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CONTENTS

THE ROLE OF NON-STARCH POLYSACCHARIDES IN POULTRY NUTRITION

M.CHOCT

Summary

The non-starch polysaccharides (NSP) of cereals can exhibit anti-nutritive activity when present in poultry diets. The high levels of pentosans in rye and ß-glucans in barley are responsible for the poor nutritive values of these cereals in broiler diets. When pentosans isolated from rye or wheat are added to broiler diets severe depressions in nutrient digestion and growth occur. The depressions are dose-dependent. Several treatments have been shown to be effective for improving the nutritive value of cereals. These include water treatment, enzyme supplementation and dietary addition of antibiotics.

I. INTRODUCTION

The cell wall of cereals is comprised primarily of complex carbohydrates which are loosely termed non-starch polysaccharides (NSP). Originally NSP were considered to make minor contributions to the nutrition of chickens and other monogastric species through limited fermentation in the lower bowel. In recent years, however, considerable evidence has been gathered indicating that cereal NSP possess anti-nutritive activity when present even at low levels (<50g/kg) in broiler diets.

To date most studies have investigated the anti-nutritive activities of the NSP from barley and rye. In barley (1-3),(1-4)-ß-glucan (30-60g/kg dry matter; Fincher and Stone 1986) causes growth depression in broilers accompanied by sticky droppings. The addition of ß-glucanases to barley-based diets ameliorates the growth depression (Gohl et al. 1978; Hesselman and Aman 1986; Classen et al. 1988) and allows barley to be used at higher levels of inclusion in broiler diets.

Rye contains high levels of arabinoxylans (approximately 100g/kg dry matter; Antoniou et al. 1981). The anti-nutritive activity of these polysaccharides was demonstrated by Marquardt and co-workers (Antoniou and Marquardt 1981; Ward and Marquardt 1987) who showed that growth of broilers was depressed when pentosans isolated from rye were added to experimental diets. Supplementation of the diets with enzymes exhibiting pentosanase activity improved the nutritional value of rye-based diets (Pettersson and Aman 1988).

Mollah et al. (1983) and Rogel et al. (1987b) reported that some Australian wheats have a low apparent metabolisable energy (AME; <13 MJ/kg dry matter) when included in poultry diets. Wet and sticky droppings were observed when broilers were fed these diets, as reported in birds fed rye (Halpin *et al.* 1936). Pentosans are also present in wheats at appreciable levels (50-80g/kg dry matter; Annison 1990a). The pentosans of wheat are similar to those of rye consisting of a (1-4)-ß-xylan chain with arabinose substituted at the O2 and O3 positions of the xylose (Fincher and Stone 1986). Choct and Annison (1990) showed that when isolated wheat pentosans were added to a sorghum-based diet containing low levels of NSP, the AME of the sorghum was depressed to an extent greater than accounted for by nutrient dilution. In addition, liveweight gain was also depressed. This paper reviews the anti-nutritive effects of cereal NSP, especially those of pentosans, in poultry diets and the various treatments available for improving the nutritive value of cereals.

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II. ANTI-NUTRITIVE ACTIVITY OF PENTOSANS

Pentosans have been isolated from several cereals including rye (Preece and MacKenzie 1952; Saini and Henry 1989), wheat (Perlin 1951; Fincher and Stone 1974; MacArthur and D'Appolonia 1980; Choct and Annison 1990), barley (Preece and MacKenzie 1952), oats (Preece and MacKenzie 1952; MacArthur and D'Appolonia 1980) and triticale (Saini and Henry 1989). Nutritional studies, however, have only been carried out with pentosans from rye (Antoniou *et al.* 1981; Fengler and Marquardt 1988b) and from wheat (Choct and Annison 1990).

(a) Rye pentosans

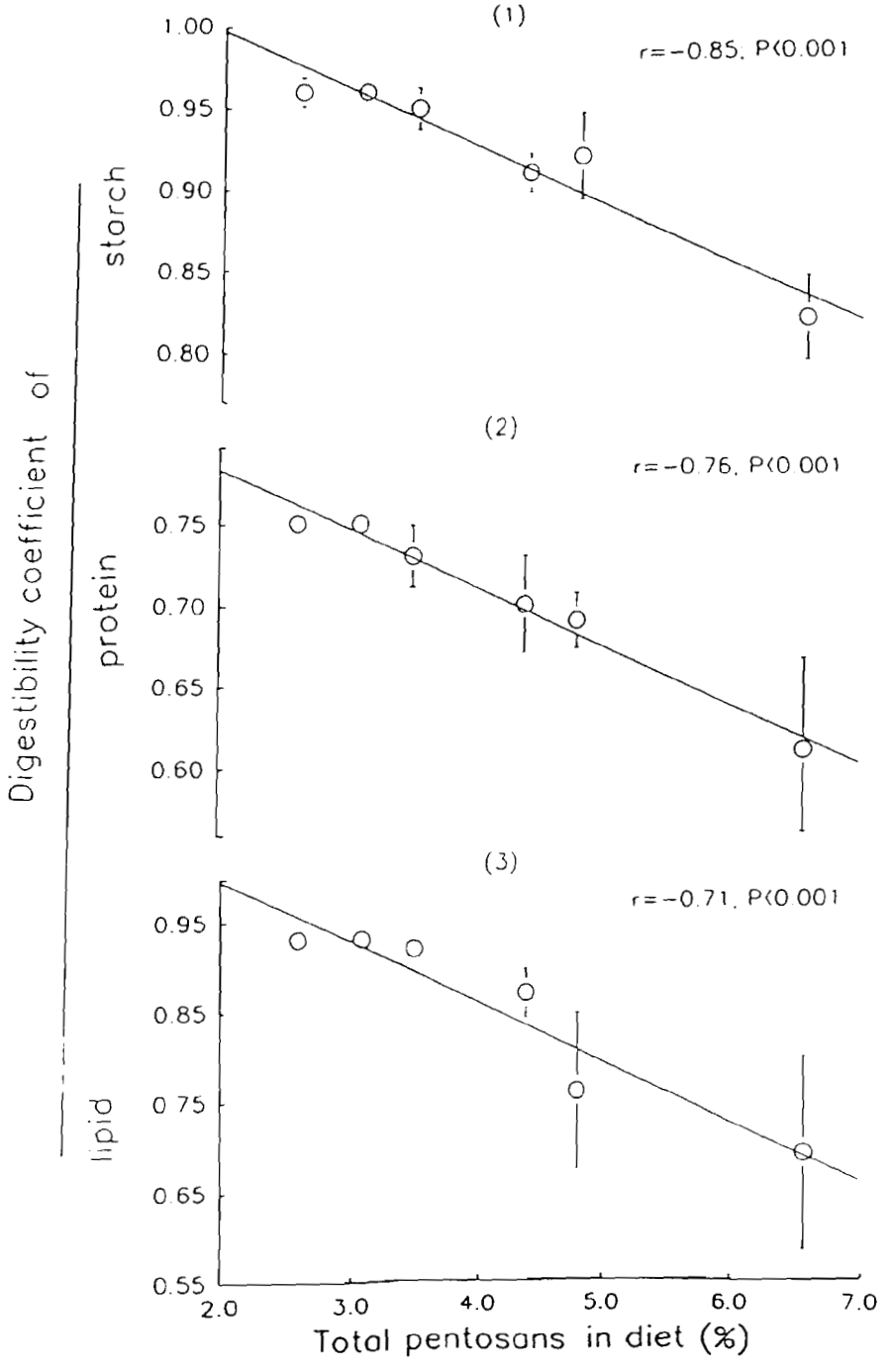
The nutrient content of rye grain is similar to that of other cereals (Jenssen *et al.* 1979). However, it is not widely used in poultry diets because of its high pentosan content. When pentosans isolated from rye were added to experimental diets significant depressions occurred in chick growth, feed utilization, and the digestibilities of fat and amino acids (Antoniou *et al.* 1981; Antoniou and Marquardt 1981). Subsequent work (Fengler and Marquardt 1988a) confirmed that the pentosans are the anti-nutritive factor in rye and suggested that the water-soluble pentosans are primarily responsible for the anti-nutritive activity.

(b) Wheat pentosans

Wheats grown in Australia have shown a significant variation in their metabolisable energy with values as low as 11 MJ/kg DM with a starch digestibility of only 80% when included in broiler diets (Mollah *et al.* 1983). To assess if the pentosans are responsible for these low-ME wheats Choct and Annison (1990) isolated two fractions of wheat pentosans, a water-extractable fraction and an alkali-extractable fraction, and incorporated them into a sorghum-based trial diet. At a level of inclusion equivalent to 30g arabinoxylan/kg the growth of broilers and their feed conversion efficiency were severely affected. Also, the AME of sorghum, the digestibility of starch and the retention of nitrogen were significantly depressed. Recently, a more detailed study was conducted (Choct and Annison unpublished data) to demonstrate the anti-nutritive effect of isolated wheat pentosans in broiler diets. When both the water-extractable and alkali-extractable fractions were added above levels equivalent to 20g arabinoxylans/kg to a commercial-type diet the AME of the diet was significantly depressed. At a level of inclusion of the alkali-extractable pentosans equivalent to 40g arabinoxylans/kg the AME of the diet was decreased from 15.05 to 12.48 MJ/kg DM and the ileal digestibilities of starch, protein and lipid were reduced from 96% to 82%, 75% to 61% and 93% to 69%, respectively. The data are shown in Figure 1. These effects were accompanied by a significant decrease in the weight gain of the broilers. At the highest level of inclusion (40g arabinoxylans/kg) of the alkali-extractable pentosans the feed intake was also affected. The severe growth depression, however, was not solely the result of the slight decrease in feed intake. There was a dramatic increase (from 1.91 to 2.70) in feed conversion ratio and the birds showed considerable gastrointestinal stress, producing copious amounts of watery excreta. It was noted that the birds appeared dull and less responsive to the environment, which suggests that the wheat pentosans not only inhibited nutrient uptake but also, directly or indirectly, caused malaise.

Wheat pentosans cause a general inhibition of nutrient digestion affecting starch, fat and protein, which indicates that they act in the same manner as the anti-nutritive NSP of rye and barley. Choct and Annison (1990)

Figure 1. The relationship between the total pentosans in diet and the ileal digestibilities of starch (1), Protein (2) and lipid (3).



showed that the pentosan content of a range of cereals was closely related to their ME values. In their more recent study, the pentosans in the diets were highly correlated ($P<0.001$) with the digestibilities of starch ($r=-0.85$), protein ($r=-0.76$) and lipid ($r=-0.71$) regardless of the type of pentosans added. This indicates that the water-extractable and the alkali-extractable fractions were very similar in their anti-nutritive activities and, probably, in their structures. Mares and Stone (1973) suggested that alkaline extraction cleaves the ester bonds which may link some of the arabinoxylans to other cell wall constituents thereby rendering them water soluble. The alkali-extractable fraction used by Choct and Annison (1990 unpublished) was almost totally soluble in water at 80°C.

The mechanism by which the pentosans exhibit an anti-nutritive effect is unclear. One explanation is that the highly viscous nature of these polysaccharides reduces the digesta passage time and impairs diffusion of digestive enzymes to their substrates and mixing with gut contents (Antoniou *et al.* 1981; Antoniou and Marquardt 1982). Viscous polysaccharides, such as pentosans and ß-glucans might also complex with digestive enzymes and reduce their activity (Ikeda and Kusano 1983). There is ample evidence that viscous polysaccharides cause physiological and morphological changes in the digestive system of rats, pigs and humans. It is apparent that the endogenous secretion of water, proteins, electrolytes and lipids can be increased markedly by NSP supplementation of the diet (Low 1989). The metabolic cost of such processes can be considerable. It has also been demonstrated that highly viscous carbohydrates decrease the accessibility of nutrients to the mucosal surface of rat intestine (Kelsay *et al.* 1978; Jenkins *et al.* 1978). There is evidence that the anti-nutritive effect of NSP is mediated by the gut microflora as dietary supplementation with antibiotics partially improves the nutritive value of rye (MacAuliffe and McGinnis 1971). This topic will be discussed later in this review. Other polysaccharides such as guar gum, pectin (Anderson and Warnick 1964; Vohra and Kratzer 1964; Patel *et al.* 1981; Grammer *et al.* 1982), xanthan gum and locust bean gum (Annison 1990b) also elicit negative effects in poultry diets.

Although the effects of these NSP are considered to be detrimental to poultry they are regarded as highly beneficial in the diets of humans because, not only do they substantially reduce nutrient absorption, especially fat and cholesterol, but they also alter the rate of glucose uptake (Jenkins *et al.* 1978) and enhance volatile fatty acid production (Kay and Strasberg 1978) in the large intestine. The net effect may be an increased tolerance to insulin deficiency and a reduced incidence of cancer and atherosclerosis (Vahouny and Kritchevsky 1982).

III. IMPROVEMENT OF THE NUTRITIVE VALUE OF CEREALS

The feeding value of nutritionally inferior cereals such as rye and barley can be substantially improved if the anti-nutritive components are eliminated or their unfavorable effects on nutrient absorption are altered. Several methods have been developed over the years to improve the nutritive value of cereals, especially those of rye and barley. These include (a) water treatment; (b) enzyme supplementation, and (c) antibiotic supplementation.

(a) Water treatment

Improvement of the nutritive value of barley, corn and wheat by a simple water-treatment was reported three decades ago (Fry *et al.* 1958; Lepkovsky and Furuta 1960). Water extraction of rye improved significantly the growth and feed utilization of chicks compared to untreated rye (Fernandez *et al.* 1973) and increased the retention of protein and the digestibilities of amino acids and fat (Antoniou and Marquardt 1982).

The positive effect of water treatment is well established and it is most likely the results of the removal of the water-soluble non-starch polysaccharides (pentosans and ß-glucans) and the activation of endogenous enzymes capable of degrading these polysaccharides. The degree of improvement is obviously dependent on the concentration of the water-soluble non-starch polysaccharides in the cereal. Thus, Adams and Naber (1969a,b) reported that the response to water treatment was consistently high for barley and wheat, and only occasional responses were seen with corn. This is because barley and wheat contain higher levels of NSP than corn or maize (Choct and Annison 1990).

Although water treatment substantially improves the nutritive value of rye and barley the performance of chicks on rye diets was still inferior to that of chicks fed comparable wheat-based diets (Antoniou and Marquardt 1982), indicating that at least part of the water-insoluble pentosans are solubilised in the gut and elicit anti-nutritive effects in a similar manner to the water-soluble pentosans (Antoniou *et al.* 1981).

(b) Enzyme supplementation

Enzyme-induced improvement of poultry diets is well documented and the subject has been comprehensively reviewed by several workers (Chesson 1987; Classen and Campbell 1990). Most research in this area has concentrated on improving the nutritive value of barley (Fry *et al.* 1958; Gohl *et al.* 1978; Classen *et al.* 1988). Barley contains a high level of mixed linked (1-3),(1-4)-ß-glucans (Henry 1985; Englyst 1989; Choct and Annison 1990), and therefore it is believed that the majority of the enzyme-induced improvement in the feeding value of barley is from the endo-ß-glucanase activity (Ricks *et al.* 1962). However, activities of other enzymes are also likely to be involved in the extensive improvement in the nutritive value of barley. Marked improvement in the nutritive value of rye-wheat based diets with supplementation of enzymes having pentosanase and ß-glucanase activities has been reported (Pettersson and Aman 1988, 1989). The dietary supplementation of enzyme preparations capable of degrading NSP also improves the nutritive value of oats (Campbell *et al.* 1986b; Pettersson *et al.* 1987; Edney *et al.* 1989), wheat (Classen and Campbell 1990), triticale (Pettersson and Aman 1988), and corn (Suga *et al.* 1978).

It is generally conceded that the improvement in performance in relation to enzyme supplementation is not due to complete hydrolysis of the polysaccharides and subsequent absorption of the released sugars (White *et al.* 1983; Campbell *et al.* 1986a; Chesson 1987). Instead, the enzymes breakdown the pentosans and the ß-glucans into smaller polymers (De Silva *et al.* 1983) and, therefore, alter the ability of these polysaccharides to form highly viscous solutions which inhibit nutrient diffusion and transport. Even if the pentosans and the ß-glucans are completely hydrolysed by the enzymes, efficient utilization of the released monosaccharides is doubtful, since the pentoses themselves are also poorly digested in poultry (Baker 1977; Longstaff *et al.* 1988).

Further research is required to elucidate the mechanisms of enzyme-substrate interactions and the fate of the resulting products in the digestive tract of chickens.

(c) Antibiotic supplementation

There is ample evidence that the anti-nutritive activity of NSP in poultry diets is related to the gut microflora of the chicken. The addition of procaine penicillin to rye-based diets resulted in marked increases in chick growth, in the efficiency of feed utilization (Moran *et al.* 1969; MacAuliffe and McGinnis 1971), as well as in feed intake and in the retention of all nutrients (Misir and

Marquardt 1978a,b). The response in performance to antibiotic supplementation may depend on the composition of the diets, notably the quantity and quality of the dietary protein (Misir and Marquardt 1978b). Also, responses were always greater in rye than in wheat, indicating that the antibiotic-induced improvement is related to the NSP content in the cereal.

The mechanism of action is not clearly defined. However, it has been hypothesized that the improvement may be the result of inhibition and suppression of intestinal microflora which competes with the host for available dietary nutrients (Misir and Marquardt 1978a,b).

The hind-gut microflora of chickens is predominantly strict anaerobes (Salanitro *et al.* 1978). A significant increase in intestinal anaerobes was observed when rye rather than corn was fed to chicks and the growth depression of the chicks were associated with a fermentative, spore-forming organism which produced large amounts of gas and butyric acid in the gut (Wagner and Thomas 1978). These authors suggested that the organism may belong to the genus *Clostridium*. Conjugation of bile salts (Campbell *et al.* 1983a; Feighner and Dashkevicsz 1988) and the production of toxins are also possible effects of deleterious microorganisms. Some clostridia are toxigenic anaerobes and might depress the growth of chicks by elaborating toxins within the intestine. It may be that when diets with high NSP (pentosans and ß-glucans) are ingested a portion of these polysaccharides is solublised in the upper gut of chickens while another portion moves down to the lower gut where it becomes a fermentable carbohydrate source for the hind-gut anaerobes to proliferate in a manner detrimental to the bird. The elimination of these deleterious microorganisms would alleviate the anti-nutritive effect of the NSP, improving the well-being of the chickens, and enhancing the utilization of available nutrients. Experiments have shown that an addition of 150mg penicillin per kg of diet containing 82% rye and 13.4% casein improved the growth of 5-week old broilers by 75% and feed intake by 33%. However, no difference was found in the AME, due to an elevated level of excreta output which increased by 57% with the antibiotic-supplemented birds. During the experiment it was noticed that the excreta of the chickens on the antibiotic-supplemented rye diet looked less watery and the chickens appeared more active (Choct and Annison unpublished data).

(d) Other treatments related to the improvement of the nutritive value of poultry diets

In addition to the treatments discussed above other attempts have also been made to improve the nutritive value of poultry diets. Gamma irradiation of rye has been reported to improve its feeding value considerably (MacAullife *et al.* 1979; Patel *et al.* 1980). This is due to the ability of irradiation to degrade the pentosans with subsequent reduction in the digesta viscosity (Campbell *et al.* 1983b). Treating cereals with acid has also been reported to be effective (Adams and Naber 1969b). Autoclaving (Antoniou and Marquardt 1982) and heat treatment (Vohra and Kratzer 1964; Patel *et al.* 1980; Verma and McNab 1982) are usually ineffective for two reasons. Firstly they fail to degrade the polysaccharides and secondly they inactivate endogenous enzymes (Antoniou and Marquardt 1982). Supplementation of fibrous materials, such as oat hulls, has been reported to improve the starch digestibility of low-ME wheats (Mollah and Annison 1981) and the digestibility of raw potato starch (Rogel *et al.* 1987a) which is normally poorly digested by chickens (Nitsan and Bartov 1972). Materials with similar texture to oat hulls, such as wheat bran and hemicelluloses isolated from oat hulls, were ineffective. Also, fine grinding of oat hulls rendered them inactive (Rogel 1985). The specific action of oat hulls is not established. Addition of NaCl to rye diets significantly improved the growth rate and feed utilization of chickens (Lee and Campbell 1983).

IV. CONCLUSION

Wheat pentosans, when included in broiler diets, exhibit anti-nutritive activity similar to that of the non-starch polysaccharides in rye and barley. The effect is manifested by depressed digestibilty of starch, protein and lipid. The net effect is a reduced growth rate and increased health problems of the chickens. Various treatments including water treatment, enzyme supplementation and antibiotic addition have proven effective for improving the nutritive value of cereals, especially the nutritionally inferior cereals. This indicates that multiple mechanisms are involved in the antinutritve activity of NSP in poultry diets. Further research is needed to elucidate the exact mechanisms of action of NSP to establish an economic and practical method of treatment.

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INTERRELATIONSHIPS BETWEEN DIETARY AMINO ACID AND ENERGY INTAKE ON PROTEIN DEPOSITION AND PERFORMANCE OF MALE BROILER CHICKENS

R.J. JOHNSON* AND R.G. CAMPBELL**

Summary

The interrelationships between dietary protein and energy intake were studied in male broiler chickens. A diet dilution technique was used to vary total lysine level, and energy intake was altered by feed restriction. Separate protein and energy dependent phases for protein deposition were demonstrated between 40 and 750 g liveweight. However maximal protein deposition in the growth phase from 750 to 2000 g was independent of energy intake. The results should enable the tissue requirement of the broiler chicken for protein deposition to be more accurately established and could form the basis for predicting the consequences of changes in protein and for energy intake on growth and carcass composition.

I. INTRODUCTION

Liveweight rather than age is a major factor determining the protein growth capacity of broilers and hence their dietary amino acid requirements. Protein deposition capacity is also influenced by energy intake and by intrinsic factors such as sex and genotype. Because all of these factors influence protein deposition capacity and the partition of energy between protein and fat, estimates of dietary amino acid requirements based solely on growth performance tend to be applicable only to the conditions under which they are established they are of limited value for the development of biological models designed to predict nutrient requirements and the consequences of change in nutrient intake on growth performance, carcass composition and economic returns.

In contrast, knowledge of amino acid availability, the capacity for protein accretion and the extent it is affected by nutrient intake enables the tissue and dietary requirements of broilers to be interrelated and for diets to be formulated to support predetermined performance levels or maximal protein accretion at minimal cost. Knowledge of the interrelationships between protein and energy intake on protein and fat deposition is critical to understanding the effects of change in the intake of either or both nutrients on growth and carcass composition. The present experiment was therefore conducted to assess the effects of seven levels of dietary protein and two levels of energy intake on rates of deposition of protein and fat in commercial male broilers growing between 40 and 750 g and 750 and 2000 g liveweight.

II. MATERIALS AND METHODS

Five hundred and fourteen male broiler chickens were allocated at day old among 14 treatments in a 2 x 7 factorial. The respective factors were feeding level (ad libitum and 0.8 ad libitum) and dietary protein (seven levels ranging from 150 to 315 g/kg). Each feeding level x protein treatment consisted of three replicates each of 12 chickens housed in raised-wire cages. Representative birds from each treatment were slaughtered when liveweight reached 750 and 2000 g. An initial group of 10 chickens were

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slaughtered at day-old at an average liveweight of approximately 40 g.

Carcass composition was determined after defeathering and protein and fat deposition rates were calculated for the liveweight periods from 40 g to 750 g and from 750 to 2000 g.

Table 1. Determined nutrient contents (g/kg) of summit and dilution diets.

	Diet	
	Summit	Dilution
Dry matter (g)	874	878
Crude protein (g)	315	136
Apparent ME (MJ)*	12.7	14.0
Total lysine (g)	18.6	04.7
Lysine: AME (g/MJ)	1.46	0.33
Methionine (g)	11.7	2.3
Threonine (g)	14.1	5.4
Aspartic acid (g)	32.0	10.7
Serine (g)	17.1	7.3
Glutamic acid (g)	58.8	25.6
Proline (g)	24.5	11.3
Glycine (g)	20.2	11.7
Alanine (g)	20.8	9.5
Valine (g)	20.1	7.5
Isoleucine (g)	13.8	5.5
Leucine (g)	31.0	13.5
Tyrosine (g)	11.6	4.4
Plenyalanine (g)	18.5	7.3
Histidine (g)	9.3	2.9
Arginine (g)	20.4	7.7

* Determined apparent metabolizable energy (AME).

The diet dilution method (Fisher and Morris 1970) was used to formulate the diets. Different proportions of a high-protein (summit) diet and a low-protein (dilution) diet were mixed to provide seven experimental diets ranging in protein from 150 to 315 g/kg and in total lysine from 5.8 to 18.6 g/kg. The summit diet had a lysine level of approximately 1.4 times the assumed requirement while all other amino acids were set at 1.8 times assumed requirement. Essential amino acids in the dilution diet were set at 0.4 to 0.5 of the assumed requirement. The procedure ensured that lysine was the first-limiting amino acid in all diets. The determinated apparent metabolizable energy (AME) and amino acid levels of the summit and dilution diets are given in Table 1. The determined nutrient content of the experimental diets mixed from the summit and dilution diets is given in Table 2.

The birds were weighed weekly and the daily feed allowance of birds on the restrictively fed treatment (0.8 ad libitum) was adjusted accordingly using a predetermined scale relating the voluntary feed intake of broilers of the same genotype to liveweight between 40 and 2000 g.

Table 2. Determined nutrient content of experimental diets (/kg).

	Diet						
	1	2	3	4	5	6	7
Apparent ME (MJ)	13.7	13.4	13.3	13.3	13.3	12.9	12.7
Crude protein (g)	150	168	183	201	234	269	315
Total lysine (g)	5.8	7.0	8.1	9.3	11.8	14.3	18.6
Lysine: AME (g/MJ)	0.42	0.52	0.61	0.70	0.88	1.10	1.46

III. RESULTS

(a) Growth performance

The results for growth performance are presented in Table 3. There were marked effects of both dietary lysine content and feeding level on growth performance although the effects of feeding level on growth rate and feed:gain were more pronounced in the period 40 to 750 g than over the heavier liveweight stage (750 to 2000 g).

Between 40 and 750 g liveweight, the growth rate and feed:gain of both restricted and ad libitum birds improved in a curvilinear fashion with increasing dietary lysine up to 14.3 g/kg (1.1 g/MJ AME). However, maximal growth rate was 30% higher for ad libitum birds. Ad libitum birds also exhibited lower feed:gain values on all diets than their restrictively fed counterparts and the minimal feed gain value for ad libitum birds (1.37) was lower than those fed restrictively (1.55).

Between 750 g and 2000 g liveweight growth rate and feed gain of both ad libitum and restricted birds improved with increasing dietary lysine up to 11.8 g/kg (0.9 g/MJ AME) and both measures of growth performance declined with each further increase in dietary lysine. Maximal growth rate differed by only 3.0% between ad libitum and restricted birds and the latter group exhibited a lower feed:gain than their ad libitum counterparts.

(b) Protein and fat deposition rates

Protein and fat deposition rates are given in Table 4. Between 40 and 750 g liveweight, protein deposition increased in a curvilinear fashion in both ad libitum and restricted birds with increasing dietary lysine content up to 14.3 g/kg (1.1 g/MJ AME). Ad libitum birds exhibited higher protein deposition on all diets and maximal protein deposition was 30% higher for the ad libitum birds compared to their restrictively fed counterparts. However, the relationship between protein deposition and protein intake (Figure 1A) showed that when protein intake was below requirement protein growth was linearly related to protein intake and independent of energy intake. In contrast, protein deposition on the two highest protein diets was determined by energy intake and independent of protein intake.

Fat deposition was higher for ad libitum than restricted birds and tended to increase with the first two increases in dietary lysine and gradually decline with further increase in dietary lysine.

Table 3. Effects of dietary lysine and feeding level on the performance of male broiler chickens between 40 and 750 and 750 and 2000 g liveweight.

Diet	Lysine (g/kg)	Feeding Level	Liveweight gain (g/d)		Feed intake (g/)		Feed:gain (g:g)	
			0-750	750-2000	40-750	750-2000	40-750	750-2000
1	5.8	A	15.7	36.9	40.2	128.3	2.47	3.85
		0.8	12.4	32.6	35.9	118.8	2.89	3.68
2	7.0	A	18.8	49.6	45.8	148.2	2.45	2.98
		0.8	15.0	42.9	36.8	123.1	2.45	2.88
3	8.1	A	24.8	61.0	49.9	145.9	2.01	2.39
		0.8	15.5	49.5	36.3	120.0	2.34	2.41
4	9.3	A	27.1	61.0	49.5	136.5	1.83	2.25
		0.8	17.7	57.1	36.6	123.4	2.07	2.17
5	11.8	A	31.6	63.8	46.6	127.0	1.48	1.99
		0.8	20.0	61.9	36.9	121.0	1.86	1.94
6	14.3	A	32.5	51.3	44.6	115.4	1.37	2.26
		0.8	22.9	53.5	35.4	108.7	1.55	2.05
7	18.6	A	31.8	57.2	45.4	118.3	1.43	2.10
		0.8	21.3	52.5	36.4	111.3	1.71	2.13
Averages								
ad libitum		(A)	26.0	54.4	46.0	131.4	1.76	2.55
		0.8A	17.8	50.0	36.3	118.0	2.03	2.46

Between 750 and 2000 g liveweight, protein deposition increased with increasing dietary lysine up to 11.8 g/kg (0.9 g/MJ AME) and declined when dietary lysine was further increased to 14.3 g/kg. Maximal protein deposition was unaffected by feeding level but on the lower lysine diets protein deposition was higher for the ad libitum birds. The relationship between protein deposition and protein intake again indicated that protein deposition was a function of protein intake and independent of energy intake, when dietary protein intake was below tissue requirement (Figure 1B).

IV. DISCUSSION

The results showed that the effects of dietary protein and energy intake on protein and fat deposition were reflected in terms of growth rate and feed gain. Between 40 and 750 g liveweight maximal protein deposition was supported by the diet containing 14.3 g/kg total lysine (1.1 g/MJ AME) which is in line with estimates of dietary lysine requirement reported previously (Gous and Morris 1985; Morris 1989). However, whilst the level of dietary lysine required to support maximal protein deposition and growth performance will vary depending on the digestibility and availability of lysine and other essential amino acids, the tissue requirement for protein and amino acids, which are provided for the genotype used in the present experiment, are independent of dietary protein quality but clearly affected by both liveweight and energy intake. This information enables the tissue and dietary protein (amino acid) requirements of broiler chickens to be interrelated and dietary formulation to be more precise.

Table 4. Effects of dietary lysine and feeding level on the rates of protein and fat deposition in the carcases of male broiler chickens growing between 40 and 750 and 750 and 2000 g liveweight.

Diet	Feeding Level	Protein deposition (g/d)		Fat deposition (g/d)	
		40-750	750-2000	40-750	750-2000
1	A	2.36	5.74	2.58	6.54
	0.8	1.91	5.00	1.21	7.20
2	A	2.81	7.59	2.63	7.12
	0.8	2.27	6.80	1.44	7.61
3	A	3.65	10.21	3.15	7.33
	0.8	2.48	8.20	1.45	5.54
4	A	4.26	10.60	3.42	6.62
	0.8	2.85	9.55	1.43	6.18
5	A	5.01	11.32	3.20	4.32
	0.8	3.26	11.38	1.25	5.09
6	A	5.20	9.00	2.84	3.22
	0.8	3.60	9.65	1.66	3.26
7	A	5.20	10.33	2.64	3.08
	0.8	3.36	9.63	1.51	3.26
Averages					
Adlibitum	(A)	4.07	9.26	2.92	5.46
0.8 A		2.82	8.60	1.42	5.44

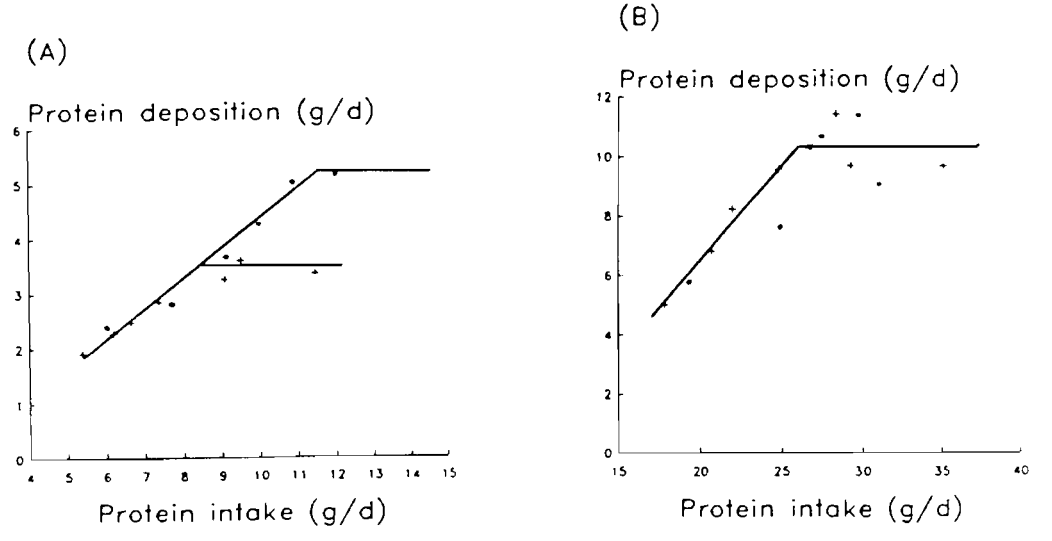

Figure 1 Relationship between protein intake and protein deposition for male broilers (A) 40 to 750 g liveweight, and (B) 750 to 2000 g liveweight. Birds were fed either ad libitum (o) or restricted to 80% of ad libitum (+).

The results for the growth stage 40 to 750 g showed that maximal protein deposition was a function of energy intake and independent of

protein intake. Consequently, restrictively fed birds exhibited lower maximal protein deposition and slower growth than those fed ad libitum. In contrast, protein deposition on the lower protein diets was related to protein intake and largely independent of energy intake. Separate protein and energy dependent phases of protein accretion (muscle growth) have been established for pigs (Campbell et al. 1983) but to our knowledge have not previously been demonstrated for broilers. From a practical aspect this finding demonstrates that there is little value in attempting to maintain near maximal growth performance by increasing the level of dietary protein (amino acids) under situations which result in a decline in feed intake.

The similarity in the dietary protein: AME ratio but marked difference in daily lysine intake required to support maximal protein deposition in ad libitum and restricted birds further demonstrates that energy intake is the major determinant of protein deposition capacity in broilers. This finding also suggests a linear relationship between energy intake and protein deposition in broilers from 40 to 750 g.

In contrast maximal protein deposition between 750 and 2000 g liveweight was largely unaffected by feeding level suggesting that over the latter stages of growth the broilers protein deposition capacity is achieved at an energy intake somewhat below ad libitum. This was further indicated by the fact the restrictively fed birds exhibited a lower feed:gain value than those fed ad libitum. Nevertheless, the situation was complicated by the marked decline in voluntary feed (energy) intake by the ad libitum birds with increase in dietary lysine above 8.1 g/kg (0.62 g/MJ of AME).

The decline in feed intake was presumably an attempt by the birds to better balance their limited protein deposition capacity (tissue requirement) and dietary amino acid intake. However, because of the consequent reduction in energy intake both protein deposition and growth performance were depressed. Deamination of the dietary protein supplied in excess of tissue demand between 750 and 2000 g would have further reduced the net energy available for protein growth on the two highest lysine diets. This was probably the major cause of the decline in protein deposition and growth performance exhibited by the restrictively fed birds on those two diets, and for the continual decline in carcase fat content with each increase in dietary lysine on both feeding treatments. Similar effects have been reported previously for growing pigs (Campbell et al. 1985).

Overall the results provide information on the relationships between protein and energy intake on protein deposition for broiler chickens. Energy intake appears to be the major factor determining maximal protein deposition and hence the broilers requirement for dietary amino acids. However, further quantitative information is required on the relationship between energy intake and protein deposition for birds over the different phases of commercial growth. This information combined with that on the amino acid composition of body protein will enable the broilers tissue requirements to be established and form the basis for more accurate diet formulation and for predicting the consequences of changes in protein and/or energy intake on growth performance and carcass composition.

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EGGS: IMPLICATIONS FOR HUMAN HEALTH

K. O'DEA and A.J. SINCLAIR

Summary

For many years health professionals have been warning the public about the dangers of cholesterol-rich foods such as eggs. In this paper we argue that the diet consumed by humans through most of their evolutionary past was a low-fat mixed diet containing a high proportion of animal foods. Under these circumstances (low-fat and low-saturated fat), dietary cholesterol may have considerably less influence on plasma cholesterol levels. In this paper, the results of experiments designed to test this question are presented; in normocholesterolaemic subjects the consumption of one egg/day was quite compatible with plasma cholesterol lowering, provided the background diet was low in saturated fat (low-fat diet or diets containing mono- or polyunsaturated fat). During the course of the dietary studies it was found that the cholesterol content of the eggs being used varied widely. A study into the cholesterol content of eggs from the major layer strains sampled from four States found that the cholesterol contents varied from 340 to 527 mg/100 g edible egg. The average cholesterol content of the 291 eggs analysed was 16% lower than the value in the Australia Tables of Food Composition (450 mg/100 g). There were significant differences between strains and smaller eggs had a lower cholesterol content due to the lower proportion of yolk and lower lipid content.

I. INTRODUCTION

It is now generally accepted by health professionals and researchers all over the world that high plasma cholesterol levels increase the risk of occlusive vascular disease (Stamler et al. 1986). However, the precise way in which cholesterol in the diet influences the level of cholesterol in the plasma is not yet fully understood. Most previous dietary studies have looked primarily at the effect of cholesterol in the context of a "normal" fat intake (i.e. approx. 40% dietary energy) but varying the relative proportions of saturated and polyunsaturated fat (PUFA) (Hegsted et al. 1985). Diets rich in saturated fat (low P/S ratio) are associated with increased plasma cholesterol levels even if the diet is itself low in cholesterol : if the diet is enriched with cholesterol this hypercholesterolaemic effect of saturated fat is enhanced. If the background diet is low in total fat or contains a high proportion of polyunsaturated fat (Schonfeld et al. 1982; O'Dea 1984), then dietary cholesterol does not lead to increases in blood cholesterol concentrations. These observations suggest that certain cholesterol-rich foods such as eggs, liver and brains, which all have numerous positive nutritional attributes, could be safely included in a low-fat diet. They also highlight the confusion surrounding cholesterol in the diet and cholesterol levels in the blood. Cholesterol is an extremely important chemical in the body, being the precursor of the steroid hormones (sex hormones, adrenal hormones) and vitamin D as well as being an integral component of all cell membranes in the body. Cholesterol can be synthesised in the liver and in most cells of the body and so we have no absolute requirement for it in the diet. If all systems are functioning optimally the body can adjust cholesterol synthesis and excretion to balance cholesterol intake in the diet, reducing it or increasing it as appropriate. High levels of saturated fat in the diet appear to interfere with this finely-tuned system and allow the cholesterol concentrations in the

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blood to rise to unacceptably high levels (Hegsted et al. 1965). This effect is now believed to be secondary to reduced numbers of LDL-receptors in the liver resulting in reduced LDL clearance (Spady and Dietschy 1985). In recent studies (Sinclair et al. 1987) we have shown that low-fat diets rich in lean red meat (500 g kangaroo meat/day) were associated with significant reductions in plasma cholesterol in normal subjects (4.5 + 0.5 mM before, 3.7 + 0.4 mM after two weeks on the diet, $P< 0.01$) and returned rapidly to baseline levels (4.5 + 0.5 mM) within one week of resuming the normal diet ($P< 0.01$). These reductions in plasma cholesterol occurred in spite of the dietary cholesterol intake remaining constant at 300 mg/day throughout the different dietary periods, and in spite of the subjects having low-normal cholesterol levels at the commencement of the study. This hypocholesterolaemic effect of meat is not restricted to extremely lean game meats such as kangaroo. In a study with maturity onset diabetics (O'Dea et al. 1989) we have shown that 10 days on a low-fat, low-carbohydrate diet which contained at least one kilogram per day of a variety of very lean conventional meats (veal, chicken, beef, pork) also resulted in consistent, marked reductions in plasma cholesterol concentrations (5.8 + 0.6 mM before, 4.9 + 0.3 mM after) despite the diet containing more than 600 mg cholesterol per day. We have also shown similar plasma cholesterol reductions in normocholesterolaemic people who consumed a low-fat diet rich in lean beef (O'Dea et al. 1990).

The diet consumed by humans through most of their evolutionary past was a mixed omnivore diet and contained a high proportion of animal foods (Eaton and Konner 1985). It is significant that in hunter-gatherer societies cholesterol-rich organ meats (liver, brains, etc.) and eggs were highly valued components of the diet, yet there is no evidence that such diets were associated with elevated plasma cholesterol levels and high risk of ischaemic heart disease. Our research on the traditional diets of Aborigines bears this out (O'Dea et al. 1980; O'Dea 1984). We have shown, for example, that Kimberley coastal Aborigines who temporarily reverted to traditional lifestyle derived two-thirds of their energy from animal foods (O'Dea 1984). In spite of these diets being relatively high in cholesterol (due to the high intakes of muscle and organ meats), plasma cholesterol levels, which were not high initially, fell (O'Dea 1984). The non-vegetable components of hunter-gatherer diets were derived from wild animals and fish, all of which have a low total fat content and a high proportion of PUFA. The traditional diet consumed by the Aborigines in our study contained only 13% energy from fat despite containing 64% energy from non-vegetable sources (O'Dea 1984). These results would suggest that moderate to high intake of cholesterol appears to have minimal effects on plasma cholesterol if the background diet is low in fat.

Although we have demonstrated that plasma cholesterol levels fall rapidly on a very low-fat diet (approx. 10% energy as fat), even if that diet contains substantial amounts of cholesterol (equivalent to more than one egg daily), the diets have been critised as being 'unrealistic' and therefore of little practical value in treating the majority of hypercholesterolaemics over the long term. An alternative approach to this very low-fat diet is to replace some of the dietary saturated fat with either mono- or polyunsaturated fat, or a combination of both. There is a large body of evidence suggesting that it is primarily saturated fat of animal origin (fat in dairy and meat products) which is the problem in terms of raising cholesterol levels. Polyunsaturated and monounsaturated fats/oils have been shown to have quite different effects on the lipoprotein lipid profile. In examining the effects of different dietary fats on plasma lipids it is important to ascertain the effects on individual lipoprotein lipid fractions. The major cholesterol-carrying lipoprotein is low-density lipoprotein (LDL), the level of which has been shown to be directly associated with increased risk of coronary heart disease. However, cholesterol is also transported on another lipoprotein, high-density lipoprotein

(HDL), which has the opposite type of association: elevated levels of HDL-cholesterol are associated with reduced risk of CHD (Mattson and Grundy 1985). In simplistic terms, LDL is primarily responsible for the transport of cholesterol into cells (via the LDL apo B receptor), while HDL transports cholesterol out of cells ("reverse cholesterol transport"). The levels of the two fractions influence the deposition and accumulation of cholesterol in artery walls, a critical factor in the etiology of atherosclerosis and occlusive vascular disease. HDL-cholesterol is comprised of several subfractions, the major two being HDL and HDL . It is the HDL subfraction which is primarily responsible for "reverse cholesterol transport" and also which is most affected by diet and lifestyle (Eisenberg 1984). Different dietary fats affect the levels of LDL and HDL cholesterol differently. Saturated fats of animal origin raise the levels of both, linoleic acid-rich vegetable oils (e.g. safflower oil, polyunsaturated margarine) lower the levels of both HDL and LDL-cholesterol, while the oleic acid-rich vegetable oils (such as olive oil) selectively lower the LDL-cholesterol and leave the HDL-cholesterol level relatively unaffected (Mattson and Grundy 1985). Very low-fat diets (usually high in carbohydrate) lower the levels of LDL and HDL-cholesterol. Diets very high in linoleic acid-rich vegetable oils do the same. However, diets containing moderate (more realistic) levels of vegetable oils lower only LDL-cholesterol levels. This may explain why Mediterranean diets are associated with particularly low rates of premature death from coronary heart disease (and all-cause mortality), even though they are not particularly low in fat (Keys et al. 1986).

We have conducted a series of studies designed to determine the effects on plasma lipoprotein lipids of the addition of one or two eggs daily to a low-fat diet and to examine the effects on these parameters of the addition of increasing levels of different fats (saturated, monounsaturated, polyunsaturated) to the diet. The second objective was to determine the cholesterol content, lipid content and fatty acid composition of Australian eggs and to examine factors which may modify them.

II. DIETARY STUDIES

Six dietary studies have been conducted in which the effect of dding either butter, olive oil, or safflower oil to low-fat diets containing either one or two eggs per day has been examined.

The subjects were normal healthy volunteers. Each study lasted for five weeks. Energy intake remained constant and weight-maintaining throughout. In the first week the subjects consumed their usual diet. In the second and third weeks they consumed a low-fat diet which contained one or two eggs daily. In order to maintain energy intake constant despite the very low-fat content and energy density of the diet, we provided a carbohydrate supplement (Polycose, administered as two drinks daily), equivalent to 20% of the total energy intake. In the fourth and fifth weeks the fat content of the diet was increased in a step-wise fashion to 20% energy in week 4, and 30% energy in week 5 by substituting the particular fat (butter, safflower oil or olive oil) for either half of the Polycose supplement (week 4) or all of it (week 5). This ensured that there were no major changes in the diet over weeks 2-5 except for the substitution of fat for a refined carbohydrate (Polycose). The subjects weighed and recorded all food and liquid consumed over the 5-week period and dietary composition was calculated using the Microdiet software package.

The results of these studies have shown that the low-fat diets containing one egg/day resulted in a 12% fall in total cholesterol, a 12% fall in the LDL-cholesterol and a 24% fall in the HDL-cholesterol. When butter was added back to the diet the total and LDL-cholesterol rose above the baseline values and the HDL-cholesterol returned to the baseline value. In contrast, adding olive oil to the one egg/day low-fat diet resulted in no change in the total

cholesterol, i.e. it remained low compared with baseline. The HDL-cholesterol also rose following the addition of olive oil. Thus the LDL/HDL cholesterol ratio was significantly improved on the olive oil diet by comparison with the butter diet.

In the experiments with two eggs/day different effects were observed. In the group fed butter, there was a reduction in the total but not in the LDL-cholesterol level in the low-fat period due largely to a fall in the HDL-cholesterol level by 20%. Addition of butter led to a significant rise in the total cholesterol level (11% above baseline) which was due mainly to a rise in the LDL-cholesterol level. In the group fed olive oil there was a significant fall in the total cholesterol level in the low-fat period due to a fall in the HDL-cholesterol; addition of olive oil resulted in no change in the total and HDL-cholesterol levels. The two groups (butter and olive oil) were again differentiated by significant differences between them in the LDL/HDL cholesterol ratios, with the butter fed group having the highest ratio.

Preliminary results with safflower oil (rich in linoleic acid, a polyunsaturated fatty acid) have shown qualitatively similar changes to those described above for olive oil.

The results of these studies are highly significant. They suggest that in normo-cholesterolaemic subjects, the consumption of one egg daily is quite compatible with cholesterol-lowering, provided the background diet is low in saturated fat. This was evident both in the low-fat diet and when olive oil was added back to the diet to raise the fat from 10% to almost 30% energy. However, in contrast these beneficial effects were completely negated when butter was added at a relatively low level (10% energy).

The results of the studies using two eggs daily were also significant. There was a small but significant fall in the total cholesterol level during the very low-fat period due mainly to a fall in the HDL cholesterol level. It appears that the cholesterol content of two eggs was almost sufficient to reverse the cholesterol-lowering effect of the very low-fat diet. When olive oil was added to the diet, however, the cholesterol levels did eventually fall below the initial baseline levels. In contrast, when butter was added to the diet, the cholesterol levels rose significantly.

These results indicate that not only the amount of fat in the diet, but also the type of fat is of critical importance in predicting the response of plasma cholesterol levels in normal subjects to increased intakes of cholesterol in the diet. Not only is it important to minimise the amount of saturated fat (as in the very low-fat diet), but oleic acid (as in olive oil) appears to have an additional beneficial effect per se. These data are consistent with the epidemiological evidence from the Seven Countries Study which suggests that the ratio of monounsaturated/saturated fatty acids in the diet is inversely related to premature cardiovascular disease and all-cause mortality (Keys et al. 1986). The major source of monounsaturated fatty acids in that study was olive oil and the major source of saturated fats was dairy products (i.e. butter fat).

III. COMPOSITIONAL ANALYSIS OF AUSTRALIAN EGGS

During the course of the dietary studies we observed in a small sample of eggs purchased from Melbourne retail outlets that the cholesterol content of eggs ranged from 355 to 544 mg/100g edible portion. This variation was of interest since the stated cholesterol content of eggs in the Australian Metric Tables of Food Composition is 450 mg/100g edible (Cashel 1985).

The aim of this aspect of our research program was (a) to verify whether there was a wide variation in cholesterol content in a large sample of eggs from different suppliers within Australia and to establish an appropriate mean value for the purpose of our dietary studies, and (b) to establish whether any variations in the cholesterol content could be

correlated with the layer strain, feed-types specific to various states or other factors.

Eggs were sampled from the major producers in five sites from four States (Victoria, New South Wales, Queensland, Western Australia); the eggs were derived from 15 different layer strains. The same strains from the same sites were sampled on two occasions, six months apart. At each sampling, six eggs of about 50-55 g were requested from each layer strain as well as information on the major feed ingredients of the layers. The age of the layers ranged from 30-77 weeks. A total of 291 eggs were examined.

Information on egg weight, yolk weight and total edible weight was recorded for all samples and the lipids were extracted from the yolk by a standard chloroform-methanol procedure. An aliquot of the extract was saponified together with 5A-cholestanol and the cholesterol content was determined by capillary GLC. The recovery of cholesterol through the procedure was routinely determined. The fatty acid composition was measured on a representative number of samples of eggs by capillary GLC. The mean results were as follows (mean + S.D.): total egg weight 58.4 + 4.7 (g), yolk weight 18.2 + 2.5 (g), percentage yolk in egg 31.1 + 2.9, egg lipid weight 6.2 + 0.9 (g), cholesterol/egg 196 + 29 (mg) and cholesterol/100 g edible 380 + 42 (mg).

The cholesterol contents varied from 304 to 527 mg/100g edible egg. The distribution of the cholesterol values was skewed towards the lower values, with 75% of eggs having less than 400 mg/100 g and 95% below the stated mean value in the Australian Tables of Food Composition. The U.S. Department of Agriculture has indicated that recent analyses of cholesterol in eggs have revealed values significantly below those in the current food composition tables (U.S.D.A. Handbook 8.1). The most likely explanation for the reduced values is the current use of improved and specific methods for the estimation of the cholesterol content; in our case we used a highly specific gas liquid chromatographic technique compared with the older, less specific, chemical methods.

Examination of the data from the 15 different strains of layers revealed that six strains (110 observations) consistently gave cholesterol values below 415 mg/100 g, whereas for the other nine strains (181 observations) approximately one third of the values were above 415 mg/100 g. The mean value for the lowest cholesterol strain was 330 + 26 compared with 418 + 21 for the highest cholesterol strain ($P < 0.05$).

Smaller eggs had smaller yolks, smaller percentage of the yolk in the egg ($P < 0.001$) and, as a consequence, less lipid and cholesterol.

The main fatty acids in the eggs were oleic acid (45.5 + 1.7%), palmitic acid (27.1 + 1.8%) and linoleic acid (13.1 + 2.0%). The proportion of saturated, monounsturated and PUFA in the eggs was found to be 34, 50 and 16% respectively. Analysis of eggs currently on the market which are labelled as rich in polyunsaturates has shown the proportions of saturated, monounsaturated and polyunsaturated to be 29, 32 and 39% respectively. While at first sight this may appear to be a beneficial change in fatty acid composition, the increase in PUFA has been primarily made at the expense of the monounsaturated rather than the saturated fatty acids. In view of the increasing appreciation of the potential protective role of oleic acid in relation to cardiovascular disease, and the questions raised on the potential for peroxidation of PUFA, this change in egg composition may not have a net beneficial effect.

IV. CONCLUSION

These data illustrate the complexity of the relationship between dietary cholesterol intake and plasma cholesterol levels and highlight the important role of the background diet (amount and type of fat) in modulating the

response to dietary cholesterol in normal subjects. The results suggest that, in such subjects, up to one egg per day is compatible with reductions in plasma cholesterol provided the diet is very low in saturated fat. This can be achieved either by consuming a diet very low in total fat, or ensuring that the fat is derived primarily from oils rich in oleic or linoleic acid. These latter diets which are not low in fat, appear to result in a more favourable lipid profile than the very low-fat diet. In future studies it will be essential to determine the responses of subjects with moderately elevated plasma cholesterol levels to similar dietary manipulations.

The second major conclusion from this work is that cholesterol intake from eggs can be minimised by selecting small eggs as they contain proportionally less cholesterol (due to relatively smaller yolks) than larger eggs.

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THE ROLE OF CYTOKINES IN THE DEVELOPING CHICKEN IMMUNE AND HAEMOPOIETIC SYSTEMS

R.L. BOYD AND T.D. OBRANOVICH

Summary

Cells of both the immune (lymphoid) and haemopoietic systems differentiate from a common precursor cell under the influence of soluble factors (cytokines) or direct cell-cell contact with stromal (structural) cells. In mammals, haemopoietic cells develop in the bone marrow, there being four major cytokines or colony-stimulating factors (CSF) involved. T lymphocytes differentiate from bone marrow precursors, in the thymus. This is a very complex process involving intricate selection mechanisms; it is primarily under the control of thymic stromal cells via their plasma membrane molecules and soluble cytokines. T cell derived cytokines (interleukins) also regulate T cell differentiation and activation. Similarly, B cells develop in the bone marrow or bursa under the influence of stromal and T cell factors. None of the mammalian cytokines are functional on chicken cells. Chickens, with the easily accessible embryo and thymus and bursa as the distinct primary lymphoid organs, represent an excellent model to investigate the mechanisms of T and B cell differentiation, in addition to haemopoiesis. Thymic and bursal stromal elements have been extensively characterized through production of mAbs. Bursal stromal cell lines have also been produced and a novel chicken cytokine (Mr 91kDa) identified which stimulates the proliferation of MHC class II positive cells, which play a major role in the immune response. The factor is also active in vivo, causing a marked increase in bursacyte number following in ovo injection. When produced in large purified quantities by recombinant DNA gene cloning, this factor(s) may be of great importance for stimulating the development of chicken immunity and hence defence against infection.

I. INTRODUCTION

The defence of the body against infection, the prevention of cancerous growth and the ability to distinguish between 'foreign' and 'self' molecules are all absolutely dependent on intact immune and haemopoietic systems. While there are many functions which require extensive collaboration between these two systems, and they both derive from a common precursor pool within the bone marrow, their cellular contents are distinct. The immune system consists essentially of two distinct forms of lymphocytes: T lymphocytes (or T cells) which develop in the thymus and B lymphocytes (or B cells) which, in avian species, develop in the bursa of Fabricius. In mammals, B cells are generated within the bone marrow or lymphoid areas of the gastrointestinal tract. Although all T cells have several features in common, within this population there are multiple sub-lineages broadly classified as T helper (T_H), T cytotoxic (T_C) or T supressor (T_S) cells. Each of these T cells express a two chain antigen receptor (TCR) stabilized as a membrane complex with at least three peptides (CD3); antigen binds to the TCR and the signal to the nucleus is transmitted by the CD3. To assist their binding to cells presenting the foreign antigen, T cells express accessory molecules. For T_H this is CD4 (which binds to MHC class II molecules and is the HIV receptor in human AIDS) and for T_C this is CD8 (which binds to MHC class I molecules). This whole process, is called associative recognition.

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The haemopoietic system is essentially all blood cells: red blood cells, granulocytes (neutrophils, eosinophils), platelets and monocytes. All haemopoietic cells are produced primarily in the bone marrow, but this can occur at other sites if the appropriate growth signals are present. Within the tissues of the body, monocytes differentiate into cells broadly classified as macrophages. Depending on their location, and hence the growth signals they receive, the macrophages themselves become very heterogeneous in their function and expression of suface markers (phenotype). Essentially they are of two classes depending on the expression or not of major histocompatibilty complex (MHC) class II molecules. Those which lack MHC class II have as their major function, the uptake (phagocytosis) of any particulate matter (eg. dead/dying cells, microorganisms, air pollutants). Those expressing MHC class II are predominantly dendritic cells which have a major role in the immune response as antigen presenting cells (APC). Their function is to uptake exogenous antigen, 'process' it into smaller peptides and present these in physical clefts formed by MHC class II or, if endogenous antigen (ie produced by virally infected APC) MHC class I molecules. This antigen presentation in the context of self MHC molecules is critical for the activation of relevant (antigen-specific) T cells. T_H recognise antigen bound to self MHC class II using both their TCR and CD4 and T_C recognise, via their TCR and CD8, antigen bound to self MHC class I. This is called 'Self MHC restriction' and is a fundamental property of T cells. They acquire this through strict selection processes during their intrathymic differentiation. In all T cells, the specificity of the TCR is generated by random associations between different genes. Those T cells which recognise self MHC class I or II are positively selected but those which do this with pathologically high affinity are subsequently eliminated (negative selection). The latter process prevents immune self destruction or autoimmunity.

B lymphocytes recognise antigen via their surface immunoglobulin. They are not self-restricted and do not require antigen to be presented by MHC molecules. Hence they do not recognise the same antigenic determinants on any given molecule as T cells do. For their activation, however, B cells require both APC and T cells. Basically an immune response is initiated thus: foreign antigen enters the body, is engulfed by APC, processed into peptides and becomes expressed on the APC surface in clefts formed by MHC class I or class II molecules; other fragments of the antigen may bind directly to B cells. T_H recognise antigen-MHC class II complexes and become activated in association with APC soluble factors (mainly interleukin 1- IL1). IL1 and T_H cytokines (interleukins 2,4,5 and 6) act in concert to stimulate antigen bound B cells to eventually differentiate into antibody secreting plasma cells. These T cells and another set of the activated B cells progress to 'memory' cells which ensure a more rapid and expanded immune response on subsequent exposure to the same antigen.

While the immune system provides persistant protection to infection, in many instances it is not required to eliminate foreign organisms. This occurs essentially through myeloid cells such as macrophages/monocytes and granulocytes which normally provide a very efficient defence through phagocytosis of the microorganisms. It is only when the infectious load is severe that the immune system is involved. In the absence of a fully mature and hence functional immune system more importance is placed on myeloid cells and the organism is obviously more prone to infection. This is particularly relevent to chickens, because their immune system is not fully developed until around three weeks after hatching. During this newly hatched period, the chickens, which obviously lack maternal protection (although in the egg they do possess maternal-derived immunoglobulin), are most susceptible to infection. On average this can result in about 10% of flocks being lost through opportunistic pathogens. This translates to an annual world wide loss of $25 billion. Enhancement of disease resistance during these first weeks is thus of

major economical importance for the poultry industry. This can be broadly achieved by two means: promoting maturation of the immune system and/or stimulation of the haemopoietic system which provides a first-line non-specific defence.

For both the lymphoid and haemopoietic systems, including myeloid cells, the development and differentiation of the effector cells from their precursors is absolutely dependent on a group of cells collectively termed stromal cells. These stromal, or structural cells exist as an intermeshed network forming the basic architecture of the thymus, bursa and bone marrow. They are essentially stationary and promote the growth and differentiation of the precursors as they percolate through the tissues emerging as mature T, B or haemopoietic cells. The mechanisms of differentiation are not fully understood but there is an involvement of cell-cell plasma membrane contact and secretion and uptake of soluble factors. Delineation of these individual stromal cells and their factors involved at each step of the maturation pathway is thus of major importance. Since major developments in the mammalian system have been made recently, they will be briefly outlined as they form the rationale behind current chicken research strategies.

II. MECHANISMS OF MAMMALIAN HAEMOPOIESIS AND LYMPHOPOIESIS

The complex effects of CSF's and interleukins on haemopoiesis and lymphopoiesis, respectively, have recently been reviewed (Metcalf 1984; Herrmann and Mertelsmann 1989; Balkwill and Burke 1989). Briefly, for T cells IL1 induces differentiation in the form of IL2 and IL2 receptor expression; IL2 is essential for T cell growth; IL4 and IL10 act as co-mitogens, and IL10 has a broad stimulatory effect on growth and differentiation (Zlotnik personal communication). For B cells, IL1 again induces expression of receptors for other interleukins; IL4 is a co-stimulator and induces MHC class II expression, as does γ-interferon, and Ig class switching; IL5 induces Ig class switching; IL6 promotes Ig secretion (Balkwill and Burke 1989). In the absence of stromal cells, none of these IL either alone or in combination with others, induce complete T or B cell differentation from immature precursors through to mature effector cells. For this, there is an absolute requirement of stromal cells (bone marrow or thymic). There are four clearly defined cytokines involved in haemopoiesis (extensively reviewed in Habenicht 1990): granulocyte colony stimulating factor (G-CSF; Mr 25kDa) so called because bone marrow cells suspended in agar in the presence of G-CSF form colonies of granulocytes. GM-CSF (21-23kDa) stimulates the growth of granulocytes, macrophages, eosinophils, megakaryocytes (platelets), mast cells and also self-renewal of precursor cells. Each of these function through binding to specific receptors (Nicola 1987) which are induced or up-regulated by IL1 (Cosman 1988; Henney 1988). Tumour necrosis factor (TNF) and IL1 induce the transcription of CSF's (Kaushanski et al. 1988). Interferons, on the other hand, generally have an anti-proliferative effect on haemopoietic cells (Gajewski et al. 1988). One fact which has clearly emerged from these studies is that the cytokines form an interacting network in their stimulation of haemopoiesis (and probably lymphopoiesis).

III. AVIAN CYTOKINES INVOLVED IN HAEMOPOIESIS

(1) cMGF. Most of our present knowledge on the effects of cytokines on haemopoiesis has been derived from the murine and human systems. The only well characterized avian cytokine affecting monocytic haemopoietic cells is chicken myelomonocytic growth factor (cMGF). This factor was first identified in Concanavalin A stimulated chick spleen supernatant which induced differentation of myelomonocytic cells transformed by

myboncogene containing E26 virus and avian myeloblastosis virus (AMV) (Beug et al. 1982). An alternative source of this factor was found in LPS stimulated chicken cell line HD11 supernatant (grown in serum free conditions). HD11 was created by transforming chicken macrophages with the myc containing MC29 virus. Conditioned medium from this cell line not only caused transformed myeloblasts to continue differentiating into macrophages, but also induced the formation of macrophages from uninfected chick bone marrow cells (Leutz et al. 1984). The protein core contains 201 amino acids which varies in its degree of glycosylation (Leutz et al. 1988). The gene for cMGF has been cloned and the factor was found to be a glycoprotein with a molecular weight of 24-29 kDa (Leutz et al. 1989). Because of its homology to murine and human IL6 and G-CSF, it was thought that cMGF may be the product of an ancestral gene. This hypothesis was rendered unlikely by showing that no biological activity could be detected when murine IL6 and human G-CSF were incubated with chicken stem cells (unpublished results cited in Leutz et al. 1989). Interestingly, a mammalian equivalent to cMGF has been postulated. Examination of human and murine genomic DNA with a cMGF probe gave rise to a band which did not correlate with murine and human G-CSF and IL-6 probes (Leutz et al. 1989).

(2) IL1. The chicken equivalent of mammalian IL1 has been identified in the supernatants of lectin- or antigen-stimulated blood monocytes, spleen macrophages, or macrophage cell lines (Hayari et al. 1982; Klaising and Peng 1987). It has not been fully characterized, but does induce the ability of thymocytes to respond to antigen/mitogen.

(3) IL2. Avian IL2 has been demonstrated in the supernatants of stimulated chicken spleen T cells (Schauenstein et al. 1982; Kromer et al. 1984). It supports the growth of activated T cells and has a molecular weight of approximately 30kDa (Fredericksen and Sharma 1987). It has also been partially purified as a 13kDa polypeptide (Schnetzler et al. 1983) but its molecular cloning has not yet been described.

(4) γ-IFN. γ-IFN or immune interferon, is produced by activated T cells, has potent anti-viral properties and has a major role in enhancing the expression of MHC class II molecules. In chickens it has a molecular weight of approximately 17 kDa (Fredericksen and Sharma 1987), but again has not been purified or cloned.

IV. IDENTIFICATION OF A NOVEL CHICKEN CYTOKINE

Based on the hypothesis that stromal cells are the major controlling element in haemopoiesis and lymphopoiesis, the methodology for isolating and culturing chicken thymic and bursal stromal cells has been developed. Initially these cells were phenotyped using an extensive panel of anti-stroma mAbs (Boyd et al. 1990; 1991; Wilson and Boyd 1990a,b). To provide a more reliable source of such cells, which is necessary for large scale production of factor-containing supernatants and sufficient supply of mRNA for gene cloning via c-DNA libraries, 11 day embryonic bursa stromal cells were infected with the Fujinama Sarcoma virus (FSV). Whereas uninfected cells died by six weeks of culture, the infected cell line (Fuji-P) was still viable after six months. From its antigenic profile, it would appear to derive from the 11 day bursa surface epithelium (data not shown).

Serum-free supernatants from the Fuji-P cells were tested for their ability to induce differentiation and proliferation of non-adherent 15 day embryonic bone marrow or bursal cells, both of which are rich sources of haemopoietic and B cell precursors respectively. There were three major effects observed: the induction of cell survival and proliferation , and increase in the percentage of cells expressing MHC class II and the development of macrophage-like adherent cells (Figure 1). Control supernatants from lung fibroblasts had no such effect, but heterogeneous bursal stromal cultures and

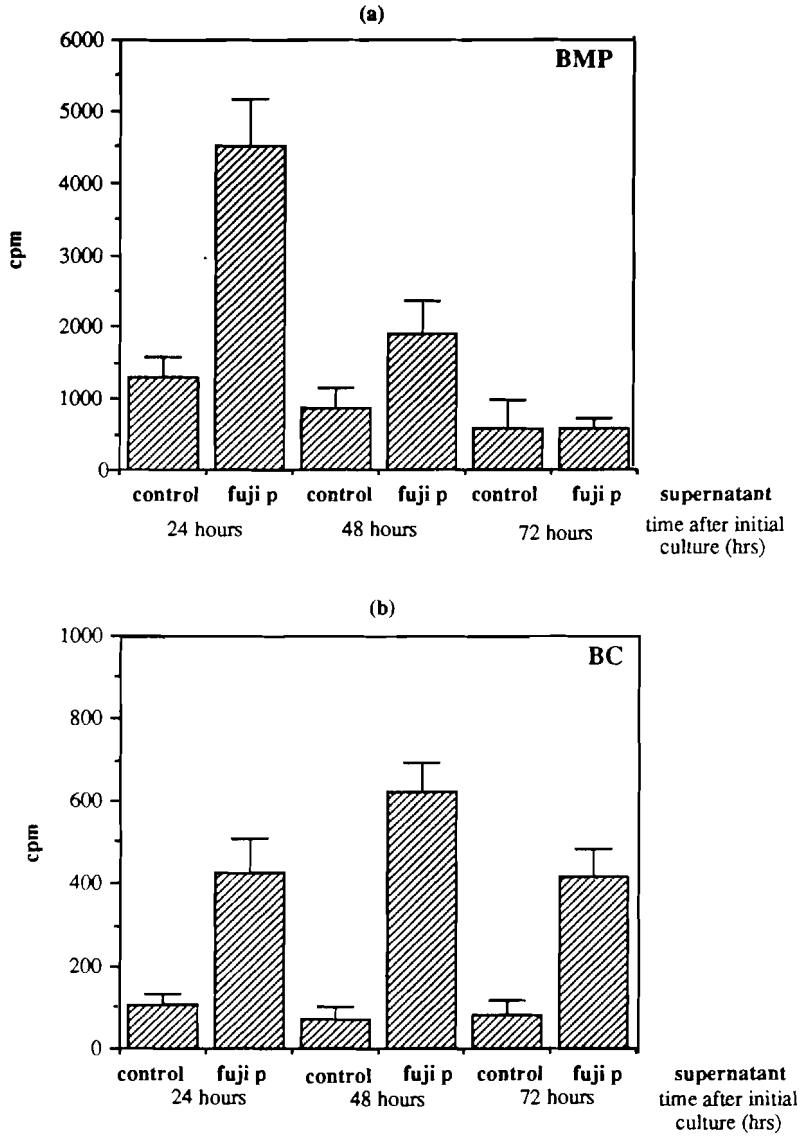

FIGURE 1: Cell proliferation (assessed by [^{125} I]-Iudr uptake into newly synthesised DNA) by (a) non-adherent 15 day embryonic bone marrow (BMP) or (b) bursa precursor cells (BC) after 24, 48 or 72 hours of co-culture with either RPMI-FCS (control) or Fuji-P conditioned medium (fuji p). Values represent the mean + 1 SD of 6 individual experiments.

another similar cell-line (Fuji-a) had similar, but reduced activity. Figure 2 shows the increase in MHC class II positive cells after 72 hours of culture. Separation of the precursor population prior to culture demonstrated that the increase is most likely due to proliferation of pre-existing MHC class II positive cells rather than induction (data not shown). Concentrated Fuji-P supernatant was also injected intravenously into 11 day embryos which were killed at day 18 of incubation. In the injected birds there was a marked increase in the number of bursal cells, but no significant effect on the spleen. This clearly demonstrated the factor(s) is functional in vivo. Initial purification of the proliferation factor by size exclusion chromatography demonstrated the active peak to have a molecular weight of approximately 91kDa; it is not yet known whether this can also stimulate MHC class II-positive cells or the development of adherence.

In summary, cell lines have been produced which synthesize at least one novel chicken cytokine which increases the development of B cells in vivo and MHC class II positive cells (probably also B cells) in vitro. There is also the induction of adherent cells which potentially could be part of the macrophage lineage of cells. Collectively, the eventual large scale production of these factors should prove very valuable in manipulating the early development of the chicken immune and haemopoietic systems. The ability to increase MHC class II positive cells may also have an important role in adjuvant therapy when given in conjunction with defined vaccines.

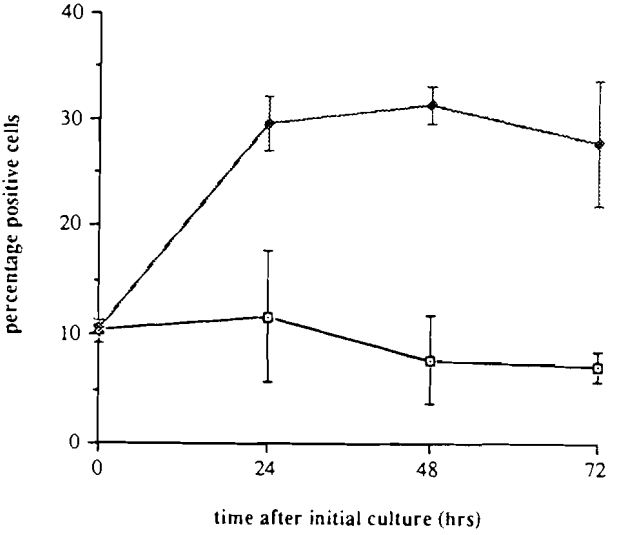

FIGURE 2: Increase in percentage of 15 day embryonic bone marrow cells expressing MHC class II after co-culture with RPMI-FCS (control) or Fuji-P conditioned medium (fuji p). Values were determined by flow cytometry using a FACScan (Becton Dickinson) and represent the mean + 1 SD of 6 experiments.

-□- control
-●- fuji p

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POPULATION STUDIES ON BACTERIAL POULTRY PATHOGENS

P.J. BLACKALL

Summary

In recent times it has been recognised that a bacterial species represents a natural population of organisms that can have a genetic diversity ranging from limited to extensive. The concept of genetic diversity within a species has important practical implications for the Australian poultry industry. This paper outlines two areas, the recognition of strains of bacteria exotic to Australia and epidemiological and pathogenicity studies of complex disease situations in which the concept of genetic diversity can be usefully applied. Some brief details of the types of techniques that can be used to measure genetic diversity (protein profiles, restriction length fragment polymorphism and multi-locus enzyme electrophoresis) are provided.

I. INTRODUCTION

Until recently, bacteriologists have been limited to identifying poultry pathogens to the species level only. At best, limited subtyping by serological properties has been possible. This has led to the impression that a bacterial species represents a uniform collection of organisms. However, in recent times, the application of new subtyping technologies has established that a species represents a natural population of organisms that can have a genetic diversity, ranging from limited to extensive, and that this diversity is organised into distinct clones (Achtman et al. 1983).

This clonal theory implies a linear descent of each clone from an ancestral cell (Achtman et al. 1983). The range of properties associated with the progenitor bacterium of a clone is preserved among most of its descendants. Due to evolutionary variation, it is accepted that members of the clone are similar, but not necessarily identical to each other (Achtman et al. 1983).

Three generalisations about the genetic diversity and structure of natural populations of bacteria have been proposed (Selander and Musser 1990). The first is that most species of bacteria are clonal in nature, which implies that rates of recombination of chromosomal genes between clones are very low, perhaps not in excess of the mutation rate. The second generalisation is that the number of clones in natural populations is relatively small. While a virtually unlimited number of clones is theoretically possible, for most pathogenic species studied to date, the actual number of clones that have been recognised are normally less than a hundred. The third generalisation is that even in the more genetically variable pathogenic species, the majority of cases of disease is caused by a small proportion of the total number of existing clones. In other words, there can be a large variation in pathogenicity between clones.

In this paper, the possible impact of studies into genetic diversity of bacterial poultry pathogens for the Australian poultry industry is illustrated. Two separate areas are covered:- the first involving quarantine aspects, in which an expanded concept of "exotic" will be presented, and the second, involving epidemiology and pathogenesis, in which the possible use of the clonal theory in studying complex disease situations will be explained. As well, some details of the techniques that can be applied to determine genetic diversity will be provided.

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II. QUARANTINE STUDIES

The Australian poultry industry has a history of biological isolation. This biological isolation, which has been enforced in modern times by strict quarantine laws, has meant that there has only been very limited opportunity for bacterial poultry pathogens to enter Australia. Expressing this in slightly different terms, it could be argued that a limited number of clones of these pathogens are present in Australian poultry i.e. that only a limited genetic diversity exists within some of the bacterial species pathogenic for chickens.

From a number of areas, Australia's traditional isolation is at threat. The increasing frequency of contact between Australia and overseas countries, combined with modern rapid transport, raises the risk of accidental or deliberate illegal importation of live poultry into Australia. The harsh rules of world trade mean that the live bird quarantine that contributed to the biological isolation of Australia can no longer be maintained. As the ability to detect birds carrying such 'common' pathogens as Haemophilus paragallinarum and Pasteurella multocida is not known, these importations could result in an increase in the genetic pool of such pathogens, thus providing the potential for increased virulence or new disease complexes.

Although the above is a theory only, there is considerable evidence to support it. Essentially the theory states that there is limited genetic diversity in Australian bacterial pathogens and that this diversity could be increased, with subsequent emergence of either entirely new diseases or new forms of old diseases, following introduction of live animals. The pig industry has already provided an example. The disease atrophic rhinitis is associated with P. multocida and Bordetella bronchiseptica. Both these organisms have been present in Australian pigs for a considerable period of time, yet the severe form of the disease was absent from Australia. Presumably the Australian isolates of P. multocida and B. bronchiseptica did not include the genetic information necessary for the production of severe, progressive atrophic rhinitis. Following the importation of live pigs from overseas, the severe form of the disease was reported in West Australian pigs (Mercy et al. 1986). Presumably, the imported pigs carried P. multocida strains with the necessary genetic information to cause atrophic rhinitis. The genetic diversity present in Australian porcine P. multocida has been extended and progressive atrophic rhinitis has now been reported in South Australia and New South Wales (Gardner et al. 1989) as well as West Australia (Mercy et al. 1986).

Already, there is mounting evidence of limited diversity in bacterial pathogens occurring in Australian poultry. Using the technique of restriction endonuclease analysis (REA), a method of directly comparing the genome of isolates, Blackall et al. (1990) have recently shown that Australian isolates of H. paragallinarum show very limited diversity in comparison with overseas isolates. Along similar lines, Morrow and colleagues (pers. comm.) have used REA to show that Australian isolates of Mycoplasma synoviae from cases of synovitis are different from isolates from overseas. As well, all Australian isolates of Bordetella avium examined to date lack the ability to produce the dermonecrotic toxin characteristically produced by overseas isolates of this organism (Blackall and Rogers 1990). Hence, imported birds carrying any of the above species, all of which occur in Australia already, could extend the bacterial gene pool. The result could be the introduction of pathogens of increased virulence.

The problem confronting the poultry industry is: How can 'overseas' strains of common pathogens be recognised? For truly exotic agents such as fowl plague, there is no problem. The absence of the agent means that any Australian isolate is a fresh introduction. However for organisms such as P. multocida, H. paragallinarum and so on, how can a 'foreign' isolate be recognised?

Population studies that provide a guide to genetic diversity could provide the answer. Techniques are now available which allow the diversity naturally present in a population to be determined. If the normal diversity is established, then the introduction of outside strains could be recognised by having characteristics outside the limited, normal diversity. The poultry industry would then be able to recognise such isolates, follow outbreaks associated with the isolates, and design suitable control programmes.

III. EPIDEMIOLOGICAL AND PATHOGENESIS STUDIES

Escherichia coli is a well known cause of extra intestinal infections in chickens (Gross 1984). The collective diseases associated with this organism are responsible for major economic losses to the poultry industry (Gross 1984).

The development of effective treatment and/or control programmes has been restricted by a major problem - not all E. coli isolates are pathogenic, some isolates are harmless commensals. The inability to rapidly identify an avian E. coli isolate as pathogenic or non pathogenic has frustrated effective treatment or prevention programmes. Traditionally, workers have used serotyping to determine the pathogenicity of avian E. coli isolates (Gross 1984). However, serotyping of E. coli is a complex, time consuming technique limited to specialised reference laboratories. In addition, several studies have indicated that there is no absolute correlation between O serovars and virulence (Sojka and Carnaghan 1961).

Recent studies on the genetic diversity amongst E. coli isolates have provided strong support for the clonal theory. Using multilocus enzyme electrophoresis as a measure of genetic diversity, Whittam and Wilson (1988) have found that, in a collection of American avian E. coli isolates, a relatively limited number of clones were associated with pathogenicity. We propose to examine Australian avian E. coli isolates to determine whether the population is organised into similar clones.

Our study on genetic diversity may allow us to establish that only certain subpopulations or clones of avian E. coli are associated with respiratory disease in Australian poultry. Having established the clones, we may be able to establish a small range of phenotypic properties, suitable for testing in diagnostic laboratories, that would identify the pathogenic clones. The ability to quickly identify pathogenic E. coli isolates would allow the epidemiology of individual outbreaks to be followed. As well, the ability to identify pathogenic E. coli isolates would indicate the appropriate treatment and control programmes.

IV. TECHNIQUES FOR ESTABLISHING GENETIC DIVERSITY IN A BACTERIAL SPECIES

A brief description of three techniques that are about to be developed at the Animal Research Institute is given in the following subsections. The common thread with all the techniques is that they are attempts to recognise natural groupings or clones within a bacterial species. The current project will apply these techniques to H. paragallinarum as an example of a pathogen with possible restricted diversity, and E. coli as an example of the use of clonal theory, in studies on epidemiology or pathogenesis. .

(a) Multilocus enzyme electrophoresis (MEE)

In MEE the electrophoretic mobility of metabolic enzymes is examined (Selander et al. 1986). MEE has emerged in recent times as a powerful tool for estimating the genetic diversity and structure in natural populations of a range of bacteria, including E. coli, Haemophilus influenzae, Bordetella spp., Neisseria spp. and Legionella spp. (Selander et al. 1986). MEE is based on the concept that the amino acid sequence of an enzyme determines the electrostatic charge of the molecule. This electrostatic charge in turn determines the mobility of the enzyme when it is subjected to electrophoresis

in a gel. Hence, changes in the amino acid sequence result in an altered mobility.

In MEE a cell free lysate is produced, typically by sonication. This lysate is then subjected to electrophoresis and specific enzyme stains applied resulting in banding patterns. The enzymes that have been examined and the stains and buffers used are described by Selander et al. (1986). The genetic diversity within the species can be calculated by statistical analysis of the banding patterns. The groups of banding patterns correspond to clones, allowing the recognition of the natural subpopulation groupings that occur within a species (Selander et al. 1986).

(b) Restriction Endonuclease Analysis (REA) and Restriction Fragment Length Polymorphism (RFLP)

REA is a method of directly comparing the genome of bacterial isolates. In this technique, bacterial chromosomal DNA is purified and digested with a restriction endonuclease enzyme which cuts the DNA strand at specific recognition sequences. The resulting fragments can be separated by electrophoresis yielding a pattern of DNA fragments. The usefulness of REA arises from the fact that the number and location of the recognition sites for a restriction enzyme are unique for each genome. Hence, the fragment patterns generated are unique for each genome. Bacterial cultures derived from the same strain will thus show similar fragment patterns. Any change in the sequence of bases in the DNA alters the distribution of the recognition sites. Hence, following restriction enzyme digestion, a different fragment pattern should result. Organisms that are genetically diverse will display different banding patterns, while organisms that show little diversity tend to have identical or very similar patterns.

A wide range of methods have been described for performing REA. The method used at this laboratory for H. paragallinarum (Blackall et al. 1990) is just one example. In the simplest approach to analysing the results of REA, the fragment patterns in the various lanes are compared visually. If the number of differences in fragments between the strains are large it is possible to state confidently that the strains are different. However, it is not possible by visual examination to quantify by how much the strains differ. In cases where only small differences are present, visual examination may also be inadequate. At the Animal Research Institute, it is proposed to use a technique in which the DNA fragment patterns will be converted to digitised data by the use of a black and white TV camera, a frame grabber, and image analysis software (Albritton et al. 1988). Subsequent computerised statistical analysis will allow a similarity matrix to be constructed and the genetic diversity of the examined organisms to be determined.

RFLP, a variation on REA, is also under development at the Animal Research Institute. In this technique, a less complex banding pattern is achieved visualising only those bands that are recognised following hybridisation or probing. Full details of the techniques involved in probing are provided in such texts as Maniatis et al. (1982).

Perhaps the most widely used probes in RFLP studies have been those based on ribosomal (r) RNA sequences. The suitability of the rRNA probes results from the fact that in bacteria, the rRNA genes appear to have changed little during evolution. Hence, a probe based on the rRNA genes Escherichia coli can be used a wide range of bacteria (Grimont et al. 1989). This is the type of probe that we will be using at this Institute.

(c) Whole Cell Protein Profiles

The sets of proteins produced by a bacterial strain are a reflection of the genome of that strain. It has been estimated that the typical microbial genome contains information that results in the production of about 2,000 different proteins. Hence, statistical analysis of protein profiles allows clusters of organisms, that is subpopulations or clones, to be recognised (Jackman 1985).

In the work about to commence at the Animal Research Institute, whole cell protein profiles will be examined using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Following electrophoresis, the separated proteins will be visualised by staining and the resultant banding pattern photographed. Detailed methods for the preparation of whole-cell proteins and the performance of the electrophoresis are given by Jackman (1985). In comparing whole-cell protein profiles of strains, the simplest approach is to rely upon a visual examination. However, as with REA profiles, it is proposed to use computerised image analysis to achieve quantification of the similarity or differences between strains.

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THE EFFECT OF SELECTION FOR GROWTH AND BODY COMPOSITION ON REPRODUCTIVE PERFORMANCE IN CHICKENS

A.T. BUNAN and R.A.E. PYM

Summary

A study was made of the effect of differing degrees of feed restriction during rearing and lay on reproductive performance of five lines of chickens selected for six generations at random (line C) or for increased abdominal fatness (line F), decreased abdominal fatness (line L), increased liveweight (line W), or increased liveweight combined with decreased abdominal fatness (line WL). Selection for increased fatness resulted in a marked decrease in mature body weight, egg production and egg weight, but no effect on feed efficiency whereas selection for decreased fatness resulted in a moderate increase in mature body weight with no effect on egg production, egg weight or feed efficiency. The responses of the W and WL lines were directionally similar with substantial increases in mature weight and egg weight, but decreases in both egg production and feed efficiency, the latter two being more marked in line WL. There were significant ($P<0.01$) line X feeding regimen interactions for egg production and feed efficiency indicating different optimal feeding regimes for the five lines.

INTRODUCTION

Moderately severe feed restriction of broiler breeders during the rearing period is a common industry practice because of the ensuing improvement in egg production, fertility and hatchability. It is customary to maintain a somewhat milder degree of restriction throughout the laying period, principally to save on feed and prevent excessive fat deposition. There is some evidence that the present emphasis in commercial broiler breeding programs for improved feed efficiency and reduced fatness may require some variation to the traditional nutritional management of broiler breeders to obtain optimal reproductive response (Pym 1985).

The results presented here were obtained from the second of three large experiments designed to determine the responses in reproductive performance and physiology to different nutritional management regimen of five lines of chickens selected for aspects of growth and body composition.

METHODS

The birds used in the study were produced from matings within five lines of birds selected for six generations at random, line C, or for increased abdominal fatness, line F; decreased abdominal fatness, line L; increased liveweight, line W; or increased liveweight combined with decreased abdominal fatness, line WL. The selection traits were measured at 8 weeks of age and abdominal fatness in lines F, L and WL was measured using the abdominal fat calipers of Pym and Thompson (1980).

A total of 96 birds from each line were used in the study. At hatching all chicks were wingbanded, vaccinated against Marek's disease and placed in a deep litter shed. Immediately following brooding, birds in each line were divided into two groups. The first group was allowed only one day on food in three (severe restriction) whilst the second

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group was allowed 1.5 days on food during the same three-day period (moderate restriction).

At ten weeks of age, the birds were transferred at random to individual laying cages and were individually fed using improvised feeders made out of two-litre plastic drink containers. The birds were given commercial chick starter (0 to 6 weeks) and pullet grower (6 to 20 weeks), crumbled diets containing 200 and 150g crude protein (CP) and 12.0 and 11.0 MJ ME/kg respectively. From 20 weeks of age all birds were given a commercial pelleted layer diet containing 165g CP, 35g Calcium and 11.5 MJ ME/kg. From 2 weeks of age birds were given 15h light per day to 21 weeks of age when this was increased to 16h per day. The individually-caged birds were subjected to the following rearing-laying nutritional regimen:

MRAL	moderate growing food restriction followed by an abrupt change to ad libitum feeding during lay;
MR-AL	moderate growing food restriction and a two-week gradual change to ad libitum feeding during lay;
MRMR	moderate food restriction during both growing and lay;
SRAL	severe growing food restriction followed by an abrupt change to ad libitum feeding during lay;
SR-AL	severe growing food restriction followed by a two-week gradual change to ad libitum feeding during lay;
SRMR	severe growing food restriction followed by an abrupt change to moderate food restriction during lay; and
SRSR	severe food restriction during both rearing and lay.

Birds severely and moderately restricted during lay were given approximately 60 and 80% respectively of the ad libitum food consumption measured in the MRAL group in each line. All birds were changed to the laying nutritional regimes at 20 weeks of age. An additional group of birds from each line were reared on the above treatments to determine effects on body composition and reproductive physiology at commencement of lay.

RESULTS

As shown in Figure 1, the high body weight lines (W and WL) were the heaviest throughout the study, the high fat line (F) the lightest and the C and L lines intermediate. The level of food restriction in the two periods was reflected, as might be expected, in body weight changes with time. The MRAL and MR-AL birds were the heaviest, the SRSR birds the lightest and those in the other groups intermediate.

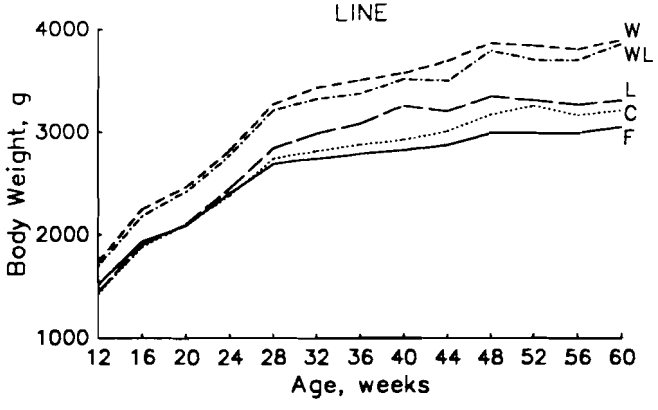

Figure 1. Average liveweight of pullets in the five lines.

As shown in the table, the L line came into lay later than the four other lines whilst onset of sexual maturity was largely determined by food allowance during rearing and lay. There were significant line differences in body fat at onset of sexual maturity with the F and W lines the fattest, the L line the leanest, and the WL and C lines similar and intermediate. Although mean differences between the feeding regimens were minimal, there was a significant ($P<0.01$) interaction between line and feeding regimen for body fat at onset of sexual maturity, due principally to the SRSR group being fattest in line WL but leanest in all the other lines. With the exception of this group, there was little variation in this trait within line across feeding regimens.

Table 1. Line and feeding regimen means for age at onset of sexual maturity (AOSM, d), body fat at onset of sexual maturity (FOSM, g/kg), hen-day egg production (HDEP, eggs/bird), average egg weight (AEW, g), average food consumption (AFC, g), feed conversion ratio (FCR, feed/doz eggs), fertility (Fert, %) and hatchability (Hatch, %)[1].

	AOSM	FOSM	HDEP	AEW	AFC	FCR	Fert	Hatch
Line (L)								
F	179^{a}	226.2^{a}	143.8^{c}	57.6^{d}	110.6^{d}	2.27^{a}	85.7	61.0^{b}
L	185^{b}	197.5^{c}	168.9^{a}	59.5^{bc}	133.9^{b}	2.31^{a}	88.5	62.6^{ab}
W	179^{a}	226.0^{a}	158.2^{b}	61.8^{a}	141.9^{a}	2.67^{b}	89.1	66.7^{ab}
WL	181^{a}	212.5^{b}	143.9^{c}	60.7^{ab}	135.7^{b}	2.89^{c}	90.2	59.6^{b}
C	180^{a}	216.7^{b}	171.9^{a}	59.2^{c}	125.5^{c}	2.17^{a}	91.0	70.9^{a}
LSD 0.05	3	4.6	10.1	1.4	2.8	0.21	7.6	9.0
Feeding Regimen (F)								
MRAL	169^{a}	217.6^{a}	175.7^{a}	59.9^{ab}	141.3^{ab}	2.49^{ab}	89.2	60.6
MR-AL	174^{b}	216.0^{a}	166.7^{ab}	59.2^{b}	142.4^{a}	2.65^{b}	91.4	64.5
MRMR	177^{b}	218.2^{a}	168.4^{ab}	60.1^{ab}	128.1^{c}	2.35^{a}	89.4	65.2
SRAL	184^{c}	215.8^{a}	162.1^{bc}	61.2^{a}	138.9^{b}	2.45^{ab}	88.0	65.7
SR-AL	184^{c}	220.8^{a}	167.2^{ab}	60.5^{ab}	139.4^{ab}	2.56^{ab}	92.3	65.7
SRMR	184^{c}	215.9^{a}	151.5^{c}	60.2^{ab}	125.1^{d}	2.38^{a}	84.7	62.0
SRSR	194^{d}	206.0^{b}	109.8^{d}	57.0^{c}	91.1^{e}	2.36^{a}	87.3	65.2
LSD 0.05	4	5.5	12.7	1.7	3.3	0.25	9.0	10.6
LXF	ns	**	**	ns	**	**	*	ns

[1] Means in each column with different superscripts are significantly different ($P<0.05$). * ($P<0.05$) ** ($P<0.01$) ns - not significant ($P>0.05$).

The C and L lines laid the greatest number of eggs, the F and WL lines the fewest, with the W line intermediate. There was a significant interaction between line and nutritional regimen for egg production to 60 weeks, as illustrated in Figure 2. In all lines the SRSR group performed the worst but there was considerably less disparity in the W line than in the four other lines. There was an indication that the WL line responded best to an abrupt increase to ad libitum feeding at 20 weeks following either severe or mild rearing restriction.

The heaviest eggs were laid by the W and WL lines, the lightest by the F line and those by the L and C lines, intermediate. Egg weight was lowest in the SRSR group but tended to be higher in the groups severely restricted during rearing followed by ad libitum

feeding during lay compared with the moderately-restricted rearing groups. The W line birds consumed the greatest amount of food from 24 to 60 weeks followed by the WL, L, C and F lines.

The heavier W and WL line birds were least efficient in converting food into eggs. There was a significant interaction between line and feeding regime for feed efficiency as shown in Figure 3. The SRSR group was the most efficient in the W line but the least efficient in the F line whilst the MR-AL and SR-AL groups, which had relatively good efficiency in the F, L and C lines, had poor efficiency in the W and WL lines.

There was no effect of line or feeding regimen on fertility but hatchability was higher in the C line than in the F and WL lines with the L and W lines intermediate. There was no effect of nutritional regimen on hatchability.

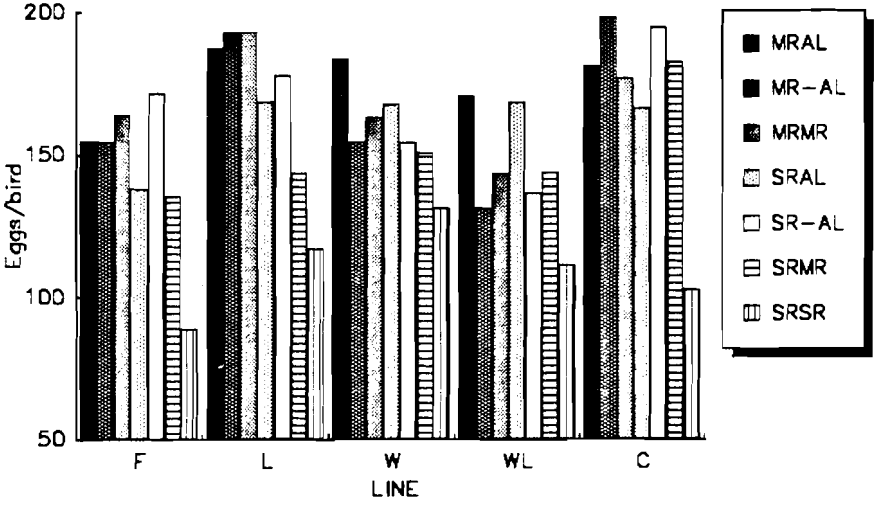

Figure 2. Hen-day egg production on the seven feeding regimes in each of the five lines.

DISCUSSION

The delay in onset of sexual maturity in the L line is likely associated with the low body fat level in this line, as suggested by Soller et al. (1984). With the exception of the SRSR group, onset of sexual maturity of birds within the lines on the different feeding regimen was determined more by body fat level than by body weight or age. In the case of the SRSR birds it would appear that additional factors were involved in determining onset of lay since these birds were generally considerably lighter (about 400g), older (about 17d) and leaner (about 10g/kg), than the mean of the other groups at onset of lay.

Body weight of the F and L line during lay diverged substantially from their similar weights at about 20 weeks. Selection for high juvenile body fatness appears to have resulted in a relatively small but plump, mature hen whilst selection for leanness at 8 weeks produced a larger, mature bird. The difference in body size is likely due to the relationship between body weight, body fatness and the onset and maintenance of egg production in the two lines. In keeping with this result, food intake in the L line was considerably greater than in the F line, although some of this difference would have been due to the higher rate of egg production in the former line.

Whilst line differences in egg production were influenced by the significant line X feeding regimen interaction, selection for either increased fatness, increased growth rate

or increased lean tissue growth rate resulted in a substantial decrease in egg production, whereas selection for leanness per se had no effect. The relatively mild depression in egg production to the SRSR treatment in the W line compared to that in the four other lines indicates the tolerance of the former line to quite severe restriction during lay. The substantially higher egg production in the groups in the WL line changed abruptly to ad libitum feeding at 20 weeks following rearing restriction compared with the similarly-reared groups changed gradually onto ad libitum feeding, indicates a high requirement in this line for tissue deposition at this stage in preparation for the onset and maintenance of egg production.

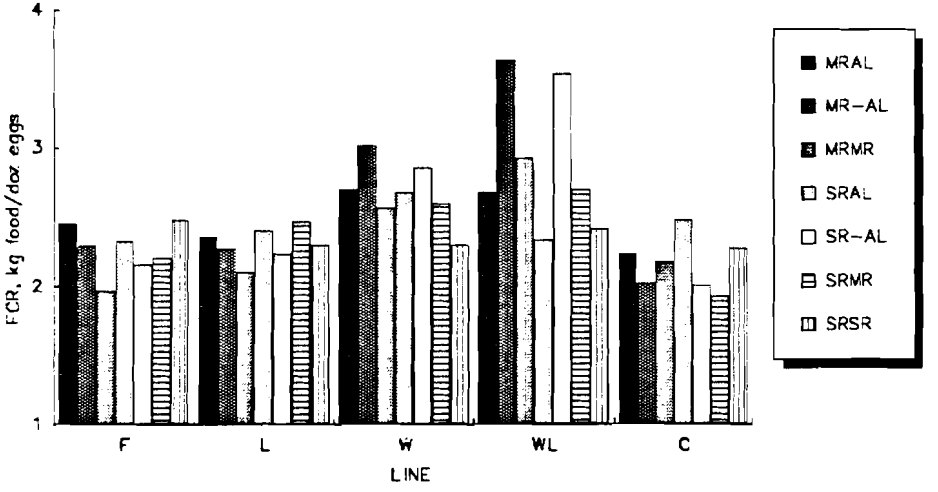

Figure 3. Feed conversion ratio on the seven feeding regimens in each of the five lines.

Line differences in feed efficiency were essentially a reflection of differences in egg production, except for the F line which was as efficient as the L and C lines, due largely to the low food intake of the former line. The relatively good feed efficiency in the F line may be due in part to reduced maintenance requirements in this line associated with their lower body weight or, possibly, to the ready availability and mobilisation of plasma lipid into the developing follicle. The opposite effect on feed efficiency of the SRSR treatment in the F and W lines indicates that the high-fat line is surprisingly intolerant of severe laying period feed restriction. The lower average egg weight of the F line is probably associated, in part at least, with their lower body weight since there was no difference in the proportion of yolk in eggs from the F, W, WL and C lines.

ACKNOWLEDGMENTS

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FACTORS INFLUENCING THE NUTRITIVE VALUE OF WHEAT

G. ANNISON

Summary

The anti-nutritive activity of wheat non-starch polysaccharides (NSP) was studied. The apparent metabolisable energy (AME) of wheat samples correlated closely with levels of water-extractable polysaccharides. Addition of glycanases improved the AME of a wheat-based diet and in vivo activity of the enzymes was detected.

I. INTRODUCTION

Structural components of cereal cell walls which possess anti-nutritive activity when present in broiler diets vary in chemical structure but all are NSP. In barley the major NSP is a (1-3),(1-4)-ß-glucan whilst in rye arabinoxylans (pentosans) predominate. The NSP are the cause of the poor nutritive value of rye and barley for broilers (Antoniou and Marquardt 1981; Classen et al. 1988)

The broiler industry has noted that wheat is of variable quality. Two surveys (Mollah et al. 1983; Rogel et al. 1987) showed that some wheats have low AME (<13MJ/Kg dry matter). Wheats have high levels (50-80 g/kg) of pentosans (Annison 1990) which, when added to diets, depress AME and growth rate (Choct and Annison 1990).

In studying anti-nutritive factors several approaches may be adopted in addition to isolating the factors and adding them back to basal diets. Their activity may be demonstrated by correlating levels in feed with the biological response or, alternatively, the effect of inactivating the factor in situ may be examined. This paper details two experiments in which these alternative approaches were used to investigate the anti-nutritive activity of wheat NSP.

II. MATERIALS AND METHODS

a) Bird management and AME assays

Commercial male broilers obtained at 1 day-old were maintained to 28 days on commercial diets prior to random allocation to treatment groups of eight birds held in individual metabolism cages. The AME was determined using a classical total collection method in which excreta and feed intake were monitored for four days.

b) Experimental diets

Diets were formulated using the ingredients and proportions (g/kg) as follows:- cereal (820); casein.HCl (134); dicalcium phosphate (26); calcium carbonate (11); vitamin premix (5) sodium chloride (3.6); choline chloride (0.4). In Experiment 2, celite (an acid insoluble ash marker, 20g/kg) was added at the expense of wheat.

c) Chemical analyses

The NSP were determined by GLC of alditol acetates of sugars after hydrolysis (Olsen et al. 1988).

The gross energies of diets and excreta were determined using an adiabatic bomb calorimeter (Gallenkamp).

Acid insoluble ash was determined as the residues of ashed samples treated with boiling 4M HCl.

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d) Enzyme Assays

ß-Glucanase activities were determined using a test kit (Biocon Australia Pty. Ltd).

Xylanase activities were determined using a Remazol-Brilliant-Blue xylan substrate (Sigma Chemicals). Release of oligosaccharides was followed spectrophotometrically (Biely et al. 1988)

e) Statistical Methods

Statistical methods as described by Steel and Torrie (1982) were used. In Experiment 1 the relationships between AME and the NSP of wheat were assessed for significance using linear correlation analysis. In Experiment 2 differences between AME and pentosan digestibility of diets were demonstrated using a least significant difference test following analysis of variance. Effects between diets for the levels of ß-glucanase were shown using Kruskal-Wallis analysis of variance. Effects between diets for the levels of xylanase were assessed by testing for the maximum likelyhood of binomial proportions after ranking the enzyme as detectable or not detectable for each bird.

III. EXPERIMENTAL

a) Experiment 1. Relationship between wheat NSP and AME.

Wheats were collected from registered seed suppliers (1989-90 season) at locations across NSW. The wheats were incorporated into diets to determine their AME. Finely ground samples (1g) of the wheats were extracted with hot ethanol (80%v/v, 20ml, 85^{o}C, 10min) and then subjected to a series of aqueous extractions (0.01M NaCl, 10 ml, 40^{o}C, 1h; 0.01M NaCl, 9.5ml, plus 0.5ml thermostable α-amylase, 85^{o}C, 1h; 0.1M NaOH, 10ml, 40^{o}C, 1h). The samples were centrifuged and the supernatants were collected between each extraction. Sugar composition of each extract was determined. Pentosans in the wheats were also determined.

b) Experiment 2. Effect of feed enzymes on wheat AME.

Six wheat diets were prepared as follows. Diet 1, control, no supplementation; Diet 2, Enzyme A (30mg/kg); Diet 3, Enzyme B (20mg/kg); Diet 4, Enzyme C (20mg/kg), Diet 5, Enzyme D (20mg/kg); Diet 6, Enzyme A (15mg/kg). The birds were trained to eat in two feeding periods each day (0900-1000 and 1600-1700) during the AME trial. Feed intake was monitored and all birds consumed normal quantities of feed (80-100g/d). The day following the collection period birds were fed exactly 50g of feed and ileal contents were collected 4 hours later following killing of the birds by lethal injection with pentobarbitone.

IV. RESULTS

a) Experiment 1

The AME values of the wheats studied ranged from 11.3-13.6 MJ/kg dry matter (DM). The AME values did not correlate significantly with the total pentosan levels which ranged from 62.6-70.0g/kg (Table 1). Highly significant correlations between the AME and the levels of pentosans and NSP removed by the first two aqueous extractions were observed.

b) Experiment 2.

Addition of commercial enzyme preparations raised the AME values of the wheat (Table 2). There were no significant differences in the efficacies of the preparations and, in the case of Enzyme A, increasing the level of inclusion did not improve the response. The digestion of polymeric pentosans was improved by all enzymes except Enzyme D. Significant increases in the levels of ileal glycanase activity were observed with elevated ß-glucanase activity in the ileum of birds fed Enzyme B and Enzyme D. Xylanase activity

Table 1. Relationship between AME (MJ/kg DM) and the levels (g/kg) of total pentosans (TP), water-soluble pentosans (WSP), water soluble non-starch polysaccharides (WSNSP) and alkali soluble non-starch polysaccharides (ASNSP) in wheats. Ranges and linear correlations are shown.

AME	TP	WSP	WSNSP	ASNSP
11.3-13.6	62.6-70.0	6.5-7.7	12.6-16.0	55.4-67.0
Linear correlation (r value) with AME				
	-0.25	-0.86	-0.91	0.16
Significance of correlation		P<0.001	P<0.001	

was greatly increased in the ileum of birds fed Enzyme A at both levels. One of the control birds was also found to have high levels of xylanase activity in the ileum.

V. DISCUSSION

The NSP form the main structural components of wheat grain. They are very diverse and include arabinoxylans, arabinogalactans and ß-glucans (Fincher and Stone 1986). Some are readily removed by aqueous extractions whilst others require more vigorous treatment with alkali. In Experiment 1 cold 0.01M NaCL and then hot 0.01M NaCl with α-amylase removed from the wheats a NSP fraction consisting mainly of pentosans which correlated negatively with AME. The small amounts of other polysaccharides extracted also possess anti-nutritive activity as an improved correlation with AME values was obtained when they were included in the regression analysis (Table 2). As the NSP which were removed using alkali extraction did not correlate with AME, it is likely that this class of NSP remains insoluble during passage through the bird and does not contribute to anti-nutritive acitivity. Since most of the pentosans are insoluble, total pentosans levels are not closely correlated to AME. This confirms previous observations (Annison 1990).

The four enzyme preparations were equally efficacious in improving the AME of the wheat. These enzymes break down the NSP, as shown by the pentosan digestibility data. The enzyme activity required to cause a significant increase in AME values is not great as the increased AME value of Diet 5 occurred although no significant effect on pentosan digestibility was detected. The birds were trained to eat during short feeding periods to reduce the variation in the digesta samples. In spite of this great variation in the amount of digesta collected, and in the enzyme activities, was observed. It can be seen from the data that elevated ß-glucanase and xylanase activities were detected in the ileum of birds fed enzyme-supplemented diets. The xylanase activity of Enzyme A was most stable but other enzymes retained activity long enough to have a beneficial effect on AME values. ß-Glucanase activity was highest in the ileum of birds fed diets supplemented with enzymes B and D. All the enzyme preparations possessed both ß-glucanase and xylanase activity when measured in vitro (data not shown). The differences noted are, therefore, the result of differences in the stability of the enzymes when added to feedstuffs and fed to birds. Trained birds store food in the crop giving the enzymes time to act at this site. The low enzymic activity in the ileum associated with some of the treatments suggests that the site of action was in the upper alimentary tract.

Table 2. Effect of enzymes on wheat apparent metabolisable energy (AME) (MJ/kg DM). Pentosan digestibility coefficient (PD), ileal ß-glucanase and xylanase activities are also shown.

Diet	AME	PD	Ileum ß-glucanase	Ileum xylanase
1.	14.26[a1] (0.02)	0.26[a1] (0.02)	2.7 (0.0-6.1;7)[2]	6.3 (0.0-47;1)[2]
2.	15.24[b] (0.12)	0.43[b] (0.02)	2.7 (0.0-5.2;7)	15.2 (0.0-30.2;6)
3.	15.72[b] (0.12)	0.39[b] (0.04)	25.3 (8.7-100.5;8)	0.0
4.	15.29[b] (0.09)	0.37[b] (0.02)	4.7 (0.3-8.7;8)	3.07 (0.0-14.3;3)
5.	15.34[b] (0.12)	0.30[a] (0.02)	27.8 (3.3-163;8)	0.0
6.	15.37[b] (0.28)	0.44[b] (0.02)	3.2 (0.0-5.8;7)	12.73 (0.0-23.0;7)
Significant effects	P<0.01	P<0.01	P<0.01	P<0.01

1. a,b. Values with different superscripts are significantly different (P<0.01). SEM is shown in brackets.
2 Range of values recorded in the group of eight birds; number of birds with detected enzyme activity (ie a number greater than 0) in each group.

The current studies indicate water-soluble NSP (mainly pentosans) play a major role in determining the nutritive value of wheat as their levels correlate with AME and their destruction in vivo improves wheat AME values.

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EFFECT OF ENZYME SUPPLEMENTATION OF WHEAT-BASED DIETS ON THE PERFORMANCE OF BROILER CHICKENS.

J. INBORR* and H. GRAHAM*

Summary

Two experiments, including 108 and 420 broiler chickens respectively, were carried out to investigate the effect of adding multi-activity enzyme mixtures to wheat-based diets. In Experiment 1, three enzyme mixtures were added at 1 g/kg to a basal diet and fed to the birds from one to 21 days of age. In Experiment 2, a single enzyme mixture was added to a wheat-based diet (1 g/kg) and fed in either mash or pelleted form between seven and 28 days of age. A maize-based diet was fed as control. Enzyme supplementation significantly improved feed efficiency in Experiment 1 and in the pelleted diets in Experiment 2 ($P<0.05$). Live weight gain was significantly ($P<0.05$) improved in both experiments by enzyme supplementation.

I. INTRODUCTION

In many countries wheat is the major cereal grain used in feed for broilers. Its high energy value in relation to price makes it very competitive in least cost formulations. In an extensive study Mollah et al. (1983) showed that the energy value of wheat was highly correlated with starch digestibility but not with starch content or hardness. These workers measured the apparent metabolizable energy (AME) value of twenty two samples of 13 wheat cultivars using growing broiler chickens. AME-values varied from 11.0 to 15.9 MJ/kg and starch digestibility correspondingly from 80 to 99%. Neither grinding nor steam pelleting improved the AME values of the wheats.

Wheat having an AME value <13.0 MJ/kg DM (approximately 11.5 MJ/kg) is usually classified as "low-ME wheat" and again it has been found that in-vivo starch digestiblilty of "low-ME wheat" is abnormally low (Annison et al. 1987; Rogel et al. 1987). Despite extensive investigations looking at starch contents, hardness, digestibility of starch isolates and amylase inhibitors, these workers could not establish the factors impairing starch digestibility and hence the ME value of wheat.

Wiseman and Inborr (1990) reported AME values between 13.04 and 15.32 MJ/kg DM for five wheat varieties grown at five different locations in the United Kingdom in 1989. The starch content of the wheat samples ranged from 650 to 692 g/kg DM. Starch digestibility was not measured, but it should be said that in some cases individual birds responded adversely and there was evidence of poor digestibility, particularly when wheat was fed in mash form. Extremely low AME values thus arising were excluded from the calculations. Furthermore, Wiseman (1989) reported that broiler chickens fed a barley-based diet utilized the food significantly better

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up to 10 days of age than when the barley was substituted with wheat.

Results from the above studies suggest that wheat may not be well utilised in the growing chick. Whether this is a consequence of digestive inadequacy (starch, protein and other nutrients being inefficiently hydrolysed by host endogenous enzymes as a result of morphological or structural barriers in wheat), qualitative and quantitative limitations of the digestive enzymes, or interference in nutrient digestion and absorption by soluble cell-wall polysaccharides (e.g. pentosans) has not been established. Whichever the case, the use of supplementary exogenous enzymes may be a way to overcome these effects. In fact, Helander and Inborr (1989) reported increased AME values of wheats up to 22% when whole diets were supplemented with various fungal and bacterial enzymes. On the other hand, enzyme supplementation of wheat-based diets to improve broiler performance has met with limited success (Broz and Frigg 1986; Pettersson and Åman 1988; Edeney et al. 1988). The poor responses may be due to the enzymes used not containing appropriate concentrations of activities needed to break down the components interfering with digestion.

The objectives of the experiments reported here were to investigate the effects of adding especially designed multi-activity enzyme mixtures to wheat-based diets on the performance of broiler chickens.

II. METHODS

Experiment 1

One-day-old broiler chicks (144) of a commercial strain were randomly allotted to pens of six birds each (Jamesway battery brooders) and fed one of the four experimental wheat-based diets for 21 days. There were six pens per treatment. Initial room temperature was 32°C and decreased with time to maintain bird comfort (automatic control system).

The diets were fed ad libitum in mash form and water was freely available from nipple drinkers at all times. Three enzyme mixtures (E1, E2 and E3) were added to the basal diet at 1.0 g/kg (Table 1). E1 contained pentosanase and cellulase from *Trichoderma longibrachiatum*, α-amylase and protease from *Bacillus subtilis* and pectinase from *Aspergillus niger*. E2 had a similar composition to E1 but with no added protease, whereas E3 contained pentosanase, β-glucanase and cellobiase (β-glucosidase) from *A. niger*.

The birds were weighed on days one and 21. All feed additions were recorded and feed not consumed was weighed back at the end of the experiment.

Experiment 2

From a total of 750 one-day-old male Hybro chicks two batches of 210 birds were divided into uniform groups and placed in cages. After a seven-day acclimatization period the birds were randomly assigned to the experimental diets with 10 replicates (cages) of seven birds per diet. Three diets - one maize-based and two wheat-based - were fed in mash form to the

first, and in pelleted form (pelleting temperature 80°C) to the second, batch of birds. The diets were formulated to be isoenergetic and isonitrogenous (Table 1). One of the wheat-based diets was supplemented with a multi-activity enzyme mixture (1 g/kg) containing pentosanase (xylanase) and cellulase from T. longibrachiatum, α-amylase from B. subtilis and pectinase from A. niger, whereas the other wheat-based and the maize-based diet formed the negative and positive controls, respectively.

During the acclimatization period all birds were fed the same feed (100 g/bird). The experimental diets were fed ad libitum from day 7 to 28 and the birds weighed at the beginning and end of the 21-day experimental period. Feed consumption was monitored and additions recorded. Feed not consumed was weighed back at the end of the experiment and recorded.

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

	Experiment 1	Experiment 2	
		Maize-based	Wheat-based
Ingredient			
Wheat	638.4	-	605.0
Maize	-	550.2	-
Soybean meal (48)	214.2	-	-
Soybean meal (45)	-	328.7	186.9
Full fat soya	-	50.0	108.5
Canola meal	30.0	-	-
Meat meal	50.0	-	-
Fishmeal	-	2.0	30.0
Canola oil	40.0	-	-
Fat blend	-	35.3	34.4
Limestone	8.0	6.8	6.3
Dicalcium Phosphate	9.7	18.4	13.5
L-lysine	-	-	2.9
DL-methionine	2.0	0.1	-
Salt	3.2	2.5	2.5
Vitamin mix	2.0	4.5	4.5
Mineral mix	2.0	4.5	4.5
Calculated nutrients (/kg)			
Crude protein (g)	222.0	211.0	211.0
ME, MJ	126.0	130.0	130.0
Lysine (g)	11.1	12.0	12.0
Methionine + Cystine (g)	8.6	8.3	8.3
Calcium (g)	9.2	8.7	8.7
Available Phosphorous (g)	5.2	4.5	4.5

[1] Enzyme mixtures added at 1.0 g/kg at the expense of wheat.

III. RESULTS

Experiment 1

Enzyme supplementation significantly improved feed utilization ($P<0.05$) of all treatments (Table 2). Live weight at day 21 and average weight gain was also significantly improved ($P<0.05$) on treatment 4 (E3). Enzyme supplementation had no effect on feed intake.

Table 2. Effect of enzyme treatment on live weight (g), average weight gain (g), feed consumption (g) and feed conversion efficiency (FCE, g:g) of chicks (0-21 days of age) fed wheat-based diets.

Enzyme	Weight at 21d	Weight gain	Feed consumption	FCE
Control	587^{b}	549^{b}	852	1.519^{b}
E1	608^{ab}	570^{ab}	825	1.434^{a}
E2	592^{ab}	554^{ab}	819	1.422^{a}
E3	619^{a}	581^{a}	869	1.451^{a}

^{ab} Mean values within columns that do not share a common superscript are significantly different ($P<0.05$).

Experiment 2

At 28 days of age, live weight of birds fed the enzyme supplemented diet was significantly ($P<0.05$) higher than that of the negative control on both mash and pelleted diets (Table 3).

Table 3. Effect of replacing maize with wheat, and enzyme supplementation of a wheat-based diet, on live weight (g), average weight gain (g), feed consumption (g) and feed conversion efficiency (FCE, g:g) of chicks (7-28 days of age).

Treatment	Weight at 28d	Weight gain	Feed consumption	FCE
Mash				
Maize	879^{ab}	781^{ab}	1712^{a}	1.95^{a}
Wheat	857^{b}	759^{b}	1648^{a}	1.93^{a}
Wheat + enzymes	903^{a}	805^{a}	1683^{a}	1.86^{a}
Pellets				
Maize	911^{b}	811^{b}	1596^{a}	1.75^{b}
Wheat	903^{b}	803^{b}	1584^{a}	1.75^{b}
Wheat + enzymes	981^{a}	881^{a}	1607^{a}	1.64^{a}

^{ab} Mean values within mash or pellets, that do not share a common superscript are significantly different ($P<0.05$).

Enzyme supplementation also improved feed conversion efficiency ($P<0.05$) in birds fed the pellets compared to both control diets. This was not the case in the mash fed birds. Birds fed the maize-based diet in mash form performed equally

to the other two treatments, whereas when fed the pellets, performance was inferior to the ones on the enzyme supplemented diet.

IV. DISCUSSION

Soluble mixed-linked ß-glucans in barley and arabinoxylans (pentosans) in rye and triticale have been found to increase extract and digesta viscosity, thus impairing nutrient digestion and absorption in poultry (Hesselman 1983; Antoniou et al. 1981; Pettersson and Åman 1989). Addition of appropriate enzymes to diets containing these cereal grains has been shown to improve nutrient digestibility and bird performance (Hesselman 1983; Classen et al. 1988; Edeney et al. 1989; Pettersson 1988). From these studies it can be concluded that gut content viscosity is an important factor influencing the nutritive value of e.g. barley, rye and triticale. The content of soluble fibre in wheat is considerably lower than in e.g. rye, and so factors other than viscosity may be responsible for the poor utilisation of e.g. starch found in so called "low-ME wheats". Relative digesta viscosity in birds fed wheat was 10 to 20 times lower than in rye-fed birds. Pentosanase addition to the diets significantly improved the performance of the rye-fed but not the wheat-fed birds (Bedford et al. Unpublished results). Similar responses have also been reported by others (Broz and Frigg 1986; Pettersson 1988).

In these experiments, addition of enzyme mixtures possessing a wider activity spectrum than in previously reported performance trials with broiler chicks, resulted in significantly higher body weights and improved feed utilisation ($P<0.05$). This indicates that factors other than fibre may limit the utilisation of wheat in the chick. Helander and Inborr (1988) reported significantly increased AME values of wheats supplemented with a combination of xylanase (pentosanase), amylase and protease. These wheats would have been classified as "low-ME" (Annison et al. 1987), and may have been more susceptible to enzyme treatment. The wheats used in the experiments reported here were feed grade wheats for use in commercial broiler feeds. Although the non-starch polysaccharide (NSP) content may vary between wheat samples (Åman 1987), these results suggest that not only fibre-degrading but also starch and protein-degrading enzymes may be needed to improve the nutritive value of wheat in broiler chickens. However, the effect of these enzyme activities on other components in the feed must not be neglected.

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PREVENTION OF SALINE WATER-INDUCED INCREASES IN EGG SHELL DEFECTS

D. BALNAVE

Summary

Various procedures have been examined as means of preventing the increase in egg shell defects which occurs when hens receive saline drinking water. The effect on shell formation appears to be permanent in affected hens so that treatments have to be preventive rather than remedial in nature. Supplementation of the diet or drinking water with ascorbic acid shows greatest promise although such supplements must be given from the time hens receive the saline water. Desalination seems the only alternative.

INTRODUCTION

During the past five years some 30 experiments have been carried out at Camden which have shown consistently that the presence of sodium chloride (NaCl) in drinking water, at between 0.2 and 2.0 g/l, significantly increases the incidence of egg shell defects and significantly decreases egg shell quality. While the problem has been clearly identified attempts to overcome it have been less successful. The present paper summarises the value of procedures with potential for alleviating the problem.

Replacement of saline water with town water

This procedure was examined in four separate studies (Table 1).

Table 1. Egg shell defects during NaCl supplementation of drinking water and prior to and after a rest from lay on town water (Balnave and Scott 1986; Balnave and Yoselewitz 1987; Balnave *et al.* 1989).

Source of drinking water	Time on NaCl	Shell defects	Town water	Shell defects prior to rest from lay	Town water	Shell defects after rest from lay
	(wks)	(%)	(wks)	(%)	(wks)	(%)
Town water	6	3.1[1]		6.8**		
NaCl(0.25g/l)	6	6.5***	4	13.2		
Town water	6	3.7**		3.8[2]		3.2[2]
NaCl(0.25g/l)	6	6.4	4	11.3	11	7.1
Town water	5	6.8***		6.1**		4.7
NaCl(0.2g/l)	5	11.3***	5	9.3***	15	6.3***
(0.4g/l)	5	15.0***	5	10.0***	15	9.1***
(0.6g/l)	5	20.5	5	13.1	15	15.1
Town water	5	6.2***		5.9[2]		
NaCl(0.6g/l)	5	11.8***	4	11.3		
(2.0g/l)	5	19.9	4	17.2		

[1] Significantly different to birds on town water: ** P<0.01; *** P<0.001.
[2] Mean value for all birds on each treatment without replication.

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The results indicate that the withdrawal of NaCl-containing drinking water had little effect on the incidence of egg shell defects. In two experiments the replacement of the saline drinking water with town water was extended through a period during which the hens were rested from lay. This gave little benefit except where relatively low salt concentrations (up to 0.25 g/l) had been used.

In a separate study hens receiving saline water during early lay showed an initial high incidence of egg shell defects which returned to control values five weeks after the withdrawal of the saline water. However, after 55 weeks of age these hens showed a much greater incidence of shell defects than hens which had received only town water throughout lay.

Removal of NaCl supplement from the diet

This procedure was attempted in one experiment the results of which are shown in Table 2. Even though this procedure significantly reduced egg shell defects at the highest NaCl concentration the incidence of shell defects was still some three-fold higher than in hens receiving town water.

Table 2. Effect of removing the NaCl supplement from the diet on egg shell defects of hens receiving NaCl supplements in the drinking water (Yoselewitz and Balnave 1989a).

Dietary NaCl supplement (g/kg)	NaCl supplement in drinking water (g/l)	Shell defects[1] (%)
2	-	6.1
2	0.5	13.1
1	0.5	12.2
2	1.0	18.1
0	1.0	16.8
2	2.0	28.1
0	2.0	21.1

[1] Treatment effect significant ($P<0.001$)

Additional dietary supplementation with calcium carbonate

Egg producers who are experiencing poor egg shell quality normally supplement the diet with additional calcium. In a short-term study 1% ground limestone added to a commercial diet had no significant influence on the high incidence of egg shell defects resulting from the prior use of saline water.

Effect of bicarbonate supplementation of drinking water

Blood measurements and metabolic studies of shell gland function, suggested that a deficiency in the supply of bicarbonate ions to the lumen of the shell gland may be the limiting factor in hens receiving saline water and a reduced activity of carbonic anhydrase in shell gland tissue supported this hypothesis (Yoselewitz and Balnave 1989). Therefore, the value of supplementing saline drinking water with bicarbonate ions was examined (Table 3). Supplementation of saline water with NH_4HCO_3 substantially

reduced the incidence of egg shell defects. This effect was greater than that shown by $NaHCO_3$ but was accompanied by a substantial reduction in water consumption, an effect not observed when NH_4HCO_3 was added to town water. This suggests that NH_4HC0_3 has limited value since low water intakes can cause losses in egg output.

Table 3. Effect of bicarbonate supplementation of drinking water on egg shell defects of hens receiving NaCl in the drinking water (Yoselewitz *et al.* 1991).

Supplement to town water	Concentration (g/l)	Shell defects (%)	Concentration (g/l)	Shell defects (%)
	Experiment 1		Experiment 2	
None	-	5.6	-	7.3
NH_4HCO_3	0.45	6.5***[1]	0.25	7.6***
NaCl	0.60	24.2***	0.60	17.5***
NaCl + NH_4HCO_3 [2]	0.60) 0.45)	16.9***	0.60) 0.25)	10.0
$NaHCO_3$	0.50	10.8***		
NaCl + $NaHCO_3$	0.60) 0.50)	20.6***		

[1]Significantly ($P<0.001$) different to hens given town water.
[2]Reduction in water consumption with this treatment.

Effect of ascorbic acid on egg shell defects

Ascorbic acid is a vitamin which is known to have beneficial effects in poultry exposed to environmental or nutritional stress. Although responses in individual experiments have been inconsistent egg shell quality is one factor which responds to ascorbic acid (Pardue and Thaxton 1986). Accordingly ascorbic acid has been evaluated as a means of overcoming the detrimental effects of saline drinking water on egg shell quality. The results of two dose-response studies in which the ascorbic acid was added to either the drinking water or the diet are shown in Table 4.

The increased incidence of egg shell defects associated with the use of saline drinking water was prevented by simultaneous supplementation of the diet or drinking water with ascorbic acid. Both routes of supplementation were equally effective in reducing shell defects to control values observed with hens receiving town water.

We have observed that supplying ascorbic acid to hens already affected by saline drinking water does not reduce the high incidence of egg shell defects. Therefore, the ascorbic acid acts as a preventive rather than a remedial treatment.

CONCLUSIONS

A large number of experiments have shown conclusively that egg shell defects increase when hens receive drinking water containing NaCl at concentrations similar to those present in many Australian bore waters. However, attempts to overcome the problem have generally proved unsuccessful. The most reliable procedure appears to be desalination of the drinking water but this could prove expensive on a large scale.

Although only a proportion of hens in a flock are adversely affected by saline drinking water the effect appears to be permanent. Any preventive

Table 4. Influence of ascorbic acid (AA) supplementation of saline drinking water (Experiment 1) or diet (Experiment 2) on egg shell defects from hens receiving saline drinking water (Balnave *et al.* 1991; Balnave and Zhang, Unpublished results).

Experiment 1		Experiment 2	
Treatment	% defects	Treatment	% defects
Town water	12.9[a]	Town water	5.3[a]
Saline water		Saline water	
+ AA (0 g/l)	27.0[b]	+ AA (0 g/kg)	16.1[b]
(0.25 g/l)	19.3[ab]	(0.2 g/kg)	8.2[a]
(0.5 g/l)	16.7[ab]	(0.8 g/kg)	8.7[a]
(1.0 g/l)	13.4[a]	(1.4 g/kg)	7.0[a]
		(2.0 g/kg)	5.2[a]

Data with no common superscripts are significantly different ($P<0.05$)

treatment must, therefore, be given from the time laying hens first receive saline drinking water. Presently, the best possibility appears to lie with the use of ascorbic acid as a supplement to the diet or drinking water. Our data suggest that the incidence of egg shell defects will be maintained at control levels when supplements of ascorbic acid are used at concentrations of 1 g /l of drinking water or 2 g/kg of diet. Lower concentrations by either route of supplementation will decrease the incidence of egg shell defects observed when saline drinking water is provided. The actual concentrations used will depend on the extent of the egg shell quality problem and the economic gains to be made from the use of ascorbic acid.

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LINSEED AND LINOLA™ MEAL IN THE DIETS OF BROILERS AND LAYERS

D.J. FARRELL* and A.G. GREEN**

Summary

In three experiments with broilers and layers, linseed and Linola™ meals were examined. Additions of vitamin B_6 to a diet with 100g linseed meal/kg did not improve broiler performance. Linseed and Linola meals did not impair chick performance at 50g/kg but depressed performance at 100g/kg. Enzyme additions to diets with 100g linseed meal/kg improved feed efficiency but not to diets with Linola meal. Performance of layers was similar on diets with graded levels of Linola and linseed meals from 0 to 75g/kg.

1. INTRODUCTION

Linseed meal has been used in livestock feeding and is a by product of flax (Linum usitatissimum). The oil in flax (linseed oil) is largely linolenic acid (52%). This oil is highly susceptible to oxidation and is not widely used in the food industry. A mutant type of flax has been developed that produces a low linolenic acid seed oil (<2%) and contains over 70% linoleic acid (Green 1988). This new oil seed grain, Linola™ is a yellow seed unlike the dark brown seed of flax. When extracted for oil, Linola meal is produced.

Linseed meal is known to be less suitable for pigs and poultry than for ruminant livestock. It contains a vitamin B_6 antagonist, linatine (Klosterman et al. 1967), and diets may need to be fortified with both vitamin B_6 and zinc. It has a high content of mucilage which reduces energy yield and may cause sticky droppings in poultry.

This paper describes three experiments with poultry aimed at evaluating Linola meal and comparing it with conventional linseed meal.

II. MATERIALS AND METHODS

Male chicks of a commercial strain were placed in compartments of electrically heated brooders with wire-mesh floors. Each compartment had individual feed and water troughs. Brooding temperature was adjusted according to age of bird and illumination was continuous. Diets were formulated to SCA (1987) specifications, using conventional ingredients and a least-cost computer program. Feed intake and bodyweight were recorded weekly.

The chemical composition of the linseed and Linola meals produced by pre-press solvent extraction, were determined following standard procedures (AOAC 1980). Amino acids were determined using an amino acid analyser. Metabolizable energy (ME) of the meals were determined using the modified rapid method of Farrell (1978).

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Data were subjected to an analysis of variance. Means were tested using the Least Significance Differences test.

The aim of Experiment 1 was to determine the response of broiler chickens to additions of vitamin B_6 (2, 5, 10, 20 and 30 mg/kg diet) to a diet containing 100 g/kg of linseed meal. The diet was offered in meal form for three weeks to each of three groups of 8 male broiler chicks starting at 1-day of age.

The aim of Experiment 2 was to measure the effects on chick performance of three inclusion levels (0, 50 and 100 g/kg diet) of linseed and Linola meal with or without the addition of three different dietary enzymes and fortified with 20 mg vitamin B_6/ kg diet. The experimental design was two levels of linseed meal and two levels of Linola meal with or without one of three enzymes. The control diet contained either no enzyme or each of the three enzymes individually included at recommended levels. There were 20 treatments x 4 replications x 8 chicks. Diets were fed in mash form and chicks were grown from 1-day-old to 21 days of age.

In Experiment 3, three groups of nine (3 birds/cage) bantamised layers (Stanhope and Parkinson 1988) were allocated at 22 weeks of age to one of seven diets. These diets contained 25, 50 and 75g/kg of either Linola meal or linseed meal and fortified with 20 mg vitamin B_6/kg in a least-cost formulated diet. A commercial layer diet (170g crude protein/kg) was used as a control.

Egg production was recorded daily, eggs were weighed weekly and egg specific gravity monthly. Feed consumption was recorded every four weeks. Prior to the end of the experiment various quality tests were made, on two occasions, to determine egg and shell quality. Birds were weighed at the start and finish of the experiment, which commenced in November 1989 and terminated in May 1990.

III RESULTS AND DISCUSSION

The ME (MJ/kg DM) was 7.42 and 6.55 for linseed and Linola meal respectively (Table 1). Crude protein (g/kg/DM) was slightly higher for Linola (375) than for linseed meal (357). Amino acid profiles were similar between the two meals.

Table 1. Metabolizable energy (ME, MJ/kg DM), crude protein (CP, g/kg DM) and some amino acid (g/kg DM) profiles for linseed and Linola meals.

	ME	CP	Lys.	Thr.	Meth.	Meth. + cyst.	Trypt.	Isol.
Linseed meal	7.42	357	14.3	14.2	7.4	13.6	6.4	16.9
Linola meal	6.55	375	13.2	13.8	6.5	12.8	5.7	16.3

The results of vitamin B_6 supplementation of a diet containing linseed meal (Experiment 2) showed no beneficial effect at any level of inclusion (Table 2). Additional vitamin B_6 supplementation (>2mg/kg) to diets containing linseed or Linola meal may be unnecessary.

Table 2. Feed intake (g/d), gain (g/d) and feed conversion ratio (FCR) of chicks grown for 21 d on a diet with 100g linseed/kg meal at additions of vitamin B6 (Expt.1).

B6 (mg/kg)	Gain	Feed intake	FCR
2	15.4	28.8	1.87
5	12.2	30.0	1.87
10	15.5	29.9	1.94
20	15.5	30.8	1.99
30	14.9	29.7	1.99
SEM	0.51	0.48	0.076

There were effects (P<0.01) of diet and enzyme on FCR, but not on feed intake (Table 3). Growth rate was influenced by diet. Inclusion of linseed or linola meal at 100 g/kg, but not at 50g/kg reduced (P<0.05) growth rate and increased FCR, without effecting feed intake. Multizyme (an enzyme mixture), cellulase and beta-glucanase all significantly improved FCR.

Table 3. Main effects of diet and enzyme type on growth rate (g/day), feed intake (g/day) and feed conversion ration (FCR) of broiler chicks grown from 1 to 21 days of age (Expt. 2).

		Linola meal (g/kg)		Linseed meal (g/kg)		LSD
	Control	50	100	50	100	(P<0.05)
Growth	28.8	27.5	20.3	27.0	23.1	2.16
Feed	44.0	44.9	42.2	43.0	42.0	2.77
FCR	1.53	1.64	2.11	1.60	1.85	0.125

	Enzyme				
	0	multizyme	cellulase	beta-glucanase	
Growth	24.1	26.2	25.6	25.4	1.93
Feed	44.3	42.5	42.8	43.1	2.48
FCR	1.87	1.67	1.69	1.75	0.112

There was a diet x enzyme interaction (P=0.09). All enzymes improved (P<0.05) FCR when added to the diet with 100 g linseed meal/kg diet. At 50g linseed meal/kg both multizyme and cellulase improved (P<0.05) FCR. Linola meal diets did not respond to enzyme additions. The reason for those differences between the two meals which are similar in proximate chemical analysis, is uncertain.

Production parameters for the layer experiment are given in Table 4. Due to an error at feed mixing, there was no 50 g Linola meal/kg treatment. For hen-day production, average egg weight and feed intake, there were no treatment effects (P<0.05). Linseed meal inclusion at 75g/kg tended to give larger eggs when compared with lower inclusions. This trend was particularly apparent in May when the egg quality tests were made. These tests did not show major differences between treatments, particularly when compared to the control diet. As expected egg specific gravity declined over the experimental period. Bodyweights of hens were not different between treatments, they increased from 1.31 kg initially, to 1.48 kg at the end. Mortality, mainly due to prolapse, was similar on all treatments at 1.3 deaths per group of nine birds, or 14%.

Table 4. Hen-day egg production, average egg weight(g) and daily feed intake(g) on linseed (LI) and Linola (LO) meal-based diets (Expt. 3).

		NOV	DEC	JAN	FEB	MARCH	APR	MAY
g/kg				Eggs laid per hen-day				
25	LO	0.81	0.73	0.71	0.65	0.66	0.57	0.55
75	LO	0.79	0.75	0.70	0.62	0.67	0.55	0.56
25	LI	0.80	0.78	0.74	0.71	0.63	0.62	0.54
50	LI	0.85	0.76	0.70	0.68	0.67	0.61	0.54
75	LI	0.82	0.79	0.78	0.70	0.65	0.63	0.51
CONTROL		0.86	0.86	0.80	0.72	0.68	0.62	0.57
SEM		0.050	0.051	0.048	0.064	0.058	0.083	0.067
g/kg				Average egg weight (g)				
25	LO	49.5	52.1	53.6	55.4	57.4	57.8	61.7
75	LO	49.7	52.8	54.8	56.8	60.0	58.8	59.2
25	LI	49.7	52.9	53.4	55.4	56.1	58.5	59.3
50	LI	47.9	53.2	53.7	54.8	56.2	58.2	57.6
75	LI	53.0	55.0	56.3	58.8	60.1	62.8	63.6
CONTROL		53.8	53.4	55.3	57.1	57.6	61.6	59.6
SEM		1.51	0.91	1.05	1.01	1.31	1.94	1.84
g/kg				Mean daily feed intake (g)				
25	LO	82.5	84.7	86.3	87.0	88.1	85.3	95.4
75	LO	81.9	86.8	88.5	87.3	94.3	93.7	99.6
25	LI	81.8	89.7	88.5	86.8	87.1	93.8	103.1
50	LI	84.8	90.2	87.8	90.2	95.8	96.6	97.2
75	LI	88.4	92.0	95.6	96.0	93.1	98.6	102.9
CONTROL		84.1	95.6	94.8	94.9	96.9	97.5	102.5
SEM		2.91	3.48	2.72	5.72	3.56	5.69	5.71

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THE EFFECT OF DIETARY COMBINATIONS OF GRAIN LEGUMES ON THE GROWTH PERFORMANCE OF BROILER CHICKENS

R.J. JOHNSON* and P.J. EASON

Summary

Field peas (Pisium sativum), lupins (Lupins angustifolius), faba beans (Vicia faba) and common vetch (Vicia sativa) were tested at various levels and in different combinations in diets for broiler chickens. Birds were grown under practical conditions on wheat/sorghum and soybean meal diets. Results showed that all the grain legumes included at 50 or 150 g/kg tended to depress growth and feed efficiency in young broilers to 21 d of age.

However, there were no significant effects to 42 d of age, except for common vetch which reduced ($P<0.05$) liveweight and increased ($P<0.05$) the feed conversion ratio. Inclusion of field peas at 150 g/kg reduced liveweight at 42 d of age by 3%. Combinations of lupins and field peas, and lupins, field peas and faba beans did not appear to have additive negative effects on broiler performance.

I. INTRODUCTION

Grain legumes are assuming increasing importance in broiler feeds within Australia, coincident with a marked increase in crop production (Johnson and Eason 1990). Although there have been a number of studies on the effects of grain legumes for poultry, there is little information on the effects of various combinations of grain legumes fed together in a single diet. This could have relevance because the predominance of antinutritional factors varies dependent on the species. As such, a combination of grain legumes in a diet may produce some form of additivity in terms of the effects of antinutritional factors on broiler growth. The present study was carried out to further examine the effects of grain legumes and of various combinations of grain legumes on broiler growth.

II. METHODS

(a) Birds and Their Management

Broiler chickens were obtained from a commercial hatchery at day-old and placed on a deep litter of wood shavings in a temperature-controlled shed. Growing conditions and management were as described by Johnson and Eason (1990).

(b) Grain Legumes

The chemical composition of the five grain legumes and soybean meal used in the growth trial is given in Table 1. Lupins (Lupinus angustifolius), were obtained from Victoria (Vic) and Western Australia (WA), field peas (Pisium sativum), faba beans (Vicia faba) and common vetch (Vicia sativa) were grown in Victoria. The commercial soybean meal was solvent-extracted (Carghill).

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Table 1. Composition of the grain legumes.

Constituent (g/kg air-dry)	Lupins Vic	Lupins WA	Field Peas	Faba Beans	Common Vetch	Soybean Meal
Dry Matter	924	927	899	893	889	886
Protein[1]	309	294	260	228	263	442
Fat[2]	65	57	11	11	8	20
Fibre[3]	183	222	44	117	68	79
Ash	27	22	23	26	25	58
GE (MJ/kg)[4]	18.4	18.3	16.8	16.4	16.6	17.5
AME (MJ/kg)[5]	9.6	7.2	10.8	11.0	12.2	10.8
Lysine	14.7	13.4	14.7	14.0	15.8	27.8
Methionine	2.5	2.4	2.5	1.9	2.3	6.5

[1]. N x 6.25. [2]. Ether extract. [3]. Acid-detergent fibre. [4]. Gross Energy. [5]. Apparent metabolizable energy (AME) determined using a rapid broiler assay (Johnson 1987).

(c) Treatments

Two samples of lupins (from Victoria and Western Australia), field peas, faba beans and common vetch were included in typical wheat-sorghum, least-cost broiler starter and finisher diets, predominantly at the expense of soybean meal. Dietary treatments are given in Table 2. The main objective was to test out two levels of lupins (Vic) and field peas, 50 and 150 g/kg, and then to combine lupins and field peas at both these levels. Each dietary treatment was fed to four replicate groups of 34 mixed-sex broiler chickens. Grain legumes and other major dietary ingredients were analysed for proximate composition, amino acids and AME prior to formulation of the diets. All diets contained a similar nutrient content. Starter diets (0-21 d) contained (/kg): 12.7 MJ AME, 12.5 g lysine, 5.6 g methionine, 9.3 methionine and cystine, 7.5 g threonine, 8.7 g isoleucine, 10 g calcium, 4.4-5.6 g available phosphorus. Finisher diets (21-42 d) contained (/kg): 13.0 MJ AME, 11 g lysine, 5.0 g methionine, 7.7 g methionine and cystine, 7.7 g threonine, 7.7 g isoleucine, 10 g calcium and 4.7-5.5 g available phosphorus. Diets were cold-pelleted with pellet diameters of 3.18 mm (starter) and 3.97 mm (finisher).

(d) Measurements

Feed intake was measured over 7-day periods. Liveweight was measured at day-old, 21 days and 42 days of age without prior feed withdrawal. Mortality was recorded and causes diagnosed. Litter moisture was determined at 39 days of age by taking random samples from each pen followed by oven-drying at 80°C for 5 days.

III. RESULTS AND DISCUSSION

Results are given in Table 3. There were no significant effects of diet on mortality. There were significant ($P<0.001$) effects of dietary inclusion of grain legumes and combinations of grain legumes on liveweight and feed conversion to 21 d of age compared with the controls. However, by 42 d of age many of these effects were markedly reduced, particularly with regard to feed efficiency.

There was a significant ($P<0.05$) reduction in liveweight at 21 d and 42 d of age in birds fed the diet which contained common vetch, associated with a lower ($P<0.05$) feed intake. Litter moisture was also higher ($P<0.05$) for birds fed the common vetch diet. Similar to our previous study (Johnson and Eason

Table 2. Dietary treatments which contained different levels and combinations of grain legumes.

Diet+	Grain Legume (g/kg)					
	Soybean Meal	Lupins (WA)	Lupins (Vic)	Field Peas	Faba Beans	Common Vetch
1	200	-	-	-	-	-
2	88	100	-	-	-	-
3	128	-	-	50	-	-
4	138	-	-	150	-	-
5	164	-	50	-	-	-
6	106	-	150	-	-	-
7	179	-	-	-	50	-
8	126	-	-	-	150	-
9	90	-	150	50	-	-
10	62	-	50	150	-	-
11	114	-	50	50	50	-
12	120	-	-	-	-	150

+Other major dietary ingredients were wheat, sorghum, fish meal, blood meal, tallow and sunflower meal.

1990), inclusion of field peas (150 g/kg) reduced liveweight at 42 d by about 3% due to a 5% reduction in feed intake. This is probably due to a heat-labile antinutritional factor (Moran et al. 1968). Inclusion of lupins (Vic) and faba beans at 150 g/kg tended to reduce liveweight at 42 d of age by about 2%. Although not significant, these results would have an economic effect on broiler production, particularly with the inclusion of lupins (Vic) at 150 g/kg which resulted in a liveweight reduction of 45 g and an increase in feed conversion to 1.75 kg liveweight of 6 points.

Faba beans included at 150 g/kg tended to depress liveweight at both 21 d and 42 d of age, due predominantly to a reduction in feed intake. This effect has been found previously and is possibly due to condensed tannins (Martin - Tanguy et al. 1977), lectins (Rabio et al. 1990) or a heat-labile factor (Campbell and Marquardt 1977). Some interaction was evident with the different grain legume combinations. Inclusion of lupins (Vic) and field peas at 150 g/kg and 50 g/kg respectively gave a 3% increase in liveweight at 42 d and a slight improvement in feed conversion compared with lupins (Vic) at 150 g/kg. However, there was no evidence of significant negative effects on performance due to the combination of two or more grain legumes in the same diet for broiler chickens.

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Table 3. The effects of dietary inclusion of grain legumes on broiler growth and efficiency.

Parameter	Diet† 1 Soy 200	2 LupW 100	3 Pea 50	4 Pea 150	5 LupV 50	6 LupV 150	7 Faba 50	8 Faba 150	9 LP 200	10 PL 200	11 LPF 150	12 Vetch 150	Sig‡	SEM§ (df=33)
Liveweight (g)														
21 d	823	802	819	797	806	810	804	786	802	787	798	778	***	5.78
42 d	2285	2272	2270	2215	2250	2240	2255	2234	2306	2256	2253	2130	*	26.52
Feed Intake (g/bird)														
0-21 d	51.4	51.4	51.3	51.2	52.0	53.5	51.8	50.9	52.2	51.6	52.8	50.0	***	0.43
21-42 d	144.8	142.2	142.6	136.9	140.5	141.8	140.2	137.8	146.4	142.7	143.4	131.5	**	2.19
Feed conversion ratio (g feed/g gain)														
0-21 d	1.39	1.43	1.39	1.43	1.44	1.47	1.43	1.45	1.45	1.46	1.47	1.43	***	0.01
21-42 d	2.08	2.03	2.06	2.03	2.04	2.08	2.03	2.00	2.04	2.04	2.07	2.04	NS	0.03
0-42 d	1.82	1.82	1.83	1.81	1.82	1.87	1.82	1.80	1.83	1.84	1.85	1.82	NS	0.02
to 1.75kg	1.67	1.68	1.67	1.68	1.69	1.73	1.68	1.68	1.69	1.71	1.72	1.71	NS	0.02
Mortality (%)														
0-42 d	7.35	3.68	2.21	5.15	2.94	1.47	4.41	2.94	4.41	3.68	4.41	3.68	NS	0.46
Litter moisture (%)														
39 d	27.9	26.6	28.3	26.1	27.0	26.7	26.6	26.3	26.4	25.4	27.6	30.9	NS	1.07

† See Table 2 for description of dietary treatments.
‡ Significances were NS, not significant, *, P<0.05, **, P<0.01, ***, P<0.001.
§ Standard error of a mean.

THE USE OF NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) TO MEASURE METABOLIZABLE ENERGY FOR POULTRY

R.J. JOHNSON*, P.J. EASON AND P.C. FLINN**

Despite the development of rapid metabolizable energy (ME) bioassays for broiler (Johnson 1987) and adult (Farrell 1978) poultry there remains, in particular, an urgent need for in vitro assays to provide an immediate estimate of ME for incoming raw materials at the feedmill level. Near-Infrared Reflectance Spectroscopy (NIRS) is based on the fact that each of the major chemical components of a feed sample has near-infrared absorption properties which can be used to differentiate one component from the others. NIRS has major advantages, in terms of speed and simplicity of sample preparation. The present study was undertaken to examine the use of NIRS to measure the ME of a range of feed ingredients for both broiler chickens and adult cockerels.

One hundred and fifteen samples of feed ingredients ranging from cereals (N=44), vegetable protein meals (N=27), animal protein meals (N=33) and cereal by- products (N=10) were assayed for apparent ME (AME) using rapid broiler and adult cockerel assays. Descriptions of the methods were given previously (Johnson 1987). Each sample was also subjected to analysis by NIRS using a variable wavelength spectrophotometer and calibration equations were developed. Results are given in the Table.

Prediction equations for in vivo apparent metabolizable energy for broilers and adult cockerels by NIRS.

		Broilers		Adult	
Ingredient	N+	R^2	SE	R^2	SE
Cereals	44	0.85	0.37	0.72	0.52
Vegetable meals	27	0.76	1.14	0.74	1.31
Animal meals	33	0.79	0.99	0.79	1.02
Cereals & by products	55	0.95	0.51	0.92	0.59
Overall	115	0.84	0.91	0.77	1.13

+ N, number of samples: R^2, coefficient of determination; SE, standard error of calibration.

These preliminary data indicate that NIRS may provide a fast and accurate measure of AME for some feedstuffs. This looks particularly encouraging for cereal grains. Further work is continuing to refine the prediction equations and to incorporate additional feed samples.

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THE EFFECT OF SALINE DRINKING WATER ON THE AVAILABILITY OF CALCIUM IN BLOOD FOR EGG SHELL FORMATION.

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Electrolytes in drinking water have been shown to have a deleterious effect on egg shell quality (Balnave and Scott 1986). We investigated the possibility that electrolytes such as sodium and chloride were reducing the availability of calcium in the blood. Such an effect would result in a reduced delivery of calcium to the shell gland where the egg shell is laid down.

Two strains of layer hens were used: Hyline reds and Tegel tinted hybrids. There were two batches of Hyline red birds (one batch of which was sampled twice) and one batch of Tegel birds. Birds were drinking either deionised water or 2 g NaCl/litre drinking water. Egg shell quality was monitored for all birds throughout the experiments. Oviposition times were determined via a video monitoring system. Blood samples were taken every two hours over a period of 30 hours and sample times normalised with respect to oviposition time. Samples of whole blood were analysed immediately for ionised calcium (AVL 984 Electrolyte Analyser) and plasma samples were analysed for total calcium (Cobas Bio Spectrophotometric Autoanalyser).

The levels of both ionised and total calcium showed a negative straight line regression from one oviposition to the next. The sigmoidal pattern of ionised calcium described by Luck and Scanes (1979) was seen in some experiments but not in others. Birds drinking saline water or producing poor quality egg shells tended to have higher levels of ionised and total calcium in their blood. This indicates that adequate levels of calcium were available for egg shell formation but were not being utilised. The levels of both ionised and total calcium in blood tended to be higher in the Hyline reds than in the Tegel tinted hybrid birds.

It is concluded that saline drinking water does not reduce the availability of calcium in blood. If anything, the levels of ionised and total calcium were higher in the birds drinking saline water or producing poor quality egg shells.

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DEPOSITION OF CYCLOPIAZONIC ACID IN EGGS

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Cyclopiazonic acid (CPA) is a mycotoxin produced by species of *Aspergillus* and *Penicillium* (Cole 1986). The principal target organs of this toxin are the liver, kidney and digestive tract. Substantial quantities of CPA have been recovered from skeletal muscle of rats (Norred *et al.* 1985) and chickens (Norred *et al.* 1987) after oral dosing. This demonstrates the possibility of human exposure to CPA through consumption of animal products and we now report the transmission of CPA into eggs.

Crossbred laying hens (White Leghorn X New Hampshire) were allocated into three groups of six, maintained in single-bird cages and fed a commercial layer ration. Daily doses of either 0, 1.25 or 2.5 mg CPA/kg liveweight were administered orally in gelatine capsules. The trial continued for four weeks and two eggs were collected from all hens each week. In a second trial groups of five hens, maintained as in trial 1, were given daily doses of either 0, 2.5, 5.0 or 10.0 mg CPA/kg liveweight for 9 days and eggs were collected each day. After collection egg white and egg yolk were separated and frozen prior to analysis for CPA (Dorner *et al.* 1990).

The production responses of the birds has been reported (Suksupath *et al.* 1989) with the most noticeable being a reduction in egg production and egg shell quality. Analysis of the eggs demonstrated the presence of CPA in all eggs from hens receiving CPA. The toxin was detected in egg white within 24 hours of dosing but was not found in egg yolk until 4 days after the initial dose. In both studies 2- to 10-fold more toxin accumulated in the egg white than egg yolk.

It may be concluded that CPA could enter human food through contaminated eggs.

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EFFECT OF CYCLOPIAZONIC ACID ON CALCIUM CONTENT IN PLASMA, EGG SHELL, UTERINE FLUID AND EGG SHELL GLAND OF LAYING HENS

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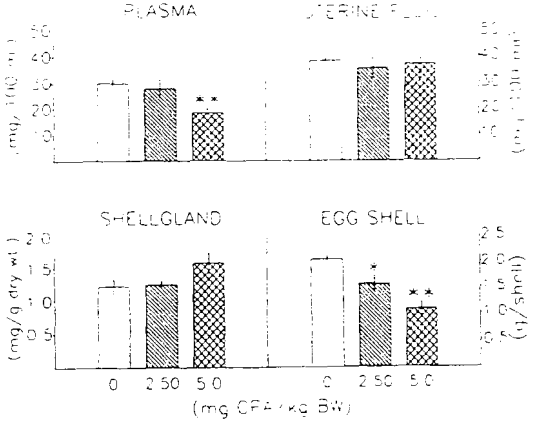
Cyclopiazonic acid (CPA), an indole tetramic acid metabolite of *Aspergillus* and *Penicillium* spp (Norred *et al.* 1985), is a potent inhibitor of Ca^{2+} uptake and ATPase activity in sarcoplasmic reticulum vesicles isolated from rat skeletal muscle (Goeger *et al.* 1988). In laying hens, this mycotoxin decreased egg production and egg shell quality (Suksupath *et al.* 1989). It is possible therefore, that CPA interferes with intracellular calcium transport during egg shell formation thus reducing egg shell quality. The present study was conducted to investigate the effect of CPA on the calcium content of tissues responsible for egg shell formation.

Laying hens (White Leghorn X New Hampshire), in their second year of production, (after a rest from lay), were allocated to three groups, placed in individual wire cages and fed a commercial layer ration. Each hen was dosed orally with 0, 2.50 or 5.0 mg CPA/kg bodyweight in a gelatine capsule. Twenty four hours after dosing, blood, uterine fluid, egg shell gland and egg shell were collected from four hens in each group. Calcium in all samples was analyzed by atomic absorption spectrophotometry.



Results show that the hens dosed with 5.0 mg CPA had decreased calcium in plasma and egg shell ($P<0.01$) but there was no significant difference in calcium content of shell gland and uterine fluid from the control birds. At the 2.50 mg of CPA, calcium contents were not significantly different from controls except the content in egg shell ($P<0.05$).

The results of the study indicate that CPA decreases circulating levels of calcium and the incorporation of calcium into egg shell.

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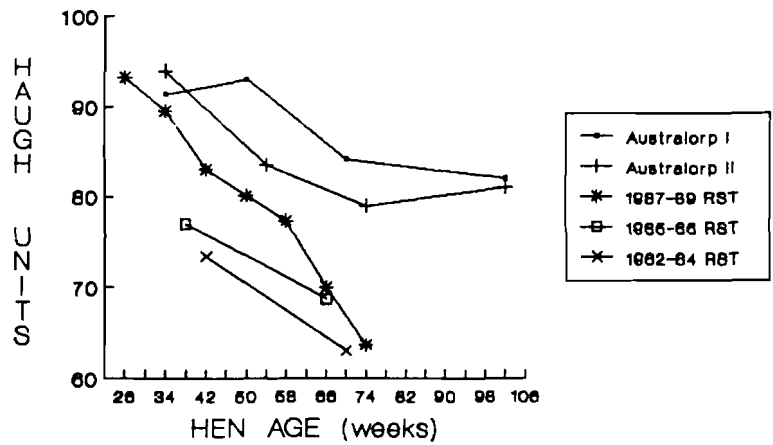
RELATIONSHIP OF EGG COMPOSITION AND FUNCTIONAL PROPERTIES TO EGG QUALITY

F.S. SHENSTONE and R.W. SLEIGH

This paper is a progress report on research on egg quality, composition and function at the CSIRO Food Research Laboratory supported by the Egg Industry Research and Development Council.

It is commonly accepted that an egg of high internal quality, as indicated by a high Haugh unit value, is better for poaching, boiling in the shell, and cake volume (Lowe 1955). Australorp lines of hens at the CSIRO Division of Animal Production consistently produce eggs with high Haugh unit values. This high quality is also maintained to an older than normal hen-age (Shenstone *et al.* 1989), as shown in the Figure.



Our investigations of the chemical basis of this high quality have focussed on two components i.e. ovomucin and lysozyme, and their interactions with other proteins in the thick white. Ovomucin is important to the gelatinous structure and viscosity of egg white. Lysozyme interacts strongly with ovomucin.

Research in this laboratory on the removal of lysozyme from commercially available egg white has shown that lysozyme is not essential for the functional properties of egg white such as gelation or foaming. For a standard whip time, the foam volume and foam stability were similar for the untreated and delysozymed-egg whites. Other studies indicate that the "poor performance" of some batches of commercial egg whites is related to the state of the constituent ovomucin.

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REMARKABLE ANTI-ULCER PROPERTIES OF EGG LECITHIN

B.A. HILLS

It has been claimed that more medication is sold for ulcers - and gastritis in general - than for any other medical disorder. The vast majority of pharmaceutical remedies have been formulated on the dictum "no acid no ulcer" despite the realisation by Davenport (1965) three decades ago that there is a "gastric mucosal barrier" to the back-diffusion of hydrogen ions secreted by the stomach, thus preventing this organ from digesting itself. This concept fell into disrepute until it was demonstrated that a hydrophobic lining of surface-active phospholipid (SAPL) - predominantly di-saturated lecithin - existed on the stomach wall (Hills et al. 1983). This theory is analogous to the use of surfactants as corrosion inhibitors by engineers, eggs having been used in the last century to inhibit rusting. Our very recent morphological studies (Hills 1990) have shown that the surfactant lining on the stomach wall and on the epithelium of the secretory glands is oligolamellar SAPL.

Attempts to fortify the natural lining by applying exogenous SAPL were successful with protection rates reaching 90% for a banana:milk mixture in rats as the best model for man. The standard tests uses the non-pylorus-ligated, rat stomach 'insulted' with 1 ml of 0.8 N HCl under anaesthesia 1 h after administration of 1 ml of the prophylactic - or 1 ml of saline for the 'controls'. Protection is then calculated as the percentage decrease in the ulcerated area of the stomachs relative to the controls, all rats being killed by cervical dislocation 1 h after the acid insult. Very recently we have tried egg lecithin as a very cheap form of di-saturated SAPL and, in solution in propylene glycol, have shown a dose-response curve reaching 95.4% protection for the 86 rats used so far. This protection rate is quite remarkable and would indicate a much less expensive form of medication (with no side-effects) for maintaining patients ulcer-free than conventional H2 blockers. Unless some form of therapy is maintained the relapse rate for gastric ulcer is 80-100% over two years.

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INTERACTIVE E. COLI/IBV INFECTIONS IN CHICKENS

G.A. ALLEN and R.C. CHUBB

Escherichia coli (E. coli) infections in chickens are triggered and/or exacerbated by infections with other respiratory pathogens, one of the most important being infectious bronchitis virus (IBV). These infections often result in colisepticaemia with invasion of E. coli causing air sac lesions, pericarditis and perihepatitis. The use of live IB virus vaccines is thought to contribute to the incidence of the disease (Jackson 1986).

In these experiments, a pathogenic strain of E. coli, 078.K80, in combination with various strains of IBV, was inoculated into the tracheas of unvaccinated, male, cross bred chickens, 4-5 weeks old. The virus isolates used were Webster's A3 (WA3) and Vic S vaccine isolates, and the nephrotropic T and B isolates of IBV. Birds were swabbed for tracheal E. coli and weighed. Blood samples were taken for culture to detect bacteraemia. Tracheal rings were examined microscopically for deciliation. Post mortems were performed. The results are summarised below.

1. Tracheal swabs showed varying loads of E. coli before inoculation. Colonisation appeared to be significantly heavier in summer than in winter.

With WA3 virus and E. coli inoculated together, there was an increase in tracheal E. coli counts about 3 weeks after infection, which persisted beyond the time of clearance in birds infected with E. coli alone or IBV alone. This effect was not seen with the other isolates of IBV.

2. Tracheal Deciliation and Bacteraemia with Different Inocula

Inoculum	Deciliation 72 hr. P.I.	Bacteraemia 72 hr. P.I.
E. coli only	Nil	Nil
E. coli + WA3	Nil	3/10
E. coli + VicS	Partial	10/10
E. coli + B	Total	10/10

3. Weight gains did not differ between the treated and untreated groups, except for a B virus + E. coli infected group, where birds were depressed and anorexic for about 21 days, with mean weight gain over this period of 180g, compared with 312g for other groups.

4. Deaths from colisepticaemia were low:- 1/10 birds in the E. coli/WA3 groups, 1/20 in the E. Coli/Vic S groups and none from other treatment groups.

It would be premature, given the variability of the work of others, to draw firm conclusions until further work validates these results.

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EFFECT OF INFECTIOUS BRONCHITIS ON THE ENERGY METABOLISM OF VACCINATED AND UNVACCINATED CHICKENS

G. AFANADOR, R. CHUBB and D.J. FARRELL

Respiration calorimetry is a sensitive technique for measuring responses of chickens to stress or a stimulus. Previously we reported observations on the energy metabolism of chickens when vaccinated against infectious bronchitis (IB) (Farrell et al. 1990). Here we provide further data from groups of 5 male broiler chickens vaccinated (V) by eye-drop at 2 days (d) of age with a high dose of VIC-S IB vaccinal strain ($10^{4.2}$-$10^{4.6}$ EID_{50}/bird) and challenged by eye-drop using a 0.025 ml suspension of a virulent nephrotoxic T IB virus at 15 d of age. Measurements were made thrice at 25°C in respiration chambers (Walker and Farrell 1976) on these and unvaccinated (UV) chicks from 15-17d and 18-21d of age.

Results (±SEM) on a bodyweight (kg/d) basis at two ages of vaccinated (V) and unvaccinated (UV) male broiler chicks challenged with IB virus

	15-17d		18-21d	
	UV	V	UV	V
Weight (g)	2434(56)	2202(84)**	2981(60)	3002(87)
Gain (g)	69(8)	89(12)	50(8)	82(7)**
Food (g)	124(4)	141(4)**	107(4)	126(3)**
Metab. (%)	74.0(0.4)	73.4(0.9)	72.5(0.6)	76.4(0.6)**
ME intake (kJ)	1572(58)	1770(46)**	1323(49)	1648(46)**
Heat prod (kJ)	979(22)	1040(21)*	942(23)	975(16)
(% ME intake)	70.0(2.1)	58.9(1.0)*	71.2(3.9)	59.2(0.9)**
RQ	0.98(0.02)	1.01(0.01)	0.93(0.02)	0.98(0.01)*
En. Ret. (kJ)	419(51)	730(33)*	381(51)	673(32)**

* $P<0.05$, ** $P<0.01$

There were differences ($P<0.05$) between the two groups particularly at 18-21 d of age. Some of these differences stemmed from the reduced food intake of the UV group. Consequently heat production and energy retention were reduced as was dietary metabolisability. When ME intake (X,kJ/d) was regressed against energy retention (Y,kJ/d) the equations were for the UV group: $Y = -765+0.87X$, RSD=72, $R^2=0.87$, n=21 and for the V group: $Y = -401+0.65X$, RSD=36, $R^2=0.89$, n=21

Equations differed ($P<0.05$) and daily maintenance energy requirements (MER) (kJ/W kg) were 884 (UV) and 620 (V). It is concluded that challenging UV chicks at 15 d of age with IB virus reduced performance significantly compared to a virulent vaccinated chick (V) and increased MER.

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TOXICITY OF FUSARIUM EQUISETI AND RELATED SPECIES IN A CHICK BIOASSAY

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The fungal genus *Fusarium* contains species known to produce highly toxic mycotoxins. Many of these toxins are associated with a number of animal diseases, most notably oestrogenic and feed refusal syndromes (Marasas *et al*. 1984). Recently, it has been established that a metabolite, fusarochromanone, produced by *F.equiseti* induces tibial dyschondroplasia (T.D.) in chickens (Walser 1987). *F.equiseti* is one of five species in section 'Gibbosum' of the genus and is one of the few species in this section to have received any detailed toxicological study. In this communication, the toxicity of *F.equiseti* and related species in section Gibbosum are compared.

Cultures of *Fusarium* species were obtained from soil samples collected in Northern and Eastern Australia. The species were isolated using a soil dilution plate technique and sub-cultured onto PDA and CLA plates for identification (Burgess *et al*. 1988). A total of 144 isolates were selected for toxicity testing in a chick bioassay.

The cultures were grown on 85 g of crushed malted whole wheat biscuits, moistened with 50 ml water and grown at 25°C in the dark for 2 weeks. Mycotoxin extraction procedures followed those of Kirksey and Cole (1974). For each bioassay, a 0.5 ml aliquot of extract in maize oil was intubated into the crop of day-old chickens. Four chickens were dosed per culture and housed in cages situated in an air-conditioned (32°C), continuously illuminated room. A commercial chick feed and water were provided *ad libitum* for the 4 days of the bioassay. The results of the bioassays are summarised in the table.

Species	No. of isolates	Average % toxicity[1]
F.equiseti	78	18
F.scirpi	6	58
F.acuminatum	15	85
F.compactum	35	70
F.longipes	10	5

[1] Average toxicity is expressed as % chick mortality after 4 days.

F.compactum and *F.acuminatum* were the most toxic species with *F.longipes* showing the least toxicity. Preliminary chemical analyses have indicated the presence of tricothecenes in toxic extracts of *F.equiseti*, *F.acuminatum* and *F.scirpi*. The procedure used in the present study only extracted fat-soluble metabolites and studies are needed to determine the possible production of water-soluble toxins, such as those causing T.D. by these *Fusarium* species.

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EFFECTS OF T-2 TOXIN ON ISOLATED CHICK CELLS

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The trichothecenes are the principal biologically active fungal metabolites produced mainly by *Fusarium* spp. (Ueno 1984). In mammalian systems T-2 toxin is perhaps the most potent of the trichothecene mycotoxins (Thompson and Wannemacher 1986). Several studies on domestic animals have indicated that T-2 toxin causes degenerative changes to the cells of the gastrointestinal tract (Mirocha 1983; Weaver *et al.* 1978; Ueno 1984). Since the cells of the intestine are reported to be sensitive to the toxic effects of T-2 toxin we have used isolated enterocytes to investigate effects which T-2 toxin has on these cells *in vitro*.

Alteration to the normal structure and functions of the membrane of enterocytes exposed to T-2 toxin have been observed. Scanning electron micrographs show unusual "holes" in plasma membranes of toxin-treated enterocytes. However, exposure to T-2 toxin allowed dye entry only after exposure to the highest concentration tested ($P<0.05$) and, contrary to expectation, no significant increase in calcium uptake was observed (see Table).

Effects of T-2 toxin on uptake of Ca^{2+} and trypan blue (TP) exclusion by isolated chick enterocytes.[1]

	control	6.4uM	32uM	160uM
$^{45}Ca^{2+}$ uptake (nmol/10^6 cells)[2]	1.59±0.12	1.62±0.11	1.79±0.10	2.02±0.19
TP exclusion (% viability)[3]	77.3±1.9	76.2±1.9	75.1±2.1	61.0±3.3

1. Isolated enterocytes were exposed to T-2 toxin for 120 min.
2. Mean and SEM of 12 observations from 4 different cell preparations.
3. Mean and SEM from 7 different cell preparations.

Unexpectedly high concentrations of toxin were required to kill isolated cells. To determine if effects observed *in vitro* adequately reflect those *in vivo*, chickens were fed comparable concentrations of T-2 toxin. No significant histological effects on intestine, liver, kidney or spleen were observed. Changes observed in the bursa of treated birds suggests that lymphoid cells may be most sensitive to the effects of T-2 toxin. Experiments to date with isolated lymphocytes have not supported this observation.

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THE INTAKE OF GREEN FEED WHEN INCLUDED AS AN ALTERNATIVE PROTEIN SOURCE FOR CHOICE-FED CHICKENS.

ELIZABETH MUDFORD, R.B. CUMMING and J.V. NOLAN

Under village conditions chickens forage for most of the day although additional feed may be supplied, usually as an energy supplement such as paddy rice. Under such conditions protein requirements are met by feedstuffs such as green shoots and insects. This study examined whether choice-fed birds can identify green feed as a source of protein.

Crossbred cockerels (4-week-old) which had been reared on a choice feeding regimen were randomised into groups of 10 and were housed in twelve 1 m^2 deep litter pens. Birds in two pens were offered one of the following feeding treatments:

1) wheat and protein concentrate ad libitum
2) wheat, protein concentrate ad libitum and lettuce.
3) wheat, limited protein (50% of that consumed by 1) concentrate and lettuce.
4) wheat, protein concentrate ad libitum and silver beet.
5) wheat, limited protein concentrate as 2) and silver beet.
6) wheat and limited protein concentrate as 2).

The wheat, lettuce and silver beet were fed ad libitum.

Wheat was fed as whole grain, protein as a pelleted concentrate, and lettuce and silver beet were finely chopped and offered fresh once daily. Feeds were offered in separate troughs with the trough positions being changed daily. Following a 14-day familiarisation period daily feed intake and weekly weight gain were recorded for 5 weeks.

Intakes of green feed (g/week) from both replicates on each treatment.

Treatment	Protein concentrate	Green feed	Week 1	2	3	4	5
2	ad. lib.	Lettuce	67	792	1266	1591	2704
			117	782	1420	2225	2835
3	Limited	Lettuce	2196	3929	3174	4811	4592
			1527	3553	3351	3803	3819
4	ad. lib.	Silver beet	171	331	873	733	1191
			436	684	645	998	1152
5	Limited	Silver beet	1042	1286	1443	2087	2133
			988	1465	1562	1842	1927

All groups offered green feed consumed the material, with more lettuce (see Table) being consumed than silver beet. Those on limited protein consumed approximately twice the amount of those receiving protein concentrate ad libitum suggesting the birds identified the green feeds as sources of protein, even though the protein contents were low - lettuce 13 g/kg and silver beet 32 g/kg. However, the body weights of birds fed the limited protein were lower than those fed the protein concentrate ad libitum and the greater intake of green feed did not increase body weight.

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SELF-SELECTION FEEDING OF BROILERS USING COLOURED FEEDS

I.K. AMRULLAH* and D. BALNAVE**

During the past decade considerable interest has been generated in the use of self-selection (SS) feeding. Many studies, including some carried out in Australia (Mastika and Cumming 1985; Sinurat and Balnave 1986), have confirmed that modern broiler strains fed separate cereal and protein-rich feeds have the ability to select nutrients so as to attain similar body weights to broilers fed complete diets. It is possible that the efficiency of SS might be improved through the use of dyes to colour the separate feeds. The results of the limited studies carried out to investigate this possibility have been equivocal (Cooper 1971; Anderson and Briggs 1948). In the present study growing broilers were allowed to self-select nutrients from separate cereal and protein feeds. Minerals and vitamins were added to both the cereal and protein feeds which had ME and crude protein concentrations respectively of 13.4 and 9.0 MJ of ME/kg and 106 and 449 g crude protein/kg. The broilers were fed the naturally-coloured feeds in separate feeders or these feeds coloured with either a green (10 g Chlorophyll-2000/kg) or yellow (5 g Xanthophyllx-050/kg) dye (Colour 1: green cereal - yellow protein; Colour 2: yellow cereal - green protein). Responses were compared with those of broilers fed a complete feed (12.5 MJ of ME and 202 g crude protein/kg). The results for the 42 day growth period are shown in the Table.

Production responses of broilers to self-selection feeding (1-42 days).

	Self-selection			Complete Diet	SEM
	Natural	Colour 1	Colour 2		
L'wt gain (g)	1651	1699	1693	1701	13.6
Food intake (g)	3593^{a}	3866^{b}	3721^{ab}	3531^{a}	24.0
FCR (g:g)	2.18^{ab}	2.28^{a}	2.20^{ab}	2.08^{b}	0.017
ME intake (MJ)	43.9^{a}	47.0^{b}	45.3^{ab}	44.0^{a}	0.31
Protein intake (g)	697^{a}	742^{b}	730^{ab}	714^{ab}	4.5

Mean values within a row with different superscripts are significantly different ($P<0.05$) as assessed by Duncan's Multiple Range Test.

The use of food dyes increased the food, ME and protein intakes of broilers fed by SS although the effects were only significant for broilers fed the green cereal - yellow protein combination (Colour 1). The yellow cereal - green protein combination (Colour 2) responses were not significantly different to those of broilers fed the naturally-coloured SS feeds. The liveweight gain of broilers fed by SS were similar to that of broilers fed the complete diet.

The results show a potential for increasing food and nutrient intakes through the use of coloured feeds. This could serve an important role where feeds of low nutrient availability are fed.

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PATTERNS OF INTAKE OF INDIVIDUAL NUTRIENTS BY FREE-CHOICE FED LAYING HENS

SRI SULANDARI, R.B. CUMMING and J.V. NOLAN

This experiment compared the patterns of intake of wheat, protein concentrate and calcium chips by trained free-choice fed hens (NH x WL) with those of similar hens fed a conventional, complete diet. There were six birds on each feeding regimen, housed individually at 18-20°C and receiving light from 0400-2000 h. The complete diet was pelleted (160 g CP, 11.7 MJ ME and 37 g Ca/kg). The choice-fed birds had three feed bins containing protein concentrate (9.98 MJ ME and 326 g CP/kg), whole wheat (13.2 MJ ME and 116 g/kg) and oyster shell chips (380 g Ca/kg) in front of each cage.

Feed consumption was recorded at 2-3 h intervals throughout the day over a 6 week period. Time of oviposition, egg production and egg quality were also determined.

Fractions of total daily consumption of metabolizable energy (ME), protein (Pr) and calcium (Ca), and relative numbers of ovipositions (RO) at different times of the day. Values are means for 42 days.

Time (h)	COMPLETE DIET				CHOICE FEEDING			
	ME (MJ)	Pr (g)	Ca (g)	RO (%)	ME (MJ)	Pr (g)	Ca (g)	RO (%)
0400-0700	0.25	3.46	1.09	5.5	0.26	4.15	0.79	5.5
0700-0900	0.17	2.38	0.58	29.4	0.19	2.45	0.19	17.2
0900-1100	0.19	2.71	0.81	43.8	0.18	2.61	0.28	41.4
1100-1300	0.13	1.80	0.43	16.4	0.14	2.05	0.15	18.0
1300-1400	0.09	1.31	0.33	2.1	0.11	1.69	0.15	10.3
1400-1600	0.14	1.97	0.59	2.1	0.17	2.85	0.43	6.2
1600-1800	0.17	2.38	0.87	0.0	0.17	2.97	1.00	0.7
1800-2000	0.14	1.97	0.92	0.7	0.12	2.26	1.18	0.7
TOTAL	1.28	17.98	5.61	100.0	1.35	21.04	4.18	100.0

The choice-fed birds consumed more protein ($P<0.001$) and more ME ($P<0.05$) and selected a diet with a higher protein:ME ratio (15.6 VS 14.0 g protein/MJ ME). The choice-fed birds also consumed less calcium ($P<0.001$) over the 6 week period, and consumed appreciably less between 0700 to 1600 h each day. Their time of oviposition was later in the day, suggesting that this may be dependent in some way on the daily distribution of calcium intake.

The total numbers of eggs laid did not differ between feeding regimes (0.93 eggs/hen day) whereas the mean egg mass was larger ($P<0.001$) for choice-fed birds (62 vs 57 g). Quality, as measured by Haugh units, proportions of shell, albumen and yolk, and specific gravity did not differ between feeding regimes. Liveweight gain did not differ significantly between the choice-fed and conventionally-fed hens, being 2.8 and 1.8 g/d, respectively.

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ELECTRONIC APPARATUS FOR CONTINUOUS MONITORING OF INTAKE OF PROTEIN, ENERGY AND CALCIUM BY FREE-CHOICE FED BIRDS

K. WOODS, J.V. NOLAN and R.B. CUMMING

Patterns of nutrient intake by choice-fed birds are affected by nutritional, environmental, physiological and behavioral factors and it is desirable to be able to determine these patterns without disrupting the bird's normal daily activities. Studies in this Department required the determination of the patterns of intake of grain, protein concentrate and calcium by birds individually choice-fed over a 24 h period and to relate these to the time of egg-laying.

Electronic apparatus was designed to enable the intake of feed of individually caged birds, via three bins per bird, to be automatically recorded at regular intervals. The time of egg-laying can also be automatically recorded.

To enable the mass of each feed bin to be recorded, several inexpensive transducers were tried. The option eventually chosen was that used in an inexpensive digital balance (Bonso, Model 323). It consists of a machined aluminium block with four strain gauge sensors attached to provide a Wheatstone Bridge whose electrical resistance varies with distortion of the block. The feed bins are mounted on the load cells which are fixed to a steel frame physically isolated from the chicken cages to minimize the transmission of vibrations.

A regulated 5 volts is applied across each bridge and the output voltage derived from opposite arms is fed via an LM324 operational amplifier, to provide approximately 1.5 volts to one of the analogue input channels of a data acquisition unit (DT100, Data Electronics Australia). The output is adjusted to indicate approximately 1-2 mV per g. The microprocessor-based DT100 has available 23 differential analogue and 8 digital input channels, and can be programmed to scan all channels at regular intervals and to store the data for later downloading to a personal computer. For each bird, individual signals from each of the three feed bins are routed to one of the analogue inputs and a 'high/low' signal from a mercury switch, triggered by egg-laying, is monitored via one of the digital inputs.

The Datataker is programmed to scan the signal inputs such that data from 100 scans (i.e. approximately 3000 data items) are collected in a desired period. In the present configuration, 6 birds can be monitored for periods of 1-3 days at intervals of 15-45 min. The unused channels are available for regular monitoring of temperature and humidity. At the end of each data collection period, the data are downloaded to a Personal Computer and processed and graphed via Lotus 1-2-3.

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CALORIMETRIC MEASUREMENTS OF THREE LINES OF BROILERS MEASURED AT THREE ENVIRONMENTAL TEMPERATURES

G.P.D. JONES and D.J. FARRELL

A major constraint to broiler production is extremes in ambient temperature. Broiler sheds are not normally maintained at a constant temperature which is influenced by diurnal changes as well as by seasonal variation. Previous work by Farrell and Swain (1977) measured the effects of temperature on the energy metabolism of one strain of broiler. The results presented in this paper describe the effects of three environmental temperatures on the energy metabolism of three strains of broiler chickens.

Three male 28 d old broilers were housed in closed-circuit respiration chambers (Pym and Farrell 1977) for 6 d with measurements made on the last 4 d. Temperatures were, 30°C, 21°C and 14°C. The experiment was repeated 4 times and the results are shown in the table.

Calorimetric measurements of three strains of broilers at three temperatures.

Temp		Strain 1	Strain 2	Strain 3
30°C	Metabolisability (%)	75.4a*	70.3b	73.5a
	MEin (kJ/kgW$^{0.75}$/d)	1578a	1526ab	1470b
	Heat prod. (kJ/kgW$^{0.75}$/d)	886a	964ab	845b
	En.ret. (kJ/kgW$^{0.75}$/d)	692a	562b	625b
	En.ret. (%)	43.9a	36.5b	40.0b
21°C	Metabolisability (%)	72.2ab	71.1b	73.4a
	MEin (kJ/kgW$^{0.75}$/d)	1870b	2105a	1891b
	Heat prod. (kJ/kgW$^{0.75}$/d)	1132b	1235a	1134b
	En.ret. (kJ/kgW$^{0.75}$/d)	738b	870a	757b
	En.ret. (%)	39.5a	41.2a	40.0a
14°C	Metabolisability (%)	73.1b	72.0b	75.2a
	MEin (kJ/kgW$^{0.75}$/d)	1941b	1970a	1998a
	Heat prod. (kJ/kgW$^{0.75}$/d)	1255b	1405a	1230b
	En.ret. (kJ/kgW$^{0.75}$/d)	686b	565c	768a
	En.ret. (%)	35.2a	28.6b	38.2a

*Values with different superscripts differ significantly ($P<0.05$).

The three strains showed similar responses at 21°C but not at 30°C. At 30°C, Strain 1 had a higher dietary metabolisability than Strain 2 and a higher energy retention than Strains 2 and 3. However, at 14°C, Strain 1 had a poorer metabolisability than Strain 3 although % energy retention was similar. As temperature decreased, % energy retained by Strain 1 decreased markedly, however Strain 3 showed no effect of temperature.

The results indicate that some of the negative biological effects of seasonal temperature variation may be reduced by careful genetic selection.

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LACK OF A BENEFICIAL EFFECT OF UNTREATED AND TREATED SODIUM ZEOLITE A (ETHACAL) ON BROILER PERFORMANCE AND pH OF THE GASTROINTESTINAL TRACT

M. EVANS and D.J. FARRELL

Residues of hydroxyl ions remaining in Sodium Zeolite A (SZA) after manufacture may be responsible for neutralising the acid in the proventriculus and gizzard with subsequent effects on protein digestion and broiler performance.

In the experiment there were 8 dietary treatments each with 4 replicates of 8 one-day-old male broilers. The factors included untreated and treated SZA at 3 levels of inclusion (1%, 2%, 3%) (6 treatments) plus 2 control diets consisting of a commercial broiler starter diet with and without kaolin. All diets were balanced for electrolytes (36 mEq/100g). SZA was treated with sulphuric acid to neutralise the residual hydroxyl ions by dissolving SZA in water, treating with sulphuric acid until the pH of the solution was 7.0, decanting and then re-drying at 40°C. Body weight and feed consumption of 256 chickens were recorded until 3 weeks of age at which stage the pH along the gastrointestinal tract (GIT) was measured in one bird from each replicate.

In birds fed untreated SZA, the pH of the crop, proventriculus and gizzard was significantly higher ($P<0.01$) than for control diets. This difference disappeared from the duodenum and onwards (Figure 1). Treated SZA also caused a significant ($P<0.05$) increase in pH but the rise was not as great as with the untreated form.

Birds fed SZA diets and the kaolin control diet had significantly ($P<0.01$) lower body weight gain (BWG) than the commercial control. The difference in BWG between the 2 control diets can be explained in terms of dietary dilution. However, birds fed SZA diets had significantly ($P<0.01$) lower BWG at 3 weeks than birds fed the kaolin control diet. This was not due to a dilution effect but to the SZA per se. Treated SZA was able to overcome these effects only at 1% inclusion. Above this, SZA reduced BWG whether treated or not.

Birds fed SZA diets and the kaolin control diet had significantly ($P<0.01$) lower feed consumption (FC) to 3 weeks than the commercial control. The difference in FC between the 2 control diets can be explained in terms of body weight which in turn is related to dietary dilution. However, birds fed SZA diets had significantly ($P<0.01$) lower FC at 3 weeks than birds fed the kaolin control diet. Again, this was not due to a dilution effect and BWG alone, but to the SZA per se. Treated SZA was not able to overcome these effects.

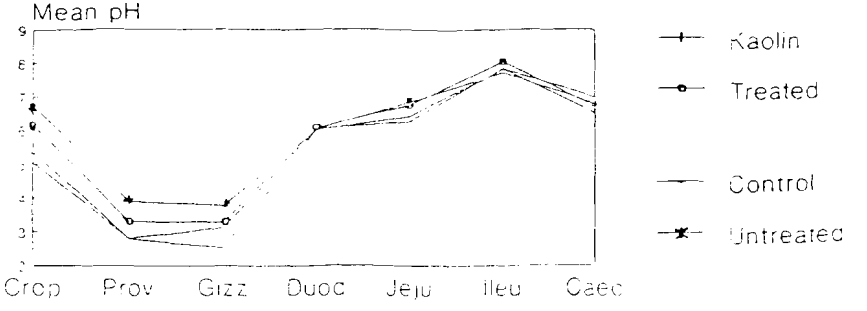

Figure 1. The effect of treated and untreated Sodium Zeolite A on pH of the chicken gastrointenstinal tract

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ENDOGENOUS AMINO ACID SECRETION IN CHICKENS FED DIETS CONTAINING DIFFERENT PROTEINS

P. SIRIWAN, W.L. BRYDEN and E.F. ANNISON

Sauer and Ozimek (1986) indicated that endogenous amino acid secretion is influenced by a number of factors including dry matter and protein intake, protein quality, levels and composition of crude fibre and anti-nutritional factors. The present experiment was designed to determine the endogenous amino acid levels in the ileum of chickens fed diets containing different protein sources. Homoarginine, arising from guanidination of lysine in dietary proteins was used as a marker for determining endogenous secretions (Siriwan *et al.* 1987).

Male broilers (5 weeks old) were fed semi-purified diets based on glucose, supplemented with vitamins and minerals and containing 20% crude protein from a single source (casein, CAS; isolated soybean, IS; meat-meal, MM; cottonseed meal, CSM; soybean meal, SBM and sunflower meal, SFM) and celite (20 g/kg) as a marker, for four days. Three hours before ileal contents were collected the birds were precision-fed with 25 g diet containing guanidinated protein. The diets and ileal digesta were analysed for amino acids, including homoarginine, and acid insoluble ash. Endogenous amino acid values (g/kg dry matter intake) were calculated from homoarginine:amino acid values in guanidinated protein diets and in ileal contents (Siriwan *et al.* 1987), and are presented in the table.

Amino acid	Dietary protein					
	CAS	IS	MM	CSM	SBM	SFM
Threonine	1.42	1.58	2.24	3.15	2.53	2.53
Valine	1.43	1.32	2.07	3.30	2.96	1.96
Methionine	0.13	0.05	0.08	0.10	0.23	0.04
Isoleucine	1.53	0.99	1.40	2.81	2.23	1.56
Leucine	0.90	1.50	1.83	2.92	2.49	2.03
Phenylalanine	0.61	1.09	1.03	1.61	1.39	1.39
Histidine	0.32	0.34	0.72	1.14	0.78	0.51
Lysine	0.62	0.71	1.69	3.70	1.45	1.62
Arginine	0.47	0.54	0.78	0.32	0.86	0.36

There was wide variation in the secretion of individual amino acids in response to the different protein sources. Purified protein sources (CAS, IS) provoked less endogenous amino acid secretion than dietary proteins of lower quality (MM, CSM, SBM and SFM).

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PRODUCTIVE RESPONSES TO LIVEWEIGHT IN HENS FED AD LIBITUM AND RESTRICTED INTAKES OF ENERGY

P.F. MANNION

Models developed from field experiments which predict the egg output responses of laying hens to dietary energy intake may underestimate the efficiency of utilisation of energy for egg synthesis. A greater requirement for maintenance energy, arising from an increase in both liveweight and carcass energy content, is confounded with the egg output responses to increasing energy intake. This stimulated the present interest in a direct examination of the relationship between liveweight and egg output and body composition variables in laying hens fed ad libitum or restricted intakes of energy.

Individually caged Hyline (WL x NH) pullets were fed either ad libitum or restricted quantities of a diet which was demonstrated in the experiment to be first limiting in energy. The restricted fed birds were offered daily 90, 81 or 72 percent of the quantity of feed consumed by the birds fed ad libitum (measured twice weekly). There were approximately 90 birds per treatment. The experiment ran from when the birds were 31 to 47 weeks of age, after which all birds in the ad libitum, 90 and 72 percent treatments were fasted for 44 h, then killed for whole carcass analysis. Data were pooled across the final 4 weeks of the experiment.

Quantitative restriction of energy intake significantly reduced egg production and egg weight, and increased feed efficiency (g egg/g feed). Liveweight was also reduced together with carcass fat, protein and energy content (kJ/g). Carcass ash content was unaffected.

In birds fed ad libitum, increasing liveweight was associated with greater feed intake and egg mass output and a decline in feed efficiency. The increase in egg mass output resulted from an increase in egg weight.

Within both ad libitum fed birds and those fed fixed but restricted amounts of energy, greater liveweight was associated with greater dry carcass weight. Fat, protein and ash accounted for 84, 12 and 2 percent, respectively of the increase in dry carcass weight from the lightest to the heaviest birds within each treatment group. The energy content of carcasses was positively correlated with dry carcass weight within all groups.

Within groups of birds in which individuals were fed the same but restricted amounts of energy, increasing liveweight was associated with a decline in egg numbers but an increase in egg weight.

The results show that heavy birds are intrinsically fatter than light birds and lay characteristically larger eggs (0.6 g/egg per 100 g increase in liveweight). The greater energy consumption of heavier birds supports their higher maintenance requirements and larger egg size while maintaining a rate of egg production equivalent to that of lighter birds. Based on the observed responses and current prices in Queensland, the nett return per dozen eggs (gross return - feed cost) increased substantially in favour of larger hens producing eggs of greater mean weight.

Estimates of the energy requirements for maintenance and egg synthesis have been made and compared with previously published data.

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PERFORMANCE OF A BANTAMISED COMMERCIAL LAYER ON DIETS WITH DIFFERENT ENERGY CONTENTS

D.J. FARRELL*, W. STANHOPE** and G. PARKINSON**

A bantamised commercial layer (bxWLxNH) was developed by W. Stanhope and G. Parkinson with a low feed intake and high egg weight to body weight ratio. It may have special dietary needs. Three treatments of groups of 12 pullets (3 per cage) were initially replicated 6 times. The diets were: diet A, High Egg Mash (165 g CP and 12.22 MJ ME/kg DM); diet B, Super All Mash (151 g CP and 11.14 MJ ME/kg DM) and diet C, a high specification custom layer mash (157 g CP and 13.44 MJ ME/kg DM). The experiment ran from September 1989 to June 1990. At the end of January the 3 groups were subdivided into 3 replicates: A(A), B(B), C(C), (A)B, (B)C and (A)C.

Production parameters from 24 to 66 weeks of age

Month		S	O	N	D	J	F	M	A	M	J
							Hen-day egg production (%)				
Diet	A(A)	76	80	83	76	71	64	70	62	50	44
	B(B)	76	85	83	78	72	64	64	61	56	52
	C(C)	77	84	81	73	74	59	53	51	49	45
	A(B)						65	63	57	54	63
	B(C)						70	68	66	64	55
	A(C)						74	72	70	69	62
	SD	7.8	7.3	7.3	7.4	7.7	7.0	7.0^{-}	5.9*	6.6*	10.2
							Feed intake (g/d)				
Diet	A(A)	84	94	112	89	86	88	95	96	99	91
	B(B)	94	100	124	103	100	105	109	112	97	117
	C(C)	89	92	110	92	90	90	87	95	93	103
	A(B)						97	103	106	106	103
	B(C)						93	93	96	97	97
	A(C)						97	102	103	108	108
	SD	3.7*	3.2*	8.0*	6.1*	8.2*	5.3	3.8*	4.6*	12.9	8.4*
							Eggweight (g)				
Diet	A(A)	48	54	55	58	51	60	55	62	65	63
	B(B)	49	52	54	58	57	61	62	62	64	66
	C(C)	46	52	58	58	56	60	61	63	63	63
	A(B)						58	59	61	62	62
	B(C)						60	59	61	63	62
	A(C)						59	61	61	63	64
	SD	1.1	2.5	3.2	1.2	2.0	1.8	3.7	2.8	3.1	1.6

Significance of effect: $^{-}$ P<0.1, * P<0.05

Egg production and egg weight were not initially influenced by diet and were similar to a normal sized (WLxNH) layer (Table). When diet C was given to group A and B egg production was improved compared to birds on diet C throughout. Feed intake was generally higher on diet B because its lower ME. Mean starting and finishing bodyweights were 1.30 and 1.57 kg respectively. Mortality (15%) was the same on all diets.

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