species	Cultivar	Ethanol extraction	FCR	AME (MJ/kg DM)	EM (g/kg)	DMd
L. angustifolius	Gungurru	No	1.78±0.13[a]	13.4±0.6[a]	720±40	0.64±0.03[a]
L. angustifolius	Gungurru	Yes	1.86±0.19[a]	12.1±0.9[b]	740±30	0.58±0.04[b]
L. albus	Kiev	No	1.64±0.07[b]	14.2±1.0[a]	700±80	0.66±0.04[a]
L. albus	Kiev	Yes	1.62±0.19[b]	13.7±1.2[a]	720±60	0.63±0.04[a]

[abc] Means with a common superscript are not significantly different (P<0.05).

IV. DISCUSSION

Diets containing ethanol treated *L. angustifolius* lupin meal had significantly lower AME than diets containing the untreated lupins. This result indicates that OS in these lupins species actually contributed to the energy in broiler diets rather than having anti-nutritive effects. This result is even more surprising considering the significant increase in ileal

viscosity in digesta of birds fed the extracted lupin meal. Increased viscosity is regarded as a cause for decreased nutrient absorption (Smits *et al.*, 1997). However, in the second experiment, the ethanol extraction of *L. angustifolius* meal resulted in a significantly higher apparent DM digestibility in comparison to the untreated meal, which is the likely cause for the increase of AME.

In diets containing *L. albus* the ethanol extraction did not significantly change AME or DM digestibility. Although not statistically significant, there was also a numerical decline in energy and DM digestibility with ethanol removal of OS. The reason for the differences between the two lupin species is not clear. The ethanol treatment might have had different effects on the nutritive value of the two lupin species due to differences in composition of di- and tri-saccharides (Saini and Gladstones, 1986) and differences in cell wall structures between the two species.

Chickens were able to utilise the higher AME of *L. albus* for a better feed conversion. However, regardless of lupin cultivar or lupin species the bird performance was not affected by ethanol treatment.

No differences in excreta moisture content were found when chickens were fed untreated or ethanol extracted lupins. A possible increase in bacterial degradation of α-galactosides in the hindgut of birds fed untreated meal was not associated with any increase in fluid retention.

The results of these two studies indicate that OS in *L. angustifolius* kernels contributed to the apparent metabolisable energy in broiler diets. OS in this lupin species are not as severely anti-nutritive as previously thought.

REFERENCES

Brenes, A., Trevifio, J., Centeno, C. and Yuste, P. (1989). Recent Advances in Antinutritional Factors in Legume Seed, pp. 374-377. Eds. J. Huisnan, T.F.B. van der Poel and I.E. Liener.

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

Saini, H.S. (1989). Recent Advances in Antinutritional Factors in Legume Seed, pp. 329-341. Eds. J. Huisnan, T.F.B. van der Poel and I.E. Liener.

Saini, H.S. and Gladstones, J.S. (1986). *Australian Journal of Agricultural Research,* **37**: 157-166.

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

Smits, C.H.M., Veldman, A., Verstegen, M.W.A. and Beynen, A.C. (1997). *Journal of Nutrition,* **127**: 483-487.

van Barneveld, R.J., Olsen, L.E. and Choct, M. (1996). *Proceedings of the Nutrition Society of Australia,* **20**: 114.

QUALITY ASSURANCE AUDITS OF MAREK'S DISEASE VACCINE HANDLING AND ADMINISTRATION PRACTICES IN AUSTRALIAN HATCHERIES

C.A.W. JACKSON

Summary

Quality assurance audits of Australian hatcheries were undertaken to provide advice to companies on the effective administration of poultry vaccines. Audits were completed through observation and interview of key hatchery personnel. From 1997, emphasis was placed on auditing Marek's disease vaccines manufactured by The Mareks Company (TMC). These vaccines differed in that they were cell-associated, dispensed in glass vials, required handling in liquid nitrogen and care in administration. The more common deficiencies recorded were inadequate vaccine records, incorrect waterbath temperature control, unnecessary removal of unwanted vials from liquid nitrogen, slow vial thawing time, failure to agitate the vaccine, failure to complete vaccination within one hour and limited reconciliation of chicks vaccinated. Repeat visits were made to hatcheries to determine if improvements had been made.

I. INTRODUCTION

Quality assurance programs have become an essential feature of poultry production in Australia (Jackson 1986; Best 1996; Jenner 1998). Quality assurance from the farm to the retail sector involves data collection to maintain the highest quality to the consumer (Coleman 1996). When Marek's disease (MD) became a major limitation to bird quality from 1993 and the need to vaccinate broiler chickens developed from 1996, a checklist of MD vaccine handling and administration procedures was designed to assist poultry companies in auditing vaccination procedures in the hatchery (Table 1). Following the manufacture of new cell-associated MD vaccines by TMC, the auditing process was strengthened by regular visitations to most Australian hatcheries to ensure that the vaccines were being administered correctly. This paper describes the auditing process and some of the major deficiencies noted in MD vaccine handling and administration in Australian hatcheries.

II. MATERIALS AND METHODS

(a) Australian hatcheries

Nineteen of 26 commercial hatcheries in Australia were audited. The hatcheries included large commercial broiler hatcheries owned by the major integrated poultry companies, smaller hatcheries owned by medium-sized broiler companies and hatcheries that specialised in layer chick production. The vaccination equipment in the major hatcheries consisted of either automatic vaccinators or EMBREX INOVOJECT ® vaccinating machines. The smaller hatcheries had a mixture of automatic vaccinators and hand vaccination syringes. At the commencement of the audits many of the smaller hatcheries were not equipped to handle cell-associated vaccines and had to purchase liquid nitrogen handling equipment as well as learning the basic procedures of handling cell-associated vaccines. Those hatcheries equipped with EMBREX INOVOJECT ® vaccinating machines were concurrently receiving advice on vaccine administration by EMBREX Inc.

Biological Technology Transfer Pty Ltd, 2 Victory Avenue, Camden NSW, 2570.

(b) Training programs

Merial Select Laboratories provided a series of videos and photographic slides illustrating vaccine handling and administration techniques for cell-associated vaccines. These visual aids together with leaflets on the handling of TMC vaccines were offered to hatcheries in a series of group discussions with hatchery staff. Staff were provided with practical demonstrations of vaccine handling techniques with the equipment that existed in their hatcheries. An initial audit of the MD vaccine handling and administration procedures was usually undertaken following the training program.

(c) Hatchery audit

Audits were undertaken by prior arrangement but were designed to coincide with the normal vaccination program undertaken at the hatchery. Observations on the methods of vaccine handling and administration were made initially without comment as to the correctness or incorrectness of the procedure. Records of vaccine batches received were examined and levels of liquid nitrogen were checked. Waterbath and diluent temperatures were measured and the handling steps were timed. The personnel responsible for vaccine handling were interviewed as to why certain procedures were being followed and noted on the audit forms. The completed audit form was discussed with the hatchery manager and a covering report was provided to the hatchery manager and the company Technical Manager.

Table 1.   Checklist of Marek's disease vaccine handling and administration procedures showing the frequency of observed deficiencies during hatchery audits.

Activity	Standard	Deficiencies[1]
	Equipment	
1. Location of hatchery	Special area for vaccine preparation	* * *
	Air control systems	
2. Vaccine supervisor	Dedicated staff member	* *
3. Vaccine storage	Liquid nitrogen storage adequate	*
4. Liquid nitrogen levels	Book for recording levels	
	Frequency of levels check	
5. Liquid nitrogen supply	Service contract exists	
6. Vaccine records	Identification of batch and deliveries	* * * *
7. Protective clothing	Face shield, gloves, long sleeves, ear noise protectors	* * *
8. Diluent storage	Room temperature at time of use	*
9. Syringes	Changed minimum daily – boiled if reused	*
10. Draw off tubes	Changed minimum daily – boiled if reused	
11. Vaccinating equipment	Cleaned daily and flushed with boiling water	
12. Needle size for vaccinating	19-20 gauge/20 mm length	*
13. Volume of vaccine	Checked daily for volume of 0.2 ml/chick	
14. Function of vaccinating equipment	Checked for faults	
	Vaccine Preparation	
15. Vaccine records	Record of batch no. and dilution	
16. Disinfectants used	Use alcohol pads on equipment or diluent ports	* *
	No contact with vaccine	
17. Diluent contamination	Pink colour check. Yellow bottles not used.	

18 Water bath temperature	Check temperature 27° – 29° C	
	Water bath with adequate heater	* * *
	Temperature maintained during thawing	
19. Diluent preparation	Diluent prepared for reconstitution	*
	Dye or antibiotics added 15 minutes before use	
20. Syringe and needle size for diluting	Prepare a 5 or 10 ml syringe fitted with an 18 gauge / 37 mm (1.5") needle containing 2.0 ml of diluent	* *
21. Vial transfer from liquid nitrogen	Canister not taken higher than container neck. Cane lifted to allow vial removal.	*
	Only vials required taken out of nitrogen	
	Vial placed in water bath immediately after removing from container	
22. Thawing of vials in water bath	Rapid thaw (30-60 sec)	* *
	Vaccine gently swirled as thawing	
	No more than two vials thawed at once	
23. Transfer to diluent	Immediately upon thawing	*
	Gentle transfer procedure	
24. Residue in vials	Vials gently rinsed	* * * *
	Check caps, rinse if required	
25. All vials diluted	All vaccine thawed mixed with diluent	
26. Vaccine mixed	Well mixed and gently rocked	* *
	Rocked every 10-15 mins. during use	
27. Speed of use	Mixture used within one hour	*
	Packs not used within one hour kept cool	
28. Syringe set up	No air bubbles	
	Vaccination Procedure	
29. Vaccination technique	Chickens held correctly	
	No hits on spinal column	
30. Vaccination speed	Syringe <1500 hour	* * *
	Accuvac <2500	
31. Vaccine spillage	Check for misses	
	Use dye	
32. Missed chickens	Re-vaccinated	
33. Vaccine usage	< 1 hour	* *
34. Full pack use	All vaccine used before breaks	
35. Cross check on usage	Compare pack number with chick numbers	* * *
36. Equipment cleanup	Vaccinators stripped and boiled or preferably autoclaved	* *

Frequency of more commonly observed deficiencies during hatchery audits (*)

III. RESULTS

(a) Training programs

Training programs focused on the safe handling of liquid nitrogen, removal of only those vials required for vaccination, rapid thawing and gentle transfer of all the vaccine to room temperature diluent. It was emphasised to hatchery staff that they were handling living cells that required gentle handling. The need for the correct needle size for transfer of vaccine and for chick vaccination was stressed. Staff were also advised on the need to administer the diluted vaccine within one hour and to frequently agitate the vaccine to maintain the cells in suspension.

(b) Quality control audit

The more commonly observed deficiencies over all hatcheries are shown in Table 1 and were each marked with an asterisk (*). A commonly observed deficiency in relation to equipment was the lack of a dedicated vaccine preparation room. Where provided, these rooms often lacked air control systems. Due to the larger number of different types of MD vaccines that were required to be held in liquid nitrogen, several hatcheries had not developed adequate recording systems to precisely identify the types and delivery dates of different batches of vaccine. The change to liquid nitrogen handling found several hatcheries deficient in the range of protective clothing.

The more commonly observed deficiencies in relation to vaccine preparation included a general lack of appreciation of the need for a high level of hygiene in the handling of MD vaccines. In particular, hatchery staff failed to appreciate that the vaccine should be handled so that hatchery air and chick dust does not contaminate the vaccine. Lifting of vials that were not required out of liquid nitrogen for more than 30 seconds was of major concern. Several hatcheries failed to thaw the vaccine within one minute due to inadequate operation of the water bath. Most hatcheries failed to rinse the vials thereby losing some 15% of the virus content.

The more commonly observed deficiency in relation to vaccination procedure was failure to administer the vaccine within one hour. This was related to the inappropriate choice of vaccine pack size for the vaccinating equipment. Several hatcheries did not undertake a cross check of the number of vaccine packs with the number of chicks vaccinated and some hatcheries did not undertake a full strip down of vaccinating equipment prior to sterilisation.

IV. DISCUSSION AND CONCLUSION

Hatcheries that had previously handled cell-free MD vaccines or cell-associated MD vaccines in plastic vials had to adopt a new approach to the thawing and the handling of TMC vaccines. Glass vials should be thawed within one minute and correct waterbath management was needed to achieve rapid thawing. Changes in diluent handling were needed, as the TMC vaccines required diluent to be held and administered at room temperature. The report on the hatchery audit was acted upon quickly by most hatchery managers. However, there was still concern remaining about the maintenance of high levels of hygiene during vaccine handling and an awareness that living cells require care in vaccine preparation and administration.

The value of an independent audit of hatchery vaccination procedures was confirmed through observation of significant deficiencies in Marek's disease handling and administration. Hatcheries responded quickly to recommendations and have adopted practices that should ensure correct vaccination of chickens.

REFERENCES

Best, E. (1996). *Proceedings Tenth Australian Poultry and Feed Convention*, Melbourne, pp 87-89.

Coleman, M. A. (1996). *Proceedings Tenth Australian Poultry and Feed Convention,* Melbourne, pp 21-24.

Jackson, C. A. W. (1986). In Poultry Health, *Proceedings No. 92 The Post-Graduate Committee in Veterinary Science*, The University of Sydney in association with Australian Veterinary Poultry Association. Sydney, pp 631-642.

Jenner, R. (1998). *Proceedings 1998 Poultry Information Exchange*. Queensland Poultry Industry and QDPI. Surfers Paradise, pp 23-27.

EFFECT OF CALCIUM FEEDING ON THE EXPRESSION OF MAREK'S DISEASE

R. D. TAYLOR, G.P.D. JONES and R.D. MURISON

Summary

Two strains of layers were fed compound or choice forms of a standard diet and given calcium as either ground limestone or coarse limestone grit fed separately and provided daily or every second day. Mortality due to Marek's Disease (MD) was monitored during rearing and laying and relative risks of death were assessed by Cox's proportional hazards regression.

Heavy MD mortality in one strain during rearing was not markedly affected by feeding treatments. Deaths due to MD in the laying phase were significantly influenced by the method of feeding and calcium provision. There were excessive losses of choice fed birds given ground calcium included in the protein concentrate. It is postulated that the lower calcium intake may have affected the immune responsiveness of the birds.

I. INTRODUCTION

Marek's Disease (MD) has seriously affected all areas of the local poultry industry. Mortalities of 25-30% have been common in flocks of the imported layer strains. There have been indications that the expression of the disease has been exacerbated by stresses on the birds due, in part, to the more variable environments met under typical Australian conditions. Interactions between nutrition and the immune system of the bird have been highlighted recently (Dietert and Miller, 1996; Husband, 1996; Klasing, 1996). Very little information is available, however, to support the direct involvement of "stress" on the expression of specific diseases such as MD, although a substantial body of anecdotal evidence from nutritionists or poultry veterinarians (M. Evans, G. Firth *pers. comm.*) suggests that this may be the case.

The greatest nutritional stress facing the laying hen is, arguably, that of calcium provision and it is generally accepted (ARC, 1975; Cheng and Coon, 1992; NRC, 1994) that the hen requires the daily availability of calcium. Provision of large particles also allows the bird to consume its calcium without the encumbrances of its energy and protein appetites.

A production experiment examined the responses of two strains of hens, during the rearing and laying phases, to the supply, in both compound and choice diets, of calcium presented as either ground limestone or as limestone grit.

II. METHODS

The parent flock MD vaccination was 0.5 dose (off label) of Maravac, 1.0 dose CR6 and 1.0 dose Herpes Virus Turkey (cell associated). The laying flock vaccination programme included 1.5 dose standard HVT (cell associated) and 0.5 dose Maravac at day-old. Management of the laying birds followed standard commercial practice.

During the rearing and laying phases a 2 x 2 x 3 factorial design was employed using two strains of bird (A and B), two feeding methods (compound and choice) and three methods of providing calcium (Ca 1, ground limestone in the feed; Ca 2, limestone grit available daily; Ca 3, limestone grit available every second day). Profiles over time of body weight and mean calcium intake and egg production and weights were modelled by spline regression, taking into account the correlations due to repeated measures from each bird, using the ASREML programme (Gilmour *et al.*, 1998).

University of New England, Armidale, NSW 2351.

The survival of all birds was monitored from 50 to 111 days (rear) and from 112 to 350 days (lay). The day of each death attributable to MD was recorded and treatments with no deaths, deemed to have no influence on bird survival, were omitted from the analysis. The survival of birds on the treatments where deaths were incurred was modelled by Cox's proportional hazards regression (Cox, 1972). The regression model is of the form; $\lambda(t) = \lambda_0(t)\ exp\ (\beta X)$ where $\lambda(t)$ is the risk of dying at a time t (known as the hazard); covariates such as treatments are denoted by X, β is the effect of the covariate which has to estimated from the data and $\lambda_0(t)$ is the unknown underlying hazard assumed to be the same for each treatment. The assumption of the model is that each bird has an underlying risk but that treatments modify this risk. Treatments (i, j) are compared by relative risk; $\lambda_i(t)\ /\ \lambda_j(t) = exp\ (\beta_i - \beta_j)$.

As the underlying or baseline hazard is unknown, its effect has to be merged with the effect of one of the treatments which by convention is taken as treatment 1. The estimates of β are differences of the log of hazard functions of other treatments to the baseline. The relative risk of two treatments (i, j) is interpreted as the odds of a death from treatment i, compared to a death from treatment j.

III. RESULTS

(a) Rearing

Birds of each strain were lighter at seven weeks of age than the breeders' suggested weights. Body weight remained significantly (P < 0.05) lighter in the strain A birds which grew more slowly (P < 0.05) when fed the choice diet than those fed the complete diet. The method of providing calcium did not influence body weight (P > 0.05).

Daily calcium intake involved significant (P < 0.05) interactions between calcium and week, feed and week, calcium and strain and calcium and feed. The profiles over time differed for each feed and calcium method; the separation of profiles for each calcium method differed between strains and the separation of calcium profiles depended on the feed type, especially for strain B. The strain A birds displayed a greater difference in their response to the different calcium methods across the feeds than did strain B.

(b) Laying

Body weight was significantly (P < 0.01) lighter in strain A than strain B and this difference continued to increase. The feeding method did not influence growth (P > 0.05). The method of providing calcium significantly affected (P < 0.05) body weights. From 22 weeks of age, ground limestone included in the feed (Ca 1) resulted in lighter birds than with the provision of limestone grit either daily (Ca 2) or every second day (Ca 3).

Point of lay (145 days) was not affected (P > 0.05) by any treatment. Egg production met the breeders' standards. A significant (P < 0.05) calcium method x spline (week) interaction occurred but the spline curves revealed no practical difference in egg production.

Daily calcium intake showed a significant (P < 0.05) calcium x spline interaction. The birds fed the Ca 2 diet maintained a greater increase in calcium intake through the peak production period than those fed either the Ca 1 or Ca 3 diets.

(c) Marek's disease mortality

Total losses through MD from 8 to 15 weeks (Table 1) were significantly different (P < 0.05) for strains A (20.8 %) and B (4.2 %) respectively. The relative risk of death due to MD was generally due to strain differences rather than feeding methods within strains.

Table 1. Days to death during rearing (n=32) and laying (n=18) of strains A and B fed with a compound or choice with calcium provided as either ground limestone (Ca 1), limestone grit *ad libitum* (Ca 2) or limestone grit every second day (Ca 3).

Strain	Feed	Ca	Days to death (rearing)	Days to death (laying)
A	Compound	Ca 1	79 82 82 85 89 91	124 139 161 225
		Ca 2	52 79 85 91 94 101 102	119 126 161 176 181 182 227 259
		Ca 3	80 86 86 91 100 102 102	133 154 176 176 204
	Choice	Ca 1	71 81 83 95 102	119 124 126 139 145 146 163 163 163 210 215 251
		Ca 2	78 85 86 86 87 92 93 100	119 124 139 147 238
		Ca 3	63 67 78 83 84 90 91 91 102	131 246
B	Compound	Ca 1	No deaths	No deaths
		Ca 2	83	No deaths
		Ca 3	No deaths	No deaths
	Choice	Ca 1	67	161 163
		Ca 2	56 74 79	233
		Ca 3	81 101	119 170 182 217

Table 2. Relative risk of deaths of strains A and B on feed (compound or choice) and calcium (Ca 1, Ca 2 or Ca 3) treatments where deaths occurred during lay. The relativity is of the treatments in the rows to those in the columns. Asterisks mark relative risks significantly different from 1.

Strain Feed Calcium	A Compound Ca 1	A Compound Ca 2	A Compound Ca 3	A Choice Ca 1	A Choice Ca 2	A Choice Ca 3	B Choice Ca 1	B Choice Ca 2
A Compound Ca 2	2.17							
A Compound Ca 3	1.26	0.58						
A Choice Ca 1	4.22 *	1.94	3.35 *					
A Choice Ca 2	1.33	0.61	1.06	0.32 *				
A Choice Ca 3	0.46	0.21 *	0.36	0.11 *	0.34			
B Choice Ca 1	0.46	0.21 *	0.37	0.11 *	0.35	1.02		
B Choice Ca 2	0.22	0.10 *	0.17	0.05 *	0.16	0.48	0.47	
B Choice Ca 3	0.97	0.45	0.77	0.23 *	0.73	2.13	2.10	4.45

Heavy mortality continued through the laying period (Table 1) and the relative risk of death (Table 2) indicated that providing ground limestone (Ca 1) free choice increased the chances of a strain A bird dying from MD. The risk of death was similarly high when the strain A birds were given a compound diet and limestone grit daily. However, the risk associated with the latter treatment was similar to the other treatments in the strain A birds.

IV. DISCUSSION

The MD "storm" at 12 and 13 weeks may indicate that in the strain A bird the stress of transfer at 7 weeks of age exacerbated the MD outbreak (Jackson and Groves, 1996). The heavy MD mortality in strain A continued into the laying period but was apparently affected

by nutrition. Influences of nutrition on MD have been raised within the industry (M. Evans *pers. comm.*), but have as yet to be documented. The excessive mortality in strain A fed by the choice method and with ground limestone included in the protein concentrate (Ca 1) raises the possibility that nutritional stress, due to an effective calcium restriction, exacerbated the disease. It was apparent that these birds were eating to a crude protein appetite (Taylor, unpublished data) and would not over-eat protein in order to attain calcium intakes similar to the birds on the alternative treatments. Although these birds may not have been in a negative calcium balance, the lower calcium intake may have placed a burden on calcium metabolism with subsequent negative effects on their immune status. The disease problem was more complex than simply a lack of freely available calcium, since compound fed strain A birds given unrestricted access to the limestone grit (Ca 2) also suffered high losses.

The presentation of two forms of the calcium component (ground and particulate) and the diet (compound and choice) indicated that the birds' appetites for energy and protein markedly affected calcium intake (Taylor, unpublished data) and that the strains varied in their response to diet presentation. Strain A did not perform optimally under the choice-feeding regime, although there were few consistent production differences found.

The erratic nature of calcium intake in birds given limestone grit as the sole calcium source, especially when provided every second day, may indicate that these birds have some difficulty in accurately meeting calcium needs, adding to the burden of production. The birds given grit every second day had lower calcium intakes than those given the grit daily which suggests either that they cannot sustain large intakes on the day of access to grit, contrary to the results of Taylor (1996), or that they may retain more of the calcium that they ingest.

V. ACKNOWLEDGEMENTS

REFERENCES

Agricultural Research Council. (1975). The Nutrient Requirements of Farm Livestock. No. 1 Poultry. Technical Reviews and Summaries. (ARC, London).

Cheng, T.K. and Coon, C.N. (1992). *Zootechnica International,* June, pp. 52-63.

Cox, D.R. (1972) *Journal of the Royal Statistical Society,* Series B, **34**: 187-202.

Dietert, R.R. and Miller, T.E. (1996). *Proceedings of the Australian Poultry Science Symposium.* Ed. D. Balnave **8**: 1-7.

Gilmour, A.R., Cullis, B.R., Welham, S.J. and Thompson, R. (1998). ASREML. NSW Agriculture, Australia.

Husband, A.J. (1996). *Proceedings of the Australian Poultry Science Symposium.* Ed. D. Balnave **8**: 14-21.

Jackson, C.A.W. and Groves, P. (1996). *Proceedings of the Australian Poultry Science Symposium,* **8**: 94-98.

Klasing, K. (1996). *Proceedings of the Australian Poultry Science Symposium.* Ed. D. Balnave **8**: 8-13.

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

Taylor, R.D. (1996). *M.Rur.Sc.Thesis,* University of New England.

DEVELOPMENT OF IMMUNITY TO *EIMERIA* SPECIES IN BROILERS REARED UNDER COMMERCIAL CONDITIONS

H. D. CHAPMAN and E. SALEH

Summary

The acquisition of immunity to *Eimeria* species was studied in broilers that were reared in two commercial broiler houses and given a drug program comprising nicarbazin followed by the ionophores salinomycin and monensin. Oocysts of *Eimeria* were found in the litter from both houses indicating that the drugs did not prevent parasite development. Selected birds were removed from each house at three weeks of age and at weekly intervals thereafter placed in battery cages, and given unmedicated feed. Three days later they were challenged with oocysts of *E. acervulina, E. maxima* or *E. tenella* and the number of oocysts produced from day five to eight post inoculation counted. For all species, a significant decrease in oocyst production was evident at four weeks of age. A progressive decrease in oocyst production occurred in older birds and, by seven weeks, oocyst production was substantially reduced (*E. acervulina, E. tenella*) or prevented (*E. maxima*). The results indicate that immunity to the three *Eimeria* species studied is acquired gradually and is not complete until the birds are seven weeks of age. Immunity developed more rapidly to *E. maxima* than the other species.

I. INTRODUCTION

Despite the widespread use of anticoccidial drugs, the parasites that cause coccidiosis (*Eimeria* species) are present on most broiler farms. Usually the numbers of organisms are insufficient to cause clinical disease but, in the absence of medication, they may increase and cause clinical coccidiosis. In the USA, there are currently two "philosophies" regarding methods for the control of coccidiosis in broilers. Most poultry producers believe that best results can be achieved by utilizing the most efficacious drugs and various drug programs have been devised with the objective of minimizing the level of flock infection and thereby maximizing flock performance. A problem with this approach is that, because of the development of drug resistant strains, few 'truly" effective drugs are currently available. An alternative is to use existing drugs but rely upon immunity development. For example, an ionophore may be included in the starter feed at less than the maximum approved level in order to permit some parasite development and then a higher concentration employed during the grower phase to prevent clinical coccidiosis. An advantage of this approach is that it may be possible to increase the duration of the withdrawal period (the period for which a drug is withdrawn from the feed prior to slaughter) with considerable savings in the cost of medication. In order for this to be successful, however, it is important to establish how rapidly immunity will develop when birds are given different drug programs and reared under commercial conditions. In this study the timing of the development of immunity to three species of *Eimeria* in broilers reared in a commercial broiler facility was investigated.

Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701.

II. METHODS

Broilers (Ross Γ x Cobb E) reared at two commercial houses (A and B) were studied. Each house contained about 19,000 birds maintained at a stocking density of 0.08 sq m²/bird. House A is a solid wall, tunnel ventilated building with evaporative cooling pads and interior mixing fans, whereas house B is a conventional building with sidewall curtains, cross-ventilation and evaporative cooling pads. A single previous flock had been reared on the same litter in house A whereas seven previous flocks had been reared on the same litter in house B. Birds received nicarbazin in the starter feed (from 0 to 15 days), and two different ionophorous antibiotics (salinomycin followed by monensin) in the grower feed (from 15 to 41 days). Finisher and withdrawal feeds were provided (from 41 to 48, and 48 to 51 days respectively) that contained no anticoccidial drug. An antibiotic (bacitracin) was provided in the starter, grower, and finisher feeds and the arsenical drug roxarsone in the starter and grower.

Forty-five birds were taken from each house when three weeks of age and at weekly intervals thereafter and randomly allocated to nine cages (five birds/cage) in a battery cage facility. They were provided with an unmedicated ration. A further 45 birds from the same hatch were reared in isolation in a separate facility to serve as susceptible controls. They were transferred to nine cages alongside those holding the commercially reared birds. Three days later, birds in three of nine of the cages were inoculated orally with 2 x 10³ oocysts of *E. acervulina*, 5 x 10³ oocysts of *E. maxima*, or 5 x 10³ oocysts of *E. tenella*. Their excreta were collected from day five to eight post inoculation and the number of oocysts present counted. For each species, oocyst production of birds from the commercial houses was expressed as a percentage of that of the susceptible controls. Data were analysed by one-way analysis of variance using the PROC GLM procedure of the SAS Institute (1988). No significant differences were found between houses and, therefore, results for both houses are combined.

III. RESULTS AND DISCUSSION

The number of oocysts produced by the birds (expressed as a % of the susceptible controls) following challenge with the different species is illustrated in Table 1. For all species, a significant decrease in oocyst production was evident at four weeks of age. A progressive decrease in oocyst production was evident by five, six and seven weeks indicating the gradual development of immunity. By six and seven weeks few or no oocysts of *E. maxima* were found but some oocysts of *E. acervulina* and *E. tenella* were still present. Thus immunity to *E. maxima* appeared to develop more rapidly, a finding consistent with previous observations (Rose and Long, 1962). There have been few other studies on the timing of the development of immunity to *Eimeria* species in commercially reared broilers. It was shown that birds reared at two farms where the coccidiostat nicarbazin was used after salinomycin developed immunity to *E. acervulina* and *E. maxima* by six weeks of age but that birds from only one of the farms developed immunity to *E. tenella* (Chapman, 1992). The present results show that immunity can develop to *Eimeria* species but that the process takes time. Several cycles of parasite development are probably necessary for this process to occur. In the USA some producers withdraw anticoccidial drugs from the feed when birds are thirty days of age. These results indicate that some protection against challenge was evident at four weeks but that this process was not complete. From a practical point of view, it is important not to withdraw drugs prematurely, since birds may not have acquired solid immunity and are therefore potentially at risk of coccidiosis.

133

Table 1. Number of oocysts produced by birds from commercial broiler houses (expressed as a percentage of that of susceptible controls) following challenge with three species of *Eimeria*[1].

Age (weeks)	Number of oocysts (% of controls)		
	E. acervulina	*E. maxima*	*E. tenella*
3	96.8[a]	107.8[a]	113.2[a]
4	60.3[b]	43.3[b]	61.4[b]
5	42.4[bc]	11.5[c]	21.3[c]
6	22.0[cd]	0.9[c]	29.6[c]
7	19.8[d]	0[c]	29.9[c]
SEM[2]	7.1	6.6	12.8
Probability	.0001	.0001	.0003

[1]Each observation is the mean of six cages each with five birds
[2]SEM standard error of the mean
[a, b, c, d] values in columns with no common superscript differ significantly (P<0.05)

REFERENCES

Chapman, H.D. (1992). *Poultry Science,* **71**: 577-580.
Rose, M.E. and Long, P.L. (1962). *Immunology* **5**: 79-92.
SAS Institute (1988). SAS/STAT® User's Guide: 1988 Edition. SAS Institute Inc., Cary, NC, USA.

Not much natural immunity until 6 weeks

Drugs allow it to develop OK (except higher synthetic after coxo).

In field — no immunity at 3 weeks even though high no. oocysts found at outset.

Never complete immunity c tenella by 7 wks

→ Thus 5-6 weeks for immunity.

GROWTH OF BODY COMPONENTS IN BROILERS

JULIAN WISEMAN

Summary

Ths paper reviews changes that are occurring in the chicken meat industry in response to customers' changing requirements and perceptions particularly in regard to fat content and meat components. An experiment is reported here in which two starter and two finished diets of high or low nutrient and energy concentrations with the same protein:energy ratio, and a commercial type diet, were fed to broilers which were killed at intervals and carcass components measured. Results of components presented are to three kg liveweight.

I. INTRODUCTION

Composition of liveweight gain is assuming increasing importance, probably for two main reasons. First, carcass fat content in poultry meat has been viewed traditionally as a relatively healthy and low fat meat in comparison with pork, beef and lamb. However broiler carcasses are not classified according to fat content in contrast to the three other species. This is probably attributable to the lack of a discrete adipose layer in broilers. The second reason is that total saleable meat is not always as important as the relative weight of the individual components. Their growth has also been linked to the means whereby nutritional practices might be able to influence their content; this has also been linked to studies of 'compensatory' growth where birds undergo an early feed restriction followed by re-feeding. This will influence both overall and component weight gain and has also been employed to avoid undue metabolic stress which is associated with the rapidly growing broiler.

II. BACKGROUND

Broiler diets are of high nutrient concentration but the incorporation of fats into these diets to achieve the necessary levels of apparent metabolisable energy (AME) together with high dietary levels of AME *per se* may contribute to excess fat growth in fast-growing broiler chickens. This has implications both for evisceration losses and the fat content of saleable meat. Previous investigations have examined the role of nutrition, in particular manipulation of the energy:protein ratio, on carcass quality (Bartov *et al.*, 1974). Feed (and dietary energy) restriction in early life followed by a refeeding period results in improved efficiency of feed utilization and reduced fat content of the carcass (Kubena *et al.*, 1974; Plavnik and Hurwitz 1985; Jones and Farrell, 1992a) and sometimes with a smaller final body weight (Jones and Wiseman, 1985), or with no difference (Deaton *et al.*, 1973; Griffiths *et al.*, 1977). Susbilla *et al.* (1994) demonstrated that early feed restriction did result in subsequent improved relative growth rate, but without any difference in feed conversion ratio and carcass fat content. Although Jones and Farrell (1992b) examined body fat accretion, most of these studies have tended to consider the effects of nutritional regime at one point in time (slaughter) rather than the pattern of these changes over time during the growth of the bird although Susbilla *et al.* (1994) did examine the pattern of internal organ growth.

The most economic means of producing chicken meat from current commercial strains of broiler chicken will probably be a compromise between achieving a satisfactory

University of Nottingham Sutton Bonington Campus, Loughborough Leics LE12 5RD, UK.

carcass and the bird's potential for maximum liveweight gain. However, fat deposition is an integral part of growth in meat-producing birds and its extent and distribution throughout the carcass is an important aspect of meat quality. Hakansson *et al.* (1978a,b) examined the distribution of lipid in tissues within broiler carcasses as influenced by dietary nutrient concentration and age but did not report data for individual fat depots.

Mathematical models are becoming increasingly useful in examining overall growth in broilers although this approach has been infrequently employed to examine carcass composition. Wilson (1977) suggested that the Gompertz equation (Laird, 1966) is applicable to avian species and Tzeng and Becker (1981) fitted this equation to growth data for broiler chickens. The latter study was aimed primarily at relating abdominal fat growth curves to total carcass fat. Knizetova *et al.* (1991) employed equations to compare the pattern of growth of live body weight between genotypes and suggested that such an approach would be of value both in selection of genotypes and feeding patterns, however growth patterns of components were not examined.

III. EXPERIMENTAL PROGRAMME

In a recent trial (Wiseman and Lewis, 1998) two starter and two finisher diets were formulated to be of high (H) and low (L) nutrient and energy concentration but with the same protein:energy ratio (g CP/ MJ AME = 17.5 and 15.4 respectively for starter and finisher). A third diet (commercial, C) was produced by mixing equal quantities of H and L in both starter and finisher phases. Each week 5 birds were slaughtered by dislocation of the first cervical vertebra mechanically plucked and dissected to isolate muscle and fat components (visceral fat, abdominal fat and skin fat) which were recorded. A Gompertz curve of the form $W = A + C \times \exp (-\exp(-B(t-M)))$ was fitted, where: W = weight at time, t (days), of live body weight or carcass component; t = age of chicken (days); A + C = the asymptotic weight approached, an estimate of the mature weight; B = the rate of exponential decay of the initial growth rate, a measure of the decline in growth rate; M = the age (days) at which growth rate is maximum.

IV. EFFECTS ON CARCASS COMPOSITION

Early feed restriction in broilers has been investigated because of the possible reductions in body fat, but not overall body weight, that might result. There were large differences in the pattern of growth of individual fat depots measured in the current study and, on a time basis, those from birds fed HH were always greater than those from LH. A more appropriate means of comparison is on a live weight basis (Table 1), which demonstrates that, at the same body weight, birds on HH were leaner (in terms of the dissectable fat depots measured) than those on LH. LL birds had the smallest fat depots followed by HL and then CC. Plavnik and Hurwitz (1985) observed that there was a lower proportion of abdominal fat (the depot most often employed to characterize overall fatness of broilers) following early feed restriction, which may also have contributed to the better feed efficiency of these birds. It was argued that the reduced fatness was attributable to impaired hyperplasia of adipocytes (as observed by March and Hansen, 1976). Jones and Farrell (1992b) concluded that the success of early feed restriction in reducing carcass fat (but no other component) was attributable to a temporary delay in fat deposition, although the older the birds, the more the likelihood that this advantage would disappear. The results of the current study support this latter observation, although LH birds were always fatter at a given weight than HH when total fat content was considered. However, there was variation with

individual fat depots where LH birds were less fat. This suggests differential responsiveness of individual fat depots to nutrient and energy intake. Hargis and Creger (1980) reduced nutrient and energy intake for the first 7 days of life whereas Arafa *et al.* (1983) reduced energy intake during the last 10 days of the growth period following a commercial starter. Both studies reported a reduction in carcass fat at 49 days of age.

Table 1.   Estimates of weight of carcass components (g) at live body weights.

Diet	Live weight (g)								
	1000	1250	1500	1750	2000	2250	2500	2750	3000
Carcass protein (g)									
HH	166	214	264	314	365	417	469	521	573
HL	170	226	284	342	400	456	510	563	612
CC	178	230	282	334	385	436	484	532	577
LH	171	225	279	333	386	438	488	536	581
LL	181	239	296	350	402	451	497	538	577
Breast muscle (g)									
HH	152	191	231	271	313	357	402	449	497
HL	149	186	224	265	307	353	402	454	511
CC	146	185	226	269	313	360	409	460	513
LH	128	166	207	249	293	338	385	433	482
LL	138	180	223	267	312	358	404	450	497
Thigh and leg muscle (g)									
HH	148	186	225	266	307	350	394	439	487
HL	150	189	229	270	313	358	405	454	506
CC	150	191	232	275	319	364	409	457	505
LH	144	184	225	268	311	356	402	450	499
LL	148	192	238	284	330	376	422	467	512
Carcass fat (g)									
HH	93	124	159	198	240	286	337	394	456
HL	82	113	147	181	217	252	288	322	355
CC	88	121	158	196	238	277	317	358	399
LH	97	134	174	217	262	309	359	410	463
LL	82	106	131	159	189	222	258	299	345

Offering diets of lower energy and nutrient concentration in the finisher phase (HL) was associated with less body fat than LH, which is to be expected as fat is generally regarded as a late maturing tissue. Furthermore, at a given weight HL birds had more white meat than LH, suggesting that feeding diets of lower energy and nutrient concentration early in life is not as effective as such an approach imposed later in life. However, the HL birds achieved specific live weights later than LH and did so with greater feed consumption (and hence poorer feed conversion ratio).

The ability of birds to compensate for early nutrient and energy restriction in terms of lean tissue weights is, according to Jones and Farrell (1992b), dependent upon the severity of this restriction. Susbilla *et al.* (1994) observed that the weight of breast and thighs at 40d of

age was not influenced by feed restriction. It is likely that the current study was more severe in this context as the content of white meat (i.e. breast muscles) from birds fed HH was always, albeit marginally, greater at the same live body weight than birds fed on LH. The effect of feeding diets of low energy and nutrient content early in life was different when considering dark meat where the small differences recorded were in favour of LH particularly at higher weights.

V. CONCLUSION

Estimation of the growth of body weight and carcass components as influenced by nutritional regimens allows an assessment of the pattern of growth over time. Decisions on which conditions are most appropriate will be influenced by the time taken to reach specific live weights if whole birds are to be marketed, or the rate of growth of individual portions if further processing is to be considered, together with feed conversion ratio and the relative costs of diets varying in energy and nutrient concentration. However, differences in carcass composition were marginal. Of additional importance (although not subject of this paper) is the overall performance of birds; energy and nutrient restriction early in life has been confirmed as being able to offer potential in that, when offered a diet with a higher concentration of energy and nutrients having been previously fed one with lower concentrations, performance of birds in terms of live weight and feed conversion ratio over time approaches that of the control group fed high concentration diets throughout.

REFERENCES

Arafa, A.S, Boone, M.A., Janky, D.M., Wilson, M.R., Miles, R.D. and Harms, R.M. (1983). *Poultry Science,* **62**: 314.

Bartov, I, Bornstein, S. and Lipstein, B. (1974). *British Poultry Science,* **15**: 107.

Deaton, J.W., Reece, F.N., Kubena, L.F., Lott, B.D. and May, J.D. (1973). *Poultry Science,* **52**: 262.

Griffiths, L. Leeson, S. and Summers, J.F. (1977). *Poultry Science,* **56**: 638.

Hakansson, J., Eriksson, S. and Svensson, S.A. (1978a). Report No.57. Swedish University of Agricultural Science, Department of Animal Husbandry.

Hakansson, J., Eriksson, S. and Svensson, S.A. (1978b). Report No. 59. Swedish University of Agricultural Science, Department of Animal Husbandry.

Hargis, P.H. and Creger, C.R. (1980). *Poultry Science,* **59**: 1499.

Jones, G.P.D. and Farrell, D.J. (1992a). *British Poultry Science,* **33** :579.

Jones, G.P.D. and Farrell, D.J. (1992b). *British Poultry Science,* **33**: 589.

Jones, R.L. and Wiseman J. (1985). *British Poultry Science,* **26**: 381.

Knizetova, H., Hyanek, J., Knize, B. and Roubicek, J. (1991). *British Poultry Science,* **32**: 1027.

Kubena, L.F., Chen, T.C., Deaton, J.W. and Reece, F.N. (1974). *Poultry Science,* **53**: 974.

Laird, A.K. (1966). *Growth,* **30**: 349.

March, B.E. and Hensen, G. (1976). *Poultry Science,* **56**: 886.

Plavnik, I. and Hurwitz, S. (1985). *Poultry Science,* **64**: 348.

Susbilla, J.P., Frankel, T.L., Parkinson, G. and Gow, C.B. (1994). *British Poultry Science,* **35**: 677.

Tzeng, R. and Becker, W.A. (1981). *Poultry Science,* **60**: 1101.

Wilson, B.J. (1977). In Growth and Poultry Meat Production, p89. Eds K.N. Boorman and B.J. Wilson, British Poultry Science Ltd., Edinburgh.

Wiseman, J. and Lewis, C.E. (1998). *Journal of Agricultural Science (Cambs).* In Press.

EFFECT OF DIFFERENT COMMERCIAL ENZYMES ON EGG AND EGG SHELL QUALITY IN FOUR STRAINS OF LAYING HEN

J.R. ROBERTS, M. CHOCT and W. BALL

Summary

Four different commercial enzyme products were added to standard commercial layer diets, based on either barley or wheat, and these diets were fed to four different strains of commercial laying hen. The strains of birds used were Isa Brown, Hy-Line CB, Tegel SB2 and Tegel HiSex. Diets were fed for five weeks prior to measurements of egg and egg shell quality. The inclusion of commercial enzyme products in the diets had no effect on egg weight. Positive effects of the enzymes were improved egg shell breaking strength and shell weight for both dietary treatments. Percentage shell was improved with use of enzymes, especially in the barley-based diet. Negative effects of the enzyme products were slightly lighter coloured egg shells and reduced albumen quality for both dietary treatments. Yolk colour was reduced for two of the enzymes, particularly for the barley-based diet.

I. INTRODUCTION

The use of enzymes in commercial layer diets has become more common in recent years (Bedford and Morgan, 1996; Gordon and Roland, 1997; Leeson and Summers, 1997; van der Klis *et al.*, 1996). Enzymes are employed 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. Some ingredients present in feed bind other feed components such as phosphorus, calcium and trace minerals. Therefore, use of appropriate enzymes will increase the availability of these feed components, many of which influence egg shell quality (Hurwitz, 1987). However, concern has been expressed about reduced egg shell quality resulting from the use of enzymes (Richards, 1998). In addition, the cereal grain on which the layer diet is based may affect egg and egg shell quality (Roberts *et al.*, 1998).

Four commercial feed enzymes were added to either a barley-based or wheat-based standard layer diet, formulated to standard commercial guidelines. Egg and egg shell quality were assessed following five weeks on the experimental diets.

II. METHODS

This study was part of an investigation into ways of reducing the incidence of wet droppings (see Choct, 1999). One thousand two hundred birds were maintained, three to a cage, in a commercial poultry house at the University of New England "Laureldale" Poultry Farm. Four strains of bird were used, 300 of each of the following strains: Isa Brown, Hy-Line CB, Tegel SB2 and Tegel HiSex. Two experiments were conducted. The first, which was conducted in April-May, 1998, used a standard commercial layer crumble diet, based on barley. There was a control group and four experimental groups. The experimental groups had different commercial enzymes added to them, as per the manufacturers' instructions. The second experiment was conducted in August-September, 1998, and was identical to the first experiment except that the layer diets used were wheat-based. Birds were fed the diets for a period of 5 weeks prior to the collection of eggs for analysis.

Divisions of Animal Physiology and Animal Science, School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351.

For each dietary treatment, 800 eggs were collected, 40 from each strain for each of the five 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), and shell weight. The percentage shell was calculated as the ratio of shell weight to egg weight, expressed as a percentage. The internal quality of the eggs was assessed as albumen height and Haugh Units as well as yolk colour. Data were analysed by two factor ANOVA and differences between means were assessed by Fisher's (Protected) Least Significance Difference test. Significance was assumed at $P<0.05$.

III. RESULTS

The effects of the different commercial enzymes on egg and egg shell quality are summarised in Table 1. Egg weight was not significantly affected by the inclusion of feed enzymes for either the barley-based or wheat-based diets. However, the colour of the shells was significantly lighter (higher reflectivity) when enzymes were added to both the barley-based and wheat-based diets. The addition of all four enzymes to the barley-based diet resulted in significant improvements in egg shell quality, as indicated by increased egg shell breaking strength, shell weight and percentage shell (ratio of shell weight to egg weight).

Table 1.   The effect of enzyme preparation on egg and egg shell quality in four strains of laying hen receiving diets based on either barley or wheat. Values are means (± SEM). P values refer to comparisons between enzymes for each diet.

	Grain	Control	Enzyme 1	Enzyme 2	Enzyme 3	Enzyme 4	P value
Egg wt (g)	Barley	64.1 (0.4)	63.5 (0.4)	63.8 (0.4)	63.0 (0.4)	63.6 (0.5)	NS
	Wheat	65.6 (0.4)	65.7 (0.4)	65.8 (0.4)	65.3 (0.5)	66.2 (0.4)	NS
Reflect (%)	Barley	42.9 (0.7)	44.4 (0.6)	45.2 (0.7)	44.7 (0.6)	45.2 (0.8)	0.0014
	Wheat	41.7 (0.6)	41.2 (0.7)	42.6 (0.6)	43.1 (0.6)	43.8 (0.7)	0.0003
Break str	Barley	33.2 (0.6)	36.7 (0.5)	36.4 (0.6)	37.1 (0.6)	37.6 (0.5)	<0.0001
	Wheat	32.6 (0.8)	34.7 (0.7)	31.9 (0.7)	35.1 (0.8)	34.0 (0.7)	0.0050
Shell wt (g)	Barley	5.55 (0.05)	5.76 (0.05)	5.83 (0.05)	5.72 (0.04)	5.82 (0.05)	<0.0001
	Wheat	5.90 (0.05)	6.02 (0.05)	5.80 (0.05)	5.93 (0.06)	5.93 (0.05)	0.0143
Shell (%)	Barley	8.67 (0.06)	8.96 (0.06)	9.15 (0.05)	9.08 (0.04)	9.16 (0.06)	<0.0001
	Wheat	9.00 (0.06)	9.16 (0.06)	8.82 (0.06)	9.09 (0.07)	8.96 (0.06)	0.0007
Albumen ht (mm)	Barley	8.63 (0.12)	8.44 (0.11)	8.21 (0.10)	7.69 (0.10)	7.58 (0.11)	<0.0001
	Wheat	7.78 (0.12)	7.33 (0.11)	7.34 (0.12)	6.80 (0.10)	7.39 (0.11)	<0.0001
Haugh units	Barley	91.0 (0.7)	90.2 (0.6)	89.0 (0.6)	86.2 (0.6)	85.2 (0.6)	<0.0001
	Wheat	85.9 (0.7)	82.9 (0.8)	82.5 (0.9)	79.7 (0.7)	83.3 (0.7)	<0.0001
Yolk colour	Barley	10.0 (0.1)	10.3 (0.1)	10.5 (0.1)	9.8 (0.1)	9.4 (0.1)	<0.0001
	Wheat	11.1 (0.1)	11.0 (0.1)	10.9 (0.1)	10.7 (0.1)	10.5 (0.1)	<0.0001

For the wheat-based diet, improvements in shell breaking strength and shell weight were observed for enzymes 1, 3 and 4 but not for enzyme 2. In addition, the percentage shell was improved for enzymes 1 and 3 but not for enzymes 2 and 4. The internal quality of the eggs,

as indicated by albumen height and Haugh Units, was reduced when enzymes were added to both the barley-based and wheat-based diets. Yolk colour was significantly reduced by the presence of feed enzymes 3 and 4. The incidence of cracked and broken eggs was generally lower when enzymes were added. Significant differences were observed among strains of bird for both of the dietary treatments. As has been described previously (Roberts *et al.*, 1997), egg weight was greater in the two imported strains than for the Australian strains and the imported strains had darker coloured egg shells (as indicated by lower shell reflectivity). Shell weight was consistently higher for the imported strains, with percentage shell generally higher also. However, the strength of the egg shells, as measured by egg shell breaking strength, was not consistently related to whether the strain was imported or Australian-bred. For the wheat-based diet, there were no significant differences among strains. For the barley-based diet, shell breaking strength was highest in the HiSex and Tegel SB2 birds, lowest in the HyLine CB, with the Isa birds intermediate. There were significant differences among strains for internal egg quality (albumen height, Haugh Units and yolk colour) although this was not related to whether the strain was Australian or imported.

Table 2: The effect of strain on egg and egg shell quality in four strains of laying hen receiving diets based on either barley or wheat and containing different feed enzymes. Values are means (±SEM). P values refer to comparisons between strains for each diet.

	Grain	Isa Brown	HiSex	HyLine CB	Tegel SB2	P Value
Egg wt (g)	Barley	65.7 (0.3)	67.2 (0.4)	59.5 (0.3)	61.9 (0.3)	<0.0001
	Wheat	67.6 (0.3)	68.9 (0.4)	61.5 (0.3)	64.8 (0.3)	<0.0001
Reflect (%)	Barley	38.9 (0.4)	37.9 (0.4)	53.2 (0.5)	47.9 (0.4)	<0.0001
	Wheat	36.9 (0.4)	37.0 (0.4)	49.8 (0.5)	46.3 (0.4)	<0.0001
Break str	Barley	35.9 (0.5)	37.1 (0.5)	34.9 (0.5)	36.7 (0.5)	0.0103
	Wheat	33.1 (0.6)	34.5 (0.7)	33.2 (0.6)	33.7 (0.7)	NS
Shell wt (g)	Barley	5.96 (0.04)	6.09(0.04)	5.24 (0.03)	5.65 (0.04)	<0.0001
	Wheat	6.16 (0.04)	6.26 (0.04)	5.36 (0.03)	5.88 (0.04)	<0.0001
Shell (%)	Barley	9.10 (0.05)	9.07 (0.06)	8.81 (0.05)	9.04 (0.05)	<0.0001
	Wheat	9.12 (0.05)	9.09 (0.06)	8.72 (0.04)	9.09 (0.06)	<0.0001
Albumen ht (mm)	Barley	8.07 (0.10)	8.08 (0.10)	8.35 (0.10)	7.94 (0.10)	0.0209
	Wheat	7.28 (0.10)	7.41 (0.11)	7.59 (0.10)	7.03 (0.10)	0.0006
Haugh units	Barley	87.6 (0.6)	87.2 (0.6)	90.8 (0.5)	87.8 (0.6)	<0.0001
	Wheat	82.3 (0.6)	82.5 (0.7)	85.4 (0.7)	81.3 (0.6)	<0.0001
Yolk colour	Barley	10.1 (0.1)	10.2 (0.1)	10.1 (0.1)	9.6 (0.1)	<0.0001
	Wheat	10.8 (0.1)	10.7 (0.0)	11.0 (0.1)	10.9 (0.1)	0.0004

There were significant differences in egg and egg shell quality between the barley-based and wheat-based diets. However, because the trial using barley-based diets was conducted in April-May, 1998 and the trial with wheat-based diets was in August-September, comparisons between the two trials must take into account the different times of year and the different ages of the birds. Most of the differences between the two trials could be accounted for by the different ages of the birds at the times the trials were conducted.

IV. DISCUSSION AND CONCLUSIONS

Benefits arising from the use of enzyme preparations in layer diets must be offset against any negative effects which the enzymes may have on the eggs produced. While concern has been expressed about negative effects on egg shell quality (Richards, 1998), studies have reported improvements in production (van der Klis *et al.*, 1997) and generally no effect on egg shell quality (Carlos and Edwards, 1998; Gordon and Roland, 1997).

The present study demonstrated improvements in egg shell quality as the result of adding commercial enzyme preparations to either barley-based or wheat-based layer diets. However, the enzymes caused a small reduction in the colour of the egg shells and a reduction in albumen quality. Minor effects on yolk colour were also observed for some enzymes.

V. ACKNOWLEDGEMENTS

REFERENCES

Bedford, M.R. and Morgan, A.J. (1996). *World's Poultry Science Journal,* **52**: 61-68.

Carlos, A.B. and Edwards, H.M. (1998). *Poultry Science,* **77**: 850-858.

Choct, M. (1999). *Proceedings of the Australian Poultry Science Symposium.* Ed. D.J. Farrell **11** (in press).

Gordon, R.W. and D.A. Roland. (1997). *Poultry Science* **76**: 1172-1177.

Hurwitz, S. (1987). In: Egg Quality – Current Problems and Recent Advances. pp. 235-254. Ed. R.G. Wells and C.G. Belyavin, Butterworths, London.

Leeson, S. and Summers, J.D. (1997). Commercial Poultry Nutrition. University Books, Guelph, Canada.

Richards, G. (1998). Alltech's 12th Annual Asia Pacific Lecture Tour, pp. 35-40.

Roberts, J.R., Ball, W. and Leary, A. (1998). *Proceedings of the Australian Poultry Science Symposium.* Ed. R.A. Pym **10**: 199.

Roberts, J.R., Leary, A, Ball, W and Nolan, J.V. (1997). *Proceedings of the Australian Poultry Science Symposium.* Ed. D. Balnave **9**: 126-129.

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

THE EFFECT OF DIETARY SODIUM SUPPLEMENTATION ON EGG SHELL QUALITY AND ELECTROLYTE BALANCE IN AUSTRALIAN LAYERS

N. GONGRUTTANANUN, J.R. ROBERTS and W. BALL

Nurat

(Thailand)

Summary

This study was conducted to determine the effects of dietary supplementation with either sodium chloride or sodium bicarbonate in Australian Hy-Line CB laying hens. Sodium supplementation has the potential to reduce cannibalistic behaviour but could result in reductions in shell quality and in the production of wet droppings. Inclusion of sodium chloride or sodium bicarbonate up to the level of 4 g Na/kg feed had no deleterious effects on egg shell quality or egg production. However, sodium bicarbonate resulted in better feed efficiency and less watery droppings than sodium chloride. Faecal moisture content increased with increased dietary sodium, accompanied by increased sodium and chloride concentrations in urine.

I. INTRODUCTION

During an experimental trial at the Laureldale Poultry Farm at the University of New England, an increased incidence of vent pecking and cannibalism was found to be associated with a lower than normal level of sodium in the diet (J.V. Nolan, personal communication). This finding resulted in the prediction that providing a higher than normal level of sodium in the diet may, conversely, reduce the incidence of vent pecking and cannibalism. However, there was concern that sodium supplementation may adversely affect eggshell quality and egg production and produce wet droppings. Hijikuro (1976) reported that manure moisture content increased with increased sodium chloride in the diet. In addition, Damron and Kelly (1987) reported that egg production and egg weight were reduced when hens were fed a high level of dietary NaCl. Howes (1966) reported that dietary $NaHCO_3$ supplementation improved egg shell specific gravity and egg shell thickness, whereas others failed to obtain similar results (Pepper *et al.*, 1968; Ferguson *et al.*, 1974; Grizzle *et al.*, 1992). Therefore, the present study was conducted to assess the effect of dietary sodium supplementation with either sodium chloride (NaCl) or sodium bicarbonate ($NaHCO_3$) on production, egg shell quality, manure moisture and electrolyte balance in laying hens.

II. METHODS

The experiment was conducted with Hy-Line CB laying hens at 38 weeks of age. Birds were divided randomly into three groups of 12 hens and housed individually. Group 1 received standard control ration (Super All Layer Feed, Ridley Agriproducts) containing 160g crude protein, 45g crude fibre, 33g crude fat, 4.0g salt and 0.2g fluorine/kg. Groups 2 and 3 had sodium added to the diet in the form of either NaCl or $NaHCO_3$ in three stages. Feed containing 2.4g Na/kg was maintained for 12 weeks, followed by 3.2g Na/kg feed for 8 weeks and then 4.0g Na/kg feed for 8 weeks. Group 2 received NaCl and Group 3 $NaHCO_3$ supplementation. The birds were maintained at ambient temperature and a 16L:8D photoperiod throughout the study. Measurements were made during the last two weeks of

Divisions of Animal Physiology and Animal Science, School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351.

each stage. Daily egg production, determined as percentage of hen-day, and feed intake were recorded on an individual bird basis. Egg shell quality measurements (shell breaking strength, deformation, shell reflectivity, shell weight, and shell thickness) were carried out on all eggs laid on two consecutive days from the last two weeks of each stage. On the last day of each stage, droppings were collected, weighed, dried at 90-95 °C for 24 h, and the percentage of moisture content calculated. At the end of each stage, 6 birds from each group were removed and placed in a cotton sling for the collection of urine samples. The urine samples were weighed, urine flow rate calculated and the osmolality and concentration of the electrolytes Na, Cl and K were determined. Blood samples were obtained from the brachial vein of the same birds on the following day. Haematocrit was determined, the remainder of the blood was centrifuged and the plasma was removed for the measurement of osmolality and the concentration of electrolytes. Results were analysed statistically by analysis of variance. Significance was assumed at P<0.05.

III. RESULTS

There were no significant differences among the three groups in hen-day production, egg weight or any aspect of eggshell quality at each stage of the study (Table 1). In stage 1, the birds in Group 3 had significantly lower feed conversion ratios than the birds in Group 2. A significant difference in the faecal moisture content among the groups was observed in stages 2 and 3, with the highest values in Group 2. In stage 3, the manure moisture content of Groups 2 and 3 was higher than that of Group 1 (Table 1). Haematocrit did not show any significant differences among groups at each stage of the study. Sodium excretion rates of Group 1 were variable whereas those of the other two groups were generally increased (Table 2). The ratios of sodium concentration in urine to that in plasma (U/P Na) of Groups 2 and 3 were significantly greater than for Group 1 in stages 2 and 3 (Table 2). In stages 1 and 3, the excretion rate of chloride in Group 2 was significantly higher than that of the other two groups. The ratios of U/P Cl among the bird groups showed a significant difference in stage 3, when the greatest value was in Group 2 (Table 2). The osmolar U/P ratio of Group 1 was variable during the study whereas that of the birds in the other two groups increased slightly. In stage 2, the birds in Group 1 showed a significantly lower ratio than the birds in Groups 2 and 3.

IV. DISCUSSION

The results of the present study indicate that the sodium level in the diet of laying hens may be increased to as high as 4 g/kg feed without any deleterious effects on egg production and egg shell quality. Higher levels than this of NaCl have been found to reduce shell quality (Damron and Kelly, 1987). Higher levels of $NaHCO_3$ than those used in the present study have been reported to increase shell quality under some conditions (Balnave and Muheereza, 1997; Howes, 1996; Makled and Charles, 1987) but not others (Pepper et al., 1968; Grizzle et al., 1992; Ferguson et al., 1974). Effects on egg shell quality of adding $NaHCO_3$ to water have also been inconsistent (Frank and Burger, 1965; Yoselewitz et al., 1990). However, in the present study, dietary supplementation with $NaHCO_3$ improved feed conversion efficiency at 1.6 and 3.2 g Na/kg feed.

Table 1.   Egg shell quality and production parameters of hens fed different dietary sodium levels (Means ± SEM).

| Experimental | Dietary sodium level   (g/kg feed) | | |
Group	1.6 (Stage 1)	3.2 (Stage 2)	4.0 (Stage 3)
	Breaking strength (N)		
1	35.31±1.24	37.99±1.34	33.02±1.70
2	31.82±1.35	35.01±1.64	33.28±1.88
3	35.54±1.46	36.60±1.49	32.10±1.88
	Shell thickness (μm)		
1	362.91±6.48	375.01±6.02	366.57±7.55
2	347.83±4.99	363.06±4.32	361.58±8.06
3	358.69±4.99	364.81±7.36	365.49±9.07
	Shell weight (g)		
1	5.25±0.10	5.62±0.12	5.33±0.14
2	4.99±0.11	5.38±0.11	5.34±0.18
3	5.22±0.09	5.34±0.15	5.32±0.17
	Egg production (%HD)		
1	89.88±1.06	77.38±2.30	78.57±4.31
2	89.88±2.40	83.93±3.30	76.79±3.77
3	84.52±3.02	83.33±2.21	75.00±4.86
	Egg weight (g)		
1	60.12±0.78	62.85±0.75	63.92±0.75
2	58.46±0.95	61.82±1.14	64.04±1.07
3	60.00±0.82	61.72±0.97	64.10±1.15
	FCR (g food/g egg)		
1	2.37^{ab}±0.07	2.63^{a}±0.10	3.07±0.21
2	2.50^{a}±0.08	2.51^{ab}±0.09	3.08±0.10
3	2.33^{b}±0.05	2.38^{b}±0.06	3.11±0.26
	Faecal moisture (g/100g)		
1	71.42±1.09	67.57^{b}±1.23	69.12^{b}±0.63
2	71.96±1.25	72.89^{a}±0.75	76.49^{a}±0.90
3	69.23±1.82	67.46^{b}±1.28	73.72^{a}±0.90

For each measurement, means within the same column with different subscripts are significantly different ($P<0.05$).

The negative effect of addition of sodium to the diet was the increase in manure moisture at 3.2 and 4.0 g Na/kg feed, especially for the NaCl. The higher concentrations of sodium and chloride in the urine of birds receiving dietary sodium supplementation (evidenced by the higher U/P ratios for Na and Cl) suggest that increased urinary excretion is contributing to the increased manure moisture. It is possible, also, that some of the sodium is remaining in the gut and increasing the faecal moisture, thus contributing to overall manure moisture. Increased faecal moisture resulting from dietary sodium supplementation has been reported by other workers (Damron et al., 1986; Hijikuro, 1975).

In conclusion, dietary supplementation with either NaCl or $NaHCO_3$ up to 4.0 g Na/kg feed could be used as a method of reducing cannibalistic behaviour in laying hens, without adverse effect on egg production or egg shell quality. However, manure moisture may be increased, especially with NaCl.

Table 2. Excretion rates and ratios of electrolytes of hens fed different levels of sodium[1]

Exptl Group	Hct(%)	Excretion rate			U/P osm	U/P Na	U/P Cl
		Osmolar	Na	Cl			
		2.4 g/kgNa	(Stage 1)				
1	27.67	0.010	0.001	0.001[b]	1.480	0.324	0.443
	±0.61	±0.003	±0.003	±0.002	±0.160	±0.064	±0.090
2	26.17	0.015	0.002	0.004[a]	1.341	0.338	0.883
	±1.25	±0.003	±0.001	±0.001	±0.162	±0.081	±0.266
3	27.17	0.011	0.002	0.002[ab]	1.187	0.269	0.574
	±0.40	±0.003	±0.001	±0.001	±0.189	±0.052	±0.178
		3.2 g/kgNa	(Stage 2)				
1	31.00	0.020	0.004	0.006	0.790[b]	0.241[b]	0.486
	±1.01	±0.006	±0.002	±0.002	±0.148	±0.060	±0.109
2	30.17	0.020	0.005	0.005	1.346[a]	0.681[a]	0.809
	±0.75	±0.007	±0.002	±0.002	±0.107	±0.137	±0.154
3	31.33	0.020	0.004	0.003	1.323[a]	0.633[a]	0.508
	±1.07	±0.001	±0.001	±0.001	±0.147	±0.135	±0.112
		4.0 g/kgNa	(Stage 3)				
1	33.42	0.022	0.003	0.003[b]	1.011	0.249[b]	0.340[b]
	±1.14	±0.004	±0.001	±0.001	±0.252	±0.075	±0.090
2	32.33	0.028	0.009	0.009[a]	1.491	0.788[a]	1.241[a]
	±0.96	±0.003	±0.002	±0.001	±0.122	±0.200	±0.126
3	32.58	0.027	0.010	0.004[b]	1.245	0.535[ab]	0.535[b]
	±1.27	±0.012	±0.006	±0.002	±0.117	±0.152	±0.132

[1]The units for Na, and Cl excretion rate are μmol/min/kg; units of osmolar excretion are μOsmol/min/kg. Hct is haematocrit; U/P is urine to plasma ratio for osmolality (U/Posm), sodium (U/P Na) and chloride (U/P Cl).
Means within the same column with different subscripts are significantly different (P<0.05).

REFERENCES

Balnave, D. and Muheereza, S.K. (1997). *Poultry Science*, 76: 588-593.

Damron, B.L., Johnson, W.L. and Kelly, L.S. (1986). *Poultry Science*, 65: 782-785.

Damron, B.L. and Kelly, L.S. (1987). *Poultry Science*, 66: 825-828.

Ferguson, T.M., Scott , J.T., Miller, D.H., Bradley, J.W. and Creger, C.R. (1974). *Poultry Science*, 53: 303-307.

Frank, F.R. and Burger, R.E. (1965). *Poultry Science*, 44: 1604-1606.

Grizzle, J., Iheanacho, M., Saxton, A. and Broaden, J. (1992). *British Poultry Science*, 33: 781-794.

Hijikuro, S. (1976). *Japanese Poultry Science*, 13: 37-41.

Howes, J.R. (1966). *Poultry Science*, 45: 1092-1093.

Makled, M.N. and Charles, O.W. (1987). *Poultry Science*, 66: 705-712.

Pepper, W.F., Summers, J.D. and McConachie, J.D. (1968). *Poultry Science*, 47: 224-229.

Yoselewitz, I. and Balnave, D. (1989). *British Poultry Science*, 30: 273-281.

EFFECT OF INTERCURRENT INFECTIOUS BRONCHITIS INFECTION ON EGG AND EGG SHELL QUALITY IN LAYING HENS

J.R. ROBERTS, W. BALL and R. CHUBB

Summary

The experiment was conducted to assess the effect of exposure to different strains of infectious bronchitis virus (IBV) on egg and eggshell quality. The birds had been vaccinated previously, according to standard commercial practice. Therefore, the experiment simulated an intercurrent infection in the field situation. Challenge with IBV of previously vaccinated laying hens produced relatively small changes in egg shell quality, although there were changes in the appearance of the eggs. These findings emphasise the importance of appropriate vaccination programs in protecting hens against intercurrent infections.

I. INTRODUCTION

Concerns have been raised in the Australian egg industry about both the duration of immunity to existing IBV vaccines such as Vic S and the ability of this vaccine to cross protect layers against other field strains of IBV. In an attempt to test these hypotheses, a study compared the effects of three different viruses; VicS, A-strain, and T-strain on egg shell quality in aged Isa Brown hens (66 weeks) which had been vaccinated previously using Vic S. IBV has been reported to have adverse effects on egg and eggshell quality, producing pale egg shells, watery albumen and consequential wrinkled or corrugated eggshells (Cavanagh, and Naqi, 1997; Jordan and Pattison, 1996; Solomon, 1991; Spackman, 1987).

II. METHODS

Ninety-six Isa Brown layers (66 weeks of age) were transferred to the isolation pens on the University of New England campus three weeks prior to the commencement of the experiment. Four groups of birds were used, 24 birds per group, control (Group 1), and innoculated with Vic-S strain (Group 2), A-strain (Group 3) or T-strain IBV (Group 4). Birds were placed, two to a cage, so that there were 12 cages in each of the isolation sheds. All birds had been raised by a professional pullet grower in the Moonbi region of NSW and vaccinated previously, according to standard commercial practice (Vic S at day old and again at 14 weeks; AE at 12 weeks). Birds were allowed to acclimatise for 2-3 weeks to ensure that the egg laying cycle had stabilised. On Day 1 of the experiment, all eggs laid were collected. Then groups 2-4 were innoculated by eye drop and the control group given a sham treatment of sterile saline. All eggs laid were then collected for days 2-8 and again on days 18-20. The following were measured prior to the innoculation of Groups 2, 3 and 4, and for 3 weeks following innoculation: feed and water intake, egg production, egg weight, gross egg shell defects, egg shell pigmentation (using a ten point colour score: 0=darkest colour, 10 = lightest colour), egg shell breaking strength by quasi-static compression, shell weight, shell thickness.

Lack of IB vacc → IB chall → ↓ egg colour. (ref Chubb ...)

Division of Animal Physiology, School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351.

III. RESULTS

The effects of strain of challenge virus on egg shell quality are summarised in Table 1. There were significant differences between groups for egg weight, shell colour, shell weight and shell thickness but no significant differences for egg shell breaking strength and percentage shell (shell weight divided by egg weight and expressed as a percentage). The most noticeable effect of viral challenge was that shell colour was lightest for the group exposed to the T strain. Table 2 summarises the effect of time in relation to viral challenge on egg shell quality. There was a significant effect of time on egg weight, shell colour and shell weight but not for shell breaking strength, shell thickness and percentage shell. Egg weight and shell weight increased over the course of the experiment and shell colour became lighter (higher colour score).

Table 1.   Effect of three challenge viruses (VicS, T, A) on egg shell quality parameters in vaccinated hens recorded at 2-8 and 18-20 days following challenge. Values are Mean (± SEM).

	Control		Vic S strain		T strain		A strain		P value
Egg weight (g)	65.1	(0.4)	64.8	(0.2)	66.7	(0.3)	64.4	(0.3)	0.0016
Shell colour score	5.0	(0.1)	4.9	(0.1)	5.2	(0.1)	4.5	(0.1)	0.0032
Breaking strength (N)	33.2	(0.4)	33.1	(0.5)	32.0	(0.4)	32.5	(0.5)	NS
Shell weight (g)	6.08	(0.04)	6.18	(0.04)	6.37	(0.04)	6.11	(0.05)	0.0005
Shell thickness	394.3	(1.7)	402.9	(2.3)	403.7	(1.4)	398.0	(2.5)	0.0060
Percentage shell	9.40	(0.05)	9.55	(0.06)	9.55	(0.04)	9.51	(0.06)	NS

Table 2.   Effects of duration after challenge with IBV on egg shell quality parameters averaged for all three virus strains (VicS, T, A). Values are Mean (± SEM).

	Day 1		Days 2-8		Days 18-20		P value
Egg weight g	64.9	(0.4)	65.0	(0.2)	65.9	(0.3)	0.0340
Shell colour score	4.44	(0.07)	4.88	(0.07)	5.07	(0.10)	0.0095
Breaking strength N	33.8	(0.7)	32.1	(0.3)	31.8	(0.5)	NS
Shell weight g	6.04	(0.06)	6.18	(0.03)	6.24	(0.04)	0.0361
Shell thickness	395.0	(3.1)	400.1	(1.3)	400.4	(1.8)	NS
Percentage shell	9.32	(0.07)	9.53	(0.04)	9.51	(0.05)	NS

However, there were no statistically significant interactions between treatment group and time during the experiment, suggesting that challenge of fully-vaccinated birds with different strains of infectious bronchitis virus has relatively little effect on egg shell quality. With respect to surface abnormalities of the eggs, calcium coated eggs occurred most commonly in the VicS group with the control group showing the lowest incidence. The T strain group had the highest incidence of lilac eggs (brown shells with a calcium layer which gives them a lilac appearance) whereas the A group had the highest incidence of speckled and calcium splashed eggs.

IV. DISCUSSION AND CONCLUSIONS

Although there were significant effects of treatment group and time on egg shell quality parameters, there were no statistically significant interactions between these two factors. This suggests that challenging fully-vaccinated birds with different strains of infectious bronchitis virus produced relatively small changes in egg shell quality, although there were changes in the appearance of the eggs. The lighter coloured egg shells found in

birds challenged with T strain IBV are similar to the findings reported by previous studies (see Cavanagh and Naqi, 1997).

These findings emphasise the importance of appropriate vaccination programs in protecting laying hens from negative effects of intercurrent infections.

V. ACKNOWLEDGEMENTS

REFERENCES

Cavanagh, D. and Naqi, S.A. (1997). In: Diseases of Poultry, 10th ed., pp. 511-526. Eds. Calnek, B.W., Iowa State University Press, Ames, Iowa.

Jordan, F.T.W. and Pattison, M. (1996). Poultry Diseases. 4th ed. W.B. Saunders Company Ltd., London.

Solomon, S.E. (1991). Egg and Eggshell Quality, Wolfe Publishing Ltd., London.

Spackman, D. (1987). In: Egg Quality – Current Problems and Recent Advances, pp. 255-282. Ed. R.G. Wells and C.G. Belyavin, Butterworths, London.

Roberts, J.R., Chubb, R., Nolan, J., Robinson, S, Ball, W and Leary, A. (1995). Recent Advances in Animal Nutrition, pp. 75-81, Ed. Rowe, J.B. and Nolan, J.V., University of New England, Armidale, Australia.

Handling can cause a loss of shell colour.

No particular effect of IB challenge (with vacc or T strain)

? There was a tendency to surface defects

THE EFFECT OF MOULT ON EGG WEIGHT IN LAYING HENS RECEIVING VARIOUS LEVELS OF DIETARY LINOLEIC ACID

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

Summary

This study investigated changes in egg weight following a moult in six strains of laying hens which were on low, control or high concentrations of dietary linoleic acid throughout lay and after the moult. Egg weight changes as a result of varying dietary linoleic acid concentrations after the moult were strain dependent. Strains of hen that responded to varying dietary linoleic acid concentrations prior to the moult also tended to respond after the moult.

I. INTRODUCTION

Several studies have demonstrated that isoenergetic or isonitrogenous diets with high concentrations of linoleic acid, as opposed to normal or control levels, produced an increase in egg size (Balnave, 1972; Mannion et al., 1992). Further studies have indicated that the effect of varying dietary linoleic acid on egg size is strain dependent (Leary et al., 1998). The Hy-Line CB hen will produce larger eggs when given a high linoleic acid diet and smaller eggs when given a low linoleic acid diet. The Isa brown hen, however, does not respond to changes in dietary linoleic acid with any resultant change in egg weight (Leary et al., 1998).

Induced moult is a common industry practice used to improve eggshell quality and production of aged hens. Specific gravity (Roland and Brake, 1982; Abu-Serewa and Karunajeewa, 1985), shell weight (Roland and Brake, 1982), percent cracks (Abu-Serewa and Karunajeewa, 1985) and shell colour (Al-Batshan et al., 1994) have been found to improve as a result of an induced moult. Egg production is also positively affected by moult (Roland and Brake, 1982; Karunajeewa et al., 1989).

The effect of moult on egg weight is variable. Induced moult has been shown to produce no significant change in egg weight in several studies (Noles, 1966; Roland and Brake, 1982; Karunajeewa et al., 1989). Egg weight has also been found to increase (Nordstrom, 1980) and decrease (Koelkebeck et al., 1992) as a result of an induced moult. This variation in the effect of moult on egg weight may be the result of strain differences. Roberts and Leary (1996) found that post-moult egg weight increased in the Hy-Line Tint hens, but did not change in the other strains investigated.

II. MATERIALS AND METHODS

Six different strains of laying hen were used in the experiment, including four imported strains, the Isa Brown, Hy-Line Brown, HiSex Brown and Lohmann Brown, and two local strains, the Hy-Line CB and Tegel SB2. Each strain was divided randomly into three groups. At nine weeks of age these groups, containing approximately twenty hens each, were given diets containing either 6, 13 or 30 g/kg linoleic acid, low, control and high, respectively. The diets had the same metabolisable energy and nutrient constraints.

Eggs were collected from the birds from point of lay to 50% production at 14 to 20 weeks of age and then at 23, 26, 31, 36, 44, 52, 60 and 68 weeks of age. Each collection

Division of Animal Physiology, University of New England, Armidale 2351.

consisted of gathering all of the eggs produced by the hens for two days. All eggs collected during these periods were weighed. At 69 weeks of age the birds were moulted by feeding only barley and shell grit for 14 days. For five days prior to the moult the birds were under continuous light, and when the feed was changed to the moult ration all artificial lighting ceased. Post-moult the birds were returned to a diet containing the level of linoleic acid they had previously received and a 16:8 light dark photoperiod. The birds lost on average 21% of their body weight during the moult. Eggs were collected and weighed at 74, 75, 77 and 81 weeks of age. The results are presented in periods instead of collections. The early-lay period consists of eggs collected at 23, 26 and 31 weeks of age, the mid-lay period consists of eggs collected at 36, 44 and 52 weeks of age and the late-lay period is eggs collected at 60 and 68 weeks of age. All eggs collected after the moult comprise the post-moult period.

III. RESULTS

Egg weight did not vary among diets either before or after the moult in the Isa brown hens except during mid-lay when the hens on the low linoleic acid diet produced lighter eggs than the hens on the control diet (Table 1). In early- and mid-lay the Lohmann brown hens on the control diet produced significantly lighter eggs than the hens on the high linoleic acid diet (Table 1). Late in lay, the Lohmann brown hens on the control diet produced the lightest eggs. Egg weights before the moult tended to be similar to egg weights recorded after the moult in both the Isa brown and Lohmann brown hens (Table 1).

Table 1.   Egg weight (g) in the Isa Brown and Lohmann Brown hens on various concentrations of dietary linoleic acid at different stages of lay (Mean ± SM).

Stage of lay	Isa Brown			Lohmann Brown		
	6 g/kg	13 g/kg	30 g/kg	6 g/kg	13 g/kg	30 g/kg
Early	58.16	59.10	58.19	61.01^{ab}	59.61^{b}	61.82^{a}
	±0.60	±0.57	±0.51	±0.68	±0.62	±0.62
Mid	63.26^{b}	65.22^{a}	63.76^{ab}	67.36^{ab}	66.70^{b}	68.83^{a}
	±0.52	±0.60	±0.62	±0.64	±0.46	±0.77
Late	65.63	65.68	66.98	69.95^{a}	67.09^{b}	71.48^{a}
	±0.58	±0.70	±0.66	±0.74	±0.71	±0.94
Post-moult	67.17	66.66	67.61	70.55^{b}	68.34^{c}	73.24^{a}
	±0.54	±0.58	±0.71	±0.85	±0.60	±0.76

Egg weights with different superscripts are significantly different from one another (P<0.05) within a strain.

HiSex brown hens on the high linoleic acid diet produced the heaviest eggs during early- and mid-lay (Table 2). The Hy-Line brown hens on the low linoleic acid consistently produced significantly lighter eggs than the other two diets, both before and after the moult (Table 2). When individual collections were studied immediately before and after the moult it was evident that overall egg weight tended to decrease in the Hy-Line brown hens for several weeks after the moult. Moult had no effect on overall egg weights in the HiSex brown hens.

Table 2.   Egg weight (g) in the HiSex Brown and Hy-Line Brown hens on various concentrations of dietary linoleic acid at different stages of lay (mean ± SEM).

Stage of lay	HiSex Brown			Hy-Line Brown		
	6 g/kg	13 g/kg	30 g/kg	6 g/kg	13 g/kg	30 g/kg
Early	60.33[b]	60.16[b]	63.07[a]	57.56[b]	59.73[a]	59.53[a]
	±0.60	±0.60	±0.66	±0.46	±0.58	±0.59
Mid	67.42[b]	66.06[b]	69.79[a]	63.35[c]	67.59[a]	66.07[b]
	±0.56	±0.65	±0.67	±0.42	±0.47	±0.55
Late	68.12	69.76	70.66	66.14[b]	70.44[a]	69.00[a]
	±0.79	±0.87	±1.22	±0.83	±0.66	±0.78
Post-moult	69.69	69.94	70.35	64.30[b]	70.04[a]	69.34[a]
	±0.89	±0.84	±0.95	±0.70	±0.57	±0.76

Egg weights with different superscripts are significantly different from one another ($P < 0.05$) within a strain.

The Hy-Line CB hens on the low linoleic acid diet consistently produced the lightest eggs (Table 3). During mid-lay and post-moult periods the Hy-Line CB hens on the high linoelic acid diet produced significantly heavier eggs than the hens on the control diet (Table 3). The Tegel SB2 hens responded consistently throughout lay to the low linoleic acid diet by producing significantly lighter eggs (Table 3). Overall egg weight in the Tegel SB2 hens was not affected by moult, however egg weight in the Hy-Line CB hens tended to decrease for several weeks after the moult.

Table 3.   Egg weight (g) in the Hy-Line CB and Tegel SB2 hens on various concentrations of dietary linoleic acid at different stages of lay (mean ± SEM).

Stage of lay	Hy-Line CB			Tegel SB2		
	6 g/kg	13 g/kg	30 g/kg	6 g/kg	13 g/kg	30 g/kg
Early	50.81[b]	51.94[b]	53.60[a]	54.66[b]	58.45[a]	59.36[a]
	±0.45	±0.44	±0.39	±0.60	±0.86	±1.11
Mid	57.74[c]	59.69[b]	60.87[a]	61.77[b]	64.58[a]	64.06[a]
	±0.34	±0.35	±0.39	±0.54	±0.61	±0.76
Late	61.42[b]	63.44[a]	64.84[a]	63.84[b]	68.31[a]	65.34[ab]
	±0.39	±0.55	±0.77	±1.11	±1.21	±1.35
Post-moult	60.74[c]	63.28[b]	64.95[a]	64.00[b]	69.77[a]	65.80[b]
	±0.63	±0.46	±0.57	±0.85	±1.05	±1.08

Egg weights with different superscripts are significantly different from one another ($P < 0.05$) within a strain.

IV. DISCUSSION

The post-moult effectiveness of varying dietary linoleic acid on egg weight is strain dependent. Significant changes in egg weight as a result of manipulating dietary linoleic acid were mostly apparent in strains of hen that responded to changes in dietary linoleic acid consistently prior to the moult. The difference, however, is that in most strains the only effect found after the moult was a reduction in egg weight by hens on the low linoleic acid diet. The exception is the Lohmann Brown hens which did not respond consistently to

varying dietary linoleic acid prior to moult, but produced significantly heavier eggs on the high linoleic acid diet, compared to the control diet, post-moult.

The effect of moult on egg weight is also strain dependent. Overall, egg weight in most strains was not affected by the moult, however the Hy-Line Brown and Hy-Line CB hens tended to produce smaller eggs after the moult. These results may be due to variations in the extent of body weight loss among the different strains. However, the Hy-Line Brown hen lost approximately 26% body weight, whereas the Hy-Line CB lost only 19% which was less than all of the other strains. These results would indicate that either the Hy-Line CB is more sensitive to body weight loss with regard to egg weight or that something other than body weight is affecting egg size in the Hy-Line CB hens after moult.

V. ACKNOWLEDGEMENTS

REFERENCES

Abu-Serewa, S. and Karunajeewa, H. (1985). *Australian Journal of Experimental Agriculture,* **25**: 320-325.

Al-Batshan, H.A., Scheideler, S.E., Black, B.L., Garlich, J.D. and Anderson, K.E. (1994). *Poultry Science,* **73**: 1590-1596.

Balnave, D. (1972). *Journal of the Science of Food and Agriculture,* **23**: 1305-1311.

Karunajeewa, H., Abu-Serewa, S. and Harris, P. A. (1989). *British Poultry Science,* **30**: 257-264.

Koelkebeck, K. W., Parsons, C. M., Leeper, R. W. and Moshtaghian, J. (1992). *Poultry Science,* **71**: 434-439.

Leary, A. M., Roberts, J. R. and Ball, W. (1998). *Proceedings of the Australian Poultry Science Symposium.* Ed. R.A. Pym **10**: 94-97.

Mannion, P. F., Neill, A. R. and Brewster, M. (1992). *Australian Journal of Agricultural Research,* **43**: 389-397.

Nole, R. K. (1966). *Poultry Science,* **45**: 50-57.

Nordstrom, J. O. (1980). *Poultry Science,* **59**: 1711-1714.

Roberts, J. R. and Leary, A. (1996). *Proceedings of the Australian Poultry Science Symposium.* Ed. D. Balnave **8**: 100-113.

Roland, D. A. and Brake, J. (1982). *Poultry Science,* **61**: 2473-2481.

RESPONSES OF ISABROWN LAYING HENS TO A PRE-LAYER DIET AND TO DIETARY PROTEIN CONCENTRATION DURING LAY

D. BALNAVE, J. GILL, XIUHUA LI and W.L. BRYDEN

Summary

IsaBrown pullets were obtained from a commercial breeder at 15 weeks of age and housed in either single-bird or multiple 5-bird cages in a new temperature controlled layer house. They were fed either a grower diet or a pre-layer diet consisting of this diet containing additional calcium (Ca) to 18 weeks of age and then maintained during lay to 56 weeks of age on diets containing either 160 or 180 g crude protein (CP)/kg.

For most of the study the daily house temperature ranged from 15° to 30°C. Mortality was low (2.25%), peak egg production was high (95-98%) and mean rates of lay at 56 weeks of age were above 88%. The pre-layer diet had no significant effect on production during lay. Feed intake and egg production were similar for hens fed both dietary protein levels during lay. Mean egg weight was excessively high at 56 weeks of age, varying between 64 g and 71 g, with the heavier eggs being obtained from hens on the diet containing 180 g CP/kg.

I. INTRODUCTION

Limited research with the recently imported brown egg laying strains has highlighted their prolific production potential and their susceptibility to disease. High levels of mortality have severely limited their economic value, the cause of death being primarily due to Marek's disease, with cannibalism also being of major concern (Cumming *et al.*, 1995, 1998; Nolan *et al.* 1998). Even allowing for this high mortality these hens produce at similar, or increased, levels to local genotypes (Kyarisiima and Balnave, 1995; Nolan *et al.*, 1996, 1997, 1998).

Attempts to evaluate the performance and to determine the dietary nutrient specifications of these overseas strains in the Australian environment have been impeded by the high mortality problem. It is apparent that in Australia the egg production, egg weight and feed efficiency achieved by those hens which do not succumb to disease or cannibalism are equatable with the performance expected from overseas studies. The present study was carried out to determine the responses of IsaBrown hens to diets containing 160 or 180 g CP/kg during lay. In addition, the effect of feeding a pre-layer diet containing 20 g Ca/kg from 15 to 18 weeks of age was also examined to determine whether this procedure influenced feed intake during the critical period between point-of-lay and peak-lay. Comparisons were made with hens housed in single-bird or 5-bird multiple cages in a new temperature-controlled commercial house which had not previously housed birds.

II. MATERIALS AND METHODS

IsaBrown pullets were purchased at 15 weeks of age in August 1997 from a commercial supplier (Baiada Poultry Pty Ltd) and housed in cages in a high-rise windowless house in which computerised control of temperature was achieved with fans and evaporative cooling pads. The birds were randomly allocated to 20 replicates of 5 pullets on each of 4 treatments. These consisted of a grower diet containing 160 g CP and 10 g Ca/kg or this

Department of Animal Science, University of Sydney, Camden, NSW 2570.

grower diet containing an additional 10 g Ca/kg fed to pullets housed in single or 5-bird multiple cages. The composition of these diets is shown in Table 1. At 18 weeks of age 10 replicates from each of these 4 treatments were fed one of 2 layer diets containing either 160 or 180 g CP/kg, the compositions of which are shown in Table 1.

Table 1. Composition of experimental diets (g/kg).

Crude protein (g/kg)	Grower 160	Pre-layer 160	Layer	
			160	180
Wheat	200.0	200.0	200.0	200.0
Sorghum	457.4	457.4	457.4	413.4
Soyabean meal	64.0	64.0	64.0	108.0
Meat meal	95.0	95.0	95.0	95.0
Millrun	100.0	100.0	100.0	100.0
Rice hulls	65.5	40.0	-	-
Tallow	9.0	9.0	9.0	9.0
Limestone	-	25.5	65.5	65.5
Sodium chloride	1.4	1.4	1.4	1.4
L-lysine	1.05	1.05	1.05	1.05
DL-methionine	1.65	1.65	1.65	1.65
Vitamin/minerals[1]	5.0	5.0	5.0	5.0
Amino acid concentration				
Lysine	7.9	8.6	7.8 ± 0.5[2]	10.3 ± 0.0[2]
Arginine	10.0	9.3	9.7 ± 0.0	10.5 ± 0.2
Threonine	5.2	5.0	5.1 ± 0.1	5.8 ± 0.1
Methionine	4.0	3.8	4.0 ± 0.2	4.2 ± 0.2
Cystine	2.7	2.6	2.7 ± 0.1	2.9 ± 0.1

[1]See Balnave and Muheereza (1998).

[2]Mean ± SEM of two mixes fed prior to peak-lay.

The hens were fed the layer diets to 56 weeks of age. Production records were maintained from 20 weeks of age when the hens achieved 40% rate of lay. A constant daily photoperiod of 16 h was used and hens were allowed free access to food and water at all times. Each replicate was treated as an experimental unit, feed intake and production being recorded for the complete group. Data were analysed as a 2^3 factorial ANOVA with the main effects being the diets fed prior to lay, the CP concentration of the diets fed during lay and the cage density.

III. RESULTS AND DISCUSSION

For most of the experiment the daily house temperature ranged from 15°C to 30°C. During mid-summer a consistent minimum night temperature of 20°C was recorded with the maximum day temperature only occasionally rising above 30°C for short periods of time. Mortality was low with no birds dying from Marek's disease. The main diagnosed cause of death was vent pecking in the multiple-bird cages. Of the nine birds which died during lay, six had been fed the pre-layer diet, six were fed the lower protein layer diet and five were in multiple bird cages.

The egg production in this study was exceptionally good with hens on all treatments peaking between 95 and 98% lay. The mean rate of lay was above 88% at the end of the study at 56 weeks of age (Figure 1). The production responses of the hens on the various treatments to 56 weeks of age are shown in Table 2. Feeding the pre-layer diet from 15 to 18 weeks of age had no significant effect on production during lay. Increasing the protein content of the layer diet from 160 to 180 g/kg had no effect on feed intake or egg production but significantly improved egg mass output through a significant increase in egg size. Egg weight was excessive with mean values between 64 g and 71 g at 56 weeks with the heavier eggs being obtained from hens fed the diet containing 180 g CP/kg. This suggests that it may be necessary to reduce the dietary concentrations of specific amino acids in order to prevent excessively large eggs being produced late in lay with these new genotypes which produce relatively large eggs at the start of lay. Individually caged hens ate significantly more feed than hens housed in multiple-bird cages. This resulted in a significant increase in daily egg mass output but a significant deterioration in feed conversion. The only significant interaction was a pre-layer x layer dietary interaction with egg production. Feeding the pre-layer diet rather than the grower diet from 15-18 weeks of age reduced the overall egg production of hens subsequently fed the diet containing 160 g CP/kg (88.0 vs 90.5%) whereas an improvement was observed with hens fed the layer diet containing 180 g CP/kg (89.8 vs 88.2%). Measurement of shell defects at the end of the study showed no major problems. The incidences of shell defects were 1.1 and 2.3% in hens fed the grower and pre-layer diets, 1.8 and 1.6% in hens fed the layer diets containing 160 g and 180 g CP/kg, and 1.6 and 1.8% in hens in the single-bird and multiple-bird cages, respectively.
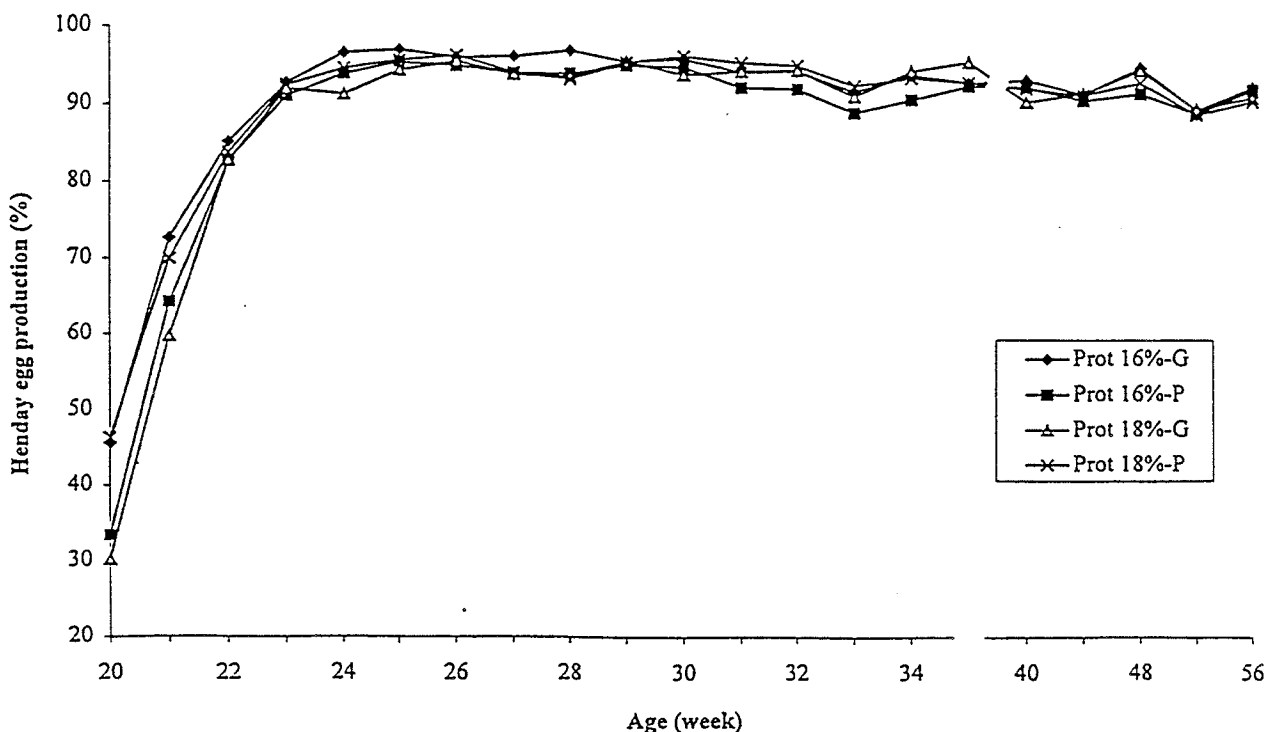

Figure 1.   Egg production of hens on the different treatments, where G = the grower diet, P = the pre-layer diet, Prot 16% = protein concentration of 160 g/kg in layer diet and Prot 18% = protein concentration of 180 g/kg in layer diet.

Table 2.　　　Production measures for main effects between 20 and 56 weeks of age.

	Feed intake (g/d)	Egg production (%)	Egg weight (g)	Egg mass (g/d)	FCR (g feed:g egg)
Grower diet	124.9	89.4	60.5	53.0	2.29
Pre-layer diet	124.7	88.9	60.0	52.2	2.30
Significance	NS	NS	NS	NS	NS
16% layer diet	124.8	89.3	59.4	51.6	2.32
18% layer diet	124.9	89.0	61.1	53.5	2.26
Significance	NS	NS	***	*	NS
Single cages	128.2	89.7	60.4	53.5	2.36
Multiple cages	121.6	88.6	60.1	51.6	2.22
Significance	***	NS	NS	*	***
SEM	1.14	0.83	0.40	0.76	0.03

* $P<0.05$; *** $P<0.001$; NS = not significant.

IV.　CONCLUSIONS

The data provide no evidence to indicate that these hens required excessively high dietary concentrations of protein and amino acids to optimise performance.

V.　ACKNOWLEDGEMENTS

REFERENCES

Balnave, D. and Muheereza, S.K. (1998). *Australian Journal of Agricultural Research*, **49**: 279-284.

Cumming, R.B., Chubb, R.C., Nolan, J.V. and Ball, W. (1995). *Recent Advances in Animal Nutrition in Australia 1995*. Eds. J.B. Rowe and J.V. Nolan. pp. 69-74. University of New England, NSW.

Cumming, R.B., Ball, W. and Nolan, J.V. (1998). *Proceedings Australian Poultry Science Symposium*. Ed. R.A.E. Pym. pp. 168-171.

Kyarisiima, C.C. and Balnave, D. (1995). *Proceedings Australian Poultry Science Symposium*. Ed. D. Balnave. p. 193.

Nolan, J.V., Cumming, R.B., Ball, W. and Thompson, E. (1996). *Proceedings Australian Poultry Science Symposium*. Ed. D. Balnave. pp. 118-122.

Nolan, J.V., Roberts, J.R., Thomson, E., Ball, W. and Cumming, R.B. (1997). *Proceedings Australian Poultry Science Symposium*. Ed. D. Balnave. pp. 161-165.

Nolan, J.V., Roberts, J.R., Thomson, E., Ball, W. and Cumming, R.B. (1998). *Proceedings Australian Poultry Science Symposium*. Ed. R.A.E. Pym. pp. 85-89.

THE NUTRITIVE VALUE OF SWEET POTATO VINES FOR BROILERS

H. JIBRIL[1], R. PEREZ-MALDONADO[2], P.F. MANNION[2], and D.J. FARRELL[1,2]

Summary

The chemical composition of sweet potato vines (SPV) when harvested fresh had high levels (g/kg) of neutral detergent fibre, ash and protein of 364, 178 and 191 respectively. The apparent metabolisable energy of the diets containing 40 and 80 g SPV/kg was higher than others. When SPV replaced lucerne meal at 0-160 g/kg diet, broilers grown to 21 d showed no differences in growth rate, food intake or food efficiency.

I. INTRODUCTION

In many countries, particularly in the low-income countries, feedingstuffs for poultry are often scarce. There is a need to identify byproducts which may be useful for poultry in these countries. Sweet potatoes are the staple food for many people in low-income countries and the vines are often discarded or fed to ruminant livestock. Occasionally they are sun dried and used to feed pigs and poultry but little is known of their nutritional value. Yield of sweet potato vines may be up to 2.6 metric tonnes of dry matter/ha (Villareal *et al.*, 1979).

The purpose of the experiment described here was to determine the chemical composition of sweet potato vines (SPV) and to include the vines at different levels in starter diets for broiler chickens.

II. MATERIALS AND METHODS

Sweet potato vines were pruned at regular intervals from a range of cultivars grown in small plots at the Centre for Amenity Horticulture, Queensland Horticulture Institute, Cleveland, Queensland. They were sun-cured in batches and hammer milled to a suitable particle size for inclusion in chick starter diets and fed in mash form. The vine meal replaced, in incremental amounts, lucerne meal included at 160g/kg. The composition of the diets formulated to nutrient specification for starter chicks (SCA, 1987) is given in Table 1.

The five diets were fed to groups of eight chickens per cage (65 cm long x 35 cm wide x 42 cm high) housed in a heated room, initially at 33°C and reduced to 22°C at 21 d. Each dietary treatment was replicated four times. Food intake and bodyweight were measured at 21d of age. During the last 4d of the experiment, food intake was measured quantitatively and all excreta were collected daily on plastic trays placed under the cages. The excreta were frozen and subsequently dried at 75°C for 48 h and subsampled and with food samples combusted in a bomb calorimeter. Apparent metabolisable energy (AME) of the diets was then calculated. Chemical composition of the dried vines and lucerne meal was determined using the methods of the AOAC (1990).

Data were analysed using a protected analysis of variance (Stastix Version 4.0). Treatment means were examined for significant effects ($P<0.05$) using the Least Significant Difference test.

[1]School of Land and Food, The University of Queensland, St Lucia, Qld 4067.
[2]Queensland Poultry Research and Development Centre, Alexandra Hills, Qld 4161.

Table 1.   Ingredients and calculated chemical composition (g/kg) of sweet potato vine (SPV) based diets on 'as fed' basis.

Ingredients	Control	4% SPV	8% SPV	12% SPV	16% SPV
Lucerne	160	120	80	40	0
Sweet potato vine	0	40	80	120	160
Sorghum	244	244	243	243	242
Wheat	250	248	245	243	240
Soybean meal	185	189	192	195	198
Fish meal	60	60	60	60	60
Meat and bone meal	33.4	33.3	33.3	33.3	33.2
Soybean oil	49.6	49.7	49.9	49.9	50
Limestone	5.0	4.8	4.7	4.5	4.3
Salt	1.3	1.3	1.4	1.4	1.4
Vitamins & minerals	7.1	7.1	7.1	7.1	7.1
D-L Methionine	3.0	3.0	3.0	2.9	2.9
Lysine HCl	0.99	0.93	0.86	0.79	0.72
Chemical composition (g/kg) (Calculated)					
Crude protein	222	221	222	222	221
AME (MJ /kg)	12.50	12.50	12.50	12.50	12.50
Lysine	12.50	12.50	12.50	12.50	12.50
Methionine	6.3	6.3	6.3	6.3	6.3
Meth + cyst	9.12	9.12	9.12	9.12	9.12
Tryptophan	2.8	2.9	2.9	2.9	3.0
Threonine	7.8	7.7	7.8	7.8	7.8

III. RESULTS

Shown in Table 2 is the chemical composition of the SPV and lucerne meal. Almost 500 g/kg of the components of SPV were neutral detergent fibre and almost 400 g/kg of the lucerne meal. The crude protein content was 190 and 160 g/kg for the SPV and lucerne meal respectively. There were no differences ($P>0.05$) in food intake, liveweight gain or food conversion ratio between diets (Table 3). However there was a tendency for growth rate to decline at the two highest levels of SPV inclusion.

Table 2.   Chemical composition (g/kg DM) of sweet potato vines and lucerne meal.

	Vines	Lucerne
Acid detergent fibre	364	352
Neutral detergent fibre	498	397
Lignin	54	69
Ash	178	100
Crude protein	191	160
Organic matter	822	900
Fat	22.5	ND[1]

[1]Not determined.

Table 3.   Food intake, live weight gain and food conversion ratio of chickens at 21 days of age on diets with sweet potato vine (SPV).

Diet (g/kg)	Food intake (g)	Weight gain (g) at 21 d	Food conversion ratio
0 SPV	867	601	1.447
40 SPV	883	610	1.449
80 SPV	870	614	1.444
120 SPV	857	584	1.469
160 SPV	831	579	1.461
Pooled SEM	26.6	21.6	0.021

The AME of the diets is given in Table 4

Table 4.   The apparent metabolisable energy (AME) of the five diets (MJ/kg DM) with different levels of sweet potato vine.

Diet	AME
0 SPV	13.81^{a1}
40 SPV	14.14^{b}
80 SPV	14.58^{c}
120 SPV	13.78^{a}
160 SPV	13.66^{a}
Pooled SEM	0.086

[1]Values with different superscripts are significantly different ($P < 0.05$)

The AME of the diets with 40 and 80 g SPV/kg was higher ($P < 0.05$) than the other three diets; this suggests that the vines had a higher AME value than the lucerne meal at these two levels of inclusion. Furthermore there was no difference in the AME of the two diets with the highest level of inclusion of SPV and lucerne meal at 160 g/kg.

IV. DISCUSSION

The chemical composition of SPV is similar to that reported by Villareal *et al* (1979). Their high ash content of 170 g/kg is also almost identical to 178 g/kg shown in Table 2 and is much higher than that of lucerne meal. However during analysis much of the ash appeared visually to be in the form of silica, suggesting some contamination of SPV with soil. At the highest level of inclusion (160 g/kg) of lucerne meal and SPV, AME values of these diets were surprisingly high (13.7-14.1 MJ/kg DM). Cilliers *et al.* (1994) reported an AME of milled lucerne hay of only 4.0 MJ/kg using adult cockerels. This would be expected to be even less for young chickens. Ravindran and Blair (1992) give an AME of 6.2 MJ/kg DM for SPV meal which is more in line with that found here. It was not possible to determine the AME value of SPV *per se*. The response to replacing lucerne meal in the diet with different amounts of SPV was not linear.

The diets were formulated to 12.5 MJ AME/kg on an 'as fed' basis. When adjusted to a dry matter basis, this value was similar to that for the 0 and 16% SPV diets of 13.6 MJ AME/kg.

We were unable to find any feeding experiments with chickens using SPV meal; the reason is probably because sweet potatoes are grown mainly in low-income countries which

do not normally have extensive research facilities and sophisticated equipment to evaluate them. According to Ravindran and Blair (1992) SPV meal is a good source of protein and pigments for poultry (Garlic *et al.*, 1974) and it contains no antinutritional factors. For intensively-farmed broiler chickens, the fibre level is too high to allow significant inclusion in diets (>5%). For village chickens, which have lower production and hence dietary requirements, SPV meal could be a useful form of protein supplement.

V. ACKNOWLEDGEMENTS

REFERENCES

AOAC (1990). Association of Official Analytical Chemists (1990). Official methods of analysis of the Association of Official Analytical Chemists 15th Edition. Association of Official Analytical Chemists, Virginia, U.S.A.

Cilliers, S.C., Hayes, J.R., Maritz, I.S. Chwaliborg, A., and Du Preez, J.J. (1994). *Animal Production,* **50**: 309-313.

Garlic, J.D., Bryant, D.M., Covington, H.M., Chamblee, D.S. and Purcell, A.E. (1974). *Poultry Science,* **53**: 692-699.

Ravindran, V. and Blair, P. (1992). *World's Poultry Science Journal,* **48**: 205-213.

SCA (1987). Standing Committee on Agriculture Feeding Standards for Australian Livestock Poultry. CSIRO, East Melbourne.

Villareal, R.L., Ison, S.C.S., Lin, S.K. and Chui, S.C. (1979). *British Poultry Science,* **15**: 117-122.

ILEAL AMINO ACID DIGESTIBILITY FOR BROILERS OF WHEAT GROWN IN AUSTRALIA

W.L. BRYDEN, L.I. HEW, V. RAVINDRAN and G.RAVINDRAN

Wheat is an economically important feed ingredient in poultry diets in Australia. Although wheat is used primarily as an energy source, its high level of incorporation in poultry diets means that it also supplies a major portion of dietary protein. Consequently, the quality of wheat protein for supporting broiler growth becomes an additional point of importance. While the variability in apparent metabolisable energy (AME) of wheat grown in Australia is well documented, limited published data are available on the amino acid availability of wheat for poultry. The present study was carried out to obtain data on variations in ileal amino acid digestibility and AME of 16 samples of Australian wheat. Correlations between the different nutritional parameters were also computed.

The AME values were determined with 5-week old male broilers (Cobb) using a classical total collection procedure described by Mollah *et al.* (1983). Following the completion of excreta collection, the same birds were used for amino acid digestibility assays. Assay diets contained wheat (918 g/kg) as the only source of protein. Diets were fortified with minerals and vitamins and contained celite (20 g/kg) as an indigestible marker. The diets were fed *ad libitum* to three pens (4 birds/pen) of broilers from 35 to 42 days of age. On day 42, digesta contents from the terminal ileum were collected and processed. Samples of diets and digesta were analysed for amino acids and acid-insoluble ash, and the apparent ileal amino acid digestibility values were calculated.

The crude protein content (N x 6.25) of wheat samples varied widely ranging from 9.30 to 17.21 g/kg. Ileal nitrogen digestibility varied from 0.72 to 0.85 and the mean ileal digestibility of the 15 amino acids from 0.70 to 0.84. In general, differences between wheat samples in digestibility of individual amino acids paralleled those in nitrogen digestibility. Lysine and threonine were the least digestible essential amino acids in wheat. A positive relationship was found between grain protein content and protein digestibility (r=0.77, P<0.001). The amino acids in high-protein wheat samples were found to be more digestible than those in low protein cultivars. In studies with pigs, Wiseman *et al.* (1994) also reported a positive correlation between protein content and protein digestibility in wheat. A significant positive correlation (P<0.001) between digestibilities of nitrogen and amino acids was also noted in this study. Nitrogen digestibility estimates were closely related to the mean digestibility of the 15 amino acids (r=0.93; P<0.001) and lysine digestibility (r=0.82; P<0.001).

The AME values of wheat samples for broilers varied from 11.4 to 13.5 MJ/kg DM. Interestingly, no relationship was evident between AME and nitrogen digestibility (r=0.08, P>0.05) or grain protein content (r= 0.06, P>0.05).

Mollah, Y., Bryden, W.L., Wallis, I.R., Balnave, D. and Annison, E.F. (1983). *Brit. Poult. Sci.*, **24**: 81.

Wiseman, J., Nicol, N. and Norton, G. (1994). In: Recent Advances in Animal Nutrition pp. 117-132. Eds. P.C.Gainworthy and D.J.A.Cole. Nottingham University Press, Nottingham, U.K.

Department of Animal Science, The University of Sydney, Camden, NSW 2570.

ILEAL DIGESTIBILITY OF TRYPTOPHAN IN FEEDSTUFFS FOR POULTRY

G. RAVINDRAN, V. RAVINDRAN and W.L. BRYDEN

Reliable values of total and digestible tryptophan in feedstuffs for use in feed formulation matrixes are paramount because tryptophan may become limiting especially when diets are based on sorghum, peas, lupins and meat and bone meal. However, since tryptophan is oxidatively destroyed during acid hydrolysis in routine amino acid analysis, its determination requires a separate analytical procedure. Several methodologies with varying analytical limitations and varying success have been reported in the literature for tryptophan determination. Based on the fundamentals reviewed, a procedure involving alkaline hydrolysis with sodium hydroxide followed by separation of tryptophan by ion exchange chromatography has been developed and validated for the routine analysis of tryptophan in feeds (Ravindran and Bryden, 1996). A compilation of digestible amino acid contents of 93 samples of 25 Australian feedstuffs for poultry has been recently published (Ravindran *et al.*, 1998), but digestible tryptophan values were not included in this database. The ingredient, diet and digesta samples from this study (Ravindran *et al.*, 1998) were analysed for tryptophan and digestibility values were calculated using acid insoluble ash as the indigestible marker. A summary of digestibility values from 52 samples involving 16 ingredients is presented below.

	Tryptophan (g/kg)	Tryptophan digestibility (%)
Maize (4)[1]	0.57 (0.54-0.62)[2]	69.7 (65.3-72.3)[2]
High lysine maize	0.85	70.2
Sorghum (5)	1.02 (0.91-1.15)	76.4 (72.3-84.4)
Wheat (18)	1.46 (1.04-1.82)	83.1 (81.2-89.1)
Triticale (2)	1.00 (0.98-1.03)	75.9 (75.6-76.1)
Millrun (1)	2.06	75.8
Soyabean meal (5)	6.05 (5.65-6.88)	84.7 (83.1-86.6)
Canola meal (3)	4.51 (3.89-5.18)	80.1 (76.9-84.4)
Cottonseed meal (2)	4.81 (4.61-5.01)	77.2 (74.7-79.8)
Sunflower meal (1)	3.74	88.1
Lupin (2)	2.76 (2.57-2.94)	78.2 (76.6-79.8)
Pea (1)	1.85	69.4
Meat and bone meal (4)	2.41 (1.90-2.75)	59.7 (54.3-66.3)
Fish meal (1)	5.14	80.6
Feather meal (1)	4.47	48.2
Casein (1)	11.75	96.7

[1]Number of samples.
[2]Range of values.

Ravindran, G. and Bryden, W.L. (1996). *Proc. Aust. Poult. Sci. Symp.* Ed. D.Balnave, **8**: 208.

Ravindran, V., Hew, L.I. and Bryden, W.L. (1998). *Digestible Amino Acids in Feedstuffs for Poultry.* Rural Industries Research and Development Corporation, Canberra, and Poultry Research Foundation, The University of Sydney, Camden.

Department of Animal Science, The University of Sydney, Camden, NSW 2570.

DIFFERENCES IN GLUCOSE METABOLISM BETWEEN BROILER AND LAYER CHICKENS

R.E. NEWMAN, J.A. DOWNING, C.M. JACKSON and W.L. BRYDEN

Recent studies from this laboratory suggest that the manipulation of glucose metabolism may result in a leaner chicken (Newman *et al.*, 1998). The aim of this study was to investigate glucose metabolism in strains of chickens differing widely in growth rate and body composition.

Day-old broiler and layer strain chickens were reared in a brooder and fed a commercial starter diet for 3 weeks. Each strain was then randomly divided into 4 groups (n=12) and fed an isonitrogenous diet containing edible tallow (50 g/kg) for 4 weeks. Two of the 4 groups were housed in individual cages and at weekly intervals, feed intake and body weight measurements were made. At the end of week 4, a blood sample was taken from each bird prior to slaughter and the abdominal fat pad and breast muscles removed and weighed. Jugular catheterisation was performed under general anaesthesia on the remaining 2 groups during week 3. Each chicken was allowed 7 days post-surgery to recover before being infused with 2-deoxy-D-^{3}H glucose (2DG-^{3}H; 50μ Ci). To estimate the clearance rate from plasma, sequential blood samples were taken via an indwelling catheter over a period of 1 h. The birds were then sacrificed and 2DG-^{3}H incorporation measured in the breast, thigh, liver and fat tissues. Plasma glucose and triglyceride concentrations were measured by enzymic analysis and plasma insulin concentrations measured by radioimmunoassay. The results are shown in the Table.

	Broiler		Layer		
Final body mass (g)	2198.8	± 75.6	772.1	±	23.9*[1]
Breast muscle (g)	314.4	± 18.0	72.4	±	3.1*
Abdominal fat pad (g)	28.6	± 2.4	4.9	±	0.7*
Glucose (mmol/L)	13.4	± 1.2	15.9	±	0.3*
Triglycerides (mmol/L)	0.34	± 0.04	0.56	±	0.04*
Insulin (ng/ml)	0.073	± 0.01	0.1077	±	0.02*

[1]Significantly different from broiler P<0.05.

The plasma clearance rate of 2DG-^{3}H from the plasma was similar for the two strains. However, there was a significant increase (P<0.05) in the uptake of 2DG-^{3}H into the breast muscle and the liver of the layer strain. These data and the differences in circulating insulin concentrations demonstrate a distinct difference in glucose metabolism between broiler and layer chickens. These differences reflect a greater efficiency of energy utilisation by broilers and may be a consequence of the higher energy requirement of this strain for muscle protein synthesis.

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

Department of Animal Science, The University of Sydney, Camden, NSW 2570.

THE EFFECTS OF BETAINE ON WATER BALANCE AND PERFORMANCE IN BROILERS REARED UNDER DIFFERING ENVIRONMENTAL CONDITIONS

R. G. TEETER[1], J.C. REMUS[2], T. BELAY[1], M. MOONEY[1], E. VIRTANEN[3]
and P. AUGUSTINE[4]

High ambient temperature (HAT) is a significant problem in many areas of the world. Though housing strategies exist, successful broiler performance hinges on a balance between heat production (HP) and heat dissipation (HD). HP directly determines bird heat load and the necessity for HD. Feed intake extent and pattern as well as ration composition and acclimatization (Weimusz and Teeter, 1992) are important factors affecting HP. If feed intake is elevated without increased HD, mortality risk will rise (Teeter *et al.*, 1987).

Alternatively, HD routes for poultry exposed to HAT are limited as nonevaporative cooling declines (Belay *et al.*, 1993). Therefore, poultry must rely upon evaporative cooling (EC). Water balance (WB) is critical for efficient EC as 1 ml of water expels 0.57 kcal while loss via urine averages 0.024 kcal/ml (Berker and Teeter, 1994). Unfortunately, blood pH, mineral retention, WB and EC are compromised during heat stress (Teeter and Smith, 1986; Teeter *et al.*, 1985), thus betaine, an osmolyte, may be needed to help alleviate WB problems.

Research was conducted to investigate the influence of betaine on WB of broilers exposed to HAT and/or cocci. Male Cobb broilers were raised in floor pens with 0 or 0.15% betaine addition to a nutritionally adequate corn-soya basal diet. At 14 days of age, half of the birds received a mixed inoculate of coccidia. At 21 days of age, birds were placed in environmental chambers; 1 bird/chamber. Stress treatments consisted of control (ambient temperature, no cocci challenge), cycling heat stress (HAT), cocci challenge and HS x cocci challenge. Each treatment was replicated seven times and overtime between 21 and 35 days of age. Betaine was added via drinking water at 0 or 0.1% (w/v) during the 21-35 day period. Betaine elevated feed efficiency (P< 0.01) to day 14. On day 21, approximately one week after cocci challenge, betaine elevated body weight while cocci reduced weight (P<0.01). Betaine effects were independent of cocci application. During heat stress, the water consumption model was significant for cocci exposure, ambient temperature and the betaine x cocci interaction. Both HAT and cocci exposure elevated water consumption (P<0.05). The betaine x cocci interaction was the result of cocci elevating water consumption in the absence of betaine (by 780%) while cocci exposure in the presence of betaine tended to be enhanced (by 47%) (P<0.1). Urine production was impacted by HAT and cocci exposure (P<0.01). Betaine improved WB (P<0.01) and was associated with lower body temperature (P<0.14), while HAT and cocci exposure reduced WB and elevated body temperature (P<0.01). Improved WB is especially important for heat stressed birds and is correlated with EC, performance and survivability. The ability of betaine to favourably affect the WB indicates that it can improve WB of birds suffering from stresses like heat stress or coccidia.

Belay, T., Bartels, K.E., Wiernusz, C.J. and Teeter, R.G. (1993). *Poult. Sci.*, **72**: 106.
Berker A. and Teeter, R.G. (1994). *J. Appl. Poultry Res.*, **3**: 77.
Teeter, R.G., Smith, M.O., Mather, S. and Mather, F.B. (1987). *Nutr. Rep. Int'l.*, **35**: 531.
Teeter, R.G. and Smith, M.O. (1986). *Poult. Sci.*, **65**: 1777.
Teeter, R.G., Smith, M.O., Owens, F.N. and Breazile, J.E. (1985). *Poultry Sci.*, **64**:1060.
Wiernusz, C. and Teeter, R.G. (1992). *Poult. Sci.*, **71**: 1101.

[1]Dept. of Animal Science, Oklahoma State University, Stillwater, Oklahoma, USA 74078.
[2]Finnfeeds, 1101 Perimeter Drive, Suite 475, Schaumburg, Illinois, USA, 60173.
[3]Finnfeeds, Kyllikinportti 2, Helsinki, Finland 00241.
[4]USDA/ARS/LPSI BARC/East, Bldg 1040, Rm. 103, Beltsville, MD USA 20705.

DIETARY ARGININE:LYSINE RATIO INFLUENCES RELATIVE RESPONSES TO DL-METHIONINE AND ALIMET [®] AT HIGH AMBIENT TEMPERATURES

D. BALNAVE[1], J. HAYAT[1] and J. BRAKE[2]

The relative efficacies of dl-methionine (DLM) and various methionine hydroxy analogue compounds, such as 2-hydroxy-4-(methylthio) butanoic acid (HMB; Alimet[®]), are a source of controversy. Studies using purified or semi-purified crystalline amino acid diets have consistently shown a low efficacy for methionine hydroxy analogues (Van Weerden et al., 1982) whereas more practical diets composed of intact proteins give similar biological performance regardless of methionine activity source (Garlich, 1985) when comparisons are made on an equimolar basis. Recently, Brake et al., (1998) have shown that the ideal amino acid pattern differs for broilers at thermoneutral and high temperatures. In particular, the optimum arginine:lysine (Arg:Lys) ratio widens at high temperatures. Since the nature of the protein source in the diet appears to influence the relative efficacies of dl-methionine and HMB, a study was carried out to determine the importance of dietary Arg:Lys ratio in this response at high temperatures.

Day-old Cobb 500 male chicks were obtained from a commercial hatchery (Baiada Poultry Pty Ltd) and grown in cage battery brooders set at 30°C in a temperature-controlled room maintained at 25°C. The brooder heat was removed at 15d and at 21d the birds were allocated at random to grower cages in five temperature-controlled rooms maintained at 32°C. Two replicates of each of nine treatments were placed in each room. The treatments consisted of three Arg:Lys ratios (1.03, 1.20 and 1.34) by three methionine sources (unsupplemented basal diet, basal diet + DLM and basal diet + HMB). Between 1 and 21d of age birds were fed a wheat-sorghum-soyabean meal- meat meal diet in which the methionine supplement was provided equally from DLM and HMB. It contained 234g crude protein (CP) and 12.55 MJ of ME/kg. The experimental methionine-limiting basal grower diet was composed of the same ingredients as used in the starter diet except that meat meal was replaced with fish meal and solka floc, an inert cellulose filler, was included. The supplements were added in lieu of solka floc. The basal grower diet contained 195g CP and 13.3 MJ of ME/kg.

Broilers fed DLM showed reductions in food intake (FI) of 3.1 and 4.6% between 21-42 and 21-48d, respectively, when the dietary Arg:Lys increased from 1.03 to 1.34, whereas the corresponding reductions in broilers fed HMB were 0.8 and 0.7%. Between 42-48d of age broilers fed HMB had significantly better FI and weight gain (WG) than those fed DLM due primarily to improvements of 7.6, 22.0 and 10.3% in FI, WG and feed conversion at an Arg:Lys ratio of 1.34. It appears that the choice of methionine activity source at high temperatures should be allied to a knowledge of the dietary Arg:Lys ratio.

Brake, J., Balnave, D. and Dibner, J.J. (1997). *Br. Poult Sci.*, **39**: (in press).
Garlich, J.D. (1985). *Poult. Sci.* **64**: 1541-1548.
Van Weerden, E.J., Bertram, H.L. and Shutte, J.B. (1982). *Poult. Sci.*, **61**: 1125-1130.

[1]Department of Animal Science, University of Sydney, Camden, NSW 2570.
[2]Department of Poultry Science, N.Carolina State University, Raleigh, N.Carolina 27695-7608, USA.

A COMPARISON OF BROILER PERFORMANCE ON DIETS FORMULATED ON A TOTAL AND DIGESTIBLE AMINO ACID BASIS

R. PEREZ-MALDONADO[1], D.J. FARRELL[1,2] and P.F. MANNION[1]

It has been suggested that formulating poultry diets on the basis of digestible rather than total amino acids gives improved performance and profitability. Here we formulated broiler diets on the basis of total (T) and digestible (D) amino acids specified from 100% to 91% of requirements. Eleven ingredients were first analysed for their apparent metabolisable energy, total and digestible amino acids. In the latter case caecectomised adult cockerels were used, and feed and excreta were analysed. The results of the analysis and other details were published recently. Separate sexes were placed in 64 floor pens, each holding 40 birds, and offered the starter (0-21 days) and finisher diets (21-42 days) which were replicated four times. Feed and water were always available.

The results shown in the table represent data combined for the sexes since there was no diet x sex interaction. Except for differences in feed conversion ratio (FCR) at the end of 21 days, there were no differences in liveweight gain (LWG) or FCR between levels of amino acid in the diet nor between total and digestible amino acid formulations at 42 days.

Diet	Amino acid formulation	% of requirement	LWG (g/bird) 21 d	FCR 21 d	LWG (g/bird) 42 d	FCR 42 d
1	T	100	726	1.39[bc1]	2192	1.78
2	T	97	728	1.39[c]	2192	1.79
3	T	94	726	1.4[abc]	2205	1.81
4	T	91	714	1.43[a]	2167	1.81
5	D	100	717	1.41[abc]	2164	1.80
6	D	97	706	1.43[ab]	2165	1.81
7	D	94	711	1.42[abc]	2142	1.81
8	D	91	701	1.45[a]	2135	1.81
SEM			14.3	0.018	27.4	0.015

[1]Values with different superscripts are significantly different (P<0.05)

It appears that published dietary amino acid requirements for broilers are overgenerous. This, to a certain extent, makes differences between total and digestible amino acids in growth experiments difficult to identify. This is particularly so when amino digestibility values of ingredients are generally high. With few exceptions, digestibility of lysine and many other important amino acids in the 11 ingredients was above 85%. A mean digestibility value of about 85% is usually taken into account when formulating diets in practice on a total amino acid basis. Only when using ingredients with unusually low digestible amino acids have differences in formulations using total and digestible been identified in poultry.

Perez-Maldonado, R., Mannion, P.F. and Farrell, D.J. (1998). *Proc. Qld Poult. Sci. Symp.,* 7: 15-1 to 15-10.

[1]Queensland Poultry Research and Development Centre, Alexandra Hills, QLD, 4161.
[2]School of Land and Food, The University of Queensland, St. Lucia, QLD, 4072.

EVALUATION OF BROILER DIETS CONTAINING GRADED LEVELS OF COTTONSEED MEAL AND FORMULATED ON THE BASIS OF TOTAL OR DIGESTIBLE AMINO ACIDS

V. RAVINDRAN and W.L.BRYDEN

Digestible amino acid values are likely to form the basis of poultry feed formulations in the future. However, published data on broiler responses to diets formulated on the basis of digestible amino acids are limited. The present study was conducted to demonstrate that differences in digestible amino acid contents will result in comparable differences in broiler performance. Broiler starter diets were formulated with graded levels of cottonseed meal (CSM) on a total AA *vs* apparent ileal digestible AA basis and evaluated in a broiler growth assay. A wheat-sorghum-soyabean meal diet, formulated to contain 12.8 MJ apparent metabolisable energy/kg, 12.5 g total lysine/kg and 0.94 g total sulphur AA/kg, served as the control diet. Diets 2 to 4 were formulated to contain 66.6, 133.3 and 200 g CSM/kg, and similar levels of total AA to those in the control diet. Diets 5 to 7 contained the same graded levels of CSM, but were balanced to contain similar levels of digestible AA. Apparent ileal AA digestibility of all ingredients was determined prior to diet formulation. Each diet was fed to five pens of eight male chicks (Cobb) from day 7 to 21 post-hatching. The performance data are summarised below.

Dietary treatment	Weight gain (g/bird)	Feed intake(g/bird)	Feed/gain (g/g)
Wheat-sorghum-soyabean meal diet (control)	597[a]	907[a]	1.52[a]
Formulation on total amino acid basis			
66.6 g cottonseed meal/kg	548[b] (92)[1]	875[b] (96)	1.60[ab] (105)
133.3 g cottonseed meal/kg	496[c] (83)	833[c] (92)	1.68[b] (111)
200.0 g cottonseed meal/kg	335[d] (56)	642[d] (70)	1.92[c] (126)
Formulation on digestible amino acid basis			
66.6 g cottonseed meal/kg	589[ab] (99)	900[a] (99)	1.53[a] (100)
133.0 g cottonseed meal/kg	582[ab] (98)	902[a] (99)	1.55[a] (102)
200.0 g cottonseed meal/kg	522[bc] (87)	886[ab] (98)	1.70[b] (112)
Pooled SEM	9.27	15.2	0.028

[a,b,c,d] Means within a column with no common superscripts are significantly different (P < 0.05).
[1] Values in parentheses refer to bird performance relative to the control.

Increasing dietary levels of cottonseed meal on a total AA basis significantly (P < 0.05) lowered weight gains and feed efficiency of broilers. Dietary inclusion of 66.6, 133.3 and 200.0 g cottonseed meal/kg lowered the weight gains by 8.7, 16.9 and 43.9%, respectively. The corresponding increases in feed/gain were 5.3, 10.5 and 26.3%, respectively. When the diets were formulated on a digestible AA basis, the performance of birds fed 66.6 and 133.3 g cottonseed meal/kg diets was similar to those fed the wheat-sorghum-soyabean meal diet (control). However, dietary inclusion of 200 g cottonseed meal/kg on a digestible AA basis resulted in lower (P < 0.05) performance compared to the control diet, suggesting the involvement of factor(s) other than poor AA digestibility.

Department of Animal Science, The University of Sydney, Camden, NSW 2570.

EVALUATION OF MEAT AND BONE MEAL IN BROILER STARTER DIETS FORMULATED ON THE BASIS OF TOTAL OR DIGESTIBLE AMINO ACIDS

V. RAVINDRAN and W.L. BRYDEN

Meat and bone meal (MBM) has long been valued in poultry diets as a source of protein and available phosphorus. The usefulness of MBM is, however, limited by wide variability in chemical composition and nutrient availability. In the present study, a MBM sample, determined to have a poor amino acid (AA) digestibility, was incorporated in broiler starter diets that were formulated on a total AA *vs* apparent ileal digestible AA basis and evaluated in a broiler growth assay. A wheat-sorghum-soyabean meal diet, formulated to contain 12.9 MJ AME/kg, 224 g CP/kg, 12.5 g total lysine/kg and 9.4 g total sulphur-amino acids/kg, served as the control diet. Diets 2 and 3 were formulated to contain 50 and 100 g MBM/kg, and similar levels of total AA to those in the control diet. Diets 4 and 5 contained the same graded levels of MBM, but were balanced to contain similar levels of digestible AAs. All diets were formulated to contain similar levels of calcium and total phosphorus. Apparent ileal AA digestibility of all ingredients was determined prior to diet formulation. Apparent ileal digestible lysine requirement was assumed to be 87% of the total requirement, and the ideal protein concept (Baker and Han, 1994) was applied to estimate the requirements for the other essential AA. Each diet was fed to six pens of eight male chicks (Cobb) from day 5 to 19 post-hatching. The results are summarised below.

Dietary treatment	Weight gain (g/bird)	Feed intake(g/bird)	Feed/gain (g/g)
Wheat-sorghum-soyabean meal diet (control)	525[a]	785	1.50[b]
Formulation on total amino acid basis			
50 g MBM/kg	511[ab] (97)[1]	797 (96)	1.56[c] (104)
100 g MBM/kg	502[b] (96)	815 (92)	1.63[d] (109)
Formulation on digestible amino acid basis			
50 g MBM/kg	531[a] (101)	780(99)	1.47[b] (98)
100 g MBM/kg	538[a] (102)	767 (99)	1.43[a] (95)
Pooled SEM	5.5	14.1	0.021

[a,b,c,d] Means within a column with no common superscripts are significantly different (P < 0.05).

[1] Values in parentheses refer to bird performance relative to the control.

Increasing dietary levels of MBM (50 and 100 g/kg) on a total AA basis significantly (P < 0.01) lowered weight gains and feed efficiency. Weight gains were unaffected by increasing levels of MBM when diets were balanced on a digestible AA basis, but an improvement (P < 0.05) in feed efficiency was observed in birds receiving 100 g /kg MBM diets. These results demonstrate the value of formulating broiler diets using digestible AA when ingredients with poor digestibilities are used.

Baker, D.H. and Han, Y. (1994). *Poult. Sci.*, **73**: 1441.

Department of Animal Science, The University of Sydney, Camden, NSW 2570.

ENZYME COMBINATIONS AND NUTRIENT DIGESTIBILITY OF WHEAT FOR BROILER CHICKENS

W.L. BRYDEN and V. RAVINDRAN

Wheat-based diets usually contain an exogenous xylanase and there is increasing evidence that an exogenous microbial phytase may also improve the digestibility of energy and amino acids of these diets (Cabahug et al., 1998). The object of the present study was to determine the influence of a commercial xylanase (Natugrain Blend™; BASF, Ludwigshafen, Germany), a microbial phytase (Natuphos®; BASF) and a protease (Gist-brocades, The Netherlands), alone and in combination, on the apparent metabolisable energy (AME) and apparent ileal nitrogen (ND) and amino acid digestibility (AAD) of a low-AME wheat (cultivar, Meering) for broilers. The basal diet (B) contained 800 g wheat/kg and 134 g casein/kg as main ingredients, and 20 g celite/kg as an indigestible marker. The following dietary treatments were tested: B, B + xylanase (X), B + phytase (Ph), B + protease (Pr), B + X + Ph, and B + X + Pr. Enzymes were added according to manufacturer's recommendations. Each diet was fed to four pens (4 birds/pen) of broilers from day 35 to 42 post-hatching. The AME of the diets was determined using the total excreta collection procedure (Mollah et al., 1983). On day 42, digesta contents from the terminal ileum were collected and the ND and AAD coefficients of the diets were calculated using acid insoluble ash as the marker. The results are summarised below.

Treatment	AME of wheat, MJ/kg DM	ND	AAD
B	12.0[a]	0.843[a]	0.805[a]
B + X	13.25[ab]	0.874[b]	0.845[b]
B + Ph	12.72[ab]	0.870[ab]	0.845[b]
B + Pr	12.36[a]	0.846[a]	0.812[a]
B + X + Ph	14.38[b]	0.890[b]	0.874[c]
B + X + Pr	14.22[b]	0.867[ab]	0.850[bc]
Pooled SEM	0.57	0.009	0.008

[ab]Means in a column having different superscripts are significantly different (P<0.05).

Supplemental X improved AME of wheat by 9.7% and the apparent N digestibility of the diet by 3.7% digestibility. The corresponding improvements due to supplemental Ph were 5.3% and 3.2%, respectively. Interestingly, supplemental Pr had no influence on the AME or ND. The AME (19%) and ND (5.6%) were further improved by the combination of X and Ph. A similar pattern was observed with AAD where Pr was without effect while the other two enzymes caused a 5% improvement which increased to 8.6% when both X and Ph were added to the diet.

The results of this study suggest that the strategic dietary inclusion of exogenous enzyme combinations will increase nutrient utilisation in wheat-based diets.

Cabahug, M., Ravindran, V., Legge, M.S., Bryden, W.L. and Selle, P.H. (1998). *Proc. Aust. Poult. Sci. Symp.* (Ed. R.A.E. Pym). **10**: 203.

Mollah, Y., Bryden, W.L., Wallis, I.R., Balnave, D. and Annison, E.F. (1983). *Brit. Poult. Sci.*, **24**: 81.

Department of Animal Science, The University of Sydney, Camden, NSW 2570.

EFFECTS OF MICROBIAL PHYTASE ON ILEAL AMINO ACID DIGESTIBILITY OF INGREDIENTS FOR BROILERS

S. CABAHUG, V. RAVINDRAN, G. RAVINDRAN and W.L.BRYDEN

The effectiveness of microbial phytase in releasing phytate-bound phosphorus and improving phosphorus bioavailability in plant feed ingredients for poultry is well established. Recent reports indicate that the addition of microbial phytase also results in significant improvements in amino acid (AA) digestibility in poultry diets (Cabahug et al., 1998). The objective of the present study was to determine the influence of microbial phytase (Natuphos[®], BASF AG, Germany) on the apparent ileal AA digestibility in three cereals (wheat, sorghum and maize), four plant protein sources (soyabean meal, canola meal, cottonseed meal and sunflower meal) and two cereal by-products (millrun and rice pollard).

Ileal AA digestibilities of the ingredients, without and with microbial phytase (1200 FTU/kg), were determined using procedures described previously (Ravindran et al., 1998). Assay diets contained the test ingredient as the only source of protein. Celite was included in all diets as an indigestible marker. Each assay diet was fed ad libitum to three pens (5 birds/pen) of male broilers from 35 to 42 days of age. At the end of the trial, digesta contents from the terminal ileum were collected and processed. Samples of diets and digesta were analysed for AA and acid-insoluble ash, and the apparent ileal AA digestibility values were calculated.

Phytase supplementation improved ($P < 0.05$ to 0.01) apparent ileal digestibility of AA in all feed ingredients. The magnitude of response varied among ingredients and among different amino acids within an ingredient. The percentage improvements were greatest in wheat and lowest in canola meal. The improvements in digestibility were higher for threonine relative to other amino acids. The mean ileal digestibility of the 15 AA in ingredients, without and with phytase, were: wheat, 77.7 and 84.6; sorghum, 74.7 and 79.4; maize, 78.0 and 80.4; soyabean meal, 82.2 and 85.5; canola meal, 78.7 and 80.7; cottonseed meal, 70.8 and 74.2; sunflower meal, 76.7 and 80.2; millrun, 70.8 and 73.4; and rice pollard, 62.1 and 66.9. Percentage increases in lysine digestibility were as follows: wheat, 10.9; sorghum, 3.7; maize, 3.2; soyabean meal, 4.0; canola meal, 0.9; cottonseed meal, 4.7; sunflower meal, 5.7; millrun, 2.4; and rice pollard, 5.1. The corresponding values for threonine digestibility were: wheat, 15.7; sorghum, 8.8; maize, 6.7; soyabean meal, 8.0; canola meal, 4.1; cottonseed meal, 6.9; sunflower meal, 5.5; millrun, 6.1; and rice pollard, 7.1. These responses are clearly related to the capacity of phytate to bind protein/AA and to the ability of the enzyme to release these nutrients during the process of hydrolysing the phytate complexes. The relatively large effect of microbial phytase on threonine digestibility suggests that the observed improvements in apparent digestibility may reflect, at least in part, reduced endogenous AA losses, resulting from the amelioration of the anti-nutritive effects of phytic acid.

Cabahug,M., Ravindran,V., Legge, M.S., Bryden,W.L. and Selle, P.H. (1998). *Proc. Aust. Poult. Sci. Symp.* Ed. R.A.E. Pym. **10**: 203.

Ravindran, V., Hew, L.I. and Bryden, W.L. (1998). Digestible Amino Acids in Feedstuffs for Poultry. Rural Industries Research and Development Corporation, Canberra and Poultry Research Foundation, The University of Sydney, Camden.

Department of Animal Science, The University of Sydney, Camden, NSW 2570.

THERMOSTABILITY OF POWDER ENZYMES : IN VITRO RECOVERIES AND IN VIVO EFFICACIES

A.M. PEREZ-VENDRELL[1], N.M. FISH[2], A.M. SABATIER[3], D. FRAPIN[3] and P.A. GERAERT[3]

Feed processing imposes strong physical constraints on feed additives. Enzymes are proteins and their structure and activity are highly sensitive to such constraints. Enzyme suppliers have thus attempted to protect as much as possible of the activity of their products. However, irrespective of the products, many studies have demonstrated that approximately 60 to 80 % of the feed enzymes incorporated in the feed prior to pelleting are destroyed at a pelleting temperature above 80°C (Gadient, 1996 ; Piironen, 1996). Even in the case of low pelleting temperatures, the irregularity of the flow often leads to sharp and sudden increases in temperature resulting in enzyme denaturation. To evaluate losses of activity of powder products, *in vitro* enzyme activity analysis is often recommended. However, discrepancies might appear between *in vitro* recoveries and *in vivo* efficacies. It was thus decided to evaluate thermostability of different commercial products through two different laboratory *in vitro* methodologies and through their true *in vivo* efficacy.

The same wheat-based grower broiler feed was conditioned and pelleted at three different sets of temperatures (65-70, 75-80 and 85-90°C) in the IRTA feed mill. Powder enzymes were included in the premix prior to pelleting at their recommended dose. Eight commercial enzyme products from different companies were tested. Feeds were then assayed for enzyme recoveries either by spectrophotometric (azo-arabinoxylan) method at the IRTA laboratory or by viscometric method at RPAN laboratory (Sabatier and Fish, 1996). Apparent metabolizable energy (AME) of the feeds was also determined in growing broilers using *ad libitum* feeding and total excreta collection (Bourdillon *et al.,* 1990).

The recovery results revealed that even at the lowest temperature most products lose at least 30 % of their activity while, at the highest conditioning temperature, losses reached 90 %. Coated or encapsulated enzyme products were also tested. *In vitro* assay results appeared to show an under recovery of activity at lower feed processing temperatures (70°C) and a greater than expected recovery of activity at higher temperatures. However, whereas *in vitro* recoveries suggested different thermostabilities, *in vivo* AME measurements showed that such products would not give further improvements compared with non-coated products.

Finally, whatever the feed form, pellets or crumbles, the easiest solution to solve the problem of such a loss of activity during feed processing is the post-pelleting spraying of liquid enzyme; the effect of enzyme being even more important in heat-processed feeds.

Bourdillon, A., Carré, B., Conan, L., Duperray, J., Huyghebaert, G., Leclercq, B., Lessire, M., McNab, J. and Wiseman, J. (1990). *Br. Poult. Sci.* **31**: 557-565.
Gadient, M. (1996). *Proc. AFTAA Seminar, March 19th 1996, Paris, France* 7 pp.
Piironen, J.T. (1996). *Proc. Austr. Poult. Sci. Symp.* Ed. D. Balnave, **8**: 146-148.
Sabatier, A.M., Fish, N.M. (1996). *J. Appl. Poult. Res.,* **6**: 61-68.

[1]IRTA, Centre de Mas Bové, Apartat 415, 43280 Reus, Spain
[2]RHODIA Ltd, Poleacre lane, Woodley, Stockport, Cheshire, SK6 1PQ, United Kingdom
[3]Rhône-Poulenc Animal Nutrition, 42 Av A. Briand BP 100 92164 ANTONY Cedex, France

ADDITIVE EFFECTS OF ß–GLUCAN AND HEAT TREATMENT ON NUTRIENT DIGESTIBILITY AND AME CONTENT IN BROILER CHICKENS

M.L. MAQUEDA DE GUEVARA[1], P.C.H. MOREL[1], G.D. COLES[2], J.R. PLUSKE[1], J.A. MONRO[3] AND D.V. THOMAS[1]

Broiler chickens fed diets containing high levels of barley generally show decreased performance consistent with lower nutrient utilisation. The anti-nutritive effect of barley is strongly related to the presence of soluble β–glucan. Most commercial broiler diets are fed in pelleted form. There is evidence that the considerable temperature rise during pelleting may further solubilise some of the NSP (Robertson *et al.*, 1997), which may increase the anti-nutritive effects of barley. We tested this proposition by adding a commercially-prepared extract of ß–glucan (~ 60 % purity) to a synthetic diet based on casein, cornstarch, sugar, 3% Cr_2O_3/kg and added vitamins and minerals. This allowed the attribution of observed effects directly to the ß–glucan. Twenty birds were arranged in a 2 x 2 factorial arrangement of treatments with the respective factors being "heat" or "no heat" treatment of the diet, and the absence or presence of ß–glucan (added at 150g/kg). Diets were fed to birds "as is", or were fed after the diet was heated to ~ 95° C for five minutes. No additional water was used in the heated treatments. Birds were housed and sampled individually in a controlled-temperature shed and fed the diets between days 15 and 21 of age. On the final three days total excreta was collected from individual birds for determination of apparent metabolisable energy (AME). At slaughter, each bird was euthanased with sodium pentobarbitone, dissected, and gross morphological measurements recorded. Digesta anterior to Meckel's diverticulum was retained for measurement of water retention, and digesta distal to the diverticulum was retained for nitrogen (N) and carbon (C) digestibility estimation. All data were analysed using the GLM procedure of SAS.

Parameter	Diet not heated		Diet heated		
	- ß-glucan	+ ß-glucan	- ß-glucan	+ ß-glucan	Pooled SEM
N digestibility (%)	97.5	96.6	96.7	95.1	0.34
C digestibility (%)	92.9	82.7	88.9	81.1	1.21
AME (MJ/kg DM)	14.84	14.00	14.20	12.61	0.579
H_2O (g/g digesta)	1.96	3.16	2.22	2.96	0.173
Contents of DSI (g)	1.6	4.3	1.3	4.3	0.38

No significant interactions were found for any parameter. A decrease in ileal N digestibility was observed in response to heating (95.9 *vs* 97.0%, P<0.001) and ß–glucan (95.8 *vs* 97.1%, P=0.002), but only ß–glucan decreased C digestibility (81.9 *vs* 90.9%, P<0.001). AME tended to decrease after heating (P=0.098) and with ß–glucan inclusion (P=0.052). Chickens fed diets with ß–glucan had more digesta (P<0.001) in their distal small intestine (DSI), consistent with a higher water holding capacity (P<0.001). These data suggest that there are both additive and independent effects of heating and ß–glucan on digestion and water holding characteristics. Heat associated with processes such as pelleting may contribute to the anti-nutritive properties of ß-glucan, especially N digestion and AME content.

Robertson, J.A., Majsak-Newman, G., Ring, S.G. and Selvendran, R.R. (1997). *J. Cereal Sci.*, **25**: 275.

[1] Monogastric Research Centre, Massey University, Palmerston North, New Zealand.
[2] Crop and Food Research, Private Bag 4704, Christchurch, New Zealand.
[3] Crop and Food Research, Private Bag 11030, Palmerston North, New Zealand.

A LIPASE PREPARATION INCREASES AME CONTENT OF FULL-FAT RICE BRAN AND BROILER CHICKEN PERFORMANCE UP TO 14 DAYS OF AGE

S. TAN, D.V. THOMAS, B.J. CAMDEN, P.C.H. MOREL and J.R. PLUSKE

Full-fat rice bran (FFRB) is a by-product available for use in the broiler industry. It has a high gross energy ($\approx$ 21 MJ/kg DM) and crude fat ($\approx$ 200 g/kg DM) content. A lower ME value of FFRB for chickens compared to cockerels was reported by Warren and Farrell (1990), an effect attributable in part to low secretion of pancreatic lipase. The hypothesis tested in this study was that an exogenous lipase preparation would improve both the AME content of FFRB and bird performance. A sample of FFRB was obtained from Thailand (CP Group, Bangkok) and kept at 4° C. A total of 270 day-old-birds and six diets (9 cages/diet, 5 birds/cage) was used between 0 and 14 days of age. A basal diet based on maize, soybean, MBM and fishmeal was prepared, and to this 90 or 180g FFRB/kg was substituted with and without 1g/kg of a lipase preparation (Alltech, Inc.). The enzyme was also added to the basal diet. Feed and water were available on an *ad libitum* basis. The AME content of the diets and FFRB was estimated between days 4 and 7 and 11 and 14 by collection of total excreta. A representative subsample was later analysed for DM and GE. Two data analyses were conducted using the GLM procedure of SAS: (1) effects of lipase in the basal diet; (2) effects of lipase and level of FFRB in diets where FFRB substituted 90 and 180g per kg of the basal diet.

Day 0-14	Basal diet		Basal + 90g FFRB		Basal + 180g FFRB		
	+ Enz	- Enz	+ Enz	- Enz	+ Enz	- Enz	SEM
Gain, (g/bird/d)	36.2 [a][1]	34.7 [b]	35.9	33.3	32.0	32.6	0.56
Food intake, (g/bird/d)	40.7	39.8	41.6	39.7	39.5	40.6	0.66
FCR, (g feed/g gain)	1.126 [a]	1.148 [b]	1.161	1.194	1.236	1.248	0.01
AME (MJ/kg DM)							
AME$_{DIET}$ d 4-7	15.41	15.31	14.91	14.65	14.36	14.06	0.15
AME$_{DIET}$ d 11-14	15.27 [a]	15.02 [b]	14.82	14.55	14.31	14.12	0.12
AME$_{FFRB}$ d 4-7	-	-	10.82	9.74	10.03	8.37	0.82
AME$_{FFRB}$ d 11-14	-	-	12.82	9.93	11.06	10.03	0.94

[1]Means without a common superscript are significantly different (P<0.05).

Significant interactions occurred between enzyme inclusion and level of FFRB for daily gain (P=0.009) and food intake (P=0.027). Birds fed 90g FFRB/kg plus lipase grew 7.1% faster and ate 4.8% more feed than birds fed 90g FFRB without lipase. When 180g FFRB/kg was added to the basal diet, performance was similar to that of birds fed 90g FFRB minus enzyme, although FCR deteriorated (P<0.001). Between days 4-7 and 11-14, AME content was higher in diets with 90g FFRB than 180g FFRB/kg (14.78 *vs* 14.21, P<0.001; 14.69 *vs* 14.22, P<0.001, respectively) and in diets containing enzyme (P=0.080). Inclusion of lipase increased the AME content of FFRB between days 4-7 (P=0.107) and 11-14 (11.94 *vs* 9.93, P=0.041), but no main effect of level of FFRB or interactions was recorded. An improvement in daily gain (P=0.011), FCR (P=0.056) and diet AME (days 4-7: P=0.11; days 11-14: P=0.002) was seen when 1g lipase/kg was added to the basal diet. These data suggest that a lipase preparation can increase AME content in FFRB and increase production between 0 and 14 days of age, with the greatest benefits being observed when FFRB was substituted for 90/kg of the basal diet.

Warren, B.E. and Farrell, D.J. (1990). *Anim. Feed Sci. Technol.*, **27**: 247-257.

Monogastric Research Centre, Massey University, Palmerston North, New Zealand.

DIFFERENCES IN IMMUNE COMPETENCE AMONGST LAYER STRAINS

S.W. WALKDEN-BROWN[1], C.W. WONG[1], J.V. NOLAN[1], A.L. GRIMA[1] and
I.G. COLDITZ[2]

In recent years there has been variation between layer strains in mortality due to causes such as Marek's disease and cannibalism (eg. Cumming *et. al.*, 1998). It is possible that differences in immune competence may underlie such strain differences. To test this hypothesis we investigated humoral and cell-mediated immunity, and lymphocyte sub-populations in 5 strains of layer birds held under commercial conditions at high production.

SuperBrown2 (SB2), Hisex, ISA, Hy-Line commercial CB (CB) and Hy-Line Brown (HLB) chicks were hatched in February 1997 (week 0) and given standard MD and IB vaccinations. At 15 weeks the birds were vaccinated against Egg Drop Syndrome (EDS) using an inactivated vaccine (Intervet®) then transferred to the University of New England 10 days later and run as a commercial layer flock (total of 3155 birds placed). In addition to live weight (LW) and mortality data (Mort.), immune variables were measured as follows:

a) Humoral immunity. Specific antibody titres against EDS were determined by ELISA in plasma samples collected at weeks 16.5, 31 and 57 (n=12/strain at each sampling).

b) Cell-mediated immunity. This was determined by the change in wattle thickness 24 h after injection of phytohaemagglutinin (PHA, 80μg in 100μl normal saline) adjusted for the change in thickness in the other wattle injected with 100μl saline (Week 57, n=12).

c) Lymphocyte phenotype. At weeks 31 and 57, blood samples from the same 8 birds from each strain were collected and the percentage of lymphocytes in the CD4+ (helper) and CD8+ (cytotoxic) classes determined using fluorescence-activated cell sorter analysis.

Strain effects are summarised in the table below (means ± SEM).

Variable	Layer strain				
	CB	Hisex	HLB	ISA	SB2
Log_{10} EDS titre	1.92 ± 0.07^{ab}	2.10 ± 0.09^{bc}	1.83 ± 0.07^{a}	1.92 ± 0.12^{ab}	2.31 ± 0.07^{c}
PHA resp. (mm)	1.05 ± 0.44^{ab}	1.52 ± 0.84^{ab}	0.83 ± 0.47^{a}	1.72 ± 0.92^{b}	1.44 ± 0.75^{ab}
CD4+ lymp. (%)	58.5 ± 1.9^{c}	52.1 ± 2.6^{bc}	49.7 ± 1.6^{ab}	54.9 ± 3.0^{bc}	48.3 ± 2.3^{a}
CD8+ lymp. (%)	15.30 ± 0.9^{a}	17.1 ± 2.1^{a}	16.2 ± 1.1^{a}	16.1 ± 0.9^{a}	20.1 ± 1.2^{a}
LW (Wk 31, kg)	1.97 ± 0.05^{a}	2.23 ± 0.05^{a}	2.03 ± 0.06^{a}	2.02 ± 0.06^{a}	2.03 ± 0.09^{a}
LW (Wk 57, kg)	2.09 ± 0.08^{a}	2.31 ± 0.04^{a}	2.30 ± 0.10^{a}	2.28 ± 0.07^{a}	2.22 ± 0.09^{a}
Mort. (Wks 16-63, %)	7.6^{a}	29.8^{c}	25.2^{c}	25.8^{c}	12.3^{b}

[abc] Means within rows not sharing a common superscript are significantly different (P<0.05).

While the specific consequences of these results cannot be determined without further work, they support our hypothesis by demonstrating significant differences in immune function between strains. The hypothesis is further supported by the observation that the HLB birds, which had the lowest mean values for our measures of both humoral and cell-mediated immunity, exhibited high levels of mortality in this experiment and had the highest total mortality among the strains compared in the study of Cumming *et al.* (1998).

Cumming R.B., Ball, W. and Nolan, J.V. (1998). *Proc. Aust. Poult. Sci. Symp.*, Ed. R.A.E. Pym, **10**: 168-171.

[1]Division of Animal Science, University of New England, Armidale, NSW 2351.
[2] Pastoral Research Laboratory, CSIRO Animal Production, Armidale, NSW 2350.

EFFECTS OF FAT SOURCES ON LEAN TISSUE DEPOSITION IN BROILERS

M. CHOCT[1], A. NAYLOR[1] and V. H. ODDY[2]

The modern broiler contains 150 to 200 g fat per kg body weight, over 85% of which is physiologically inessential. Fatness in poultry has three major attributes: a) it depresses feed efficiency; b) some adipose tissues are of little economic value, ie, abdominal fat is removed by evisceration, thus decreasing processing yield; and c) consumption of saturated fat is associated with increased incidence of cardiovascular risks in humans. Increased fat content in the chicken meat is therefore undesirable both economically and socially. Nutritional manipulations taken to counter excessive body fatness include feed restriction, changing protein to energy ratio and manipulation of the balance of individual amino acids. Although some of these measures have yielded favourable results their practical use has been limited. The current study was undertaken to examine the effect of various fat sources on lean tissue deposition in broiler chickens.

Fish oil, linseed oil, lard and safflower oil was added to a commercial type broiler diet at 20 and 40g/kg levels. It consisted of cereals (sorghum and barley), protein sources (soybean meal, meat meal and cottonseed meal), and vitamin and minerals. Each diet was fed to 6 individual birds for 42 days. On day 42, all birds were weighed and feed was withdrawn. On day 43, all birds were killed by cervical dislocation and the weight of the empty body, abdominal fat pad, breast muscle and viscera was recorded. The abdominal fat pad weight differed between treatments due to fat sources with linseed oil giving a significantly heavier fat pad compared with fish oil and lard (P<0.05).

Fat source	% Fat	Fat pad (g)	% Fat	Fat pad (g)
Fish oil	2	19.6 (1.44)[b][1]	4	21.6 (2.15)[a]
Lard	2	18.9 (2.67)[b]	4	24.6 (2.51)[a]
Linseed oil	2	28.7 (2.99)[a]	4	26.8 (4.25)[a]
Safflower oil	2	22.6 (3.35)[ab]	4	20.7 (2.99)[a]

[1]Means (± SEM) within a column bearing the same superscript do not differ significantly P<0.05.

Addition of 20 g fish oil or 20g lard/kg to broiler diets markedly (P<0.05) reduced abdominal fat pad weight with no effects on weight gain and feed conversion efficiency of the birds. At 40g/kg, however, the current trial failed to detect a significant (P>0.05) difference between abdominal fat pad weights of birds fed various fat sources. An increase in glucose uptake into the muscle tissue and a decrease in plasma triglyceride concentration due to the effects of n-3 fatty acids is a possible explanation for the current result (Newman *et al.*, 1998).

Newman, R.E., Dowling, J.A., Dehon, J.A. and Bryden, W.L. (1998). *Proc. Aust. Poult. Sci. Symp.*, Ed. R.A.E. Pym, **10**: 210.

---

[1]School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351.
[2]Cooperative Research Centre for Beef and Cattle, University of New England, Armidale, NSW 2351.

CALCIUM-45 ACCRETION IN BONE AND EGGSHELL OF LAYERS

J.V. NOLAN and W. BALL

Layers have a prodigious requirement for calcium (Ca) and it is understood that they will exhibit efficient renal reabsorption of filtered Ca and efficient storage in bone at times of the day when Ca is not being used for eggshell formation. Several studies have investigated aspects of Ca deposition into bone and eggshell using ^{45}Ca, eg. Clunies and Leeson (1994) but apparently none involving short-term changes including excretion.

Hens (15 Hy-line layers at 68 weeks of age) were moved on day 0 from an open-sided production shed (48 weeks into lay) to single–bird cages indoors where they continued to be offered free access to a commercial layer diet (11.5 MJ/kg, 17% crude protein). Ovipositions were recorded from day 1 to day 13 so that likely times of oviposition on days 14 and 15 could be predicted. On day 14 or day 15 each hen was injected intravenously with ^{45}CaCO$_3$ solution (15μCi in 0.5ml physiological saline) at a strategic time relative to the time of previous oviposition. The birds were slaughtered 2 h later so that, taken together, the birds provided a series of "2 h windows" on the complete oviposition cycle. At slaughter, eggshell, if present, and excreta produced during the previous 2 h was collected. The excreta and clean eggshell were treated with 0.1M HCl and, after centrifugation, the radioactivity in the supernatant fraction was determined by scintillation counting. The values in the figure show the fate of the injected radioactivity and its distribution in eggshell, excreta or tissues (by difference) at strategic times during egg formation.
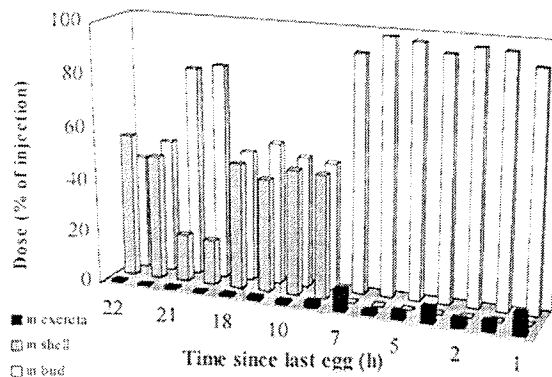
Only 8 hens had eggs with hard shells at slaughter. In these hens, 17-51% of the tracer was deposited in eggshell during the preceding 2 h. Also, renal reabsorption of Ca was apparently very high and Ca was efficiently retained in tissues. In hens at earlier stages of egg formation, more than 90% of ^{45}Ca was stored in tissues, but significantly, there was a small "leakage" from the body via excreta (via gut and/or kidneys) at times when there was no shell deposition. The results show that there are marked changes in the partitioning of blood Ca during egg formation with the major shift occurring after the time of eggshell formation. There was a large flux of Ca through the tissues, and hence bone, during shell deposition, rather than simply depletion from blood and replacement from bone stores. The increased excreta ^{45}Ca at the closing and onset of shell deposition are also noteworthy, and may warrant further investigation.

Clunies, M. and Leeson, S. (1994). ^{45}Calcium dynamics of hens laying thick- or thin-shelled eggs. *Can. J. of Anim. Sci.*, 74: 541-532

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

Egg shell form'n begins around 10 hrs 177
after last oviposition –

RELATIONSHIP BETWEEN EGGSHELL ULTRASTRUCTURE AND HATCHABILITY

J. RUIZ[1], C. LUNAM[1], P. GROVES[2] and P. GLATZ[3]

There is limited information concerning the relationship between the proportions of the eggshell laminae and hatchability. This is in spite of the fact that the palisade lamina determines most of the pore length, which is likely to influence the rate of gaseous movement across the eggshell during incubation (Ar *et al.*, 1974). Furthermore, the eggshell is the primary source of calcium for the developing embryo with the mammillary lamina providing most of the required calcium after day 10 of incubation (Dieckert *et al.*, 1989). The cuticle provides physical protection to the embryo by preventing the penetration of foreign micro-organisms across the eggshell (Bruce and Drysdale, 1991).

To investigate the effects of specific calcified laminae and the cuticle on hatchability, we compared the relative proportions of each lamina in two imported broiler breeder lines (standard and reversed-cross) having different hatchability levels.

Fifteen eggs were randomly selected from a single morning collection period of three hours at 38 weeks of age (hatchability peak). For each egg, the cross-sectional length of each lamina was measured from three equatorial shell pieces using a Siemens ETEC scanning electron microscope. Differences between the cross-sectional length of each lamina was compared between the breeding lines using analysis of variance (SSPS Inc., 1995).

There was no difference in the proportions of either calcified laminae or cuticle in the two imported lines. Similarly, no significant differences in the proportions of the individual lamina between the lines were observed.

Laminae	Total cross-sectional length (% mean ± sd)		Probability
	Standard line	Reversed-cross line	
Cuticle	0.76 ± 0.31	0.78 ± 0.25	0.829
Vertical crystal	4.37 ± 0.36	4.45 ± 0.38	0.577
Palisade	67.83 ± 1.44	68.74 ± 1.20	0.068
Mammillary	27.22 ± 1.37	26.38 ± 1.09	0.074

These results suggest that the decrease in hatchability observed in the reversed-cross line is not related to the proportions of either the calcified laminae or cuticle. To determine the effects of age of the hen on eggshell ultrastructure and hatchability, further research is currently in progress to examine the relationship between the eggshell laminae and hatchability over the entire laying period for both imported broiler breeder lines.

Ar, A., Paganelli, C. R., Greene, D. and Rahn, H. (1974). *Condor,* **76**: 153.

Bruce, J. and Drysdale, E. M. (1991). Avian Incubation: Poult. Sci. Symp. Ed. S. G. Tullet, **22**: 257. Butterworth-Heinemann, UK

Dieckert, J. M., Dieckert, M. C. and Creger, C. R. (1989). *Poult. Sci.,* **68**: 1569-1584.

[1]Department of Anatomy and Histology, Flinders University, Bedford Park, SA 5042.
[2]Baiada Poultry Pty Ltd, PO Box 21, Pendle Hill, NSW 2145.
[3]SARDI, Roseworthy Campus, University of Adelaide, Roseworthy, SA 5371.

EFFECTS OF CALCIUM SUPPLEMENTATION ON SHELL QUALITY IN BROILER BREEDERS

J. RUIZ[1], C. LUNAM[1], P. GROVES[2] and P. GLATZ[3]

There is extensive information concerning dietary calcium supplementation and shell quality in commercial egg layers but limited for broiler breeders. Calcium supplementation is often necessary for adequate shell formation in broiler breeders (Hogden, 1990). Poor shell quality due to calcium deficiency in broiler breeders has been associated with excessive egg weight loss, higher embryonic mortality and increased susceptibility to contamination (McDaniel *et al.*, 1979). This study investigated the effect of dietary calcium supplementation on shell quality in a single broiler breeder line.

There were two experimental diets; a standard diet containing calcium at 28g/kg of feed and a high calcium diet containing an extra amount of feed in the form of limestone (28g/kg plus 40gCa/kg) were provided to a total of 100 females divided into 10 replicates for each diet. One hundred eggs were collected over a period of 4 days between 0900 and 1200 h at 38 weeks of age (hatchability peak). The following shell quality parameters were measured: egg weight, shape index (breadth x 100/length), shell weight, shell thickness and percentage shell (shell weight/egg weight). Differences in the shell quality parameters between dietary treatments were assessed using analysis of variance (SPSS Inc., 1995).

Supplementation of the diet with calcium significantly reduced egg weight, surface area and breadth. In contrast, calcium supplementation significantly increased shell thickness ($P<0.05$) and percentage shell. No significant variation in the other parameters were observed between breeder lines.

Shell quality parameters	Diets (mean ± sd)		P Value
	Standard	High calcium	
Egg weight (g)	68.71[a] ± 4.79	66.93[b] ± 4.47	0.007
Shell weight (g)	6.07 ± 0.54	6.07 ± 0.49	0.990
Shell (%)	8.85[a] ± 0.69	9.08[b] ± 0.59	0.011
Shell thickness (μm)	357.14[a] ± 27.43	365.21[b] ± 23.38	0.026
Surface area (cm^2)	78.65[a] ± 3.86	77.20[b] ± 3.65	0.007
Shape index	75.97 ± 3.40	75.82 ± 3.00	0.738
Length (mm)	59.32 ± 2.06	58.82 ± 2.20	0.096
Breadth (mm)	45.02[a] ± 1.38	44.55[b] ± 1.11	0.008

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

The reduction in egg weight and increased shell thickness suggest that supplementation of calcium results in smaller egg size. This statement is supported by the significant reduction in surface area and decreased breadth and width of eggs from hens on the higher calcium diet.

Hogden, A. (1990). *Proc. 1990 Poultry Information Exchange, Qld.* p. 1. ANA, The Gold Coast, Qld.

McDaniel, G. R., Roland, D. A. and Coleman, M. A. (1979). *Poult. Sci.*, **58**: 10.

[1]Department of Anatomy and Histology, Flinders University, Bedford Park, SA 5042.
[2]Baiada Poultry Pty Ltd, PO Box 21, Pendle Hill, NSW 2145.
[3]SARDI, Roseworthy Campus, University of Adelaide, Roseworthy, SA 5371.

EFFECTS OF DIETARY PROTEIN AND LINOLEIC ACID ON SECOND-CYCLE PERFORMANCE OF FOUR STRAINS OF LAYING HENS

D. ROBINSON and M.J. DATUGAN

An objective of second-cycle management in the current Australian market is to maximise egg number while minimising egg weight. Nutrition in the period immediately following rest inducement is often considered to be especially important in achieving satisfactory second-cycle performance. In the first laying cycle egg weight responds to both dietary protein concentration (Morris and Gous, 1988) and, in at least some strains of bird, linoleic acid (LA) concentration (Mannion et al., 1992; Leary et al., 1998).

Following a study of the effects of protein and LA levels on first-cycle performance of two local and two imported strains of layer (Robinson and Datugan, 1998), birds were moulted at 64 weeks of age by feeding barley for 18 days and were re-allocated to post-moult treatments, equal numbers of experimental units from each previous treatment to each new treatment. A total of 176 units of 6-8 birds were used. Moulted birds received one of two recovery diets for a 23-day period (R1 145 g/kg protein, 8.8 g/kg LA; R2 178 g/kg protein, 20 g/kg LA), following which they received one of three post-recovery diets which were fed until 102 weeks of age (P1 156 g/kg protein, 8.2 g/kg LA; P2 156 g/kg protein, 20 g/kg LA; P3 178 g/kg protein, 20 g/kg LA).

Barley intake in the moult inducement period was higher (P<0.01) in local than in imported strains, and higher (P<0.05) in birds that received 165 g/kg as opposed to 183 g/kg protein diets in the first laying cycle. Differences in barley intake had little effect on bodyweight loss, which averaged 19.5%. Post-moult performance criteria were unaffected by first-cycle laying diet. Following the moult R2 birds resumed lay sooner and achieved a higher peak (P<0.05) than R1 birds. In the recovery period R2 birds laid heavier eggs, ate more food, gained more weight and produced fewer downgraded eggs than R1 birds (P<0.05). Recovery period diets had no effect on overall post-recovery performance other than feed intake, for which there was an interaction (P<0.05) with strain and post-recovery diet. In the post-recovery period, the average increase in egg production of moulted over unmoulted birds was 19.2% in local strains (P<0.01) and 14.3% in imported strains (P<0.01; strain difference P<0.01). The proportion of downgraded eggs was reduced (P<0.05) but egg weight was unaffected by moulting. In the moulted treatments, diet P1 was associated with higher feed intake (P<0.01), poorer feed conversion (P<0.05) and (in imported strains only) lower egg weight (P<0.05) than other diets, and tended to be associated with higher rates of lay. P2 birds of all strains tended to lay heavier eggs than P1 birds (2.1g difference in Hyline Brown birds, P<0.01). P3 imported strains laid 5% more eggs (P<0.05) and produced 6% more total egg mass (P<0.05) than P2 imported strains. These results suggest that in the second laying cycle dietary LA concentration affected egg weight in all four strains while protein concentration affected egg number in imported strains only. A high-protein post-moult recovery diet improved early performance but was of little benefit in the longer term.

Leary, A.M., Roberts, J.R. and Ball, W. (1998). Proc. Aust. Poult. Sci. Symp. Ed. R.A.E. Pym, **10**: 94-97.

Mannion, P.F., Neill, A.R. and Brewster, M. (1992). Aust. J. Agric. Res., **43**: 389-397.

Morris, T.R. and Gous, R.M. (1988). Br. Poult. Sci., **29**: 93-99.

Robinson, D. and Datugan M.J. (1998). Proc. Aust. Poult. Sci. Symp. Ed. R.A.E. Pym, **10**: 197.

Queensland Poultry Research and Development Centre, PO Box 372, Cleveland, Qld 4163.

VALUE OF DUCKWEED (*Lemna* sp) AS A FEED SUPPLEMENT FOR LAYING HENS

S.C. SLIPPERS, SARAH HUGHES-GAMES and J.A. FOLI

Duckweeds are considered valuable feed supplements for poultry (Leng *et al.*, 1995). Hence, duckweed (*Lemna gibba*) was investigated as a partial substitute for layer mash (160 g crude protein/kg) in the diet of laying hens. Lohman Brown pullets (20 weeks old) were set randomly in a battery cage situated over a fish pond, without provision of artificial light. Birds in two tiers (n = 40/tier) were used as controls and fed layer mash (100 g/day). A like number of birds received fresh duckweed (average 25 g/day; range 10 to 40 g/day; equally divided between morning and afternoon feeds, on weekdays only) and layer mash (average 97.5 g/day: range 95 to 100 g/day). Birds in both treatments received once-weekly a pulse dose of calcitic grit supplement (3 g/week). Shell thickness (by micrometer) and yolk colour intensity (scored visually with the new Roche colour fan, El Boushy and Raterink, 1992) were recorded on a limited number of eggs sampled randomly from every tier (n = 24/treatment; three eggs/tier/week during weeks 49, 52, 53 and 55). Body weight and shell thickness data were statistically analysed by one-way ANOVA, while egg counts and yolk colour score data were analysed by chi-square without *a priori* hypothesis (Schefler, 1980). Duckweed feeding had no significant effect on body weight, production of damaged eggs and shell thickness (P > 0.05), but significantly improved total egg production and yolk colour intensity (P < 0.05), at lower cost due to a saving on use of layer mash. The improved yolk colour intensity has potential for further exploitation in quality-conscious market niches. These promising results beg further investigation of the value of duckweed as a feed for laying hens.

Shown in the table are performance results over 42 weeks (from 20 to 62 weeks of age)

Parameter		Control	Duckweed	SD
Mean body weight at 20 weeks	(g)	1 451[a]	1 453[a]	121
Mean body weight at 62 weeks	(g)	1 790[a]	1 754[a]	172
Total egg production	(n)	18 579[a]	19 177[b]	
Total sound : damaged egg production	(n:n)	18 477 : 102[a]	19 061 : 116[a]	
Mean weight of sound-shelled eggs	(g)	58.6	58.0	
Mean egg shell thickness	(micron)	589[a]	584[a]	44

Yolk colour score distribution was as follows:

Roche colour fan number →		7/8	8/9	9/10	10/11	11/12
Control[x]	(%)	92	8			
Duckweed[y]	(%)	25	17	21	29	8

[a, b] Values in the same row with unlike superscripts differ significantly (P < 0.05)
[x, y] Distribution of yolk colour intensity differ significantly (P < 0.05) between treatments.

El Boushy, A.R. and Raterink, R. (1992). *Wld. Rev. Anim. Prod.*, **27** (1): 50.
Leng, R.A., Stambolie, J.H. and Bell, R. (1995). *Liv.Res. Rur. Development*, **7** (1): 13 pp.
Schefler, W.C. (1980). Statistics for the Biological Sciences, 2nd ed. Addison-Wesley, Reading.

Department of Agriculture, University of Zululand, P/ Bag X1001, Kwadlangezwa 3886, RSA.

ECONOMICS OF DUCKWEED (*Lemna* sp) AS A FEED SUPPLEMENT FOR LAYERS

TEBEGO MAGOLEGO, S.C. SLIPPERS, SARAH HUGHES-GAMES and J.A. FOLI

It was reported at this meeting that duckweed feeding significantly increased egg production and yolk colour intensity (Slippers *et al.*, 1999). Both traits are of economic importance to egg producers and to consumers. Consumer preference for yolk colour intensity varies from market to market. In Sweden, pale egg yolks are acceptable, whilst in Germany more intense yolk colour (minimum 13 on the Roche colour fan) is desired, which is also the preferred colour intensity for industrial use of eggs (El Boushy and Raterink, 1992). Here we have used the performance results reported by Slippers *et al.* (1999) for economic comparison of the effects of duckweed feeding of layers, when eggs so produced are marketed either as a commodity or as a speciality product (high yolk colour intensity).

Production results and margin over feed cost of duckweed-produced eggs marketed as commodity or speciality products are given in the table.

Parameter		Control	Duckweed	Duckweed
Total number of sound-shelled eggs	(n)	18 477	19 061	19 061
Total consumption of layer mash	(kg)	2 361	2 306	2 306
Marketing approach		Commodity	Commodity	Speciality
Total income from egg sales[1]	(ZAR)[4]	6 251.4	6 448.9	7 100.2
Total cost of commercial feed[2]	(ZAR)	3 196.1	3 121.6	3 121.6
Total cost of duckweed feeding[3]	(ZAR)	0.0	574.9	574.9
Total margin over feed cost	(ZAR)	3 055.3	2 752.5	3 403.7
Margin over feed cost per hen housed	(ZAR)	38.2	34.4	42.6

[1]Producer price of eggs assumed as ZAR 4.06/dozen for commodity sales plus a 10% price premium for speciality sales.
[2]Retail price of layer mash assumed to be ZAR 1.35/kg.
[3]Labour requirement for duckweed feeding assumed to be 0.5 hour/day for 210 days at a cost of ZAR 5.48/hour.
[4]South African rand.

The extra labour cost associated with duckweed feeding negated the income advantage of the higher egg production and reduced feed bill, when the eggs are sold as commodity products. By selling duckweed-produced eggs as speciality products, at a 10% price premium, the tables are turned in favour of duckweed supplementation.

El Boushy, A.R. and Raterink, R. (1992). *Wld. Rev. Anim. Prod.*, 27:50.
Slippers, S.C., Hughes-Games, Sarah and Foli, J.A. (1999). *Proc. Aust. Poul. Sci. Symp.*, (in press).

Department of Agriculture, University of Zululand, Kwadlangezwa, South Africa.

DETERMINATION OF APPARENT METABOLIZABLE ENERGY CONTENT OF BOVINE RUMEN CONTENT BY RAPID ASSAY WITH MATURE MUSCOVY DUCKS

Q.E. NYOKA[1], S.C. SLIPPERS[1] and J.E.J. DU TOIT [2]

The rapid assay procedure for determining metabolizable energy of poultry diets (Farrell, 1978) was applied in a study of rumen contents as a potentially useful poultry feed supplement in cash-strapped smallholder farm systems of Kwazulu-Natal (KZN) Province. Muscovy ducks, after chickens, are the most abundant poultry species kept by smallholder households in KZN. Muscovies of both sexes (36 week-old) were equally divided within treatments and used as experimental models. Dietary treatments consisted of a standard mixture of yellow maize meal and commercial broiler finisher mash, fed *ad libitum* (control); the standard mixture offered at 60% of the pre-determined *ad libitum* intake (restricted control); or the standard feed at restricted control level plus one of the three forms of freshly-collected rumen contents, offered *ad libitum* (i.e. fluid, solid or unfractioned material). The dry matter content of the rumen content fractions were (g/kg), 20 (fluid), 213 (solid) and 143 (unfractioned).

Parameter	Feed Portion	Dietary Treatments					Prob. of F[1]	SEM
		Control	Restricted control	Fluid fraction	Solid fraction	Unfrac-tioned		
Replicates (n)		6	8	5	6	6		
DMI (g)	Standard feed	221.29[a]	106.67[b]	87.92[b]	99.53[b]	102.45[b]	**	9.83
	Rumen content			3.81[a]	36.20[b]	56.61[c]	**	5.55
	Total feed	221.29[a]	106.67[b]	91.73[b]	135.73[bc]	159.06[c]	**	9.34
AME (kJ/g)	Standard feed	15.05	15.00	15.02	15.02	15.02	n.s.	0.05
	Rumen content			18.80	9.16	12.87	n.s.	1.76
	Total feed	15.05[a]	15.00[a]	14.98[ac]	13.41[b]	14.23[c]	**	0.14
AME$_n$ (kJ/g)	Standard feed	14.63	14.54	14.58	14.58	14.58	n.s.	0.04
	Rumen content			18.40[a]	8.95[b]	12.46[ab]	*	1.53
	Total feed	14.63[a]	14.54[a]	14.51[ac]	13.03[b]	13.80[c]	**	0.13

[1] ** = (P < 0.01); * = (P < 0.05); n.s. = (P > 0.05).

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

Dry matter intake (DMI) and apparent metabolizable energy (AME and AME$_n$) are shown in the table. AME and AME$_n$ values of the control and restricted control were similar (P > 0.05). Hence, their pooled means were applied to the standard feed component of rumen content treatments, to determine the AME and AME$_n$ contents of rumen content fractions by difference. These values differed significantly between the different rumen content fractions (P < 0.01), being highest for the fluid and lowest for the solid material. However, the AME and AME$_n$ values of the fluid fraction are probably unreliable, due to the very small dry matter intake, coupled with high variability (within-group SEM of 5.33 and 4.30 respectively). Fresh rumen contents appear to be of limited value as an energy-supplying feed supplement for muscovy ducks.

Farrell, D.J. (1978). *Br. Poult. Sci.*, **19**: 303.

[1]Department of Agriculture, University of Zululand, Kwadlangezwa, South Africa.
[2]Department of Animal Science, University of the Free State, Bloemfontein, South Africa.

EFFECTS OF VARYING DIETARY ENERGY AND LYSINE ON GROWTH, FEED EFFICIENCY AND CARCASS FAT CONTENT OF WHITE PEKIN DUCKS

C.W. SELL and R.G. PACKHAM

Variation in the carcass fat content of White Pekin ducks has been associated with dietary regimes, genetic strain, processing techniques and rearing conditions (Walters *et al.*, 1994).

Day-old-ducklings of mixed sexes were fed a commercial starter diet for two weeks. Following this, two energy levels (12.97 MJ ME/kg and 11.72 MJ ME/kg) and three dietary lysine levels (7.0, 8.0 and 9.0 g/kg) were offered to 240 White Pekin ducks divided into four replicates, over a four week period. Birds were killed at 6 weeks of age and representative minced samples were analysed for total carcass fat.

Source	Growth 15-42d (g gain / bird)	FCR 15-42d (g feed / g gain)	Carcass fat (%)
Energy-High	2455.9[a1]	2.64[a]	35.5
Energy-Low	2422.2[b]	2.89[b]	35.4
SEM	16.4	0.045	
Lysine-High	2435.9	2.73	35.4
Lysine-Medium	2465.1	2.76	35.6
Lysine-Low	2425.9	2.82	35.4
SEM	20.1	0.055	
Sex - Male	2514.4[a]		35.4
Sex - Female	2386.7[b]		35.5
SEM	16.4		

[1]Superscripts (a-b) indicate that means differ significantly ($P<0.05$).

The results indicate that growth and feed conversion ratio (FCR) of White Pekin ducks were significantly ($P<0.05$) affected by dietary energy concentration, and that sex of bird influences growth (separate feed intakes could not be measured). Carcass fat was not influenced significantly by the dietary lysine inclusions. Lysine levels had no significant effect on growth, feed conversion or fat content, although a trend appeared to suggest that the lysine to arginine ratio in duck is important for growth. This needs further exploration.

The experiment investigated the effects of varying nutrient density of diets to obtain a response in carcass fat. There were no significant differences ($P<0.05$) between either male or female birds, between energy levels or between dietary treatments of lysine in reducing carcass fat.

Walters, B. S., Maurer, A. J., Rodgers, G. E. and Kohl, H. (1994). *Poult. Sci.*, **73**: 322-325.

Faculty of Environmental Management and Agriculture, University of Western Sydney-Hawkesbury, Richmond, NSW 2753.

FORMULATING DIETS FOR LAYING HENS WITHOUT A VITAMIN AND MINERAL PREMIX GIVES LESS NUTRIENT EXCESSES

J.G. DINGLE[1] and Y.L. HENUK[1,2]

It is now routine to add premixes at levels recommended by the premix manufacturer so that all of the bird's requirements will be met by the premix, ignoring the nutrients already in the feed. This has led to more expensive feed (Waldroup, 1998) and pollution of the environment. Organic poultry farming discourages the addition of supplements to feeds. In developing countries, supplementation with vitamin and mineral premixes may be expensive or unavailable. Diets formulated without a vitamin and mineral premix may be nutrient deficient (McDonald, 1996); however any saving on excess vitamins and minerals in a ration would be advantageous. To examine the level of excess nutrients with a normal premix, and whether it would be possible to supply the vitamin and mineral requirements of laying hens without a premix supplement, two layer diets were formulated to meet NRC (1994) requirements either with or without a standard vitamin and mineral premix (Rabar Pty Ltd., Beaudesert, Qld. 4286 Australia).

Ingredient (g/kg)	+ Premix	− Premix	Nutrient	NRC (1994)	+ Premix	− Premix
Sorghum	672.2	675.0	Vitamin A (IU/kg)	3,000	80,000	10,280
Millrun	101.9	19.7	Vitamin D_3 (IU/kg)	300	300	300
Rapeseed meal	7.8	45.9	Vitamin E (IU/kg)	5.0	14.1	9.6
Meat & bone meal (450 g CP)		32.9	Vitamin K_1 mg/kg)	0.5	0.5	0.5
Meat & bone meal (520 g CP)	11.4	30.5	Vitamin B_1 (mg/kg)	0.7	4.0	3.1
Lucerne meal	20.2	50.5	Vitamin B_2 (mg/kg)	2.5	5.4	2.5
Fish meal (70 g CP)	108.1	82.8	Vitamin B_3 (mg/kg)	2.0	21.7	5.4
Fish oil		0.3	Vitamin B_6 (mg/kg)	2.5	6.1	3.2
Maize oil	0.8	3.3	Vitamin B_{12} (mg/kg)	0.004	0.059	0.068
Limestone	75.7	60.2	Biotin (mg/kg)	0.1	0.1	0.2
Premix	2.0		Niacin (mg/kg)	10.0	41.5	41.0
Total	1000.1	1001.1	Folacin (mg/kg)	0.25	0.25	0.20
Feed cost (A$)	216.10	210.77	Choline (mg/kg)	1,050	1,039	1,166
			Manganese	20.0	125.6	38.0
			Zinc (mg/kg)	35.0	82.6	37.6
			Iron (mg/kg)	45.0	369.8	332.0
			Selenium (mg/kg)	0.06	0.12	0.07
			Iodine (mg/kg)	0.035	0.057	0.090

It was possible to formulate a complete layer diet without a vitamin and mineral premix. The unsupplemented diet contained fewer nutrients in excess requirement, and the levels of excess were lower than in the supplemented diet. The supplemented diet was dearer and contained over 20 times the required amount of some nutrients, thus potentially being more damaging to the environment.

McDonald, M. (1996). *Australasian Poul.,* **7** (2): 10-11.
NRC (1994). Nutrient Requirements of Poultry 9th edn. National Academy Press, Washington, D.C.
Waldroup, P. (1998). *Asian Poultry Magazine*, July/August, 32 - 34.

[1]School of Veterinary Science and Animal Production, The University of Queensland, Gatton College, Qld. 4345 Australia.
[2]Faculty of Agriculture, The University of Nusa Cendana, Kupang, N.T.T. 85361 Indonesia.