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IMPROVEMENT OF FEED UTILISATION THROUGH THE USE OF ENZYME PRODUCTS

H.L. CLASSEN and G.L. CAMPBELL

Summary

Utilisation of many feedstuffs by poultry is limited by the lack of endogenous enzymes necessary for hydrolysis of fibre components. Poultry are also sensitive to the anti-nutritional effects of factors contained in feed ingredients. Dietary enzymes offer a mechanism whereby nutrient utilisation can be enhanced and/or negative effects reduced or eliminated. Enzyme use is well established for diets containing barley and has increased for other feedstuffs as well. Realisation of the potential of this technology to improve feeding value requires the establishment of specific substrates and enzyme requirements, knowledge of substrate effects and factors affecting substrate importance. In addition, the stability of enzyme supplements is essential for successful incorporation into commercial feeding programs.

I. INTRODUCTION

The potential for using enzymes in poultry diets to improve the nutritional value of feedstuffs has been recognized for years. Of particular significance is research related to enzyme use in barley diets (Fry et al. 1958; Willingham et al. 1959; Rickes et al. 1962). Despite this relatively long history, commercial exploitation has only been significant during the last decade. With the development of commercial and laboratory enzyme preparations, research on their use has also increased dramatically. The scientific basis for enzyme addition is established for some feedstuffs while for others it is not. In addition, the potential for using dietary enzymes to improve the nutritional value of other ingredients remains to be examined.

Enzyme addition to barley diets is the primary commercial application and also is the best understood from a scientific standpoint. In this paper the theory and application of dietary enzymes in barley diets will be examined to demonstrate key aspects of enzyme use that need to be considered for other feed ingredients. Other uses of dietary enzymes, current and potential, will also be discussed. For a review of dietary enzymes see Chesson (1987).

II. ENZYME USE IN BARLEY DIETS

(a) Substrate: structure

A key aspect of the scientific application of enzymes is knowledge of the substrate(s) of importance. In barley, the primary substrate is ß-glucan, a polysaccharide component of cell walls. ß-Glucan, primarily located in barley endosperm, is a polymer of glucose containing a mixture of ß 1-3 and ß 1-4 linkages. The presence of ß 1-3 linkages leads to fractions of the ß-glucan which are water soluble and possess viscous characteristics in solution. It is the soluble fraction which is considered of primary importance in determining the nutritional value of barley although it should be recognised that there is not a clear distinction between soluble and

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insoluble portions. Some solubilisation of the latter fraction may occur under the rigorous conditions in the gastrointestinal tract. Poultry lack endogenous enzymes necessary for the breakdown of ß-glucan and therefore it remains intact to a large extent as it passes through the digestive tract. Some hydrolysis is likely to occur in the hindgut as a consequence of microbial action but is of unknown nutritional importance.

(b) Substrate: antinutritional effects

A second important factor to consider is the mechanism whereby the substrate influences feedstuff nutritional value. After ingestion of barley, ß-glucan becomes solubilised in the digestive tract resulting in increased digesta viscosity (Burnett 1966; Salih et al. 1990). The increase in digesta viscosity is considered to be the major factor influencing the nutritional value of barley samples. The exact effect(s) of viscosity has not been established but possible mechanisms include reduced diffusion of endogenous enzymes and nutritional substrates and increased feed passage time. Viscous substances affect diffusion rates and therefore it is conceivable that reduced enzyme-substrate interaction would have a negative impact on nutrient assimilation (Fengler and Marquardt 1988). Feeding barley has been shown to reduce feed passage time in rats (Gohl and Gohl 1977) and chickens (Salih et al. 1990). With other viscous carbohydrates this can increase the depth of the unstirred water layer adjacent to the epithelial lining of the small intestine, which is considered to be a rate limiting step in absorption (Johnson and Gee, 1981). Changes in digesta flow rate result in increased microbial numbers in the small intestine (Salih et al. 1990). A negative effect of microorganisms is implicated by the enhanced response of birds fed barley to antibiotic supplementation (Moran and McGinnis 1965; Classen et al. 1985). Deconjugation of bile salts (Campbell et al. 1983; Feighner and Dashkeviscz 1988) and the production of toxins are possible effects of deleterious microorganisms. Increased feed passage time might also influence total feed intake. Since barley diets are frequently lower in energy it is important that birds consume more feed to meet nutrient requirements; therefore, a reduction in feed intake would have a negative effect on performance.

ß-Glucan is also likely to act as a physical barrier to endogenous amylase and therefore reduce the utilisation of starch encapsulated within endospermal cells (Hesselman and Åman 1986). Breakdown of ß-glucan via enzyme addition resulted in enhanced hydrolysis of starch in the anterior region of the small intestine which may result in more efficient starch utilisation since microbial action on the starch is reduced.

Regardless of the exact mechanism of action feeding barley and, therefore, ß-glucan reduces nutrient assimilation, growth rate and the efficiency of feed utilisation. In addition, undigested ß-glucan results in sticky droppings which can have an adverse effect on the environment of intensively housed poultry. Increased litter moisture and hence ammonia production has the potential to directly damage the respiratory tract, feet, legs and breasts of litter-housed stocks, and indirectly influence resistance to respiratory disease. Faecal condition can also increase the occurrence of dirty eggs and reduce the ease of manure storage and removal.

(c) Substrate: Hydrolysis

Hydrolysis of ß-glucan is necessary to alleviate its negative effects on the feeding value of barley. ß-glucan destruction has primarily been accomplished by the use of enzyme sources containing endo-ß-glucanase activity, but other treatments such as gamma irradiation (Classen et al. 1985; Campbell et al. 1986, 1987) are also effective. However, at the present time the use of enzyme supplements is the most cost effective and acceptable method.

Sources of enzyme activity are variable but generally are derived from various types of microbial cultures (Chesson 1987). Although the degree of response to enzymes has been variable, a positive effect on barley feeding is consistent when proper precautions are taken to assure adequate levels of the enzyme activity.

The majority of the enzyme-induced improvement in barley feeding value is from endo-ß-glucanase activity (Rickes et al. 1962). However, other enzyme activity will probably increase the response. Recently, purified ß-glucanase was used to break down the cell wall carbohydrates of the cereal grain, rye (GrootWassink et al. 1989). Although the pure enzyme improved the feeding value of rye, crude enzyme preparations were more successful. It stands to reason that optimum hydrolysis of a complex carbohydrate interwoven into cell walls requires multiple enzymes. Related studies in plant physiology and biochemistry are essential for understanding this complex situation. In barley, pentosans also contibute to cell walls (Forrest and Wainwright 1977) and pentosanase activity is likely required to act together with endo-ß-glucanase (de Silva et al. 1983). Release of ß-glucan from barley cell wall is apparently influenced by ß-Glucan solubilase (Yin and MacGregor 1989) but the importance of this enzyme(s) in feeding barley is unknown. Finally exo-enzymes may provide additional benefit by complete hydrolysis to monomeric glucose. The degree of hydrolysis necessary to achieve beneficial results is an important consideration in enzyme use. It is generally conceded that the improvement in performance demonstrated by enzyme use in barley diets is not due to complete hydrolysis of the molecule and the absorption of the glucose (White et al. 1983; Campbell et al. 1986; Chesson 1987). Instead, relatively minor hydrolysis alters the ability of ß-glucan to form viscous solutions and act as a barrier to endogenous enzyme activity. Considering the relatively short time frame for enzyme-substrate interaction in the digestive tract and the relatively low level of enzyme supplementation, this would appear to be an important factor in the success of enzyme applications. However, recent reports in swine indicate that ß-glucan is essentially totally digestible and that enzyme supplementation facilitates this breakdown (Graham et al. 1989). Again further research is required to determine the fate of ß-glucan breakdown products in the avian digestive tract.

(d) Factors Influencing Enzyme Response

Variation in the level of substrate or in this case, ß-glucan, can influence the enzyme response. Genotypic effects on ß-glucan content are well established (Campbell et al. 1989). Similarly, regional variations in the nutritional value of barley are known to occur (Willingham et al. 1960; Burnett 1966) and appear to be attributed largely to climatic effects on ß-glucan content. Moisture stress, brought on by hot, dry conditions during crop maturation elevates both acid soluble and total ß-glucan content (Aastrup 1979). Stage of maturity at harvest also plays a role in the ß-glucan problem (Hesselman and Thomke 1982). Campbell et al. (1989) examined the extract viscosity of sixteen barley cultivars grown at five locations in Western Canada. Differences in extract viscosity among locations were most apparent for high-viscosity cultivars while those exhibiting low viscosity were more uniform across locations. Lowest viscosities were noted for the most Northerly location (Beaverlodge, Alberta) which coincides with cooler temperatures and increased rainfall in this area.

Campbell et al. (1989) compared the feeding value of barley cultivars with different levels of soluble ß-glucan as judged by extract viscosity. Low-viscosity barley consistently resulted in higher growth rate and more efficient feed conversion than high-viscosity samples. Comparison of the feeding value of high- and low-viscosity barleys without and with enzyme supplementation demonstrated that high-viscosity barley responds more to

enzyme addition but that even low-viscosity samples were significantly improved by enzyme addition. Productivity of broilers fed the two barley types was similar after enzyme addition indicating the importance of enzyme use in reducing variability among barley samples. Similarly, comparisons of the response to enzyme addition of nine Saskatchewan-grown barley samples revealed that the coefficients of variation were reduced from 11.9 to 3.3% in the case of growth rate and from 5.2 to 2.7% for feed to gain ratio by the addition of enzyme (Classen et al. 1988). Elimination of ß-glucan as a source of variability among barley samples has important implications in the more accurate formulation of poultry diets.

Naturally occurring endo-ß-glucanases are present in barley and it has been suggested that the relative level influences barley feeding value. In comparisons of high- and low-viscosity barleys (Campbell et al. 1989) enzyme levels tended to be higher in low-viscosity cultivars. However, variability in viscosity due to environment did not match enzyme levels. Water soaking barley prior to feeding also improves its nutritional value (Fry et al. 1958); activation of endogenous or microbial enzymes may be responsible for this effect (Willingham et al. 1959).

The adverse effect of feeding barley to chickens is age dependent. Salih et al. (1990) studied the interaction of bird age with diets based on barley, barley plus enzyme or wheat. They found that the major negative effect of feeding a high ß-glucan barley was during the first four weeks of life for both broiler and egg production stock. After four weeks, growth rate and other production parameters were similar for birds fed the three experimental diets. Digesta viscosity was higher for the barley-fed birds regardless of age and irrespective of the improvement in performance. On the other hand, feed transit time was only lower for barley-fed birds when performance was poor and did not differ from other treatment groups at later ages. This implies that the negative effect of viscosity on young birds is related to feed transit time. Older birds appear capable of more readily transporting viscous material in the gastrointestinal tract and, therefore, production is not adversely affected.

As performance is affected to a lesser degree by feeding barley to older birds, enzyme addition is likely to be less effective. However, research with both turkeys and broilers indicates that beneficial effects are still seen beyond four weeks of age (Campbell et al. 1984; Salmon et al. 1986). In addition, enzyme supplementation is necessary for litter-housed birds to improve the litter quality and the air environment. A high incidence of breast trimming at processing can occur if enzymes are not added to broiler diets (Campbell et al. 1984). Use of enzymes in barley diets for laying hens is less clear. Results range from no effect (Berg 1959, 1961; Bustany and Elwinger 1988) to a small, but significant, improvement which may not be ß-glucanase-dependent (Classen and Campbell, Unpublished data). Enzyme addition for laying hens may still be warranted to improve manure consistency and handling in deep pit cage barns and to reduce the potential of soiled eggs.

(e) Enzyme Stability

Dietary enzymes are exposed to hostile environments during feed processing and passage through the digestive tract. Examples include high temperature during pelleting, low pH in the proventriculus and gizzard and the effect of proteolytic enzymes. Enzyme activity necessary for ß-glucan hydrolysis can be found in a wide range of plant and microbial sources. Although the essential endo-ß-glucanase activity is present, specific enzyme characteristics are likely variable. As an example, a crude enzyme source has recently been shown to contain different forms of pentosanase which vary in pH and temperature stability (GrootWassink et al. 1989). Therefore biological as well as laboratory testing of enzyme activity is essential prior to

acceptance of an enzyme source. In general, when acceptable biological activity is shown, caution is still necessary in regard to heat stability. High pelleting temperatures are not recommended unless clear evidence to the contrary is provided by the enzyme manufacturer. Failure to follow such guidelines eliminates the usefulness of enzyme addition and also reduces the acceptance of this technology by poultry producers.

III. ENZYME USE FOR OTHER POULTRY FEEDSTUFFS

Cell wall polysaccharides can have large effects on the nutritional value of feedstuffs, as evidenced by ß-glucan in barley, and therefore are potential substrates for dietary enzymes. Since cell wall polysaccharides vary with feed ingredients, enzyme preparations with specific activity are required for beneficial effects. It is improbable that complete hydrolysis to monosaccharide constituents is possible within the time frame of feed passage through the digestive tract; therefore, total utilisation of these fractions is doubtful. In addition, monosaccharides released by enzyme action may be poorly available or cause undesirable nutritional effects (Longstaff et al. 1988). Partial destruction of cell wall material to eliminate anti-nutritional effects or provide enhanced availability or access to nutrients are more likely routes of action. Other examples of positive responses to enzyme addition have been shown for poultry feedstuffs.

Oats, like barley, contains relatively high levels of ß-glucan. Therefore, hydrolysis of ß-glucan also improves its nutritional value for young birds (Campbell et al. 1986, 1987; Petterson et al. 1987; Edney et al. 1989). However, feeding oats does not always have a negative influence on poultry production. Differences in response may be due to variability in the nature or content of ß-glucan.

Broiler chicks fed rye as a cereal grain grow poorly due to pentosans (arabinoxylans) found primarily in endosperm cell walls. Pentosans are viscous like ß-glucan and appear to affect productivity in a similar way. Enzymes with pentosanase activity are known to improve the feeding value of rye although most responses are not sufficient to make diets with rye as the sole cereal grain a practical alternative (Petterson and Åman 1988, 1989; GrootWassink et al. 1989). Further research is required to obtain more effective enzyme sources or to design the proper enzyme cocktail to overcome the pentosan effect.

Wheat endosperm cell walls also contain pentosans but the level and viscosity of extracts is markedly lower than for rye. Enzyme use in wheat based diets has met with variable results but has on numerous occasions resulted in an improvement in feeding value. Unpublished studies at the University of Saskatchewan and the University of Nottingham have shown that enzymes from different sources were capable of increasing the apparent metabolizable energy of wheat by up to 24%. Improvements in growth rate and feed conversion of broilers fed wheat-soybean rations also indicate a potential commercial benefit, although in these studies the beneficial response cannot be definitely attributed to the wheat. Cellulase (Trichoderma viride), when combined with a diet containing high levels of wheat bran, caused an increase in the digestibility of cell wall components (Nahm and Carlson 1985). Some Australian wheats have been shown to have lower metabolisable energy than expected which is apparently due to reduced starch digestibility (Mollah et al. 1983). Based on the research with barley it would not be unexpected to find genetic and environmental differences in nutritional value which affect the response to enzyme addition. Clearly additional research is required to determine the reason for the variable but frequent improvement in the feeding value of wheat by the use of enzymes and

the relevance to the feed industry.

Triticale cultivars are the result of interspecific crosses between rye and wheat which tend to resemble wheat more than rye in terms of nutritional value. Petterson and Åman (1988) reported small but significant improvements in the growth and feed conversion of broilers when diets containing three different triticale cultivars were supplemented with an enzyme source containing high levels of ß-glucanase and pentosanase activity. The wide range of crosses used to produce triticale cultivars makes it likely that variations in response to enzyme supplementation will occur.

Diets based on corn and various protein supplements have also shown responses to enzyme products. Suga et al. (1978) found that a multiple enzyme cocktail improved broiler body weight gain and feed conversion by 5 and 2 percent, respectively. Classen and Campbell (unpublished data) also noted a significant improvement in broiler feed conversion when an Aspergillus niger-derived enzyme source was added to corn-soybean meal starter and grower feeds.

The presence of various types of non-starch polysaccharides in protein supplements also offers promise for appropriate enzyme supplementation either during the processing procedure or as an addition to the diet. Anti-nutritional effects have been suggested for oligosaccharides in soybean meal (Leske et al. 1988), cell wall carbohydrates in Canola and soybean meal (Ward and Reichert 1986) and viscous mucilages in some cultivars of Canola, mustard and linseed (Vose 1974; King et al. 1982; Muralikrishna et al. 1987)

Fungal phytase (Aspergillus ficuum) increases organic phosphorus utilisation when incorporated into chick diets. Three grams of a crude extract per kilogram of diet were sufficient to result in complete hydrolysis of naturally occurring phytate in a corn-soybean meal diet (Nelson et al. 1971). This represents a substantial quantity of enzyme to be added to the diet, which is reflective of the mode of action of phytase. Unlike ß-glucanases or pentosanases, where only minor damage to the substrate will reduce viscosity and have the desired effect, phytate degradation will be a comparatively slow process. Weighing the cost of phytase against the value of phosphorus this would appear economically prohibitive in terms of improved phosphorus availability. Minimal damage to phytate may alter its solubility profile and ability to interfere with mineral metabolism, in which case phytase supplementation may be viable if the result was to reduce skeletal abnormalities. Areas where phytase supplementation is being investigated are concerned with soil pollution, a large part of which is due to excessive phosphorus in animal waste. Increasing phosphorus utilisation via enzyme supplementation could conceivably reduce this problem.

IV. CONCLUSIONS

The use of dietary enzyme sources is well established for poultry barley based diets and is a widely acknowledged method to improve the nutritional value of other feed ingredients. Establishment of specific substrates (and therefore enzyme activity), substrate effects, factors affecting substrate importance and stability of enzyme cocktails are required to fully exploit this technology. The renewed research interest in this area and the advent of genetic manipulation of microorganisms makes major advances likely in the near future. The economic value of adding enzymes must be decided on a case by case basis by determining the probability of improved performance or efficiency or the benefit to the agricultural industry.

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COMPLEX POLYSACCHARIDES IN BROILER DIETS

G.Annison

SUMMARY

Many non-starch polysaccharides (NSP), but not all, have an anti-nutritive activity when they are present at low levels (<5%) in broiler diets. The apparent metabolisable energy (AME) of the diets is reduced and growth of birds may be depressed. This paper reviews the literature detailing the action of NSP and reports the results of studies looking at the physico-chemical properties of NSP which may be responsible for their biological activity.

Addition of various NSP to a basal sorghum diet caused a marked depression in AME and growth in some cases. The anti-nutritive activity was unrelated to the viscosity of the NSP when measured in vitro. Depolymerisation of xanthan gum did not ameiliorate the depression of AME. The decrease in starch digestibility which was also noted did not appear to be due to α-amylase inhibition as xanthan gum did not affect the in vitro or in vivo activity of this enzyme.

I. INTRODUCTION

Plant polysaccharides have different biological activities when they are present in diets for broiler chickens. For example, it is now well established that the non-starch polysaccharides (NSP) of barley and rye are responsible for the poor nutritive value of these cereals. In barley (1-3),(1-4)-ß-glucan (at approximately 40g/kg) causes growth depression accompanied by sticky droppings. The addition of ß-glucanases to barley diets ameiliorates the growth depression (Gohl et al. 1978; Hesselman and Aman 1986; Classen et al. 1988) and allows barley to be used at higher levels.

Rye contains high levels (approximately 10% dry wt.) of arabinoxylans (pentosans). The anti-nutritive activity of these polysaccharides has been demonstrated by Marquardt and co-workers (Antoniou and Marquardt 1982; Ward and Marquardt 1987). Growth depression in broilers was caused by the addition to experimental diets of pentosans isolated from rye. Enzyme supplementation of rye diets with pentosanases improves their nutritional value (Peterson and Aman,1988).

Mollah et al. (1983) reported that some Australian wheats have low AME values when included in poultry diets. Wet droppings were observed when broilers were fed these diets. This is also found with birds fed rye diets. Pentosans are also present in some wheats at appreciable levels (approximately 7% dry wt.) and, therefore, may contribute to the low AME wheat phenomenon.

Examination of the literature reveals that many NSP have anti-nutritive activities when added to broiler diets (Table 1). At low levels (<50g/kg) of inclusion growth depression, inhibition of fat digestion and nitrogen and dry matter retention have been observed. Some NSP, however, have no detrimental effect and the reason for their lack of activity is unclear. Differences must be due to the different physico-chemical properties of the polysaccharides or their breakdown products.

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Table 1. The effects of polysaccharides in broiler diets.

Polysaccharide	Level (g/kg)	Effect	Reference
Cellulose	to 260	Increased growth; reduced nutrient digestibility.	Saito et al.(1959).
Cellulose	60-420	Energy dilution growth depression at >6%.	Sibbald et al. (1960).
Cellulose	210	No detrimental effect.	Begin (1961).
Guar gum	20	Depressed growth and	Anderson and Warnick
Locust gum	20	feed efficiency;	(1964).
Guar gum	20	Growth depressed 25-30%.	Vohra and Kratzer
Locust gum	20	"	(1964).
Tragacanth	20	"	
Carrageenin	20	"	
Pectin	40	"	
Okra	20	Growth depressed 15%.	
Psylium husk	20	"	
Cellulose	20	No detrimental effect.	
Methyl cell'se	20	"	
Carb.met.cell.	20	"	
Dextrin	20	"	
Dextran	20	"	
Linseed mucin	20	"	
Ghatti gum	20	"	
Agar agar	20	"	
Cellulose	30	Used as control	Griminger and Fisher
Citrus pectin	30	Depressed growth, lipid	(1966).
Scleroglucan	30	cholesterol digestibility.	
Rye pentosans	30	Growth and feed efficiency depression.	Antoniou and Marquardt (1981).
Citrus pectin	40	Depressed growth,	Patel et al.(1981).
Guar gum	20	liver fat and serum cholesterol	
Guar gum	20	Depressed growth and	Grammer et al.(1982).
Pectin	20	tibial ash.	
Arabic gum	20	No effect.	
Pectin	40	Depressed growth, liver fat and serum cholesterol.	Bishawi and McGinnis (1984).
Rye pentosans	30	Reduced fat and dry matter retention	Fengler and Marquardt (1988).
Wheat pentosans	30	Reduced AME, growth, and starch digestion.	Choct and Annison

Plant NSP are diverse in structure and have very different physical and chemical properties. They vary greatly in their viscosity and ion exchange, hygroscopic and surface activity properties in aqueous systems. The anti-nutritive activity of the NSP has been attributed mainly to their ability to increase the viscosity of the digesta with suggestions that this inhibits diffusion of digestive enzymes and nutrients. The diffusion of NaCl and glucose has been shown to be inhibited by increases in viscosity in a model system using dialysis tubing to represent the gut (Fengler and Marquardt 1988). Nutrient movement in the gut is also by means of convective transport as a result of peristalsis. Edwards et al. (1988) reported that increases in viscosity inhibit convective transport of NaCl after studies also using dialysis tubing as a model system.

It has been argued that increases in viscosity will also inhibit the activity of digestive enzymes. In solutions of NSP the diffusion of enzymes and substrates may be hindered by the increased viscosity which is a sieving effect. The rate of catalysis may subsequently be affected. The kinetics for the reaction, however, will also be affected as the chemical activities of both reactants and products will be increased due to their exclusion from part of the solvent by the NSP. Laurent (1971) demonstrated that addition of a neutral polymer decreased the Km (concentration of a substrate at which the rate of an enzyme reaction is half the maximum) of the depolymerisation of hyaluronic acid by a bacterial lyase and thus acted in an opposite way to a competitive inhibitor.

Inhibition of the breakdown and absorption of nutrients may also be caused by NSP through more direct interactions. The polysaccharides may bind the digestive enzymes or their substrates. They may also bind to the surface of the gut thus inhibiting the transport of nutrients from the lumen as well as interfering with the surface resident enzymes. There is evidence that NSP inhibit fat digestion (Table 1). The digestion of fat and some fat soluble nutrients (ie. cholesterol and Vitamin D) depends on micellar formation which may be inhibited by surface active properties of the NSP.

Enzymic supplementation of barley diets has been shown to be beneficial. The depolymerised β-glucan is similar to cellulose which appears to be inert in the chicken gut. This may be due to a lack of endogenous or microbial cellulases which allows the fragments to pass straight through the lower intestine as large inert oligosaccharides. The fragments of other polysaccharides may be less well tolerated and may have anti-nutritive activities of their own. The β-galactose oligosaccharides, for example are not absorbed in the small intestine and pass into the large bowel where they may subsequently be fermented by the microflora to cause diarrhea and flatulence (Saini 1986). Indeed, the gut microflora seem to play a role in the anti-nutritional activity of rye pentosans as antibiotics have been shown to partially reverse the growth depression caused by rye diets (Wagner et al. 1977).

The continued use of high levels of cereal grains in poultry diets, in conjunction with the increased use of novel grains, both legume and non-legume, means that investigations into the specific effects of NSP on growth and performance are warranted This paper details experiments investigating the biological activity of NSP in broiler diets.

II. METHODS

Bird management. In all experiments 5-6 week old commercial type male broilers were used. The birds, obtained at 1 day-old, were maintained on commercial starter and finisher diets and were housed in brooders and group cages. During experiments birds were held in individual metabolism cages.

Experimental diets. Diets were formulated by adding polysaccharides in place of sorghum to the basal diet detailed in Table 2..

Table 2. Composition of sorghum basal diet (g/kg)

Sorghum	820	Premix	5.0
Casein.HCl	134	NaCl	3.6
Dicalcium phosphate	26	Choline chloride	0.4
Calcium carbonate	11		

AME assays. The AME of trial diets was determined using the classical total collection method. Birds were randomly allocated to equal live weight treatment groups. There were eight birds in each group and 48 individual metabolism cages allowed 5 trial diets and a control diet to be assayed in each experiment. Birds were fed trial diets ad libitum for 7 days. Excreta were collected daily and feed intake was monitored over the last 4 days of the trial. The excreta from each bird was dried at 80°C overnight. The gross energy of the trial diets and the pooled excreta from each bird was determined using bomb calorimetry.

Starch digestibility. The starch contents of diets and excreta were determined using a method based on that described by Aman and Hesselman (1984).

Viscosity measurements. The relative viscosities of the polysaccharides used in the trial diets were determined using an Ostwald viscometer. Values are expressed relative to those obtained for distiled water. The polysaccharides were dissolved in distilled water (100mg/100ml.) at room temperature or with gentle warming. Locust bean gum was the only polysaccharide which did not dissolve under these conditions. It was also insoluble in 0.1M NaOH.

Depolymerisation of Xanthan gum. Xanthan gum (100g) was suspended in 3l of HCl (0.1M, 0.5M or 1M) and held at 100°C in a domestic-type slow cooker for different times. After cooling the preparation was dialysed (molecular weight cut off approximately 12000) exhaustively against running tap water to remove the acid and small molecular weight products. The retentate was dried in a forced air oven (80°C, 24hr).

α-Amylase activity. α-Amylase activity was determined using the following two methods. To determine the effect of xanthan gum on a-amylase wheat starch granules (10mg) were incubated in citrate buffer (10ml, 4mM $CaCl_2$, pH5.2, 40°C) with α-amylase (Boehringer Mannheim, E.C.3.2.1.1., 0.1ml). Aliquots (1ml) were removed periodically, added to methanol (9ml) and centrifuged. The supernatant (5ml) was removed and evaporated to dryness. The residue was re-suspended in citrate buffer (pH4.6, 10ml.), incubated with amyloglucosidase (Boehringer Mannheim, E.C.2.3.1.3., 0.1ml., 55°C, 5hr.). The glucose content of the mixture was determined using the glucose oxidase method. The α-amylase activity in gut contents was measured by following the increase in reducing power of an incubation mixture containing starch with 3,5 dinitrosalicylic acid as described by Osman (1982).

III. EXPERIMENTAL AND RESULTS

The Effect of NSP on AME and broiler growth. Sodium alginate, xanthan gum, locust bean gum, low viscosity methyl cellulose and high viscosity methyl cellulose were added to the basal diet (30g/kg) in place of sorghum. The AME and starch digestibility of the diets were determined. The body weight gain of the broilers during the trial period was also monitored (Table 3). The ileal starch contents of the control and 30g/kg xanthan gum treatment group were assessed. The results are shown in Table 4.

Table 3. Effect of viscous non-starch polysaccharides (30g/kg) on liveweight gain, AME and starch digestion.

Diet	Liveweight gain (g)	AME (MJ/kg DM)	Starch digestib'ty coefficient	Relative vis.[1]
Control Diet	449[a]	13.58[a]	0.980[e]	-
Xanthan gum.	168[c]	11.28[b]	0.958[f]	8.0
Locust bean gum.	224[c]	11.57[b]	0.958[f]	-[2]
LVMC[3].	430[ab]	13.25[a]	0.958[e]	1.6
HVMC[3].	332[b]	13.50[a]	0.993[e]	2.0
sodium alginate.	455[a]	13.29[a]	0.986[e]	12.0

1. Viscosity of a 0.1% solution relative to water.
2. Insoluble in water and 0.1M NaOH.
3. Low and high viscosity methyl cellulose
a,b,c. Values with unlike superscripts are significantly different (P<0.01).
e,f. Values with unlike superscripts are significantly different (P<0.05).

Table 4. Starch content of ileal digesta of birds fed xanthan gum (30g/kg).

Diet	Starch (% wet weight)	Starch (% dry weight)	Starch (g)
Control	7.0[a]	16.9[a]	0.30[a]
Xanthan Gum.	2.5[b]	18.3[a]	0.39[a]

a,b. Values with unlike superscripts are significantly different at (P<0.05)

Xanthan gum and locust bean gum caused a marked and highly significant (P<0.01) depression in weight gain over the trial period. The high viscosity methyl cellulose also depressed growth but to a lesser extent. The AME and starch digestibility of the diets with xanthan gum and locust bean gum were significantly reduced also. These effects were not related to the viscosity of solutions formed by the polysaccharides as sodium alginate had no effect on any of the measured parameters.

The effect of depolymerised xanthan gum on AME. Three xanthan gum hydrolysates (A,B,and C) were prepared with the following treatments: 0.1M HCl, 100°C, for 1 hour, 0.5M HCl, 100°C for 1 hour and 1.0M HCl, 100°C for 4 hours respectively. The viscosity of each preparation and their effect on the AME and starch digestibility of the basal sorghum diet when added at 20g/kg was determined. The results are shown in Table 5.

Partial depolymerization of xanthan gum with hydrochloric acid greatly reduced the relative viscosities of the solutions formed by the polysaccharides (cf. Table 3 and Table 5). The preparations, however, retained the anti-nutritive activity of native xanthan gum causing a depression in the AME and starch digestibility of broiler diets when included at 20g/kg of diet.

Effect of xanthan gum on α-amylase activity. The effect of xanthan gum on the rate of release of maltose from wheat starch ganules was investigated. Wheat starch granules (10mg) were incubated with α-amylase in buffer (10ml) with and without xanthan gum (0.1% w/v). The release of maltose from the starch is shown in Figure 1. Also α-amylase activity in the small intestine of control broilers and broilers fed a diet containing xanthan gum was determined. The results are shown in Table 6.

Table 5. The effect of xanthan gum hydrolysates (30g/kg) on AME and starch digestibility.

Diet	AME (MJ/kg DM)	Starch digestibility coefficient	Relative viscosity[1]
Control	12.73[a]	0.983[a]	-
Hydrolysates			
A (0.1M HCl, 100°C, 1 hr.)	12.00[ab]	0.968[ab]	ND[2]
B (0.5M HCl, 100°C, 1 hr.)	10.75[c]	0.955[b]	4.25
C (1.0M HCl, 100°C, 4 hr.)	10.74[c]	0.948[b]	2.65

1. Viscosity of a 0.1% solution relative to water.
2. Value not determined as polymer did not readily dissolve.
a,b,c. Values with unlike superscripts are significantly different (P<0.05).

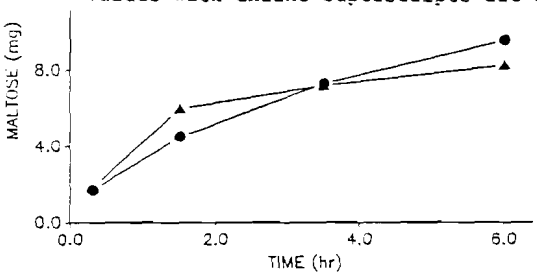

Fig.1. Effect of xanthan gum (0.1% w/v) on the release of maltose from starch by α-amylase.

Table 6. The influence of dietary xanthan gum on the α-amylase activity (mg of maltose released/min) of jejunal and ileal contents in broilers

Diet	α-Amylase activity (mg maltose/min) Jejunum	Ileum
Control	249(22)[1]	161(34)
Xanthan gum (30g/kg)	177(34)	110(34)

1. Standard error in brackets.

Xanthan gum did not inhibit the release of maltose by α-amylase from starch granules. (Fig.1). Compared to controls there was no significant difference in the α-amylase activity in the birds fed xanthan gum. The levels measured were highly variable., which made statistical testing difficult.

Response of AME to NSP mixtures. The AME of trial diets containing xanthan gum (20g/kg), xanthan gum(10g/kg) + locust bean gum (10g/kg) and locust bean gum (20g/kg) were determined. The results are shown in Table 7.

The AME of trial diets was depressed by inclusion of xanthan and locust bean gum at 2%. There was no evidence of an interaction between the two polysaccharides which altered their activity in lowering the nutritive value of the basal diet.

Table 7. Effect of non-starch polysaccharides mixtures on AME of diets.

Diet	AME (MJ/kg DM)	Starch digestibility. coefficient
Control	12.89[a]	0.989[a]
Xanthan gum (20g/kg).	12.05[b]	0.978[ab]
XG.(10g/kg) + LBG (10g/kg).	11.66[b]	0.957[b]
Locust bean gum(20g/kg).	11.40[b]	0.950[b]

[a,b]. Values with unlike superscripts are significantly different (P<0.05)

IV. DISCUSSION

The variability in biological activities of NSP in poultry diets is illustrated by the results in Table 3. The significant (P<0.01) depression in AME caused by xanthan and locust bean gum was accompanied by depression in growth. Both of these effects are not due to simple energy dilution which is the probable cause of the small non-significant effects observed for the other NSP. The depression in AME was associated with an inhibition in starch digestibilty which is to be expected with trial diets containing the high levels of cereals and suggests that the of addition of xanthan gum and locust bean gum to diets provides a model for the low AME wheat phenomenon described by Mollah et al. (1983)

The lack of correlation between the AME of the diets and the viscosity of the added NSP was unexpected and suggests that other physico-chemical properties are responsible for the anti-nutritive effect. Alginate is a polyuronate very similar in structure to the polygalacturonate of citrus pectins which have been shown to have a growth depressing effect in broilers (Patel et al. 1981; Bishawi and M^{c}Ginnis 1984) and therefore would have been expected to show a similar activity considering that it also had a high viscosity. Alginate is considered to be biologically inert and is used in human food products as is xanthan gum. The reason why one should be active and the other not is unclear.

Further evidence that the anti-nutritive activity of xanthan gum is not due solely to its ability to form viscous solutions is provided by the results in Table 5. The three xanthan gum hydrolysates also depressed the AME of the basal diet by inhibiting starch digestibility. The hydrolysates did not produce solutions as viscous as the native xanthan gum but, due to the dialysis procedure used in their preparation, they would still have consisted of relatively long molecules which possibly retained other properities responsible for the anti-nutritive activity.

Polysaccharides may interact in solution to produce effects which neither will achieve alone. Dietary polysaccharides from different feedstuffs may also interact to produce biologically active species. When xanthan gum is mixed with locust bean gum in equal proportions in solution the viscosity is greater than would be expected (Kovacs 1973). This synergism is due to specific interactions between the polysaccharides. The data in Table 7 do not indicate that synergism between these two gums took place to depress AME to a greater extent than expected from their individual activities.

The starch content in the ileum of control birds and birds fed xanthan gum differed significantly only on a wet weight basis (Table 4). This is either a result of the hygroscopic nature of the polysaccharide or a general upset of the gut system causing the secretion of water into the lumen. The

total starch content values were similar in control and treatment birds indicating that the reduced starch digestibility of the diet containing xanthan gum was a result of an increase in the rate of passage of the digesta. The in vitro experiments did not show that xanthan gum has an inhibitory effect on α-amylase. The gum did not significantly depress the α-amylase activity in the jejunum or ileum (Table 6). The range of values obtained, however, was great, which may be because the amount of material obtained from the birds was highly variable.

The present studies confirmed that not all NSP possess anti-nutritive activity. This was shown clearly with the different responses to xanthan gum and sodium alginate. Also, the anti-nutritive activity of xanthan gum and other NSP appears to be independant of their ability to form viscous solutions. Low-viscosity xanthan gum fractions retain activity and some high viscosity NSP appear to have no activity. The anti-nutritive activity of the NSP is associated with a depression of starch digestion which has been noted to occur with low-AME cereals (Mollah et al. 1983; Annison and Johnson 1989). No evidence was found, however, that the in-vivo and in-vitro activity of α-amylase was inhibited by the xanthan gum.

Attempts have been made in the past to include dietary fibre levels in ME prediction equations (Fisher 1987). In the future the task that confronts nutritionists is to be able to more closely predict the responses of poultry to specific NSP, in terms of both digestion and ultimately on growth and performance. Antagonism and synergism between various NSP require clarification. Such an approach could provide answers to industry problems (eg.low-AME wheat) and an improved basis for the prediction of ME.

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PROGRESS TOWARDS THE MODIFICATION OF GROWTH BY GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTORS IN CHICKENS

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Summary

Research over the past decade has established that plasma concentrations of growth hormone (GH) in chickens reflect the pulsatile and episodic release of this hormone from the pituitary gland. This pattern is only evident during the first few weeks after hatching when the growth rate is highest; subsequently, pulses cannot be detected and blood concentrations of GH are low. The exogenous administration of chicken GH (cGH) has produced either no effect or a modest stimulation of weight gain and food conversion efficiency, with positive results obtained only when the endogenous pulsatile release of cGH was mimicked.

The somatomedin hypothesis presented as an explanation for the GH stimulation of growth in mammals argues that the main role of GH is to increase the circulating concentrations of insulin-like growth factor-I (IGF-I) which is the actual mediator of growth. Recently, chicken IGF-I (cIGF-I) and cIGF-II have been characterised and research directed towards an understanding of their roles commenced. Chicken IGF-I has been shown to increase in plasma at an age when cGH is decreasing, and does not respond acutely to cGH pulses. Moreover, no sex or strain differences in cIGF-I concentrations were detectable that might have explained alterations in growth rate. Although these results question the somatomedin hypothesis in chickens, an evaluation of IGF-I growth effects must await the outcome of chronic IGF-I administration experiments.

I. INTRODUCTION

In mammals, pituitary-derived GH acts on liver to produce and release IGF-I into the blood (Van Wyk 1984). Although most of the growth promoting effects of GH seem to be mediated by IGF-I, it is still not clear how much of this IGF-I has been derived from liver rather than being synthesised by those target tissues that also contain GH receptors. Certainly the plasma levels of IGF-I fall in response to situations that reduce growth, including poor nutrition, diabetes, infection and other types of trauma (Van Wyk, 1984). They also increase upon exogenous administration of GH or when growth has been stimulated by the expression of a GH transgene (Mathews et al. 1988). A regulatory loop is completed when high plasma IGF-I levels act on the pituitary and hypothalamus to reduce GH secretion (Van Wyk 1984; Baxter 1986; Tyrrell et al. 1988).

Exogenous GH and IGF-I have been shown to stimulate growth in virtually all studies with hypopituitary mammals, but the results are less consistent when the pituitary gland is present. This distinction has at least two component factors. First, GH does not restore growth if nutrition is inadequate or during catabolic states, probably because GH receptors are both reduced in number and fail to initiate IGF-I synthesis. Importantly, under these conditions growth can be at least partly restored by

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circumventing the GH block via the direct administration of IGF-I (Scheiwiller et al. 1986; Schalch et al. 1989).

Second, a species difference exists with respect to GH efficacy. Thus dramatic anabolic effects of injected GH on growth have been demonstrated with pigs (Etherton et al. 1987), but growth results with rats, cattle and sheep have been harder to demonstrate (Tyrrell et al. 1988; Ronge and Blum 1989; Steele and Elsasser 1989). The differences may be caused by the failure to reproduce the pulsatile and episodic release of GH, by compensatory responses that reduce GH efficacy, or perhaps because the animals are at a stage in development when they do not respond to GH by an increase in growth. On this last point it is noteworthy that although cattle growth is not usually increased by GH, the hormone substantially increases milk yields when the animals are lactating (Tyrrell et al. 1988).

In this overview of GH and IGF I shall cover recent research directed towards improved growth rates of chickens and also indicate the similarities or differences between the GH/IGF-I situation in chickens and in mammals.

II. GROWTH HORMONE IN CHICKENS

The administration of mammalian GH preparations to hypophysectomised chicks generally produces only modest or no effects on growth, even though growth is retarded by pituitary removal in an analogous way to that established for mammals (King 1969; Scanes et al. 1984, 1986). Likewise, no effects of GH could be demonstrated in sex-linked dwarf chickens (Marsh et al. 1984). On the other hand, growth stimulation was observed in an autosomal recessive dwarf chicken line (Marsh et al. 1984). Three explanations have been advanced for the poor responses: (1) chicken growth is poorly regulated by GH; (2) mammalian forms of GH may not be active in poultry; or (3) the dose patterns tested may not be appropriate. The good correlation between growth rate and GH levels in normally-growing chickens (Vasilatos-Younken and Zarkower 1987) and turkeys (Vasilatos-Younken et al. 1988a) and the modest effects demonstrated by GH administration in some studies suggests that birds are responsive to GH. However, recombinant cGH injected three times a day between 2 and 24 days of age at 0.5 mg/kg/injection had little effect on growth parameters in chickens other than to reduce the growth-inhibitory responses of the saline-injected control group (Burke et al. 1987). In another study, Scanes et al. (1986) found that quite low doses of natural cGH (10 µg/d) given daily by injection for the first two weeks after hatching produced a 10-15% increase in body weight but only in male chicks.

Recently Vasilatos-Younken and Zarkower (1987) and Johnson (1988) have demonstrated that endogenous GH levels in chickens are pulsatile only until about eight weeks from hatching, with the pulsatile pattern lost earlier in female birds. It was hypothesised that the loss of pulsatility might explain the lower growth rate of females and that if exogenous GH is to be effective, it may need to be administered in an episodic manner that mimics the situation in vivo. Support for this strategy comes from experiments with rats where episodic GH administration had a greater effect on growth than continuous infusion (Clark et al. 1985). Accordingly, Vasilatos-Younken et al. (1988b) measured growth responses from 8-11 weeks in chickens, a period when endogenous pulses of GH had ended, and compared a 90 min pulse pattern of cGH administration to continuous infusion of the same total dose. The experiment demonstrated improved body weight gain, increased food conversion efficiency and decreased body fat, but only in those animals that received the hormone episodically. Interestingly, cartilage growth and IGF-I levels were increased to about the same extents

in the two treatment groups.

It is premature to conclude from the studies of Vasilatos-Younken et al. (1988b) and Burke et al. (1987) that improved growth in chickens is only achieved when cGH is administered episodically and after normal growth rates have slowed. While such a conclusion fits the data, the results must be tested more extensively.

III. CHICKEN IGF-I AND IGF-II

The concentrations of IGF-I in chicken plasma have been reported to be much lower than in mammalian species (Wilson and Hintz 1982; Daughaday et al. 1985), although since the assays use human IGF-I as the reference peptide and anti-human IGF-I antisera, the low levels detected could rather reflect poor antibody or receptor cross-reactivity. In order to resolve this point, Dawe et al. (1988) and Ballard et al. (1990) have purified IGF-I and IGF-II to homogeneity from chicken serum and used the pure growth factors to validate an assay for cIGF-I. Our results show that cIGF-I is detected in radioimmunoassays (RIA) with three different antisera prepared against human IGF-I (hIGF-I), and that the cross-reactivities were about 50% relative to hIGF-I. Moreover, a similar small potency difference was found in a cell culture bioassay. The concentration of IGF-II in chicken serum was found to be low and cIGF-II cross-reacted so poorly in the IGF-I RIA that any contribution of this second growth factor to measured IGF-I levels would be minimal. The assay for IGF-I in chicken plasma was also tested for interference by the IGF binding proteins remaining after acid-ethanol pretreatment. No significant interference could be detected (Ballard et al. 1990).

The purified cIGF-I and cIGF-II peptides were completely sequenced in order to establish differences from the corresponding mammalian peptides (Kallincos et al. 1989; Ballard et al. 1990). Chicken IGF-I was shown to be a 70 residue peptide with 8 amino acid substitutions in relation to human IGF-I (Fig. 1a). Clearly these substitutions must have little significance for the immunological or biological activities of the growth factor since cIGF-I and hIGF-I have similar potencies. Interestingly, the mammalian IGF-I peptides are highly conserved, with the porcine, bovine and human peptides being identical and only one, three and four substitutions occurring in ovine, rat and murine IGF-I respectively (Ballard et al. 1990).

Sequence analysis of cIGF-II shows a 66-residue peptide with nine amino acid substitutions compared with the human homologue. The amino-terminal residue of mammalian IGF-II is also deleted (Fig. 1b). These differences do not appear to contribute substantially to the biological activity of cIGF-II because the peptide has a very similar potency to ovine IGF-II (Kallincos et al. 1989) or to bovine IGF-II (Dawe et al. 1988) in a range of biological assays. However, although the chicken and mammalian IGF-II peptides have similar activities, those chicken tissues so far investigated do not contain the specific type 2 IGF receptor that occurs on most mammalian cell types (Rechler et al. 1980; Kallincos et al. 1989). The significance of this difference is unclear because no definitive role in IGF action has been ascribed to the type 2 IGF receptor.

IV. PHYSIOLOGICAL CHANGES IN IGF-I CONCENTRATIONS IN CHICKEN PLASMA AND THEIR RELATIONSHIP TO GH LEVELS

The plasma concentration of IGF-I in chickens increases about two-fold between one and seven weeks after hatching, with most of the increase occurring between weeks 1-3 (Johnson et al. 1989; Ballard et al. 1990).

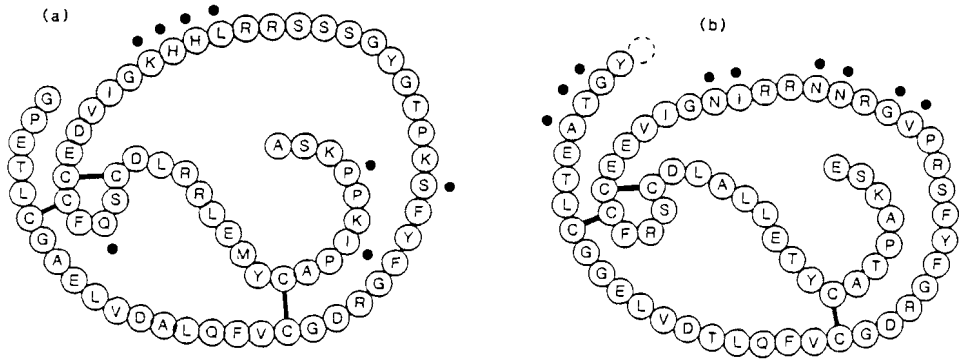

Fig. 1. Amino acid sequences of (a) cIGF-I and (b) cIGF-II. The single letter amino acid code is used with residues that differ from the respective human growth factors indicated by dots.

The concentration in female birds of 20.3±1.1 (SEM) ng (human equivalents)/ml at 1 week was higher than in males (15.8±0.9 ng/ml), but was reversed by 7 weeks where the respective concentrations in females and males were 34.1±1.2 and 41.3±1.3 ng/ml respectively (Ballard et al. 1990). These values represent the means from two commercial strains of broiler chickens and four strains selected for weight gain, food conversion efficiency or food consumption as well as randomly-selected controls. There were no strain differences in IGF-I concentrations even though growth rates were disparate.

In another study using only commercial broiler strains (Johnson et al. 1989), the sex difference was also observed in young birds, as well as during the period of declining IGF-I concentrations that occurs near sexual maturity and in birds aged six months. It is pertinent to note that the low plasma IGF-I at sexual maturity contrasts with a sharp increase in plasma IGF-I at puberty in several mammalian species (Baxter 1986).

The increase in plasma IGF-I in chickens between one and seven weeks occurs at the same time as GH is falling (Fig. 2a, see also Vasilatos-Younken and Zarkower 1987; Johnson 1988; Johnson et al. 1989). This observation would be surprising if plasma IGF-I in chickens was under the direct positive control of GH. A comparison between GH and IGF-I in plasmas collected at 10 min intervals demonstrated no pattern (Fig. 2b, see also Johnson et al. 1989). Rather, the IGF-I levels were relatively constant in the presence of several GH pulses. This result contrasts with the situation in rats where IGF-I levels increase transiently after a GH pulse (Baxter et al. 1983). However, plasma concentrations of IGF-I are apparently increased following chronic administration of cGH to chickens (Vasilatos-Younken et al. 1988b), with the proviso that the RIA and extraction methods used may have detected IGF binding proteins instead of or as well as IGF-I (see below). A similar qualifier applies to an earlier

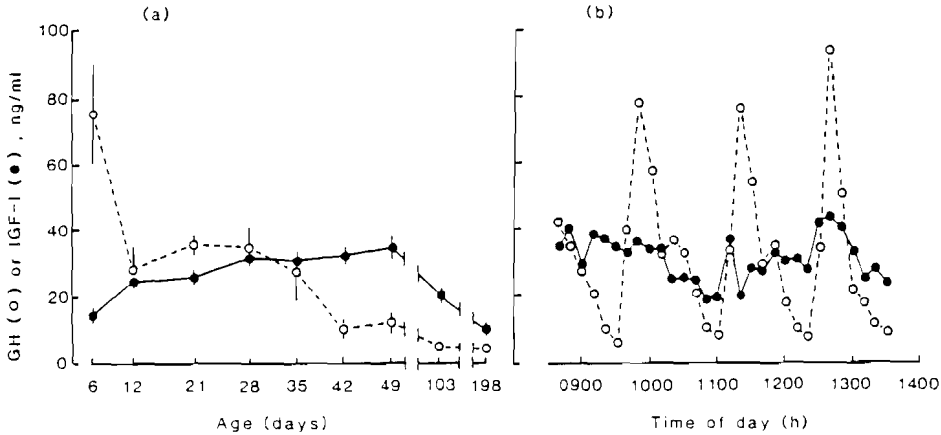

Fig. 2. Lack of any direct relationship between plasma GH and IGF-I in chickens: (a) changes in plasma GH and IGF-I with age in male birds; (b) levels of GH and IGF-I at 10 min intervals in a single animal 29 days after hatching.

study where IGF-I levels were reduced in hypophysectomised or dwarf chickens and were partially restored after injection of cGH (Huybrechts et al. 1985).

Analysis of plasma IGF-I in chickens over a 24h period gives no indication of a diurnal rhythm (Ballard et al. 1990). However, as in mammals, the plasma concentration falls markedly upon fasting (Ballard et al. 1990). Few responses of cIGF-I to manipulations of other hormones have been reported, except for a decrease following corticosterone (Buyse et al. 1987), a response also demonstrated in mammals (Unterman and Phillips 1985).

The response of plasma IGF-I to changing external or homeostatic conditions in chickens is generally similar to that occurring in mammals

Table 1. Similarities and differences between plasma IGF-I responses in chickens and mammals

	Chickens	Mammals
Increase after birth/hatching	Yes	Yes
Increase at sexual maturity	No	Yes
Diurnal rhythm	No	Slight
GH-related rhythm	No	Yes (rats)
Increase after exogenous GH	Yes	Yes
Decrease on hypophysectomy	Yes	Yes
Decrease on starvation	Yes	Yes
Higher levels in young females	Yes	Yes
Decrease with glucocorticoids	Yes	Yes

(Table 1). The differences in chickens include a lack of an increase at sexual maturity, the absence of a rhythm relating to GH levels and the much lower plasma concentrations.

V. ADMINISTRATION OF IGF-I TO CHICKENS

The restricted availability of IGF-I has limited an examination of the response of chickens to the administration of exogenous growth factor. Francis et al. (1990) have recently compared the plasma clearances of radiolabelled hIGF-I and cIGF-I in chickens as a first step towards an evaluation of IGF-I treatments. These experiments show that cIGF-I is cleared with a half-life of 54 min over the first 90 min, somewhat slower than the 33 min found with hIGF-I tracer. Such half-lives are shorter than observed in rats (Zapf et al. 1986) or in lambs (Francis et al. 1988a), possibly because the IGF-I in chickens does not bind predominantly to a high

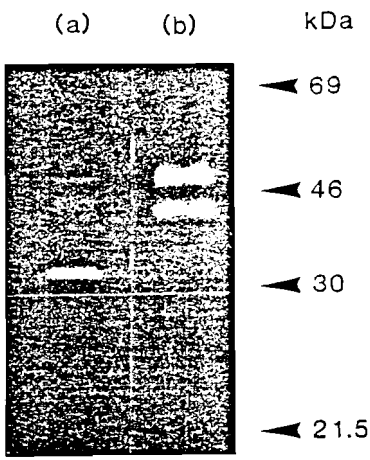

Fig. 3. Detection of IGF binding proteins in (a) 10 µl of chicken plasma, and (b) 1 µl of human plasma that have been electrophoresed, blotted on to nitrocellulose, probed with labelled hIGF-I and subjected to autoradiography. The position of molecular mass markers (kDa) in parallel lanes is indicated.

molecular weight binding protein in blood (Francis et al. 1990). In this context the differences in binding proteins between chicken and human plasma are readily shown with the ligand-blotting technique (Fig. 3), which detects binding proteins with labelled IGF-I after first separating the plasma proteins by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferring them to a nitrocellulose sheet (Hossenlopp et al. 1986).

The reduced concentrations of IGF binding proteins in chicken plasma may also explain the much lower circulating IGF-I levels as compared to mammals, because IGF-I that is not bound in rats or lambs is cleared from the circulation (Zapf et al. 1986; Francis et al. 1988a).

A rapid clearance of IGF-I from the blood does not necessarily mean that attempts to increase IGF-I levels in chickens will not produce an increased growth response. Indeed, when IGF-I is complexed to binding proteins in blood it is probably being withheld from the potential tissue sites of action. Thus IGF-I administration to chickens may actually lead to a more effective delivery of the growth factor. This logic applies equally

to exogenous IGF-I treatment and to IGF-I increased endogenously due to the expression of an inserted transgene. Such approaches, together with the development of IGF-I muteins that bind poorly to plasma binding proteins (Francis et al. 1988b; Ross et al. 1989; Cascieri et al. 1989), will provide useful information in the near future on the modification of growth in chickens by the insulin-like growth factors.

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EFFECTS OF INCREASING PHOTOPERIOD LENGTH ON BROILER PRODUCTIVITY AND HEALTH

H.L. CLASSEN

Summary

Recent reports have described the effects of lighting programs (increasing) for broiler chickens which are characterized by initially short photoperiod length and gradual increases to continuous light prior by marketing at six weeks of age. The hypothesis was that short photoperiod lengths would reduce feed intake and growth rate during a critical stage when proportional increases in size are largest. In turn, slower growth rate would reduce metabolic disease such as leg abnormalities and sudden death syndrome (SDS) which are major causes of loss in the broiler industry. Increasing the photoperiod was hypothesized to stimulate the development of reproductive hormones even though broilers are marketed prior to sexual maturity. In particular, increases in male sex hormones (androgens), which are anabolic in nature, could cause compensatory growth and normal body weights at marketing. In comparisons to continuous light, increasing programs reduced three week weight but not market weight. At the same time, skeletal disease, SDS and other forms of mortality were significantly reduced. Androgenic hormone output was increased as indicated by comb size and plasma androstenedione, but no relationship could be shown between plasma hormone and growth rate. Other effects of increasing programs include apparently increased bird activity and superior or equal feed efficiency. If kept beyond six weeks of age, growth rates are superior for broilers on increasing lighting programs.

I. INTRODUCTION

Poultry species have long been known to respond to photoperiod or daylength changes. In terms of reproduction or egg production, increasing or long photoperiods stimulate sexual maturity and maintain high production levels. In addition, changes in photoperiod length have a more pronounced effect on reproduction than exposure to constant photoperiods of different length. With poultry raised for meat, the nature of photoperiod manipulation has been different presumably because of sexual immaturity at marketing. The emphasis has largely revolved around maximization of feed intake during a 24 hour day. Consequently, popular lighting programs for chickens and turkeys are 23 hours light:1 hour dark (23L:1D) or 24L:0D. The role of darkness in the management of broiler chickens has yet to be determined, however, considerable evidence suggests that the use of continuous or near-continuous light should not be recommended for broiler chickens (Classen 1988; Classen and Riddell 1989). Shorter day-length improves bird health and reduces stress but also results in slower growth rate than continuous light (Freeman et al. 1981; Robbins et al. 1984; Whitley et al. 1984). Intermittent lighting programs, which utilize repeated light and dark periods within a normal 24 hour day have also been shown to improve bird health while maintaining equal or superior growth and food conversion to birds given continual light (Buckland et al. 1971, 1973, 1976; Ononiwu et al. 1979; Malone et al. 1980ab;

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Wilson et al. 1984). Very few attempts have been made to use photoperiod to stimulate physiological responses in broiler chickens. This paper describes broiler lighting programs using day-length which is initially short and increases during the broiler's lifetime.

II. INCREASING LIGHTING PROGRAMS

Seven experiments utilizing approximately 32,000 birds have examined the effects of changing photoperiod length for broiler chickens. Examples of the lighting programs are shown in Table 1; in most experiments a 23L:1D (23H) lighting program served as a control.

Table 1. Experimental lighting programs

Trial day	Intensity (lux)	23H	6H	INC	INC+1
0	20	24L:0D	24L:0D	24L:0D	24L:0D
3	20	23L:1D	6L:18D	6L:18D	6L:8.5D:1L:8.5D
7	5	23L:1D	6L:18D	6L:18D	6L:8.5D:1L:8.5D
14	5	23L:1D	6L:18D	10L:14D	10L:6.5D:1L:6.5D
21	5	23L:1D	23L:1D	14L:10D	14L:4.5D:1L:4.5D
28	5	23L:1D	23L:1D	18L:6D	18L:2.5D:1L:2.5D
35	5	23L:1D	23L:1D	23L:1D	23L:1D
42	5	23L:1D	23L:1D	23L:1D	23L:1D

In three experiments, the 23H or conventional program was compared to a regime (6H) where the daylength was six hours from three to 21 days and then increased to 23 hours in one step (Classen and Riddell 1989). When the data from all three experiments was combined (Table 2), the birds on the 6H program weighed less at 21 days but gained more weight for the remainder of the experiment thereby reaching a weight equal to the 23H broilers at 42 days.

Table 2. Comparisons of conventional lighting (23H) to lighting programs characterized by an increase in photoperiod

	Body weight gain (kg) 0-21 days	21-42 days	0-42 days	Feed intake (kg)	Feed/ gain	Mortality (%) SDS[1]	Other	Leg problems (%)
23H vs 6H								
23H	0.604	1.247	1.858	3.568	1.88	2.64	2.99	3.40
6H	0.569	1.275	1.844	3.451	1.86	1.85	1.75	1.26
23H vs INC								
23H	0.630	1.255	1.885	3.513	1.82	1.75	1.61	5.12
INC	0.576	1.314	1.890	3.482	1.82	1.04	1.29	2.73
23H vs INC and INC+1								
23HR	.633	1.250	1.883	3.480	1.82	2.78	1.93	7.70
INC	.563	1.337	1.900	3.461	1.80	1.82	1.50	3.53
INC + 1	.593	1.336	1.929	3.540	1.81	2.24	1.07	4.44

[1] SDS - sudden death syndrome.

Overall, feed to gain ratio was not affected by the lighting programs. The major benefit of the 6HR program was a 63% reduction in skeletal disease or leg problems. Mortality due to sudden death syndrome (SDS) and other causes was also reduced in a significant manner. A consistent effect of the 6H program was an increase in the size and redness of male combs. This suggests that the change from 6 to 23 hours of daylength stimulated the broilers to produce male sex hormone which is responsible for comb growth.

Since a large increase in photoperiod length increased hormone output, it was proposed that a gradual increase might be even more stimulatory. The INC program, described in Table 1, was compared to the 23H regime in three experiments and the results were similar to those described for the 6H lighting program (Table 2). There was no effect on growth rate and feed conversion but gradually increasing the daylength (INC) reduced leg problems by 47% and mortality by a smaller but significant amount. In one of the experiments there was a significant increase in final body weight for birds on the INC program with the majority of the response in the males.

Body weight was reduced during early life using the 6H or INC programs and therefore an experiment was designed to see the effect of an additional hour of light in the middle of the dark period of the INC program (Classen et al. 1990). Broilers raised on this program (INC+1), grew more rapidly than INC birds up to 21 days and were numerically heavier than both INC and 23H broilers at 42 days (Table 2). INC and INC+1 resulted in similar levels of mortality and leg problems which were significantly lower than for birds on the 23H program. The nature and incidence of skeletal disease are shown in Table 3. Angular deformities (valgus and varus) are present in the highest numbers and are affected in a pronounced way by lighting treatment. Other apparent differences due to lighting program are shown for spondylolisthesis and enlarged stifle joints.

Table 3. Effect of lighting program on the nature and incidence of skeletal disease[1]

	23H				INC				INC + 1			
	C[2]	T	N	TOTAL	C	T	N	TOTAL	C	T	N	TOTAL
Arthritis	2	0	0	2	4	0	0	4	5	0	0	5
Enlarged stifle joints	0	0	30	30	0	0	14	14	0	0	10	10
Rotated tibia (left)	4	0	0	4	1	0	0	1	1	0	0	1
Rotated tibia (right)	0	1	0	1	1	0	0	1	1	0	0	1
Spondylolithesis	7	3	6	16	1	0	1	2	0	3	7	10
Valgus (left)	4	6	16	26	1	2	12	15	0	2	11	13
Valgus (right)	1	4	4	9	2	3	1	6	1	6	14	21
Valgus (bilateral)	2	8	8	18	3	3	8	14	1	4	8	13
Varus (left)	1	7	8	16	0	0	4	4	0	3	3	6
Varus (right)	1	4	5	10	0	0	1	1	0	0	1	1
Varus (bilateral)	5	2	2	9	0	0	2	2	0	0	0	0
Miscellaneous	0	2	3	5	0	0	2	2	0	1	2	3
TOTAL	27	37	82	146	13	8	45	66	9	19	56	84

1 Number of birds affected (1872 broilers per lighting program).
2 C - culled; T - trimmed; N - noted.

The benefits of increasing lighting programs would likely be greater for broilers kept to larger weights where leg problems pose a more important problem. To test this hypothesis, two increasing programs with different rates of photoperiod increase were compared to constant light (Classen et al. 1989). The two increasing treatments resulted in similar performance and both produced heavier market weight and reduced skeletal disease and mortality in comparison to birds given constant light (Table 4). More gradual increases in day-length and a minimum night of six hours (Increasing 2) resulted in less mortality due to causes other than skeletal disease and SDS.

Table 4. Effect of lighting program on performance of broilers to 63 days of age

	Age				
	0-21d	21-42d	0-42d	42-63d	0-63d
Body weight gain (kg)					
Constant	0.666[a]	1.516	2.182[a]	1.432[b]	3.612[b]
Increasing 1	0.632[b]	1.515	2.147[b]	1.546[a]	3.701[a]
Increasing 2	0.627[b]	1.534	2.162[ab]	1.534[a]	3.683[a]
SEM1	0.004	0.004	0.006	0.014	0.017
Feed to gain ratio (kg/kg)					
Constant	1.517[a]	2.012	1.850	2.618	2.119
Increasing 1	1.424[b]	1.974	1.803	2.534	2.082
Increasing 2	1.429[b]	1.959	1.800	2.624	2.091
SEM	0.008	0.018	0.013	0.046	0.014
Skeletal disease (%)					
Constant	1.76	2.33[a]	4.10[a]	3.06	7.56[a]
Increasing 1	0.72	0.72[b]	1.44[b]	1.28	2.88[b]
Increasing 2	1.04	0.72[b]	1.76[b]	1.92	3.53[b]
SEM	0.21	0.21	0.30	0.36	0.57
Sudden death syndrome (%)					
Constant	2.64[a]	1.68	4.33	1.29	5.94[a]
Increasing 1	1.36[a]	1.68	3.05	0.32	3.37[b]
Increasing 2	0.88[b]	0.75	1.63	0.21	4.65[ab]
SEM	0.23	0.22	0.34	0.22	0.46
Other mortality (%)					
Constant	0.72	0.64	1.36	1.13[a]	2.74[a]
Increasing 1	0.40	0.96	1.36	1.29[a]	2.89[a]
Increasing 2	0.32	0.32	0.64	0.00[b]	0.96[b]
SEM	0.11	0.13	0.17	0.21	0.34

[a,b] Means within a column and main treatment bearing different superscripts are significanlty different (P<.05).

[1] Standard error of the means.

In comparisons to intermittent lighting programs where light and dark periods are repeated every 24 hours (1L:3D), increasing lighting again reduced skeletal problems and mortality but not to the same degree as comparisons to continuous light. This provides additional evidence of a beneficial effect of exposure to darkness (Classen and Riddell, unpublished data).

III. PHYSIOLOGICAL EFFECTS

Reduced skeletal disease is the most pronounced and consistent effect of increasing lighting programs but the exact reason for this effect has not been

established. Potential explanations for the superior skeletal development include one or all of the following:

1. Reduced early growth rate
2. Increased vigorous activity (exercise)
3. Metabolic factors associated with an extended period of darkness

Increasing lighting significantly alters the growth curve of broiler chickens. The increasing programs decrease early growth, but result in compensation later so that final body weights are equal or superior to continuous long lighting treatments. Reduction in growth rate has previously been shown to decrease the incidence of leg abnormalities of different types (Haye and Simons 1978; Riddell 1983; Riddell et al. 1983). Similarly, metabolic disease such as SDS and ascites are also decreased with slower growth.

Broilers are markedly more active when given increasing lighting programs. Again decreased skeletal disease has been reported in birds which exercise vigorously.

In a preliminary student experiment, plasma alkaline phosphatase was found to increase dramatically in the environmental day and decrease during the night for broilers given increasing lighting. In contrast, no circadian rhythm was found for broilers exposed to continuous light and enzyme activity was never higher than that found during the night of the increasing program. This evidence plus the results of comparisons to intermittent lighting suggest that scotophase induced metabolic changes (circadian rhythms) may be essential for proper skeletal growth and modelling. Circadian rhythms in bone growth, which are light dependent, are known to occur in mammals (Simmons 1962, 1968; Hansson et al. 1974).

Recent research with mice supports the concept that darkness, and more specifically the hormone melatonin, are associated with increased immune function and animal livability (Pierpaoli and Maestroni 1987). The majority of mortality in the present experiment was non-infectious but a reduction in stress associated with lighting programs having dark periods (Freeman et al. 1981) may influence both infectious and non-infectious disease.

Increased androgen production in chickens given the experimental treatments is suggested by larger combs. Also plasma androstenedione was higher for females on an increasing program; male hormone levels were numerically but not significantly higher than broilers given constant or decreasing light (Robinson et al. 1988). The separation of hormone level was only noted at five weeks of age and therefore potential anabolic effects of increasing lighting would more likely occur in birds kept for longer periods of time. The results for broilers grown to 63 days of age support this hypothesis. However, the importance of bird mobility in later growth cannot be ruled out. Attempts to correlate androstenedicne level with growth rate have failed to demonstrate a relationship.

IV. LIGHTING PROGRAM RECOMMENDATIONS

Although the 6H, INC and INC+1 programs all produced beneficial effects it would appear that the latter two may be superior. To reduce the snock of changing to 6 hours of light, it may be beneficial to gradually reduce daylength to that level during the first seven days of the broiler cycle. Birds on these programs are very active and can be more difficult to handle at marketing. To overcome potential problems, broilers should be exposed to continuous light several days prior to loadout. Currently recommended lighting programs for broilers and roasters are shown in Table 5. Light infiltration will likely reduce the benefits of the lighting programs but they

are recommended even in barns without light control. Consideration should be given to reducing light infiltration during the first two to three weeks when day-length is shortest and the need for ventilation is lowest.

Table 5. Current lighting program recommendation

	Light Intensity	Photoperiod*	
Age (d)	(lux)	Broiler	Roaster
0	20	24.0L:0.0D	24.0L:0.0D
4	20	18.0L:6.0D	18.0L:6.0D
7	5	6.0L:8.5D:1.0L:8.5D	6.0L:8.5D:1.0L:8.5D
14	5	10.0L:6.5D:1.0L:6.5D	9.0L:7.0D:1.0L:7.0D
21	5	14.0L:4.5D:1.0L:4.5D	12.0L:5.5D:1.0L:5.5D
28	5	18.0L:6.0D	15.0L:4.0D:1.0L:4.0D
35	5	24.0L:0.0D	18.0L:6.0D
42	5	To market	21.0L:3.0D
49	5		24.0L:0.0D
			To market

* L - light; D - dark.

V. CONCLUSIONS

Major improvements in bird health can be obtained without decreased production parameters by using an increasing photoperiod lighting program. Further research is required to determine the reason for improved health, but what ever the effect, financial gains from a reduction in bird loss due to leg problems and mortality can be accrued through the use of these programs. Also using increasing programs can be justified on the improvement of bird welfare alone.

VI. ACKNOWLEDGEMENTS

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THE PRESENTATION OF V4 NEWCASTLE DISEASE VACCINE ON FEED

R.C. CHUBB and R.B. CUMMING

It has been established that the Australian V4 Newcastle disease virus is suitable as a vaccine given by 'eyedrop'. Recently, it has been shown that a heat resistant variant, when mixed with feed, can also infect chickens, inducing haemagglutination inhibition (HI) antibodies and a resistance to challenge with virulent virus (Ideris et al 1987). ACIAR is using a similar variant as a prototype vaccine (A. Webster Pty. Ltd., Claxton and Leonard 1987) for field trials in a number of Asian countries . Results in the field have been variable. We have therefore been investigating the different methods of vaccine presentation using the latter vaccine.

The vaccine was mixed with diluent and fed according to the instructions ie. 1 vial/50ml diluent, 5ml vaccine/100 gm of feed. The birds were 3-4-week old crossbred cockerels, reared and housed in isolation, and trained to eat the feed vehicles for three days prior to vaccination. The diluents used were distilled water (DW), phosphate buffered saline (PBS), and skimmed milk (SM) made up as instructed with DW. Feed vehicles were starter crumbles, paddyrice, corn (maize), cracked corn (maize) and wheat. Groups of at least ten birds were starved overnight before vaccination with 10 gm of feed per bird during the next day.

Antibodies (HI) were induced in birds fed vaccine mixed with either crumbles or paddyrice. Mixing the vaccine with the other feed vehicles did not induce HI antibodies. Birds rarely produced HI antibodies before three weeks after vaccination. For example, using DW as diluent and all the feed vehicles, no birds had HI antibodies at 15 days post-infection (PI). At 18 days PI, two of ten crumble-treated birds and two of ten paddyrice-treated birds had HI antibodies. At 25 days PI, 7 of 10 crumble-treated birds and 6 of 10 paddy rice-treated birds had HI antibodies. Later experiments included revaccination at three or four weeks PI. Again, only when crumbles or paddyrice was used as a vehicle were HI antibodies produced. It was also apparent that the diluent could affect HI antibody production. For example, using crumbles, at 3 weeks PI, birds with DW as diluent had 6 of 10 with HI antibodies, whereas none were seen with PBS as diluent. After revaccination two more weeks elapsed before 2 of 10 birds in the PBS group had HI antibodies compared with 8 of 10 birds in the DW group.

These, and other experiments, suggest that this particular feed vaccine may have presentation problems. Not all grains can be used as vehicles without some protection of the virus. Using conventional diluents, only crumbles and paddyrice have been successful. Dilution of the vaccine with PBS had a deleterious effect on the ability of the vaccine to induce HI antibodies compared to dilution with DW or SM. There is some evidence to suggest that differences of degree may exist between DW and SM, depending on the vehicle. Revaccination seems to increase the number of birds with HI antibody, but does not affect the overall titre of the antibody.

This work suggests that there is a need for, and strict adherance to, a presentation protocol for this vaccine to be effective.

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ABILITY OF BIRDS TO DISCRIMINATE AGAINST ERGOT-CONTAMINATED FEED

W.L. Bryden, A.H. Liu and W.J.K. Bakau

The initial response of animals suffering a toxic insult is often to reduce food intake. With some mycotoxins, especially the trichothecenes, pigs will refuse to eat contaminated feed (Bryden 1989). It has been argued that toxic secondary metabolites or mycotoxins of fungi act as a chemical defence system to deter potential predators. Such a system would be particularly relevant to fungal sclerotia or ergots which act as nutrient-storage and survival or dormancy capsules for the fungus (Wicklow and Cole 1982). There is very little information on refusal of feed contaminated with mycotoxins in poultry and in the present study the ability of poultry to discriminate against feed containing the sclerotia of Claviceps purpurea was assessed.

Three week old cross-bred cockerels were allocated into groups containing five birds and randomly assigned to treatments. The feeder on each pen was divided with a partition and one half was filled with the basal grower diet and the other half of the feeder was filled with the basal diet containing 0, 10 or 20 g ergot/kg. Other groups were offered the diets containing ergot only. There were five replicates of each treatment, and chickens were allowed free access to the feeders for 21 days.

In the second experiment commercial cross-bred laying hens, aged 55 weeks and housed in single bird cages were offered a layer diet containing either 0, 5, 15 or 45 g ergot/kg. In addition, other birds were offered two feed containers; one containing uncontaminated feed and the other, feed containing ergot. Six hens were monitored for 14 days on each treatment.

The addition of ground ergot to mash diets resulted in a significant ($P<0.05$) decrease in the performance of both growing chicks and laying hens. The results with chicks showed that ergot decreased weight gain, depressed food intake and caused a corresponding rise in food conversion. Chicks fed both levels of ergot developed gangrenous lesions of the digits, but this lesion did not occur in chicks offered a choice between a contaminated and uncontaminated diet. These chicks also grew at the normal rate. Although laying hens did not develop gangrenous lesions ergot did decrease food intake and egg production with birds consuming the diet containing 40 g ergot/kg ceasing to lay after seven days. However, laying hens offered a choice of diets continued to perform at the normal rate.

The results of the experiments establish that when offered a choice between an uncontaminated diet and a diet contaminated with ergot, poultry are able to discriminate against the toxic diet and modify their food intake accordingly. The underlying mechanism of this ability is not known but it is interesting to note that a number of alkaloids contained within the ergots of C.purpurea are antagonists of neurotransmitters (Bryden 1989).

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THE SIGNIFICANCE OF CEREAL NON-STARCH POLYSACCHARIDES IN POULTRY DIETS

M.CHOCT and G.ANNISON

The poor performance of broilers fed rye-based diets and barley-based diets is due to the high level of pentosans in rye (Antoniou et al. 1981) and the β-glucan content of barley (Gohl et al. 1977). The anti-nutritive effects of these non-starch polysaccharides (NSP) are manifested by the depression of nutrient digestion and absorption (Antoniou et al. 1981). It has been reported that approximately 25% of wheat cultivars grown in the eastern part of Australia show an unexpectedly low metabolisable energy (<13 MJ/kg DM) when used as the main energy source in broiler diets (Mollah et al. 1983). Other studies showed that starch digestibility of broiler diets containing low-ME wheats was significantly depressed (Rogel et al. 1987). In the present studies an experiment was conducted to examine 7 commonly available cereals (Table) for apparent metabolisable energy (AME) and starch digestibility (SD) using 4-5 weeks old commercial broilers in individual cages (8 birds/treatment) with subsequent measurements of pentosan and β-glucan levels. The results are shown in the Table.

CEREALS	AME (MJ/kg DM)	SD (%)	PENTOSANS (%)	β-GLUCANS (%)	β-GLUCANS+ PENTOSANS (%)
RICE	17.36	99.27	0.00	0.04	0.04
MAIZE	15.83	99.48	4.26	0.12	4.38
SORGHUM	15.77	99.11	2.84	0.10	2.94
WHEAT	14.32	96.64	6.05	0.50	6.55
TRITICALE	13.83	--	6.97	--	--
BARLEY	11.92	96.55	7.55	3.32	10.87
RYE	11.34	95.01	8.90	1.15	10.05

A highly significant correlation ($P<0.001$; $r=-0.95$) was found between AME and pentosan levels in cereals. Furthermore, when the summed pentosan and β-glucan levels are regressed against AME the correlation is higher ($P<0.001$; $r=-0.98$). These data confirm and extend the observations of Annison and Johnson (1989) who also noted the strong correlation between pentosan levels and energy metabolisability in cereals. The current investigations indicate that the NSP also correlate closely with AME, which suggests that at high levels they may inhibit starch digestibility. The low-ME phenomenon experienced in the Australian wheat industry may, therefore, be due to a varying level of non-starch polysaccharides, especially pentosans, among cultivars.

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EFFECTS OF CALCIUM AND PHOSPHORUS ON VITAMIN D STATUS IN CHICKENS

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Studies with rats have shown that dietary factors can enhance the metabolic destruction of vitamin D and lead to vitamin D-deficiency rickets (Clements et al. 1987). Experiments were conducted to determine the effect of changes in the level of dietary calcium and phosphorus on the requirements for vitamin D in broiler chickens.

Day-old male broilers were raised on diets containing (i) adequate calcium (10.0 g/kg) or low calcium (3.0 g/kg) with adequate phosphorus (7.0 g/kg available P), or (ii) diets containing graded levels of phosphorus (10.0, 7.5, 5.0, 2.5 g/kg available P) and adequate calcium (10.0 g/kg). All birds were given oral doses of vitamin D_3 three times per week. After 21 days plasma levels of vitamin D metabolites were measured by competitive protein binding assay.

Plasma concentrations of vitamin D_3 metabolites

	Calcium or Phosphorus (g/kg)	D_3 dose (μg)	25(OH)D_3 (n, +sem) (nmol/l)	1,25(OH)$_2D_3$ (n, +sem) (pmol/l)
(i)	10.0 Ca	40	46.2 (16, 4.8)	266 (7, 26)
		20	37.2 (12, 3.0)	
		5	28.8 (16, 2.1)	247 (6, 19)
	3.0 Ca	40	32.8 (15, 2.3)	713 (5, 17)
		20	26.3 (15, 2.5)	
		5	16.6 (17, 1.6)	593 (6, 39)
(ii)	10.0 P	40	41.6 (8, 3.2)	275 (5, 34)
	7.5 P	40	40.1 (6, 5.1)	267 (6, 38)
	5.0 P	40	41.5 (7, 1.2)	234 (5, 20)
	2.5 P	40	34.0 (6, 3.5)	355 (5, 46)

The plasma concentrations of 25-hydroxyvitamin D_3 (25(OH)D_3) in chickens on the low-calcium diet were significantly lower ($P<0.001$) than in chickens on the normal calcium diet. Plasma 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2D_3$) levels were significantly elevated ($P<0.001$) in chickens on the low-calcium diet and this may account for their reduced 25(OH)D_3 status. The plasma clearance rate of 25(OH)D_3 was determined in both groups by measurement of the elimination half-time ($t_{1/2}$) of a tracer amount of ^{3}H-25(OH)D_3. The clearance rate was only slightly faster in calcium-deficient chickens ($t_{1/2}$=1.42 days) compared to chickens with adequate calcium ($t_{1/2}$=1.65 days). Therefore, calcium deficiency increases the utilisation of vitamin D only by increased conversion to 1,25(OH)$_2D_3$ and not by enhanced destruction of 25(OH)D_3 to inactive metabolites.

Dietary phosphorus levels had minimal influence on vitamin D_3 metabolite concentrations. Plasma 25(OH)D_3 and 1,25(OH)$_2D_3$ concentrations were slightly depressed and elevated respectively in birds fed the diet containing 2.5 g/kg phosphorus. Therefore, phosphorus deficiency or excess phosphorus do not appear to directly modify vitamin D status in broiler chickens.

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REPRODUCTIVE PERFORMANCE AS INFLUENCED BY NUTRITIONAL MANAGEMENT IN CHICKENS SELECTED FOR ASPECTS OF GROWTH AND BODY COMPOSITION

A. T. BUNAN

Commercial broiler breeding programs now place emphasis on increased lean tissue growth rate but there is limited information available as to the consequences of such selection on reproductive performance. An experiment was conducted to assess the effects of nutritional management on reproductive performance of 480 pullets from five lines of chickens selected for high (line F) or low (line L) abdominal fat, high body weight (line W), high body weight combined with low abdominal fat (line WL), or at random (line C).

Birds were given one of three rearing-laying feeding regimes viz. restricted-restricted (RR), restricted-ad libitum (RA) or ad libitum-ad libitum (AA). The rearing and laying diets contained 10.5 and 11.2 MJ ME and 150 and 160g CP/kg respectively and restricted birds were fed approximately 75 and 90% of ad libitum food consumption during the rearing and laying periods respectively. Changeover of treatments and diets was at 20 weeks and egg production was measured to 60 weeks. The results are shown in the table.

Bodyweight (BW,g), age (A,d) and body fat (F,g/kg) at onset of sexual maturity, hen-day egg production to 60 weeks (HDEP,eggs/bird), fertility (Fert,%) and hatchability (Hatch,%) in pullets from the five lines on three rearing-laying nutrient allowance regimes (R = restricted, A = ad libitum)

Regime	Line	BW	A	F	HDEP	Fert	Hatch
RR	F	2787^{h}	190^{bcd}	22.7^{abc}	101.3^{cde}	92.3^{a}	56.3^{abc}
	L	3132^{g}	194^{ab}	21.5^{bcde}	106.1^{cde}	89.0^{a}	63.3^{ab}
	W	3701^{bc}	198^{a}	24.0^{a}	98.6^{de}	83.3^{ab}	54.3^{abc}
	WL	3541^{de}	188^{de}	24.0^{a}	93.2^{ef}	90.0^{a}	54.3^{abc}
	C	3014^{g}	193^{bc}	22.4^{abcd}	118.9^{ab}	91.3^{a}	67.7^{a}
	Mean	3235^{r}	192^{p}	22.9	103.7^{p}	89.2^{p}	59.2^{p}
RA	F	3136^{fg}	185^{e}	20.2^{de}	102.9^{cde}	84.3^{ab}	56.3^{abc}
	L	3246^{f}	191^{bcd}	20.5^{cde}	127.5^{a}	81.3^{abc}	54.0^{abcd}
	W	3771^{b}	188^{de}	23.5^{ab}	97.5^{def}	65.3^{c}	47.0^{c}
	WL	3634^{cd}	189^{cde}	23.9^{a}	112.5^{bc}	92.3^{a}	69.0^{a}
	C	3133^{fg}	189^{cde}	22.4^{ab}	118.9^{ab}	72.0^{bc}	44.0^{c}
	Mean	3133^{q}	188^{q}	22.1	111.9^{p}	79.1^{q}	45.9^{q}
AA	F	3437^{e}	153^{fg}	24.6^{a}	86.8^{fg}	63.7^{c}	23.7^{d}
	L	3571^{cd}	159^{f}	19.5^{e}	101.3^{cd}	83.0^{ab}	49.7^{b}
	W	4024^{a}	152^{g}	23.5^{ab}	77.1^{gh}	92.7^{a}	52.0^{bc}
	WL	4014^{a}	148^{h}	23.3^{ab}	69.6^{h}	72.0^{bc}	63.0^{ab}
	C	3543^{de}	149^{gh}	22.8^{abc}	108.2^{bcd}	94.0^{a}	41.3^{c}
	Mean	3718^{p}	150^{r}	22.7	88.7^{q}	81.1^{pq}	45.9^{q}
LSD 0.05		132	5	2.4	12.5	5.1	15.6

With the exception of the high-fat line F, there appears to be a characteristic level of body fat rather than weight which determines the onset of sexual maturity in each line. Egg production in all lines was improved by rearing period restriction while in contrast to the F, W and C lines, the L and WL lines benefitted from full feeding during lay. Mean fertility and hatchability were better on continued restriction although hatchability in line WL improved with full feeding in lay in contrast to line C. Full feeding during rearing resulted in a drastic reduction in hatchability in line F.

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SELECTION TO OPTIMISE ECONOMIC RESPONSE IN GROWTH AND FOOD UTILISATION EFFICIENCY IN JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA)

D.B. ETSE

Genetic improvement in food utilisation efficiency in commercial broiler chickens has been hampered by a lack of information on the genetic and phenotypic parameters associated with the relevant traits and by the complexity of the various alternative selection strategies. Japanese quail (Coturnix coturnix japonica) are a useful model for chickens in selection studies because of their much shorter generation interval and lower maintenance costs. In this study phenotypic and genetic parameter estimates for growth, food intake and food utilisation efficiency were determined by sib analysis in Japanese quail. Measurements were made of 14-day body weight (14dW), 26-day body weight (26dW) and 14-to 26-day food consumption (FC) in 866 quail produced from matings between 40 males and 120 females sampled from the F_2 generation of an original mating between two commercial quail strains. Weight gain (WG) and FCR were calculated from the above measures.

Six selection lines were established from the base population. They were selected for increased 26dW (line W), increased FC (line F), decreased FCR (line E), an index combining 14dW, 26dW and FC (line I_1), a multi-stage index with the same traits but with initial selection and culling on 14dW (line I_2) or at random (line C). Results are shown in the table.

Heritability and genetic (above diagonal) and phenotypic (below diagonal) correlation estimates (sire+dam, sexes combined) for 14-d weight (14dW), 26-d weight (26dW) and 14-to 26-d weight gain (WG), food consumption (FC) and FCR from sib analysis of 866 Japanese quail. Standard errors in parenthesis. Response (g) to selection for one generation in the five lines is shown below.

Trait	14dW	26dW	WG	FC	FCR
14dW	0.46 (0.08)	0.89 (0.04)	0.59 (0.13)	0.83 (0.08)	0.12 (0.26)
26dW	0.80	0.52 (0.07)	0.89 (0.04)	0.98 (0.02)	-0.36 (0.27)
WG	0.31	0.82	0.39 (0.08)	0.94 (0.04)	-0.77 (0.32)
FC	0.55	0.78	0.71	0.29 (0.07)	-0.48 (0.25)
FCR	0.22	0.17	-0.48	0.23	0.08 (0.04)
Line					
W	4.5	9.0	4.0	10.7	0.01
F	4.0	6.8	2.3	12.6	-0.05
E	1.2	2.9	1.6	2.4	-0.06
I_1	5.2	8.5	2.2	6.1	-0.03
I_2	3.1	3.3	0.1	1.0	0.02

Responses and heritability and genetic and phenotypic correlation estimates were in reasonable agreement with those obtained in chickens (Pym and Nicholls 1979), although the heritability of FCR was considerably lower in the present study. From these preliminary data, selection on I_1 resulted in similar response in 26dW but considerably reduced response in FC compared to the W line selected for 26dW alone. Selection on I_2 resulted in a relatively low response in all traits at this early stage of the selection study.

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LACK OF EFFECT OF ZEOLITES ON PERFORMANCE AND EGG SHELL QUALITY OF TWO STRAINS OF LAYING HENS

M.EVANS and D.J.FARRELL

Both natural (Clinoptilolite) and synthetic (Ethacal) zeolites are used in the diets of laying hens (see Evans, 1989).

In the experiment there were 16 dietary treatments each with 4 replicates each with 10 birds. The factors included 2 strains of bird (New Hampshire x Australorp [NHxA], and New Hampshire x White Leghorn [NHxWLH]), 4 zeolite types (no zeolite, Ethacal (0.75%), Z1, Z3 (2.5%)), 2 phosphorus levels (0.6 %, 1.2 %) and two limestone particle sizes (powder, 2mm chips). Z1 and Z3 are natural bimineralic zeolites mined at the 'Escott' prospect near Werris creek, N.S.W. A total of 1280 birds of 37 weeks of age were used in the experiment and were recorded until 68 weeks of age for 1 three week period and 14 two week periods.

Dietary treatment did not affect hen day egg production (HDEP), egg weight (EW), egg mass (EM), mortality (MO), or body weight change (BWC). Specific gravity (SG) of eggs were significantly ($P<0.05$) reduced by the higher phosphorus (1.2 %) level in the diet up to 52 weeks of age but not thereafter. The SG was reduced from 52 weeks of age when limestone chips were fed compared to limestone powder ($P<0.05$). Prior to this, the calcium source did not significantly influence SG. Calcium source significantly ($P<0.01$) affected daily feed consumption (DFC). Birds of both strains on diets with limestone chips had a higher DFC than birds fed diets with limestone powder.

There was a significant ($P<0.01$) strain effect on EW, EM, HDYS, DFC, and feed efficiency. There was no difference between strains for HDEP or BW. The superiority of NHxWLH was due to a lower DFC and larger EW. This strain also laid eggs with a significantly ($P<0.01$) higher SG.

Eggs were sampled at 43 and 61 weeks of age to determine haugh units (HU), shell weight (SW) and shell thickness (ST). There were no significant effects of the dietary treatments on any of these measurements with the exception of ST which was significantly ($P<0.05$) reduced on the high phosphorus level in eggs sampled at 43 weeks but not at 61 weeks. The NHxWLH had a significantly ($P<0.01$) greater SW and ST at both 43 and 61 weeks.

Samples of eggs were taken at 48 and 61 weeks of age for 7 consecutive days and classified and grouped into 5 shell defect categories for statistical analyses. These were gross cracked eggs, major shell defects, minor shell defects, hairline cracks and total defects. There was no significant affect on any category due to dietary treatments, with the exception that in eggs sampled at 48 weeks only, limestone chips significantly ($P<0.05$) increased the incidence of major shell defects. This was not seen in eggs sampled at 61 weeks. The NHxWLH had a higher ($P<0.01$) incidence of minor shell defects than the NHxA.

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CALORIMETRIC MEASUREMENTS ON CHICKENS VACCINATED WITH INFECTIOUS BRONCHITIS

D.J. FARRELL, R. CHUBB, E. THOMSON and YANG AIJUN

Infectious bronchitis is a problem in the Australian poultry industry. This paper describes continuous calorimetric measurements on groups of 9 male broiler chickens to determine effects of eye-drop vaccination at 1-d-old with Vic S (10^6 EID 50 per ml) and on groups of 5 similar birds vaccinated at 7 or 8d of age and measurements made for the next 3 to 8d.

The effects of vaccination and sham vaccination of broiler chicks at 1 or 7 or 8d of age on ME intake, heat production, energy balance (EB) (all in kJ/kgW per d) and RQ in 3 experiments.

	Age (d)	ME intake	RQ	Heat production	Energy balance
		Experiment 1			
Control	1-3	2294	0.96	1324	970
Infected		2177	1.05	1125	1052
Control	4-7	2360	1.01	1253	1106
Infected		2287	1.05	1202	1086
Control	8-11	2217	1.05	1165	1053
Infected		2352	1.06	1214	1137
Control	12-14	1849	1.05	1043	806
Infected		1918	1.03	1075	843
		Experiment 2			
Control	1-3	1936	1.00	1159	777
Infected		2144	1.03	1126	1019
Controls	4-6	2302	1.07	1184	1118
Infected		2258	1.05	1193	1065
Controls	7-10	2595	1.06	1200	1395
Infected		2495	1.06	1237	1257
		Experiment 3			
Control	1-4	1829	1.03	1187	643
Infected		2034	1.03	1174	860
Control	5-7	1777	1.01	1119	658
Infected		1999	1.01	1161	837
Control	8-11	1711	1.02	1172	538
Infected		1432	0.98	1171	261
Control	12-15	1821	1.04	1108	731
Infected		2334	1.02	1188	1147

Although no attempt was made to pair feed birds, neither heat production nor ME intake differed markedly between the control and infected birds. If anything infected birds in the period immediately following vaccination at 1d reduced heat production and had a higher EB. Only in experiment 3 when birds were vaccinated at 8d was intake reduced between d8 and d11 even though heat production was the same as the controls.

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RECENT DEVELOPMENTS IN THE MANIPULATION OF BODY FAT IN POULTRY AND SOME OF THE CONSEQUENCES

D.J. FARRELL, G.P.D. JONES and YANG AIJUN

Summary

Excess fat in broilers is still a problem despite genetic improvement in some commercial strains. Calorimetric measurements are reported showing a higher heat production in lean birds compared to their fat counterparts. Chemical manipulation of body fat by dietary inclusion can reduce carcass fat but usually there is also a decline in growth rate. These chemicals do not change the heat production of birds when compared before and after dietary inclusion. Short-term dietary manipulation of chicks at 6-7d of age for short periods (4-6d) to achieve zero growth frequently gave reduced abdominal fat pad size, some times less total body fat and often improved food efficiency. Calorimetric measurements on birds up to 35d of age that had been restricted for short periods to maintenance showed differences in heat production compared to control birds up to 35d of age. Sequential determinations of body fat in live birds using a tritiated water dilution method show the changes that occur following food restriction of broiler chicks. It is concluded that real progress is unlikely to be made in this area of reduced fat in broilers until consumer resistance generates much increased research effort.

I. INTRODUCTION

Much has been written recently about the problem of excess fat in the carcass of broiler chickens and the topic has been reviewed by McLeod (1982), Lin (1981), Cherry (1987), Pym (1987) and Jones et al. (1989).

Only one of four strains of commercial, 49-day-old male broilers currently grown in Australia has a total carcass fat content below 10% (Table 1) although growth rate of each strain is similar.

Table 1. Growth rate and fat content of four current Australian commercial strains of male broilers (Jones and Farrell, unpublished data)

Strain	Bodyweight (W,g)	Food efficiency	Body fat (% W)	Fat pad (% W)
A	2358	1.79	8.8	1.01
B	2403	1.90	15.6	2.31
C	2344	1.92	14.2	1.58
D	2415	-	15.0	2.01

Undoubtedly, the ultimate solution to excessive levels of

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carcass fat lies in the hands of geneticists (as shown in Table 1). At present it must be recognised that selection pressure for desirable traits, such as lean tissue deposition, may inadvertently introduce undesirable breeding characteristics (Gous 1986).

In the short term, dietary manipulation of body composition is a viable alternative. This can be used successfully, not only with experimental broiler lines but with commercial broilers regardless of existing carcass fat levels.

The purpose of this paper is to provide an up-date on recent developments to tackle the problem of excess carcass fat in broilers. Other factors that are associated with changes in carcass fat are also described; these relate mainly to energy metabolism.

II. CALORIMETRIC MEASUREMENTS ON LEAN AND FAT LINES

Experimental lines of broilers (25-42d of age) selected for fatness or leaness (Pym 1985) showed in two experiments (A and B) significant differences in heat production (Table 2) when food intake was the same (Yang and Farrell, unpublished data). This would be expected because the net availability of metabolisable energy (NAME) is higher for protein deposition (k_p) than for fat synthesis (k_f) (Webster 1980).

Table 2. Comparisons of the mean (±SEM) heat production, metabolisable energy intake (ME), energy (E) balance and nitrogen (N) balance of groups of three fat and lean birds (aged 25-38 d) with sexes combined (Experiment A 3d) and groups of three female birds (aged 28-42 d) (Experiment B 5d)

	Experiment A (3d)			Experiment B (5d)		
	Fat	Lean	SEM	Fat	Lean	SEM
Body weight (kg)	2.33	2.33	0.018	2.03	1.99	0.016
Weight gain (g)	202	194	14.1	397	380	5.2
Food intake (g)	542	526	8.0	883	879	4.8
Metabolisability (%)	74.2	74.8	0.69	71.8	71.9	0.18
ME intake (kJ/d/W)	1051	1047	22.2	1094	1124	2.5
Heat production kJ/d/W	714[1]	744	6.3	747[1]	790	4.2
Energy balance kJ/d/W	337	273	22.2	347	333	4.4
kJ/d/$W^{0.75}$	402	322	24.6	413	391	5.6
E efficiency (%)	30.0	25.3	1.31	31.0	28.8	0.41
N intake (g/d/W)	2.51	2.48	0.050	2.77	2.83	0.012
N excretion (g/d/W)	1.23	1.20	0.018	1.40	1.43	0.016
N balance (g/d/W)	1.28	1.29	0.035	1.37	1.40	0.010
N efficiency (%)	50.1	51.9	0.52	49.0	49.3	0.45

[1]Fat line significantly ($P<0.05$) different from lean line.

The regression of energy retention (Y, kJ/d per $W^{0.75}$) against ME intake (X, kJ/d per $W^{0.75}$) in Experiment A

(Equations 1 and 2) indicated that NAME for weight gain was 0.54 for the lean broilers. This was lower ($P<0.05$) than that for the broilers selected for fatness (0.84).

Lean line $Y = -354 + 0.54X$ ($R^2=0.84$, RSD=53, n=13) (1)
Fat line $Y = -635 + 0.84X$ ($R^2=0.90$, RSD=61, n=13) (2)

MacLeod et al. (1988) found a significantly higher fasting heat production for lean broilers compared to their fatter counterparts. The observed differences in NAME between fat and lean birds is likely due to the energy costs of amino acid turnover associated with protein synthesis (Buttery and Annison 1973) and to the low energy cost of lipid deposition (Annison 1974).

III. CHEMICAL MANIPULATION OF BODY FAT

(a) Beta agonists

A group of compounds, the beta-adrenergic agonists, used to reduce body fat and increase lean deposition in livestock have received much attention (see Hanrahan 1987). Their exact mode of action is uncertain, but they seem to have the effect of depressing lipogenesis and markedly reducing tissue protein degradation with a small increase in protein synthesis (Buttery and Dawson 1988). These beneficial effects of beta agonists appear to be much reduced in broiler chickens (Hanrahan 1987). Cimaterol had variable results in lines of broilers genetically selected for fatness and leanness (Yang 1989), but was more effective in the experimental lean line of females than in males by reducing body fat at 0.2 and 0.4ppm from 10.8% to 8.7% and 8.5% respectively. In the fat line, males given 0.6ppm cimaterol had a reduced abdominal fat pad (3.4% to 2.2%). Corresponding reductions for females from 3.3% to 2.4% on 0.4ppm were not significant but concomitant with a decline in bodyfat. Liveweight and food intake were reduced on dietary inclusions of cimaterol. However Yang (1989) observed no change in growth performance or fat pad size in either males or females in a commercial strain of broilers on a diet with 0.5ppm cimaterol.

(b) Lipolytic agents

Thyroid hormones and other lipolytic agents can regulate adipose metabolism (Fain 1980). In chickens the methylxanthines (theophylline and caffeine) exert their lipolytic action by potentiating the effects of the catecholamines on cyclic AMP in the adipose tissue (Langslow and Hales 1971). Jones et al. (1989) reported results which confirmed the positive effects of these agents in reducing body fat in broilers and also in reducing growth performance through a reduction in food intake. Further results are reported in Table 3. Only iodinated casein at either 50 or 100ppm was able to sustain growth rate while still reducing fat pad size. Iodinated casein in combination with theophylline or caffeine depressed growth but only with theophylline were the abdominal fat pad and carcass fat reduced.

Table 3. The effects of iodinated casein (C) and/or theophylline (T) or caffeine (K) on the growth performance, food efficiency (FCR), abdominal fat pad (AFP) and carcass fat content of male commercial broilers grown from 28 to 49 d of age in two experiments (C and D)

	Control	0.05% T	50ppm C	0.05% T +50ppm C	100ppm C	0.05%T 100ppm C	SEM
			Experiment C				
Final weight(g)	1855^{a1}	1750^{b}	1908^{a}	1675^{c}	1862^{a}	1679^{bc}	14.2
Weight gain(g)	1133^{a}	1001^{b}	1183^{a}	931^{b}	1134^{a}	950^{b}	12.9
FCR	2.29^{a}	2.19^{ab}	2.00^{d}	2.15^{bc}	2.06^{cd}	2.19^{ab}	0.018
AFP(%)	1.16a	0.80^{b}	$0.71b^{c}$	0.54^{c}	0.79^{b}	0.53^{c}	0.011

	Control	50ppm C	50ppm C +0.05%K	0.05% K	0.1% K	SEM
			Experiment D			
Final weight(g)	2152^{a}	2164^{a}	2003^{b}	2018^{b}	1838^{c}	14.0
Weight gain(g)	1323^{a}	1337^{a}	1175^{b}	1158^{b}	1008^{c}	13.2
FCR	1.99^{a}	1.98^{a}	2.07^{a}	2.06^{a}	2.06^{a}	0.021
AFP(%)	1.30^{a}	1.06^{b}	1.27^{a}	1.05^{b}	0.70^{c}	0.013
Carcass fat (%)	10.2^{a}	9.6^{a}	9.7^{a}	9.7^{a}	8.1^{b}	0.19

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

(c) Calorimetric measurements

The introduction of various chemical agents to the diet of 21-35 day old female broilers produced no marked changes in heat production (Table 4).

Table 4. Effects, 3d prior to (d1-3) and following (d4-6) the inclusion of theophylline, iodinated casein and cimaterol in the diet on daily heat production (kJ/W or $kJ/W^{0.75}$) of female commercial broilers aged from 21 to 35 d

Day	Control		Theophylline (0.05%)		Iodinated casein (50ppm)		Cimaterol (0.5ppm)	
	(W)	($W^{0.75}$)	(W)	($W^{0.75}$)	(W)	($W^{0.75}$)	(W)	($W^{0.75}$)
1	818	957	773	923	840	987	823	939
2	778	919	754	906	814	965	792	926
3	767	912	744	897	805	961	771	910
4	763	912	699	847	798	959	785	932
5	724	868	698	849	775	937	756	904
6	745	898	711	868	757	921	752	905
SEM	11.8	14.9	5.8	7.7	7.3	9.1	8.3	10.0

IV. DIETARY MANIPULATION

We have reported several experiments in which we have successfully reduced body fat of broilers by drastically depressing growth for several days starting at 6-7d of age (Jones and Farrell 1987, 1989; Farrell et al. 1989, Jones et al. 1989). The underlying principle of this manipulation is to reduce fat accretion during this time to zero. It is known that in the chicken both hyperplasia and hypertrophy of adipose cells continues until at least 12 weeks of age (Hood 1982). Both hyperplasia and hypertrophy of the adipocytes is affected by food restriction (Jones and Farrell 1989).

When fat deposition in female commercial broilers was studied over a 10 week period using a tritiated water technique (Johnson and Farrell 1988) it was observed that the rate of fat deposition was bi-phasic (Fig. 1) and was similar to that observed by Leeson and Summers (1980).

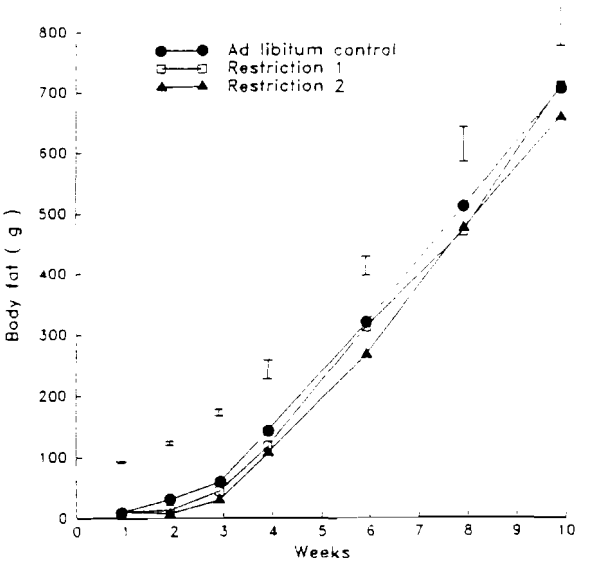

Figure 1. The deposition of carcass fat, determined using a tritiated water technique, in female commercial broiler chickens fed ad libitum or subjected to food restriction to 20% ad libitum intake for 4 d or to 25% ad libitum intake for 6 d commencing at 7 days-of-age.

Fat deposition increased slowly to 3 weeks of age, during which time adipocyte hyperplasia would be dominant. After 3 weeks of age the rate of deposition increased commensurate with adipocyte hypertrophy being mostly responsible for fat deposition.

Our results showed that there may be additional benefits in restricting food consumption of chicks at an early age. Jones et al. (1989) reported improved feed efficiency and, on

occasions, growth rate compared to unrestricted controls. On the other hand we (Jones and Farrell 1987; 1989) and others (Plavnik and Hurwitz 1985; Plavnik et al. 1986) have also reported a small reduction in final bodyweight as a consequence of early food restriction.

The derived data indicate that food restrictions decreased carcass fat. However, these reductions were generally not significant.

Recently we have repeated measurements (See Jones et al. 1989) on a different strain of broiler chickens in which 4 replicates of 8 unsexed birds were restricted at 7d of age using a food allocation of 20% of ad libitum for different periods or diet dilution with rice hulls. At 48d growth rate was the same ($P>0.05$) on all treatments (Table 5), despite significantly lower intakes by birds on all experimental treatments compared to the unrestricted controls (1). Both food allocation restrictions gave a significant improvement in feed efficiency. The abdominal fat pad (g or %W) was smaller in birds on treatments 2,3 and 5 compared with the control birds. Those on treatments 3 and 5 had less total fat than had the control birds.

Table 5. Effects of dietary restriction of unsexed broilers at 7d of age fed ad libitum (1), 20% of ad libitum for 4d (2), or 2d on, 2d off, 2d on (3) and 35% broiler crumbles/65% rice hulls (4) or 40% broiler crumbles/60% rice hulls (5) for 2d on, 2d off, 2d on.

	Treatment					
	1	2	3	4	5	LSD 5%
Bodyweight (48d)	2315^{a1}	2219^{a}	2231^{a}	2211^{a}	2188^{a}	139.6
Intake	4668^{a}	4189^{b}	4343^{b}	4363^{b}	4284^{b}	274.9
FCR	2.15^{a}	2.01^{b}	2.07^{b}	2.10^{ab}	2.11^{ab}	0.078
Total fat (%)	16.9^{a}	15.3^{ab}	14.8^{b}	15.5^{ab}	14.4^{b}	1.74
Abd. fat pad (%)	2.57^{a}	2.11^{b}	2.09^{b}	2.26^{ab}	2.01^{b}	0.39
Dressed wt. (%)	70.0^{a}	68.8^{ab}	68.1^{b}	-	-	1.68

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

Previously Jones et al. (1989) reported an enhancement in growth following a 2d x 2d food restriction but this was not observed here. The savings in food were 478g and 325g per bird on treatments 2 and 3 respectively. Only about 40% and 50% of this could be explained in terms of reduced growth thus giving net savings of about 280g and 157g per bird.

The effect of early food restriction in broilers is to increase the heat production of the bird. Calorimetric measurements on female broilers (Table 6) show increased heat production of restricted-fed broilers up to 35 d of age.

Table 6. The influence of food restriction beginning at 7d of age on the heat production ($kJ/W^{0.75}$) of commercial female broiler chickens.

Age (d)	No of birds	Restriction-1 (20% ad lib. 4d)		Ad libitum		Restriction-2 (20% ab lib. 2 x 2d)
4-6	8	3776	NS[1]	3649	NS	3705
7-10	8	3766	***	5044	**	4655
11-14	8	5372	**	4998	NS	4829
17-21	5	5973	**	5586	*	5810
24-28	4	5348	*	5161	**	5424
31-35	3	4786	*	4478	*	4745

[1]NS = not significant; * = $0.01<P<0.05$; ** = $0.001<P<0.01$; *** = $P<0.001$.

Another approach (Morris pers comm.) to combatting the problem of excess carcass fat is to increase the important essential dietary amino acids that may otherwise limit lean tissue deposition but not necessarily growth. The assumption therein is that the capacity for maximum lean deposition in poultry exceeds that of maximum dietary energy intake, such that there is little surplus energy to support fat deposition. In the broiler this is possible (Bartov 1979) and it does highlight the importance of protein:energy ratios in order to improve carcass conformation, particularly at the end of the growing period (Bartov 1987).

Recent work by Campbell et al. (1987) showed that carcass fat in broilers substantially decreased with a high lysine:apparent metabolizable energy ratio (1.5g:MJ), while protein deposition reached a plateau well below this ratio. We have shown that increasing the methionine and lysine levels to 15% above recommended levels (NRC 1984), however, produced no effect on fat deposition (Farrell et al. 1989). When supplementation was combined with food restriction more favourable results were obtained, particularly with methionine supplementation; this improved FCR and decreased the size of the abdominal fat pad in commercial broilers.

V. CONCLUDING REMARKS

There are exciting future prospects in attempts to reduce carcass fat in commercial broilers using a number of techniques. As mentioned in previous reports some of these manipulations may have to be geared to specific strains of broilers and possibly even to sexes within strains. Opportunity for success should be greater with the fatter strains of broilers but we have consistently reduced fat pad size in the leanest commercial strain of broiler (A) (Table 1).

Several of the underlying changes that may occur as a consequence of short-term food restriction need to be studied in more detail. Another important area is a study of some of the changes that occur in broiler breeder stock as a consequence of selection pressure. This is an area which has been neglected by research workers. Finally, it is only when

consumer pressure is applied to poultry producers for a leaner bird, which will presumably fetch a premium price, will real progress be made in efforts to reduce carcass fat in broilers.

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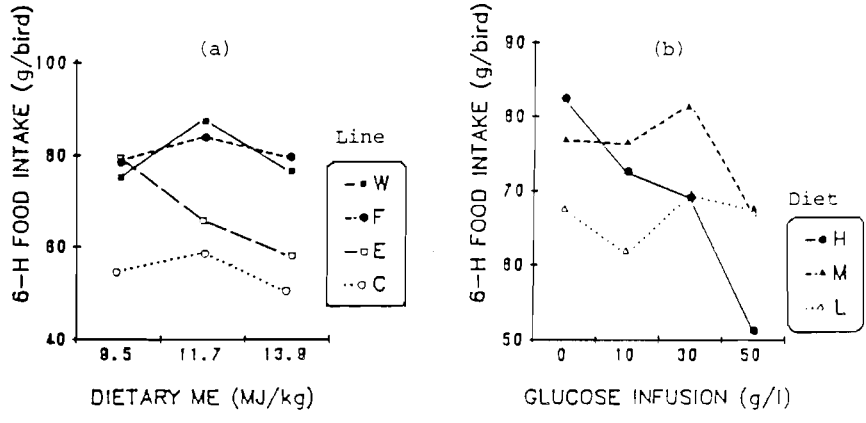
THE GLUCOSTATIC MECHANISM OF APPETITE REGULATION IN CHICKENS SELECTED FOR ASPECTS OF GROWTH AND FOOD UTILISATION EFFICIENCY

S. ISKANDAR and R.A.E. PYM

Strong evidence that glucose plays an important role in controlling food intake in chickens was presented by Shurlock and Forbes (1981). The present study was undertaken to determine whether genotype had an influence on the glucostatic mechanism of appetite regulation in chickens. Birds used in the study came from four lines selected for 12 generations for increased weight gain (line W), increased food intake (line F), decreased FCR (line E), or at random (line C). Four solutions ranging in glucose concentration from 0 to 50g/l were infused at 33±1.2ml/h for three hours into the hepatic portal system of 49-d old male birds from the four lines following 18h starvation. The birds were then given one of three diets ranging in ME from 9.5 to 13.9 MJ/kg with a constant ratio between energy and the other nutrients. Food intake was measured after 6h. A total of 96 birds were measured in the study, each bird being tested on one diet following glucose infusion at the four levels, with a 24h recovery interval between each infusion.

Whilst there were significant ($P<0.05$) effects on intake of line, diet and glucose level there were significant ($P<0.05$) line X diet and diet X glucose level interactions as indicated in the figure below. The line X glucose level interaction was, however, not significant.



Food intake (g/bird) of the four lines on the three diets (a), and as affected by glucose infusion concentration on the three diets (L= 9.5, M= 11.7 and H= 13.9 MJ ME/kg) (b)

There was a reduction in food intake with increasing dietary nutrient density in the E line but not in the other lines indicating that the former line was not eating to gastrointestinal tract capacity. The dietary difference in response to glucose infusion level indicates a certain threshold hepatic glucose concentration below which satiety signals are not generated. There was no apparent effect of line on the glucostatic mechanism of appetite control.

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EFFECTS OF DIETARY INCLUSION OF FIELD PEAS, LUPINS, NARBON BEANS AND CHICKPEAS ON THE GROWTH PERFORMANCE OF BROILER CHICKENS

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Summary

The availability of grain legumes for inclusion in broiler diets is dramatically increasing in Australia. The occurrence of anti-nutritional factors in legumes necessitates a vigilant policy with regard to their level of inclusion in diets for broiler chickens due to possible growth, efficiency, litter condition and pigmentation problems. The present study involved a broiler growth trial to measure the effects of dietary inclusion of field peas (Pisium sativum), lupins (Lupinus augustifolius), narbon beans (Vicia narbonensis) and chickpeas (Cicer arietinum) on the growth and feed efficiency of broiler chickens. Legumes were incorporated into practical wheat/sorghum diets essentially at the expense of soybean meal, and nutrient levels were maintained between diets. Broiler starter (0-21 days) and finisher (21-42 days) diets were fed to four replicate groups of 34 birds for each of twelve diets. The field peas, lupins and narbon beans were included at approximately 80, 140 and 200 g/kg, the two varieties of chickpeas were included at 200 g/kg, and the control diet contained 200 g/kg soybean meal. Diets which contained narbon beans depressed ($P < 0.05$) growth and feed intake. Growth and efficiency of the broilers fed diets containing the other legumes were not significantly different from the control treatment.

I. INTRODUCTION

Three factors are clear with regard to the use of grain legumes in the Australian poultry industry. The first is that Australia relies heavily on importations of protein supplements for stockfeed, principally fish meal (27,000 tonnes) and soybean meal (40,000 tonnes) valued at approximately SA33 million in 1988/89. The second factor is that the production of grain legumes in Australia has increased dramatically over the last decade, from about 0.2 to over 1.75 million tonnes (Sidiropoulos,L. pers.comm). This expansion in production has seen an increased usage of grain legumes by the stockfeed industry as medium contributors to both the protein and energy components of diets. Price and availability considerations would result in a relatively high level of inclusion for grain legumes in broiler diets.

This leads to the third and final factor of relevance, that nutritionists use a wide spectrum of maximum inclusion levels in broiler diets which is to some degree independent of the price and availability of the grain legumes. The reason for this is that grain legumes contain a number of anti-nutritional compounds such as protease inhibitors and haemagglutinins which could potentially reduce broiler performance. Litter condition and skin pigmentation problems have also been observed with grain legumes in broiler diets.

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These three factors indicate a need for continued assessment of the use of grain legumes in poultry diets. Given their potential future importance to the poultry industry, there is a surprising lack of information on legumes in broiler diets under local conditions. The present paper describes an experiment which measured the response of broilers under practical conditions to different and increasing quantities of grain legumes in the diet.

II. METHODS

Birds and their management

Day-old broiler chickens were obtained from a commercial hatchery and placed on a deep litter of wood shavings in a temperature controlled shed. The shed was fan-ventilated with 48 floor pens each with an area of 3.73 m^2. Thirty-four mixed-sex (equal numbers) chickens were placed in each pen, giving a stocking density of 0.093 m^2 per bird at day-old and approximately 24 kg/m^2 at 42 days of age, after correction for feed and water containers. Infra-red heating lamps were provided until the birds were 28 days of age, and ambient shed temperature was maintained at 25°C until 21 days and 20°C thereafter. Water and feed were provided *ad libitum*. Birds were treated with an antibacterial in the water for the first three days after hatching to protect against yolk-sac infection.

Grain legumes

The chemical composition of the grain legumes used in the growth trial is given in Table 1. Field peas (*Pisium sativum*, cultivar Dundale), lupins (*Lupinus augustifolius*, cultivar Yandee) and narbon beans (*Vicia narbonensis*) were obtained from the Department of Agriculture at Walpeup. Chickpeas (*Cicer arietinum*) of both the Kabuli type (cultivar Kaniva) and Desi type (cultivar Dooen) were obtained from Victoria.

Table 1. Composition of the grain legumes used in the broiler experiment.

Constituent (g/kg air-dry)	Soybean meal	Lupins	Field peas	Narbon beans	Chickpeas Kabuli	Chickpeas Desi
Dry matter	878.3	923.9	898.9	874.8	874.8	880.5
Protein[1]	433.5	309.2	260.2	234.3	195.5	205.3
Fat	20.5	64.7	12.1	11.4	63.0	40.4
Fibre	63.6	183.0	44.1	118.0	32.8	108.9
Ash	52.6	26.9	22.9	28.3	22.7	23.5
GE(MJ/kg)[2]	17.58	18.38	16.78	16.52	17.60	17.14
AME(MJ/kg)[3]	9.46	9.60	10.82	10.92	12.55	10.35
Lysine	28.2	14.7	17.0	15.6	13.9	14.3
Methionine	7.0	2.5	2.3	2.1	3.3	3.3

[1] N x 6.25; [2] Gross energy; [3] Apparent metabolisable energy.

Metabolisable energy determinations

Apparent metabolisable energy (AME) was determined on all major ingredients and mixed diets using a rapid broiler assay (Johnson, 1987). This assay is a modification of the Farrell rapid bioassay on adult cockerels (Farrell, 1978) except that broiler chickens, previously trained over a 7-day period to rapidly consume feed, are used. For the ingredients a test diet was offered composed of equal parts of a sorghum basal (per kg containing sorghum 989.5 g, dicalcium phosphate 6.5 g limestone 2.9 g and vitamin/minerals 1.1 g) and the test ingredient. Birds were allowed to consume feed for a 1 hour period followed by a 42 hour excreta collection period. AME values for ingredients are given in Table 1.

Treatments

Field peas, lupins, narbon beans and two types of chickpeas were included in typical least-cost broiler starter and finisher diets, predominantly at the expense of soybean meal. The peas and narbon beans were each included at three levels of 80, 140 and 200 g/kg, the lupins were included at three levels of 80, 130 and 180 g/kg due to difficulties in attaining the specified AME level within a constraint of 60 g/kg of added fat, while the two chickpea types were each included at 200 g/kg. The control diet contained soybean meal at a level of 200g/kg. There were four replicates of 34 birds allocated to each of the twelve dietary treatments. Separate starter (1-21 days of age) and finisher (21-42 days of age) diets were formulated according to the recommendations. Starter diets contained (/kg) 13.0 MJ AME, 12.5 g total lysine, 6.0 g methionine, 9.3 g methionine + cyst(e)ine, 10g calcium, 5.5 g available phosphorous. Finisher diets contained (/kg) 13.0 MJ AME, 10g total lysine, 4.5 g methionine, 7.3 g methionine + cyst(e)ine, 10 g calcium and 5 g available phosphorous.

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 42 days of age by taking random samples from each pen followed by oven-drying at 80°C for 5 days. At 42 days of age two birds (one male and one female) from each pen were slaughtered to determine organ (liver, pancreas) and abdominal fat pad weights. Analyses of variance were carried out using a randomized complete block design.

III. RESULTS AND DISCUSSION

Mortality and growth performance

Results are given in the Table 2. Mortality was not significantly influenced by diet, but birds fed the diet which contained 200g/kg of narbon beans tended to have a higher mortality. Birds on the narbon bean diets had lower ($P < 0.05$) liveweight gain and feed intake than birds on the other diets. The observed significant differences in feed conversion for birds on the narbon bean diets were removed when feed conversion was expressed on a common liveweight basis, to 1.75 kg (see Table 2), indicating that the reduction in liveweight was due primarily to a reduction in feed intake. There were no significant differences between the control diet and those diets which contained field peas, lupins or chickpeas. However, increasing the inclusion level of field peas tended to slightly reduce feed intake (3 % at the highest inclusion level) and liveweight at 42 days of age (2.7 % at the highest inclusion level).

Table 2. The effects of dietary inclusion of grain legumes on broiler growth and feed efficiency.

Parameter	Diet													
	1	2	3	4	5	6	7	8	9	10	11	12	Sig*	SEM
	Soy	Field peas			Lupins			Narbon beans			Chickpeas			(df=33)
		80	140	200	80	130	180	80	140	200	Kabuli	Desi		
Liveweight (g)														
21d	785	785	778	785	796	784	798	722	695	621	801	793	***	11.3
42d	2316	2290	2234	2229	2252	2237	2293	2109	2050	1886	2300	2251	***	28.0
Feed Intake (g/bird)														
0-21d	50.3	50.5	49.6	49.0	50.2	50.2	51.2	45.5	43.9	37.7	50.0	50.7	***	0.8
21-42d	149	150	144	142	146	146	151	134	132	117	148	145	***	2.4
0-42d	99.0	99.7	96.2	94.9	97.5	98.1	100.2	89.4	87.3	76.3	98.6	97.6	***	1.5
Feed conversion ratio (g feed/g gain)														
0-21d	1.42	1.43	1.42	1.39	1.40	1.42	1.42	1.41	1.41	1.37	1.38	1.42	NS	0.02
21-42d	2.04	2.09	2.07	2.06	2.10	2.12	2.12	2.03	2.04	1.94	2.07	2.09	***	0.02
0-42d	1.83	1.86	1.84	1.82	1.85	1.88	1.87	1.82	1.82	1.74	1.83	1.86	***	0.02
to 1.75 kg	1.68	1.71	1.70	1.67	1.69	1.72	1.71	1.71	1.73	1.71	1.67	1.71	NS	0.02
Mortality (%,0-42d)	4.4	2.9	2.9	2.9	4.4	2.2	5.2	2.2	3.7	8.1	3.7	2.2	NS	0.45
Litter moisture (%)	26.7	25.7	25.7	26.4	24.3	26.5	25.7	25.1	26.9	26.9	24.8	23.0	NS	3.5
Abdominal fat (%)	2.9	3.3	3.2	3.1	3.2	3.2	3.2	3.2	2.7	2.3	2.8	3.4	NS	0.4

* Significances were NS, not significant; *** $P < 0.001$.

Narbon beans included at 350g/kg caused a cessation of growth in young pigs (Davies, 1986), but an initial evaluation in broiler chickens with inclusion to 100g/kg did not significantly affect growth or efficiency, although liveweight at 21 days was reduced by about 3% (Eason et al., 1987). The present study with higher inclusion levels and with greater bird numbers has shown that raw narbon beans could not be used at more than 50 g/kg in broiler diets. No information is available on the anti-nutritional factor in narbon beans responsible for the observed depression in feed intake and growth. The presence of an heat-labile anti-nutritional factor in field peas has been shown previously (Moran et al., 1968), and the present results indicate that this factor may tend to depress feed intake and growth of broilers in cold-pelleted diets. Different results may be obtained in steam-pelleted diets. The finding that dietary inclusion of lupins resulted in comparable growth and performance to the soybean meal control supports previous Australian studies (Smetana, 1973; Yule and McBride, 1976).

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FEARFULNESS AND SOME PHYSIOLOGICAL CONSEQUENCES OF CHRONIC DISTRESS IN THE DOMESTIC FOWL

R. Bryan JONES

Summary

The relationships between underlying fearfulness and selected physiological consequences of distress were examined in hens and pullets. The tonic immobility (TI) fear reactions and heterophil/lymphocyte (H/L) ratios of adult hens were prolonged at 4 and 11 days after implantation of osmotic infusion minipumps releasing corticosterone. Chronic elevation of plasma corticosterone may thus predispose hens to react more fearfully to alarming stimulation. Plasma corticosterone concentrations and H/L ratios were increased at 20 and 44h respectively after frustration of feeding began. H/L ratios rose progressively during the 68h frustration period whereas circulating corticosterone levels remained virtually constant after 20h. There was a non-significant but consistent trend towards greater adrenocortical responsiveness to this stressor in hens classed as high (long TI) rather than low (short TI) fear-responders. Fasting and/or frustration of feeding for 72h increased H/L ratios in Brown Leghorn pullets and destabilised their TI hierarchies. Positive intra-individual correlations were found between pre-treatment TI durations and subsequent H/L responses to fasting/frustration. Simple behavioural measures, such as TI and open-field responses may have predictive value concerning subsequent adrenocortical and leucocytic responsiveness to stressful stimulation.

INTRODUCTION

Despite its complex nature, the motivational, defensive aspects of fear are widely recognized (Jones 1987a; 1987b; 1989a). It is regarded here as an adaptive psychophysiological response to perceived danger, with fear behaviour ideally functioning to protect the animal from injury. Fear is an important component of stress and animals generally show closely integrated behavioural and neuroendocrine responses to aversive stimulation (Dantzer and Mormede 1973; Harvey et al. 1984). For example, exposure to an alarming event activates the brain and neuroendocrine system and thereby generates a fluctuating internal fear state (Jones 1987a). The intensity of this induced fear state is sensitive to factors such as past experience and hormonal status and it will also vary with conceived changes in the nature and potency of the frightening stimulus. The initial response is characterised by increased activity of the sympatho-medullary system and the release of catecholamines. These cause secondary physiological changes, such as increased heart rate, peripheral vasoconstriction and the mobilization of energy stores, which prepare the animal for a fight or flight reaction. This may be followed by activation of the hypothalamo-pituitary-adrenocortical axis. The release of adrenocorticotrophic hormone (ACTH) from the pituitary gland stimulates the adrenal cortex to secrete corticosteroids. These amplify the metabolic

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effects of the catecholamines and protect against over-reaction by suppressing normal defense mechanisms. Behavioural fear reactions may be shown during one or both of these stages and, if successful, they may eliminate the threatening properties of the situation and thus reduce both fear intensity and the associated neuroendocrine activation. Underlying fearfulness and neuroendocrine responsiveness to stressors vary between and within populations of chickens (Siegel, P.B. 1985; Jones 1989a) and may influence the intensity of the induced fear state. Thus, for example, birds which have been classified as fearful in one situation are also likely to show more exaggerated overt fear reactions to a wide variety of potentially alarming stimuli than are their less fearful counterparts (Faure 1982; Jones 1987b).

The biological significance of fear and of short-term pituitary-adrenocortical activation is clear. However, they may severely harm a chicken's welfare and performance, particularly if intense or prolonged. For example, fear responses such as panic and violent escape, which are inappropriate to an intensive environment, may waste energy and injure birds or their companions (Jones 1985; 1989a). High underlying fearfulness is also associated with delayed maturity and reduced growth, egg production and eggshell quality (Bessei 1984; Jones and Hughes 1986; Hemsworth and Barnett 1989; Jones 1989a). Similarly, chronically elevated adrenocortical activity or corticosterone treatment depressed growth and reproductive capacity and altered resistance to disease (Siegel 1987).

A clearer understanding of the behavioural and physiological components of fear and distress is important. Stress not only elevates plasma corticosterone concentration (Beuving 1980; Harvey et al. 1984) but also increases the ratio of circulating heterophils to lymphocytes (Gross and Siegel 1983). The present report focusses on the possible existence and nature of any interrelationships between fearfulness (estimated using the tonic immobility (TI) reaction), plasma corticosterone (C) concentration and heterophil/lymphocyte (H/L) ratio. TI is a state of reduced responsiveness to external stimulation induced by brief physical restraint and its duration is positively related to the antecedent fear state (Gallup 1979; Jones 1986). The close associations between a bird's TI reaction and its responses in other potentially frightening situations support its validity as an index of general, underlying fearfulness (Jones 1987c).

(a) Effects of corticosterone (C) infusion

Short- and long-term exposure to frightening stimulation are known to elevate plasma C concentrations in chickens (Beuving 1980; Jones and Harvey 1987). However, the influence of circulating C levels on the elicitation and expression of fear in birds is not clear. The TI reactions of 26 adult White Leghorn hens were, therefore, measured one day before and 4 and 11 days after implantation of osmotic infusion minipumps releasing either C solution (15 ug/hr) or polyethylene glycol vehicle alone (Jones et al. 1988). Blood (1ml) was also withdrawn at these points. Plasma C concentrations were measured using a specific radioimmunoassay (Beuving and Vonder 1981) and H/L ratios were calculated.

Pre-treatment TI reactions, C concentrations and H/L ratios were similar in both groups (Table 1). Infusion of vehicle alone exerted no apparent behavioural or endocrine effects and the increases in H/L ratios in the control group probably reflected low-level leucocytic responses to discomfort and tissue damage caused by the operation. In contrast,

Table 1. Tonic immobility (TI), plasma corticosterone concentrations and heterophil/lymphocyte (H/L) ratios in White Leghorn hens before and after implantation with minipumps delivering either corticosterone (C) or vehicle (V) (means ± SEM)

	Duration of TI(s)		Corticosterone (ng/ml)		H/L ratio	
	C	V	C	V	C	V
1 day pre-implantation	265.8 ±36.2	277.3 ±36.7	1.16 ±0.17	1.10 ±0.22	0.11 ±0.02	0.15 ±0.02
4 days post-implantation	555.7 ±83.2	287.1* ±68.8	2.82 ±0.26	0.85** ±0.13	1.14 ±0.09	0.29** ±0.03
11 days post-implantation	639.8 ±91.2	213.3* ±41.3	2.93 ±0.36	0.87* ±0.15	1.75 ±0.23	0.48** ±0.05

P values derived from analysis by the Wilcoxon matched-pairs signed-ranks test; *P<0.02; **=P<0.01

circulating C levels were significantly and similarly elevated at 4 and 11 days after implantation of the C pumps. The induced C concentrations were within a physiological range, i.e. they fell below those previously found after the application of various stressors (Beuving 1980). The progressive increase in H/L ratios with sustained infusion of C supported previous findings obtained when C was administered either in the diet or via implanted pellets (Gross and Siegel 1983; Davison et al. 1986).

The prolonged TI reactions found in those hens receiving C suggest that, not only may chronic elevations of circulating C impair growth and reproductive performance (Siegel 1987), but they may also predispose chickens to react more fearfully to alarming stimulation. Interestingly, broilers showed prolonged TI after transportation, (Cashman et al. 1989), a procedure known to elevate plasma C levels (Freeman et al. 1984), and hens which consistently laid eggs with abnormal shells, a stress-related phenomenon, were more fearful than normal layers (Jones and Hughes 1986). Naturally-increased pituitary-adrenocortical activity also induces behavioural inhibition indicative of heightened fearfulness in ducks, rats and pigs (see Jones et al. 1988). Positive relationships have also been reported between circulating cortisol levels and the appearance of panic and anxiety disorders in man (Goldstein et al. 1987; Kopp et al. 1989). However, it is not yet clear whether C exerts its effects directly or via a negative feedback effect on either the hypothalamo-pituitary-adrenocortical axis or on the release of other brain ACTH-like peptides.

(b) Responses to distress in hens showing short or long TI fear reactions

The possibility that a hen's underlying fearfulness might influence its

subsequent adrenocortical and leucocytic responses to challenge has been tested (Beuving et al. 1989).

The TI reactions of 50 individually-caged, 36-week-old White Leghorn hens were measured and ranked from low to high. Those 10 birds showing the shortest immobility reactions (76.9±5.2, mean ± SEM) and the 10 scoring the longest durations (818.1±46.1) were classified as low (LF) or high (HF) fear responders respectively. There was no overlap between groups in this character.

An indwelling polyethylene cannula was inserted into each bird's brachial artery (Beuving et al. 1989). This allowed remote collection of blood and the hens were not overtly disturbed either by its presence or by the withdrawal of blood.

A single dose (0.1 IU/kg) of porcine ACTH was injected via the cannula on the following day and blood samples were withdrawn at 0, 6, 10 and 14 minutes respectively. As expected (Etches 1976; Beuving and Vonder 1986), ACTH administration significantly increased plasma C concentrations but the mean maximal elevations were similar in both the LF (12.5±0.8 ng/ml) and the HF (13.0±1.4) groups. Variations in adrenocortical sensitivity to ACTH were, therefore, unlikely to have accounted for the divergence in TI responses between groups. Of course, the LF and HF hens may have released different amounts of catecholamines and/or ACTH in response to capture and TI induction.

ACTH injection exerts only a transient effect on circulating C (Beuving and Vonder, 1986) and a subsequent 48h recovery period was considered sufficient to allow any carry-over effects to disappear. Blood samples were then taken immediately before feeding was frustrated by blocking each hen's access to its food hopper with a clear perspex plate. The inaccessible food remained visible. Further blood samples were collected remotely after 20, 44 and 68h and plasma C concentrations and H/L ratios were measured. Main effects were compared using analyses of variance after the data was partitioned into two segments, i.e. 0 vs 20h samples (no stressor vs stressor) and the 20-44-68h samples (stressor constantly present).

The increased plasma C concentrations and H/L ratios illustrate the stressful nature of the denial of access to visible food (Table 2). The C levels were again well within a physiological range but the sustained nature of the adrenocortical and leucocytic modifications suggested that the birds failed to adapt to this stressor. Consistent with previous reports (see Siegel, H.S. 1985), adrenocortical activation preceded leucocytic effects. However, whereas H/L ratios continued to rise with progressively longer periods of frustration, the elevated C concentrations found after 20h then remained virtually constant. Such constancy might be interpreted in various ways: a) the observed adrenocortical response may have been sufficient to cope with this particular stressor, b) the perceived aversiveness of the frustration/fasting regime might have reached its ceiling within 20h, c) adrenocortical activation may have been modulated by contrasting effects of habituation and metabolic demand and d) the sensitivity of plasma C concentration as an index of stress may diminish after a certain point, at least during this type of chronic procedure. H/L responses to frustration of feeding were significantly higher in HF rather than LF hens ($P<0.02$) but this may have simply reflected pre-treatment group differences, because there was no significant time x group interaction. High-fear hens also showed higher plasma C levels at each post-treatment sampling point than did low-fear hens. Although the latter differences

Table 2. Plasma corticosterone and heterophil/lymphocyte (H/L) responses to frustration of feeding in low- (LF) and high-fear (HF) hens (means ± SEM)

Duration of feeding frustration	Plasma corticosterone concentrations (ng/ml)		H/L ratios	
	LF	HF	LF	HF
0h	0.92±0.14	0.84±0.07	0.34±0.04	0.54±0.07
20h	1.93±0.36	2.59±0.30	0.41±0.07	0.62±0.11
44h	2.14±0.26	2.70±0.42	0.84±0.15	1.06±0.10
68h	1.84±0.30	2.69±0.71	1.00±0.07	1.46±0.19

failed to reach significance ($P>0.1<0.2$), I consider them relevant in view of their consistency and the fact that pre-frustration concentrations were virtually identical in the two groups. Previous findings have also suggested that underlying fearfulness and adrenocortical activation are positively associated. Firstly, for instance, chicks selected genetically over several generations for high activity in a novel environment were not only less fearful in a variety of situations than those of the corresponding inactive line but they also showed lower resting and stress-induced plasma C concentrations (Faure 1980). Secondly, the adrenocortical response to capture and transport was highest in those hens which had shown the greatest home-cage avoidance of the experimenter (Broom et al. 1986). It is conceivable that fearful chickens are alarmed more frequently and more profoundly than their less fearful counterparts. Consequent differences in the degree of neuroendocrine activation may, in turn, perpetuate or exaggerate the behavioural dichotomy.

(c) Chronic distress, fear and leucocytic responses

Chronic fasting or frustration of feeding are known stressors which increase plasma C levels (Scanes et al. 1980; Beuving et al. 1989). The H/L ratios and TI reactions of individually-caged Brown Leghorn pullets (17-18 weeks old) were measured before and after their exposure to one of three treatments lasting approximately 72h (Jones 1989). Firstly, control birds were allowed to feed ad libitum. Secondly, birds were fasted, i.e. their food hoppers were completely emptied. A third group was frustrated by denying them access to visible food with a clear perspex plate.

Pre-treatment H/L ratios were similar in all the groups and remained virtually unaltered in the ad libitum control group (Table 3). In contrast, H/L ratios were markedly increased following 72h periods of either fasting or frustration of feeding, again illustrating the stressful nature of these procedures. Of course, the effects of deprivation and of frustration cannot be entirely separated using this sort of approach. Not only were the frustrated birds also deprived of food but fasted pullets may also have experienced frustration because their assumed expectation of finding food in the hoppers was thwarted.

Table 3. TI durations and H/L ratios in Brown Leghorn pullets before and after a 72h regime of either ad libitum feeding, fasting or frustration of feeding (means ± SEM)

	Ad libitum feeding (n=15)	Fasting (n = 14)	Frustration of feeding (n=15)
Duration of TI(s)			
Pre-treatment	348.2 ± 70.9^{a}	314.0 ± 64.8^{a}	307.4 ± 95.2^{a}
Post-treatment	191.9 ± 39.4^{b}	164.2 ± 43.0^{b}	172.7 ± 38.7^{b}
H/L ratios			
Pre-treatment	0.051 ± 0.004^{a}	0.048 ± 0.004^{a}	0.054 ± 0.006^{a}
Post-treatment	0.050 ± 0.005^{a}	0.301 ± 0.041^{b}	0.295 ± 0.039^{b}

Kruskal-Wallis one-way analyses of variance and Wilcoxon matched-pairs, signed-ranks tests were used for across and between group comparisons respectively. Values with different superscripts are different at $P<0.001$.

All three groups showed similar TI responses prior to treatment. Immobility durations were reduced upon retesting, presumably through habituation, but this effect was relatively homogeneous. Both fasting and frustration might have been expected to increase general arousal but neither procedure seemed to affect TI because the mean responses of control and stressed birds were similar. Previous manipulations of arousal via food deprivation, frustration induced by non-delivery of an expected food reward, or by amphetamine injection also either failed to affect chicks' TI reactions or attenuated them (see Gallup 1979). It would, therefore, appear that TI and, presumably, underlying fearfulness, are not affected in any direct, systematic way by nonspecific variations in arousal.

However, an interesting picture emerged upon the examination of intra-individual relationships rather than overall effects. Birds were ranked within groups on the basis of their TI durations, both before and after treatment and the degree of association between pre- and post-treatment ranks was assessed within groups (Spearman rank correlation coefficient). A significant, intra-individual correlation was found across TI tests in the control group (Spearman $rs = 0.64$, $p<0.02$). This suggested that its members had maintained relatively stable positions in a fearfulness hierarchy. On the other hand, the TI hierarchy was totally destabilised in the fasted and frustrated groups ($rs = 0.284$ and 0.128 respectively). There is no clear reason for such destabilisation but it may reflect the success or otherwise of whatever coping strategies might have been adopted by the birds.

Infusion of C prolongs TI in adult White Leghorn hens (Jones et al. 1988) and fasting/frustration is known to elevate circulating C concentrations (Beuving et al. 1989). There is thus no obvious explanation for the apparently paradoxical failure of the present stressors to influence

TI while simultaneously elevating H/L ratios. However, destabilisation of the TI hierarchy and differences between studies in strain, age and duration of treatment may have been important factors. Exposure to stressors also weakens the correlation between plasma C concentrations and H/L ratios (Gross and Siegel 1983). Indeed, the implantation of minipumps releasing only vehicle increased H/L ratios without affecting circulating C levels (Jones et al. 1988).

Since fasted and frustrated pullets showed similar behavioural and leucocytic profiles both before and after treatment, the data from the two groups was pooled. The birds were ranked from low to high, i.e. from 1 to 29, according to their pre- and post-treatment immobility durations and their H/L responses to stress. Pre-treatment TI durations and subsequent H/L responses to fasting or frustration of feedng were positively correlated within individual birds ($r_s = 0.48$, $t = 2.81$, $p<0.01$). Leucocytic responses to chronic stressors may, therefore, be more pronounced in pullets with high rather than low underlying levels of fearfulness, although causality is not necessarily implied. Post-treatment TI reactions and H/L elevations were not significantly associated and this may have reflected the destabilisation of the TI hierarchy.

Two main conclusions may be drawn regarding the relationship between general, underlying fearfulness and the physiological consequences of distress. Firstly, chronic elevation of plasma corticosterone concentrations may predispose hens to react more fearfully to alarming stimulation. Secondly, hens and pullets classified as high (long TI) rather than low (short TI) fear responders tended to show greater increases in circulating corticosterone concentrations and heterophil/lymphocyte ratios with chronic distress, or at least that associated with fasting/frustration of feeding. Simple behavioural measures, such as tonic immobility, might thus have predictive value concerning subsequent adrenocortical and leucocytic responsiveness to stressful stimulation. This potentially important notion merits further study.

Other potentially fruitful areas of research include: a) the assessment of fear in genetic lines selected for low or high adrenocortical sensitivity to ACTH injection and/or to various stressors and b) the effects of procedures designed to reduce fearfulness, e.g. artificial selection, socialization or environmental enrichment (Jones 1989) on behavioural and physiological responsiveness to chronic distress.

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NUTRITIONAL MODELLING FOR PROFITABLE EGG PRODUCTION

I LITTLETON

There are several compelling arguments supporting the need to adopt modelling techniques to determine the most economic diet specification for a laying flock.

Current laying strains vary widely in performance potential and body weight. In the 34th Random Sample Layer Test, peak production rates varied from 49g/day to 57g/day and live weight ranged from 1.2kg to 1.5kg at 18 weeks of age. Deregulation of the NSW egg industry will provide for a wider differentiation in pricing between the types of markets available to egg producers. There is an increasing trend to alternative supplies of feed. These include on-farm feed mixing and the supply of custom-mixed diets on a "cost plus" basis which can lead to marked differences between farms in the cost of increasing the daily supply of nutrients, such as amino acids, to a laying flock.

Too often the economic impact of achieving a small improvement in egg output from a laying flock has not been correctly evaluated. A field trial conducted with Hyline 300A layers at Seven Hills demonstrated an improvement of 1.5g/day in daily egg mass production could be achieved by feeding a higher specification feedmix. The higher specification feedmix cost an additional $14.50 per tonne but the increased production improved returns by 3.72 cents per dozen eggs at the April 1989 egg price of 180 cents/kg + 1.5 cents/kg for each g of mean egg weight above 45g. An improvement of 1.37 cents per dozen eggs was obtained at the October 1989 price of 163 cents/kg. These gains are important - the net income for egg production for the year ending June 1989 ranged from 8.3 - 12 cents/dozen.

The Reading Model (Fisher *et al.* 1973) is a useful tool for predicting the responses of a laying flock to varying inputs of a limiting nutrient. With the assistance of the Egg Industry Research Council the NSW Agriculture & Fisheries has developed a computer program utilising the model to assist in advising egg producers of the most profitable feedmixes for a particular situation of feed ingredient costs and egg prices.

The program utilises data on the bodyweight, production potential, environmental temperature, feed ingredient costs and egg prices to establish the most profitable diet specification. An interface with a least cost feedmix formulation package then produces the most profitable feedmix on a per unit of energy basis.

As an example, the most profitable feedmix for a client who consigned his eggs to the NSW Egg Corporation during October 1989 at a price of $1.58/kg had an ingredient cost of $194/tonne and contained 10.8 MJ of metabolisable energy (ME)/kg and 8.1g lysine/kg. If that client direct marketed and was able to achieve a margin above marketing costs of 10 cents/dozen on eggs above 55g then the optimum feedmix would have cost $198/tonne and contained 10.3 MJ of ME/kg and 8.3g lysine/kg. Those solutions were based on a mean house temperature of 18°C. A mean house temperature of 22°C would require more dense diets of 11.2 MJ of ME/kg costing an additional $10/tonne.

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OATS REDUCE EGG CHOLESTEROL

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Concern with cholesterol in egg yolk has resulted in a decline in egg consumption and attempts have been made to produce eggs with lower cholesterol levels. Cholesterol levels circulating in the blood of humans has been reported to be influenced by the daily intake of saturated fatty acids (Miles 1989) and soluble fibre (Van Horn et al. 1986). The consumption of oat bran and oat meal have been shown to reduce blood cholesterol in humans (Van Horn et al. 1986) but often the effect is small (<5% reduction).

The present study was designed to measure the responses in egg cholesterol resulting from the use of whole oats and/or rice pollard in diets of laying hens. The fat component in both these feedstuffs contain a high proporiton of unsaturated fatty acids. Oats contain less total, but more soluble, fibre than rice pollard.

Two replicates of ten 45-week old laying hens were fed one of four diets. Diet 1 contained (g/kg): sorghum 317, wheat 420, soyabean meal 80, meat meal 100, L-lysine 0.4, DL-methionine 0.6 and vitamins and minerals 82. In Diets 2 and 3 respectively rice pollard or oats (150 g/kg) replaced an equivalent quantity of wheat and in Diet 4 both were substituted in lieu of wheat at similar concentrations. Egg cholesterol was measured using the method of Ishikawa et al. (1974). The results are shown in the table.

Responses to dietary inclusion of oats and/or rice pollard

Dietary supplement	Egg weight (g)	Yolk weight (g)	Egg cholesterol (mg/g)	(mg/yolk)
None	59.6	17.5	14.8	259
Rice pollard	60.4	17.7	14.0	249
Oats	61.7	17.5	12.9	226
Oats + rice pollard	59.0	17.8	13.1	233
LSD (P<0.05)	NS	NS	1.4	28

NS Non-significant

Shane (1989) has indicated that the economics of dietary manipulation of egg yolk lipid do not appear to offer any commercial benefit at the present time. However, in the present study the egg yolk cholesterol concentration and total egg cholesterol were reduced significantly by including oats in the diet in lieu of wheat. Oats reduced egg cholesterol levels by approximately 10%.

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EVALUATING INCUBATION PRACTICES

ROBERT E. MORENG

Summary

Attempts by humans to reproduce the chicken and other poultry through the application of artificial incubation may be looked upon by all as the epitome of success in the field of animal reproduction. This may not necessarily be based upon the ability to duplicate the process of the hen, but for the ability to multiply the capacity of the hen many times. The advantages gained for the poultry industry have been enormous. Those involved in other areas of animal reproduction should be envious of our success. In order to copy the hen attempts have been made to duplicate the conditions considered necessary for co-ordinated embryonic development. This has been accomplished through a great deal of effort to combine the major biological components with designs for mass production. Perhaps the hen would find this story amusing as her incubation procedures are derived from natural instincts and produce a high percentage of chicks from the fertile eggs she sets upon. How much progress have we really accomplished? What future lies ahead? Will incubation technology keep pace with commercial production requirements both scientifically and economically?

INTRODUCTION

Early history of the development of artificial incubation (Landauer 1976) reveals many attempts to duplicate the processes of the bird and provides a very interesting tale demonstrating how a relatively uncomplicated procedure in the confines of the natural environment of the hen has been interpreted into a relatively complicated procedure in an artificial set of man-made conditions. Romanoff (1960) stated: "From the very beginning of our knowledge of embryology, the avian embryo has occupied a unique position among the higher vertebrates. It has an extra-uterine existence. All its requirements, except oxygen, are provided within the egg, which is by far the most self-contained organism, and is capable of developing in a terrestrial environment. Also, because of its universal availability, it undoubtedly has been an object of embryological observations from earliest historical time".

The development of the hen's egg from blastoderm to chick is one of the most fantastic procedures followed by the eye of man, from an apparent quiescent stage to life itself. The miracle of life can be demonstrated easily in the educational process from the earliest days of schooling through advanced tetralogical studies, where the response of the embryo to the influence of drugs and various chemicals has been demonstrated. In addition, the avian embryo has proved in its own way to be a useful medium for the mass production of vaccines and other laboratory products that support human welfare.

The basic study of embryology includes a careful examination of the avian embryo through the fourth day of incubation since up to this stage of development the embryo shows similarities to other animals, comparable, for example, to about 20 days in the pig and 10 weeks in the human. From this

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point forward differentiation proceeds as the embryo becomes "bird like" or moves in whatever direction its genetic code dictates to become a dog, pig, horse, cow, etc. The embryo provides the basis of life itself in many more forms than is appreciated.

Incubation of the egg with the objective of producing a live, healthy and normal-appearing offspring is the objective of a large segment of the commercial poultry industry which depends heavily on hatching many multiples of that egg. The production of the fertile egg is a very important step to consider in this whole process and comprises another phase in itself. To the industry the quality of eggs and hatchability are of the utmost importance. The number of eggs in and the number of viable chicks out, "garbage in garbage out" is the computer analogy, is only a portion of the story facing our incubator manufacturers and hatchery operators and for a period of 21 days everything must be "text book perfect" for the purpose for which the machine is intended.

(a) History of artificial incubation

The literature reveals that artificial incubation was practised in China between 246 and 207 B.C. utilising incubating ovens during the Chin period. According to Landauer (1976) this involved a system of moving eggs from one incubator to the other to provide uniform distribution of heat. He also notes that the early Egyptians also utilised various methods of artificial incubation. This latter effort led to the development of incubator ovens but attempts to transfer this knowledge to Italy in 1644 by transporting incubators (ovens), and the artisans to operate them, were generally unsuccessful, possibly due to differences in climatic conditions.

Methods of applying a reliable, steady flow of heat which could be closely regulated provided a challenge that required a great deal of energy and time to refine. The major steps towards solving these problems appear to have culminated in the accomplishments of Reamur and by Huzard in the 1700's who built and developed an incubator capable of handling 6000 eggs. Thereafter refinements continued into the 1800's. It is interesting to note that progress along the long path towards the development of the modern incubator appears to have moved slowly and Landauer (1976) states "While the general use of incubation equipment had to await the proper historical setting, it should be noted that the unsolved problem of artificial incubation served as a potent stimulus to the invention (thermostatic devices, self-regulating lamps) and the improvement (thermometer, hygrometer) of instruments which found many important applications in other fields. It is clear that the history of an idea, even if it concerns a very concrete and applied technique, is quite different from the history of specific inventions made relative to the same subject".

The development of the incubator since this period in time has also progressed at a relatively slow pace because of the persistent problem of providing a suitable environment for large numbers of eggs. A gradual evolution from hot water to hot air heat, from solid fuel sources to electric heat, from still air to forced draft ventilitation, and from humidity by use of water pan to evaporative pad and on to more precise humidity control by spray-fogging devices, occurred. Egg turning was accomplished by numerous techniques but the ultimate accomplishment probably was attained when the mechanically-timed mechanism was developed to move eggs through a 90°C arc at specified times throughout the incubation period. Since these basic innovations there have been refinements in incubator design to improve the efficiency of operation and reduce the labour involved in setting and transferring eggs. Roll-in, roll-out tray racks and plastic flats for setting eggs are among the most obvious improvements. The larger units, with less initial cost per egg capacity as well as the lowest operating expense, have

led as sales advantages. Of course, the computer has been introduced as well to monitor and regulate conditions. However, questions exist concerning the value returned for value invested. All in all, egg handling, setting and chick harvesting are major items of labor and expense.

Now let us pause for a moment and go back to consider the lonely hen. If she could watch this development from a great platform nest in the sky she, no doubt, would find amusing the attempts of man, the superior being, to duplicate this relatively simple process in her life. Many have studied the incubation of eggs by the hen under natural conditions and the general conclusion is that the chicken is not nearly as accomplished as man in providing ideal conditions. The hen, while attentive, does not appear to be as precise and accurate as man in his attempt to establish the "mechanical hen". No doubt man has missed some factors in this attempt to transpose but many have been identified. Some have been set aside because of the very fact that implementation would cost more than the benefits gained. Landauer (1967) states "The problem of hatchability is of great economic importance as well as of outstanding scientific interest". Proudfoot (1969) notes "Small improvements in the hatchability of chicken and turkey eggs can result in impressive economic gains". Christensen and Bagley (1989) describe the hatchability of turkey eggs as being notoriously poor, with mortalities between the 24th and 28th day of incubation accounting for the greatest proportion of all deaths. Why? Is it because we haven't really learned how to incubate the egg of the turkey?

(b) Incubator conditions

Examination of the basic conditions identified for incubation may be appropriate at this point.

1. Temperature; 37.5°C (99.5°F) for incubation then decrease to 36.9°C (98.5°F) during the hatching period. The control of temperature is quite accurate and precise through the use of modern temperature control methods. Monitoring and other techniques have been designed to minimize variations in temperature within the incubator but variations throughout the chamber will occur. Minimal air trapping, current eddies, temperature pockets and diversions can easily exist within forced air machines. Temperature interacts with other conditions of incubation too and these implications must be considered.

Differences in temperature gradients appear to be a minor factor in the incubation practices of various wild birds although it is generally agreed that temperature is the major factor involved in the incubation procedure. According to Freeman and Vince (1974) "Under artificial conditions attempts are made to maintain incubator temperatures within quite narrow limits through the incubation period and this aim is consistent with high hatchability. However, in the wild, where hatching success is also high, incubation temperatures are less constant. Fluctuations in nest and egg temperatures are found even in species where one or the other parent is on duty at the nest for at least 95% of the time". Lundy (1969) notes that "Intermittent cooling of eggs for short periods below the normal incubator temperature of 37.5 to 38°C in various types and sizes of incubators always results in increased hatchability if the results are compared with uncooled controls or with controls cooled less frequently --". Russian workers have also suggested that "intermittent cooling is more valuable during the first than during the last week of incubation". This is a subject which requires further study, along with the need for knowledge regarding the special temperature requirements of specific genetic lines since the physiology of new and rapid-growing stocks may well include changes in embryo metabolism.

2. Ventilation; gaseous exchange must take place at a rate which will satisfactorily remove the carbon dioxide and supply adequate oxygen over

the period of embryonic growth. This factor is also associated with air movement, not only in and out of the incubator but also around the eggs themselves. Kaltofen (1969) studied the effects of air movements on the hatchability and weight loss of chicken eggs and, using air speeds from 0.08 to 3 m/s, detected no unfavourable influence of high air speeds. Accordingly this author reported that with "stirrer speeds" of 60, 120 and 180 rev/min the best hatching results were always obtained in the incubator with the highest "stirrer speed". He further concluded that this was due to a greater accumulation of heat between the eggs with lower speeds. Lundy (1969) has stated that there is a continuous relationship between oxygen supply and partial pressure, the embryo being highly sensitive to decreasing oxygen concentrations below 15 per cent (pO_2 = 114 mmHg) and above 40 per cent (pO_2 = 304 mmHg). The embryo is much more sensitive to excess carbon dioxide than to a deficiency of oxygen. The sensitivity apparently decreases with the age of the embryo and with the chick at time of hatch. We know that at high altitudes the lower atmospheric pressures change the availability of gases and their rate of exchange between the embryo and its environment. By utilising chambers to simulate specific conditions we have recently shown in our laboratories in Colorado that fewer turkey eggs hatch at 1524 m than at sea level.

3. Turning, as well as the position of the egg, should be given careful consideration. The frequency and duration as well as the direction of rotation and position of the egg are important. The literature appears to be well supplied with evidence showing that eggs are best incubated large end up compared to small end up. These two positions have been most studied during artificial incubation in modern incubators as eggs apparently are more easily handled in these positions. Does the hen make this distinction throughout the period of incubation? Should the egg be rotated rather than turned? Does the hen really turn her eggs or does she rotate or roll them and, if so, how often and to what degree? This movement not only accommodates the embryo by providing a means by which adherence to the shell and associated membranes is minimized, but this should also provide a means by which the egg is moved through the various temperature gradients existing in the nest surrounding the hen herself. Both of these functions should be considered of importance to the developing embryo. Funk and Forward (1960) reported improved hatchability from multiple turning but this was considered economically unacceptable by the industry.

4. Relative humidity of the incubator environment will influence the rate at which water is lost from the egg during embryonic development and this relates directly to hatchability. Water loss from the egg is directly related to egg temperature, egg weight, shell weight and the porosity of the shell. According to Lundy (1969) water loss is related to hatchability and about 10 to 12 per cent loss is optimal for best hatchability. Excessive water loss results in the embryo's environment becoming dehydrated to a point where the embryo cannot move freely within the egg due to the enlarged air cell. Where the egg has excess water, and the air cell does not develop sufficiently for the lungs to function, the embryo may actually drown. Optimum humidity is generally accepted to be in the range between 60 and 65%. There is little evidence to suggest that humidity exerts additional influences on the developing embryo although there have been suggestions that consideration should be given to the application of Fick's First Law of Diffusion which states that "The amount of water an egg loses during incubation is the product of the surface arc of the egg, the vapor pressure gradient across the shell and the permeability of the shell".

(c) Fertile egg to chick

How many commercial hatchery workers have knowledge of the basic

processes that take place within the egg between the time the egg is placed in the "setter" and the time the chick is removed from the "hatcher"? Should they really be expected to understand these processes? There are those who would argue that the incubator operator must be familiar with the machine that is being utilized and that it must be fine-tuned as a good musician tunes his instrument. It must perform at its maximum. The point of the discussion might, however, be that the incubator operator of the future would simply "throw a switch" and, as long as fertile eggs are supplied on schedule, the chicks could be boxed as they emerged from the machine on completion of the incubation period. The necessary knowledge should be derived from the scientific community and, when they truly know what is required to produce a chick, manufacturers should obtain competent and understanding engineering that can provide the mechanical support.

Perhaps the basis for success is in the statement made many years ago by Martin (1939) in introducing the subject of incubation research: "The desired trend of future research in incubation is for embryologists, geneticists and nutrition workers to study further the causes of developing embryos. This should assure more uniformity and greater economy of hatching". Christensen and Bagley (1989) summarize the problem as seen today as follows: "Hatchability defines a broad sequence of events - when the egg is fertilized, all the chemicals and mechanisms are present to form a new poult with the notable exception of oxygen - heat must be applied to synchronize the development - the ability to synchronize heat, oxygen and the biochemistry of the egg to produce an embryo from the egg within a given time frame." Romanoff (1967) more directly stated "The developing avian egg in its entirety is the most complex physiochemical system. At all stages, it maintains a constant equilibrium between the embryo and its extraembryonic structures - membranes and non-embryonic components of the egg". When one considers the complexity of the system within the developing egg's environment the necessity for absolute balance and synchronization may be understood and the guidance system for this development of life must be highly regarded beyond the "application of heat". We must recognise heat as the stimulating force to initiate this mechanism but the process must be supported as it advances. A refined genetic coding mechanism must be followed, synchronized with an appropriate environment conducive to this development. How this feat may be accomplished by the hen herself may be considered as something of a miracle.

The main accomplishment has been an economic advantage through the attainment of the ability to incubate large numbers of eggs over a relatively co-ordinated period of time with a hatching percentage similar to that of the hen. It is assumed that this has been accomplished at a minimum labor cost and at a maximum economic advantage based upon the financial investment in all factors involved.

(d) Future requirements

New innovations are required in incubation practices which will more completely meet the basic environmental conditions by providing greater uniformity to each and every egg. New techniques make feasible some of those previously considered unpracticable because of a lack of available methodology. We should continue to reach into the pool of available technical information in other fields. Although the computer has provided the basis for monitoring conditions in some of the newer incubator innovations, more sophisticated application is required and the chamber must not only be engineered to monitor but also to perform. We must implement methods and techniques that will provide more uniform conditions throughout the incubation chamber so that all eggs experience similar temperature and humidity ranges. Eggs must be subjected to accurate fluctuations so that they may be cooled and warmed at specified stages for specified time periods during the course of

development. This no doubt will require heat techniques for efficient exchangers as well as mechanisms for heating, cooling and distributing gases, and accurate techniques for locating and measuring these changes. Perhaps microwave-type heating of incubating eggs is not too far from reality.

The incubator of the future must be designed to provide ventilation through minimal air movement within the incubating chamber and, at the same time, to provide the gaseous environment to bathe each egg with the appropriate level of oxygen for the particular stage of incubation, as well as to control carbon dioxide level at the optimum for normal respiration and growth. This design should incorporate a humidity system which could moisturise the incubator air, saturate or desert-dry as required, to fog or spray and sanitise egg shells, and to infuse components through the shell pore system into the embryonic circulatory system. These latter will include nutrients, vaccines, medicinal components, hormones and other growth-stimulating and protective compounds. At Colorado we have been investigating the use of a liquid medium for containing the fertile egg and the developing embryo. This type of environment could respond accurately to the requirements of the embryo when the proper bath solution is utilised, and it would provide an accurate means by which compounds could be incorporated into the developing embryo. As one considers the advantages of using a liquid incubating environment many potential problems arise which must be considered step by step as progress toward completion is addressed.

The incubator-hatcher should be pressurised to provide a hyperbaric environment. Eggs should be turned through multiple axes and rotated in order to maximize embryonic development. Single-stage incubation pods within a multiple-stage incubator room or chamber, down-free hatching chambers with audio- and photo-stimulating procedures and mechanical "harvesting" of chicks would also be utilized. Incorporated into this would be a chamber with a spray-fog for nasal vaccine administration which would also have the capability to identify chicks by sex. This might all be accomplished through a continuous flow system in which the eggs would move from setting to hatching.

Perhaps the words of Freeman and Vince (1974) provide the basis for an appropriate close to this discussion. This statement also appeared in the concluding remarks to their publication: "In the life-cycle of an animal any stage is the product of earlier ones and at the same time it must necessarily influence further stages"____"the newly-hatched chick is a product of incubation and the adult is, in turn, the product of the newly-hatched chick as further influenced by the environment. Hatching should not, therefore, be considered as an end-point for it is simultaneously a beginning. It is perhaps nothing more than a convenient reference point".

The incubation systems of the future must be considered in the context that they supply some of the steps towards the production of poultry meat which provides a protein food source for an expanding world population. It is, therefore, pertinent to the total effort that the process of incubation moves forward in basic techniques as well as industrial production. The industry today is rapidly approaching the point where the reproductive phases must keep pace with the productive phases. This necessitates some co-ordinated and industralized approaches toward artificial incubation which presently exhibits some serious signs of having reached a plateau.

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ADVANCES IN THE CHEMOTHERAPY OF COCCIDIOSIS IN BROILER CHICKENS

S.W. PAGE

Summary

Resistance of the pathogenic species of Eimeria to currently available anticoccidial medications is rapidly emerging as an important problem impeding optimum broiler production worldwide. This has resulted in a critical need for safe and effective alternative drugs. Diclazuril, a benzeneacetonitrile, is a novel broadspectrum anticoccidial compound only recently synthesised and developed for use in broiler chickens. Dose titration studies have revealed anticoccidial activity against some species of Eimeria at feed inclusion rates of only 0.1ppm. Optimum coccidiocidal activity is demonstrated against all pathogenic Eimeria of chickens at 1 ppm. Floorpen studies and field trials throughout the world have substantiated the efficacy of this compound. A major feature of the action of diclazuril is to inhibit oocyst production and excretion, thus providing a powerful weapon in the epidemiological management of coccidiosis.

I. INTRODUCTION

Coccidiosis remains one of the most important diseases affecting the health and productivity of chickens used for broiler production (Groves 1986). Since the introduction of the sulphonamides almost 50 years ago,

Table 1. Anticoccidial families

Decade of introduction	Anticoccidial family	Example
1940's	Sulphonamides	*Sulphaquinoxaline
1950's	Nitrofurans	*Furazolidone
	Organic arsenicals	Roxarsone
1960's	Carbanilide	*Nicarbazin
	Nitrobenzamides	*Zoalene (DOT)
	Thiamine analogues	*Amprolium
	4-hydroxyquinolines	Buquinolate
	Substituted benzoic acid	+*Ethopabate
	Pyridinols	Clopidol
	Pyrimidines	+*Pyrimethamine
1970's	Quinazolinones	*Halofuginone
	Guanidines	*Robenidine
	Polyether ionophores	*Monensin
		*Lasalocid
1980's	Polyether ionophores	*Narasin
		*Salinomycin
		*Maduramycin
	Combinations	*Narasin/Nicarbazin
	Purine analogues	Arprinocid
1990's	Benzeneacetonitriles	Diclazuril

*Commercially available in Australia,
+Used as combination only: ethopabate + amprolium, pyrimethamine + sulphonamide

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prophylactic in-feed medication has become established as a critical vehicle for coccidiosis control. Table 1 lists the various classes of anticoccidial compounds that have become available since the 1940's. As pointed out by McDougald (1986) many of these drugs were limited in their usefulness because of unacceptably low efficacy, incomplete spectra of activity, toxicity or the development of resistance.

As can be seen in Table I, anticoccidial chemotherapy in the 70's and 80's has been dominated by the polyether ionophores. Not unexpectedly, reliance on a single class of compound, sharing similar modes of action, has resulted in the emergence of specific resistance, shared by all members of the class (Jeffers 1989). In order to extend the useful life of existing anticoccidial drugs it becomes increasingly important to find safe and effective alternative compounds with unique modes of action. The following review summarises many of the key aspects of the novel coccidiocidal compound diclazuril, a benzeneacetonitrile, the structural formula of which is set out in Figure 1.

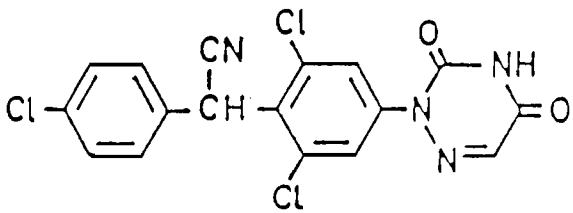

Figure 1. Structural formula of diclazuril

II. EFFICACY

The anticoccidial activity of diclazuril has been described in the chicken (Braem 1989; Vanparijs et al.1989 c,d), turkey (Vanparijs 1989b) and rabbit (Vanparijs et al. 1989a). Evaluation of the activity of diclazuril in chickens against E.acervulina, E.tenella, E.maxima, E.necatrix, E.brunetti and E.mivati (mitis) has been undertaken in controlled battery and floorpen experiments as well as under commercial conditions in field trials.

Table 2 sets out the results of dose titration studies (Vanparijs et al. 1989d) undertaken in Belgium using birds with single species infections. The results are expressed as the composite anticoccidial index (McManus et al. 1968). When it is considered that a score of 180 is associated with good coccidiosis control it can be seen that even at 0.1ppm, the activity of diclazuril against E.maxima is acceptable. E. mivati (mitis) is the dose-limiting species and at the commercially recommended rate of 1ppm, the activity of diclazuril against all major pathogenic species of Eimeria is at or above 180.

Table 2. Anticoccidial indices (ACI)* obtained in dose titration battery trials with diclazuril

Eimeria species	Dose Rate of Diclazuril in feed (ppm) 0.0	0.1	0.5	1.0	5.0
tenella	104	170	200	200	198
acervulina	102	142	181	190	201
necatrix	106	160	194	197	197
brunetti	96	101	182	193	196
maxima	126	184	191	193	194
mivati(mitis)	105	-	159	180	196

*ACI = % survival + % relative weight gain - dropping index - oocyst index

The results of initial battery studies have been confirmed in 13 floorpen trials conducted in 6 countries (Braem 1989). The results of an Australian replicated floorpen study (Page 1989) carried out using mixed sex broiler chickens infected with field isolates of *E.acervulina* and *E.tenella* are presented in Table 3. In all parameters measured diclazuril demonstrates significant activity even at 0.5ppm. Additional studies have now been completed and will form the basis of later communications.

Table 3. Summary of results of Australian floorpen study

Treatment	MTLS 7DAI	Oocyst count (opg) 7DAI	ALW(g) D42	FCR D42	Mortality(D42) C	T
IUC	3.62a	95017a	1504a	2.079a	10.8a	15.1a
diclazuril 0.5ppm	0.00b	4333b	1880b	1.877b	0.0b	6.3b
diclazuril 1.0ppm	0.08b	67b	1905b	1.868b	0.0b	7.4b
diclazuril 2.0ppm	0.07b	0b	1893b	1.872b	0.0b	8.8b

IUC : infected untreated controls DAI: day after infection
MTLS : mean total lesion score ALW: average live weight
FCR : feed conversion ratio
C : coccidial T : total
Means having a common letter are not significantly different (P>0.05)

III. SITE OF ACTION

Although diclazuril at 1ppm has an almost absolute effect in the prevention of oocyst excretion by all major species of *Eimeria*, differences between the species have been observed in relation to the presence of coccidial lesions. For example, lesions due to *E.tenella* and *E.acervulina* are completely prevented, while slight lesions can occasionally be observed with the other pathogenic species. These findings have suggested a species-specific mechanism of action, the elucidation of which has been the subject of a series of histological and ultrastructural studies (Maes et al. 1988, 1989; Verheyen et al. 1988,1989). These eloquent investigations have identified the development stages in the coccidial lifecycle that are sensitive to diclazuril and have clearly demonstrated that the action is coccidiocidal. A summary of the results is presented in Figure 2

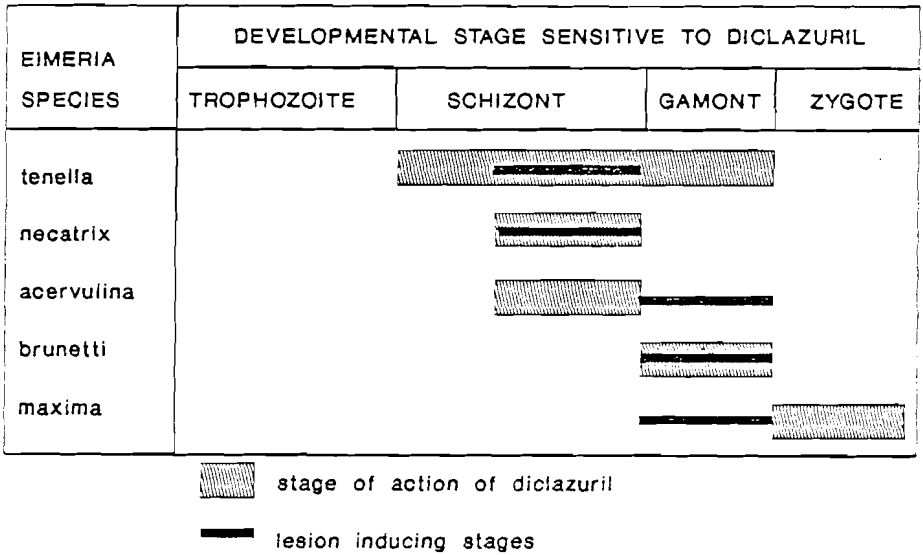

Figure 2. Site of action of diclazuril against endogenous stages of different Eimeria species of the chicken

Clearly, the action of diclazuril precedes the lesion-inducing stages of E.tenella and E.acervulina. However, lesions due to E.maxima appear during gametogeny - prior to diclazuril's action against the zygotes of this species. This explains the possible presence of lesions but absence of oocysts. In all situations the coccidial life cycle is completely interrupted.

IV. IMMUNITY

The implications of medication of birds with diclazuril on the development of immunity to coccidial challenge is naturally of great importance. Development of the immune response to Eimeria infections is complex (Rose 1986), but appears to have been provoked, at least in part, by the time of early schizogeny. With an appreciation of the site of coccidiocidal action of diclazuril, it is only in the case of E.tenella that diclazuril acts in early schizogeny. Specific investigation of the immune response of diclazuril - treated and E.tenella - infected birds to rechallenge with E.tenella when medication has been withdrawn, suggests that sufficient first generation schizonts develop to induce protective immunity (Maes et al. 1989).

V. SAFETY

Knowledge of the circumstances in which the use of a chemotherapeutic agent is associated with adverse effects allows the formulation of rational directions for use and the introduction of appropriate precautions. A review of the adverse effects of compounds in

common use in poultry production has recently been published (Reece 1988). It was noted that many of the widely used anticoccidials have particular safety limitations. However, identification of these limitations has permitted the recommendation of necessary safeguards and continued use. The safety profile of diclazuril has been evaluated extensively in target and non-target species (Table 4), in controlled laboratory experiments and under conditions of normal commercial use (Janssen Pharmaceutica, unpublished results). These studies have revealed no adverse effects on growth rates, feed conversion efficiencies, mortality and a broad range of biochemical and haematological parameters. Similarly, a study of the effect of continuous exposure of broiler breeders to diclazuril-medicated feed (1 and 5ppm) discerned no negative effects on egg production, shell quality, hatchability or semen quality.

Table 4. Studies of oral toxicity of diclazuril in target and non-target species

Species	Dose rate		Dosing	Number of	
	mg/kg b.wt.	mg/kg feed	frequency	Animals	Result
Chicken	5000		single dose	20	NAE*
Chicken		25	continuous day 0-37	300	NAE
Chicken		5	continuous day 0-42	3150	NAE
Turkey		100	continuous 7 days	10	NAE
Duck (Peking)		100	continuous 7 days	10	NAE
Duck (Peking)		10	continuous day 0 - 56	100	NAE
Horse	1		daily, 6-7 days	7	NAE
Cattle	10		single dose	1	NAE
Dog	10		single dose	2	NAE

*NAE = no adverse effects

Diclazuril at the recommended use rate has been included in rations of broilers concurrently medicated with each of a variety of antibacterials (tylosin tartrate, oxytetracycline hydrocholoride, ampicillin, erythromycin, furaltadone, tiamulin), anticoccidials (monensin, narasin, lasalocid, salinomycin, maduramycin, halofuginone, robenidine, nicarbazin, amprolium, sulphaquinoxaline plus pyrimethamine), growth promoters (virginiamycin, zinc bacitracin, avoparcin, nitrovin and roxarsone) and anthelmintics (flubendazole). No adverse interactions have been observed.

The overall safety in use of diclazuril is affirmed by commercial use for more than 12 months in the successful production of more than 5 million birds.

VI. RESISTANCE

As emphasized by Long and Jeffers (1986) the selection of Eimeria resistant to anticoccidial drugs has been one of the major limitations to coccidiosis control by chemoprophylaxis in the past. Indeed the rapid emergence of resistance after relatively little commercial use considerably reduced the anticoccidial utility of buquinolate in the 1970's and arprinocid in the 1980's (Chapman 1982). However, alternative approaches to coccidiosis control are not at present sufficiently developed to replace chemotherapy. For this reason, considerable reliance is placed on anticoccidials which will continue to form the cornerstone of control well into the future (Long 1987; Chapman, 1988). The need for new anticoccidial agents is acute, especially to reduce the resistance selection pressure imposed by current compounds. It is vital also that new agents are used judiciously and that their propensity to select resistance is assessed prior to commercial use. Laboratory selection studies of the development of resistance to diclazuril have been undertaken both in the U.K. and in The Netherlands. (Janssen Pharmaceutica unpublished results) Because of the effect of diclazuril in inhibiting oocyst output, selection trials have had to be run at dose rates considerably lower than recommended commercially. Nevertheless, the studies suggest that specific diclazuril resistance will not be selected rapidly.

VII. ACTIVITY AGAINST RECENT FIELD ISOLATES

The sensitivity of recent field isolates of coccidia to diclazuril has been described in the United Kingdom (Chapman 1989), The Netherlands (Vertommen and Peek 1989) and Australia (Groves 1989). Chapman (1989) examined the sensitivity of fifteen isolates of E. tenella to diclazuril, a number of polyether ionophores and the combination of narasin and nicarbazin. All strains were classified as sensitive to diclazuril, while varying numbers of strains were found to be resistant to each of the other anticoccidials. Similar results were reported by Vertommen and Peek (1989) who tested mixed isolates of E. acervulina, E. tenella and E. maxima obtained from broiler farms throughout The Netherlands. In Australia, Groves (1989) examined the activity of diclazuril, a number of ionophores and the combination of narasin and nicarbazin, against a composite field isolate containing predominantly E.tenella, with smaller proportions of E. acervulina type oocysts and E. maxima. Diclazuril was the only compound to prevent lesions due to E. tenella. Consistent with the site of action (Section III) lesions due to the other species were observed. The sensitivity to diclazuril of field isolates from throughout the world is not surprising, considering the absence of prior exposure to anticoccidials with this mode of action. However, these preliminary results do suggest that cross resistance with previously used drugs is unlikely.

VIII. CONCLUSION

The availability of diclazuril with broadspectrum anticoccidial activity and a novel mechanism of action comes at a time when resistance to the widely used polyether ionophores is increasing. Diclazuril integrated into broiler health programmes should offer the ability to effectively and safely control coccidiosis and enable optimum welfare and productivity.

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THE INHERITANCE OF PLASMA INSULIN-LIKE GROWTH FACTOR-I AND CORRELATIONS WITH PERFORMANCE TRAITS IN MEAT-TYPE CHICKENS

R.A.E. PYM*, F.M. TOMAS** and R.J. JOHNSON***

There is increasing interest in alternative selection strategies aimed at improving the efficiency of lean tissue growth in meat-type chickens. Because of the mediating role of insulin-like growth factor-I (IGF-I) in protein synthesis and cartilage development (Scanes 1987), plasma concentration of IGF-I may be a suitable and useful trait for inclusion in a selection program for increased lean tissue growth in broilers. The present study was designed to provide information on the genetic and phenotypic parameters associated with IGF-I in a population of meat-type chickens.

Heritability and genetic and phenotypic correlations between plasma IGF-I (measured at 42d), 56-d liveweight (56dW), 28 to 56-d food intake (FI) and feed conversion ratio (FCR) and abdominal fatness (AF) at 56 days, were determined by sib analyses in a population of 327 pedigreed chickens produced by matings between 18 cockerels and 72 pullets from a line of chickens randomly bred for eight generations from an Australian commercial broiler strain. Results are shown in the table.

Heritability and genetic (above diagonal) and phenotypic (below diagonal) correlation estimates (sire+dam) for 56dW, FI, FCR, AF and IGF-I from sib analysis of 327 meat-type chickens. Standard errors in parenthesis.

Trait	56dW	FI	FCR	AF	IGF-I
56dW	0.49 (0.13)	0.88 (0.05)	-0.21 (0.22)	0.72 (0.12)	-1.05 (0.42)
FI	0.90	0.55 (0.13)	0.21 (0.21)	0.75 (0.11)	-1.10 (0.43)
FCR	0.18	0.12	0.73 (0.14)	0.09 (0.22)	-0.30 (0.37)
AF	0.64	0.68	0.17	0.49 (0.13)	-0.43 (0.39)
IGF-I	0.02	-0.02	-0.07	-0.02	0.10 (0.08)

Plasma IGF-I concentrations (ng/ml) were also measured in ten 35-d old male chickens sampled from each of five lines selected for six generations for increased abdominal fatness (31.30±1.37), decreased abdominal fatness (33.73±1.52), increased 56-d liveweight (33.43±1.17), increased 56-d liveweight combined with decreased abdominal fatness (32.75±1.59), or at random (33.49±0.91). There were no significant differences in plasma IGF-I between the lines.

The large genetic correlation estimates between plasma IGF-I and the other traits are the likely result of a mathematical artifact caused by the low sire and dam variance components for this trait, as indicated by the low heritability estimate. These variance components become the denominator in the calculation of the genetic correlation. The lack of any line differences in plasma IGF-I in the second study combined with the low heritability estimate from the first, suggests that plasma IGF-I, measured using the present methods and techniques, may not have a role as a selection trait in meat chicken breeding programs.

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PROTEIN TURNOVER IN CHICKENS SELECTED FOR DIFFERENT ASPECTS OF GROWTH AND BODY COMPOSITION

R.A.E. PYM* and F.M.TOMAS**

Protein turnover in the whole body accounts for about 25% of maintenance energy requirements in the growing chicken and muscle protein turnover is a large component of this. In a previous study (Tomas et al. 1988) we reported that selection for improved food utilisation efficiency in chickens reduced the rate of muscle protein breakdown. In the present study, protein breakdown and synthesis rates were measured in lines of chickens selected for six generations for increased (line F) or decreased (line L) abdominal fatness, increased 8-week liveweight (line W), increased 8-week liveweight combined with decreased abdominal fatness (line WL) or at random (line C).

Measures of protein breakdown and synthesis rates were made on ten male chickens from each of the above five lines at 35 days of age. The birds were placed in single cages at 21 days of age and given a diet free of animal protein and containing 195g crude protein and 13.0 MJ ME/kg. Excreta from individual birds was collected quantitatively on days 34 and 35, dried and subsequently analysed for N^t-methylhistidine (3MH), to provide a measure of myofibrillar protein breakdown. On day 35 the birds were injected with a measured amount of radioactively labelled amino acid (L-[2,6,^{3}H]phenylalanine) and after a precisely measured interval, samples of breast and thigh muscle were taken and immediately placed in liquid nitrogen for later measure of the specific radioactivity of amino acid incorporated into muscle to provide an estimate of the rate of muscle protein synthesis. Measures were made of 21-to 35-day growth, food intake and FCR and of whole-body protein and fat composition at the two ages. Results of the study are shown in the table.

Growth rate (GR, g/d), FCR, lean tissue growth rate (LTGR, g/d), 3MH excretion (µmol/100g lean body mass) and fractional rates (%/d) of protein breakdown (K_d), synthesis (K_s) and accretion (K_a) in ten male chickens sampled from each of the five lines.

Line	GR	FCR	LTGR	3MH	K_d	K_s	K_a
F	15.2^{ab}	3.66	10.89^{bc}	0.71	1.57	3.78	2.21
L	16.4^{ab}	3.49	12.92^{ab}	0.71	1.58	4.06	2.49
W	15.7^{ab}	3.66	11.88^{abc}	0.78	1.73	3.94	2.21
WL	17.1^{a}	3.41	13.31^{a}	0.76	1.68	4.14	2.46
C	14.4^{b}	3.71	10.61^{c}	0.81	1.80	3.87	2.07
$LSD_{0.05}$	2.6	0.38	2.22	0.17	0.36	0.66	0.47

Although lean tissue growth rate was greater in the L and WL lines than in the C line, there were no significant differences between the lines in the fractional rates of protein breakdown, synthesis or accretion. Whilst line differences in synthesis rate, although not significant, were in the expected direction, there was no logical relation between selection criteria and protein breakdown rate in the lines.

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The Physiological Basis of Poor Egg Shell Quality in Laying Hens: The Effect of Saline Drinking Water on Electrolyte Balance and Renal Function

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Summary

Electrolyte balance and renal function were assessed in laying hens receiving normal levels of sodium chloride (NaCl) in feed or in water, and in hens receiving 2g NaCl/litre in drinking water in addition to the normal NaCl supplement in feed. Birds drinking 2g NaCl/litre were divided into those producing good quality egg shells and those laying eggs with poor quality shells. Plasma sodium levels and the fractional excretion of sodium, chloride and potassium were significantly elevated in birds drinking 2g NaCl/litre as compared with the control animals.

I. INTRODUCTION

The presence of electrolytes such as sodium (Na^+) and chloride (Cl^-), in drinking water, has been shown to have a deleterious effect on egg shell quality at concentrations as low as 200 mg NaCl/litre (Balnave and Scott 1986; Balnave and Yoselewitz 1987). In addition, the same quantity of NaCl presented in feed does not adversely affect egg shell quality.

The physiological mechanism by which NaCl affects the quality of egg shells requires further investigation. The present study was designed to investigate the electrolyte balance in laying hens receiving NaCl in either feed or water. Birds receiving 2000 mg NaCl/litre drinking water were divided into those producing egg shells of good quality and those producing poor quality shells.

II. METHODS

Laying hens were housed, individually, in commercial layer cages, in a room in the University of New England Animal House. They were maintained at 22°C and a 16L:8D photoperiod. Four groups each of six hens were investigated. Group 1 (control) animals (Body weight 1.67±0.07 kg) were fed a layer mash with NaCl supplement (2g/kg) and given deionized water as the drinking solution. These birds were laying eggs with good shells. Groups 2 and 3 received feed containing the NaCl supplement and were provided with 2g NaCl/litre drinking solution. Group 2 birds (1.91±0.09 kg) were laying eggs with good shells and Group 3 birds (1.65±0.09 kg) were laying eggs with poor quality shells. Birds in Group 4 (1.83±0.09 kg) received feed without the NaCl

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supplement, a drinking solution of 1g NaCl/litre and were laying eggs with good shells. Results of previous studies indicated that the overall intake of NaCl by Group 4 would be equivalent to that of Group 1. Birds were 51-58 weeks of age at the time of the experiments.

Renal function was assessed by standard clearance experiments, employing the "constant infusion" technique. Catheters were placed, under local anaesthesia, into both brachial veins. A cloacal cannula, fashioned from a 1.5 ml plastic microcentrifuge tube, was sutured into the cloaca, under local anaesthesia, for collection of ureteral urine. The bird was then restrained in a cloth sling, suspended from a wooden frame. A solution of 0.1% inulin plus 0.1% PAH (para-aminohippuric acid) in 2.5% mannitol (180 mOsm/kg) was infused into one brachial vein at the rate of 0.2 ml/kg/min (following a priming injection of 10mg inulin plus 10mg PAH in 1 ml). Blood samples were collected from the opposite brachial catheter and ureteral urine was collected, from the cloacal cannula, into pre-weighed plastic vials which were re-weighed to determine volumes. Two clearance periods, each of 30 minutes, were conducted.

Some blood was placed immediately into an AVL 984 Electrolyte Analyser for the measurement of ionised calcium (Ca^{++}), Na^{+} and K^{+}. A heparinised haematocrit tube was filled for the determination of haematocrit and the remainder was centrifuged to separate the plasma. Urine samples were mixed thoroughly and an aliquot diluted 1:1 with 0.5M LiOH to dissolve the uric acid precipitate. The remaining urine was centrifuged and the supernatant analysed for osmotic concentration. The osmotic concentration of plasma and urinary supernatant was analysed using a Wescor Model 5100 Vapour Pressure Osmometer. Plasma and diluted urine samples were analysed for inulin by the method of Waugh (1977); PAH by the method of Brun (1957) ; chloride by chloridometry (Radiometer Model CMT10) and Na^{+} and K^{+} by an AVL Electrolyte Analyser. Total calcium in urine was measured on a Cobas Bio Spectrophotometric Autoanalyser.

III. RESULTS

There were no significant differences in the body weights of the birds among the four groups. The osmotic pressure (O.P.) and concentrations of Na^{+}, K^{+}, Cl^{-} and Ca^{++} in the plasma of the hens are shown in Table 1. Values with the same superscripts are significantly different from each other.

Osmotic pressure and the concentrations of K^{+}, Cl^{-} and Ca^{++} were not significantly different between groups. However, mean plasma Ca^{++} tended to be the highest in the hens laying poor quality egg shells. The plasma Na^{+} of Group 1 was significantly lower than that of Groups 2 and 3. Plasma Na^{+} was significantly higher in Group 2 than in Group 4.

TABLE 1 Plasma Electrolytes of Hens on the Different Treatments

		Group 1	Group 2	Group 3	Group 4
O.P.	mOsm/kg	292.1 ±1.3	288.6 ±2.8	292.9 ±0.9	290.0 ±1.57
Na^+	mM	148.9 ±0.4^{AB}	151.4 ±1.0^{AC}	150.6 ±0.4^{B}	150.0 ±0.9^{C}
K^+	mM	3.60 ±0.08	3.54 ±0.10	3.65 ±0.10	3.61 ±0.08
Cl^-	mM	119.9 ±0.8	118.1 ±1.1	120.5 ±0.6	119.8 ±1.5
Ca^{++}	mM	1.449±0.021	1.474±0.013	1.420±0.022	1.399±0.023

$^{A}P<0.05$ $^{B}P<0.02$ $^{C}P<0.02$

Renal function parameters are summarised in Table 2. Values with the same superscripts are significantly different from each other.

TABLE 2 Renal Function of Hens on the Different Treatments

		Group 1	Group 2	Group 3	Group 4
GFR	ml/min/kg	2.48±0.10	2.65±0.09	2.78±0.24	2.66±0.14
ERPF	ml/min/kg	37.3 ±4.1	33.6 ±3.2^{A}	59.8 ±15.2	49.1 ±4.9^{A}
ERBF	ml.min/kg	54.1 ±5.8	47.1 ±4.1^{B}	83.9 ±21.1	70.9 ±7.5^{B}
F.F.	%	7.07±0.71	8.36±0.83^{A}	6.10± 1.36	5.77±0.53^{A}
V	mls	0.16±0.01	0.21±0.02	0.17± 0.02	0.16±0.03
O.C.	ml/min	0.16±0.01	0.19±0.02	0.17± 0.02	0.18±0.02
F.W.C.	ml/min	0.00±0.01	0.01±0.02	0.00± 0.02	-0.02±0.02
F.E. Na^+	%	0.24±0.04^{AD}	1.01±0.35^{A}	0.82± 0.16^{DC}	0.38±0.13^{C}
F.E. K^+	%	13.39±1.86^{AC}	18.6 ±1.26^{A}	21.83± 3.15^{C}	19.01±2.92
F.E. Cl^-	%	0.79±0.13^{AD}	1.57±0.29^{A}	1.46± 0.15^{D}	1.02±0.26
F.E. Ca^{++}	%	3.29±0.43	3.60±1.47	6.17± 1.38	6.45±2.34

$^{A}P<0.05$ $^{B}P<0.02$ $^{C}P<0.05$ $^{D}P<0.01$

Glomerular filtration rate (GFR) and urine flow rate (V) were not significantly different between groups. Effective renal plasma flow (ERPF) and thus effective renal blood flow (ERBF) and filtration fraction (F.F.) were variable. The ERPF and ERBF were significantly higher, and the F.F. significantly lower, in Group 4 than in Group 2. Osmolar clearance (O.C.) and freewater clearance (F.W.C.) were not significantly different between groups. However, the fractional excretions of sodium (F.E. Na^+), chloride (F.E. Cl^-) and potassium (F.E. K^+) were higher for Groups 2 and 3 than for Group 1. The fractional excretion of Na^+, Cl^- and K^+ were not significantly different between Groups 1 and 4. The fractional excretion of Ca^{++} was highest in Groups 3 and 4 although these differences were not statistically significant.

IV DISCUSSION

The results of this study reveal some changes in the electrolyte balance of Groups 2 and 3. Plasma Na^+ levels were significantly elevated in both groups, as compared with the

control Group, 1. This suggests that the birds consuming NaCl in their drinking water are regulating plasma Na^+ at a slightly higher level than the control animals. There was no consistent effect of saline drinking water on plasma Cl^- levels. Yoselewitz et al. (1988) reported elevated plasma levels of both Na^+ and Cl^- in birds under similar conditions of salt intake.

The higher fractional excretion of Na^+ and Cl^- in Groups 2 and 3 reflects the greater intake of Na^+ and Cl^- in these groups. The similar results for Groups 1 and 4 suggests that the intakes of Na^+ and Cl^- in these two groups were equivalent. The fractional excretion of K^+ was lowest in the control Group 1 and similar in the other three groups where NaCl was supplied in the drinking water. The increased fractional excretion of K+ in the latter three groups is most likely due to an increase in the delivery of Na^+ to the distal tubules of the kidneys producing an increased secretion of K^+. The large, if non-significant, increase in Group 4 birds may reflect a greater problem when NaCl is included in drinking water rather than in the diet. The higher fractional excretion of Ca^{++} in Group 3 as compared with Groups 1 and 2 may be associated with the poor quality egg shells produced by this group.

The additional intake of NaCl in Groups 2 and 3 was not sufficient to cause any change in glomerular filtration rate (GFR). The changes occurring in renal plasma flow (ERPF) are difficult to interpret. There is very little information available on the factors affecting renal plasma flow in avian species. The presence of the renal portal system means that varying amounts of venous blood may either perfuse or bypass the kidneys. The lowest renal plasma flow was recorded in Groups 1 and 2 with Group 3 having the highest value (although with a high standard error).

Laying hens receiving 2g NaCl/litre in their drinking water show only minor changes in electrolyte balance as compared with the control group. Their higher fractional excretion of Na^+, Cl^- and K^+ reflects the increased intake of Na^+ and Cl^- in these birds.

V. ACKNOWLEDGEMENTS

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EFFECT OF INCUBATION CONDITIONS ON SUBSEQUENT COMPETITIVE BEHAVIOUR PATTERNS AND ITS RELEVANCE TO THE CHICKEN INDUSTRY

L.J. ROGERS

Summary

Although there has been considerable study of the behaviour of chickens after hatching and the long-term consequences of early learning on later social and sexual behaviour, little consideration has been given to the possibility that incubation conditions may influence the development of the embryo in such a way that posthatching social behaviour and survival is altered. It is known that light exposure of eggs during the last days of incubation determines the direction of lateralisation in the brain, and that this occurs as a consequence of the orientation of the embryo in the egg, which permits the right eye to receive light while the left eye is occluded. It is reported here that groups of chicks hatched from eggs which have been exposed to light, and so all have brain lateralisation in the same direction, form a more stable and stratified social hierarchy than groups of chicks hatched from eggs incubated in darkness. In more rigid or stable hierarchies the lower ranking individuals compete less successfully for food and may have a greater risk of dying due to starvation. Recommendations for controlling incubation conditions are therefore made.

I. INTRODUCTION

Chickens are a precocious species, which is apparently one of the reasons why they were domesticated in the rather distant past. The chick hatches from its egg with almost fully developed visual and auditory systems (Sedláček 1972; Freeman and Vince 1974), a brain capable of forming stable, long-term memories (Horn 1985) and motor abilities much in advance of those of newly hatched or born altricial species. Pecking behaviour begins immediately, although these pecks are mostly given with the beak closed and probably driven by curiosity rather than being specifically for feeding. Within the first two days of posthatching life the chick gains the ability to stand upright on its legs and so can walk and run efficiently (Hess 1959; Gottlieb 1961), and within an early sensitive period, beginning immediately after hatching, it undergoes perhaps the most important learning in its life. In the natural situation, it learns to recognise and follow the hen, learning which ensures its proximity to the hen, which is important for its protection and for learning its feeding preferences (Tolman 1967). This early learning to recognise and follow the hen is more rapid and stable than later learning and, therefore, it is referred to by the special term, 'imprinting'.

Imprinting is an aspect of behaviour which has been extensively studied by some of the most important early researchers in the field of ethology (e.g. Lorenz 1935; Hinde 1962; Sluckin 1966; Bateson 1966). The optimal time for a chick to imprint on a visual stimulus is 13 to 18 hours after hatching (Hess 1959), and at around this age it will imprint on any conspicuous object, such as a striped box or a flashing light. The optimal time for auditory imprinting precedes this (Boyd and Fabricius 1965), a developmental sequence which reflects the fact that the auditory system of the chicken develops in advance of the visual system (Sedláček 1972).

Indeed the auditory and visual systems are functional even before hatching. Auditory potentials can be recorded from neurones in the embryo's forebrain by day 16 of incubation (Sedláček 1972) and functional visual connections between the eyes and the forebrain are present by day 18 of incubation (Freeman and Vince 1974).

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After day 19 of incubation there is a marked increase in the embryo's behavioural response (bill-clapping) to light stimulation (Oppenheim 1968). Despite the fact that the optimal time for imprinting in the visual and auditory modalities occurs after hatching, it is possible to imprint chicks to either auditory or visual stimuli prior to hatching. Newly hatched chicks are attracted by sounds similar to those which they themselves emitted when in the egg (Kruijt 1964). Guyomarc'h (1974) has shown that exposing the eggs to one of their species-specific calls increases the posthatching response to that sound, and Gottlieb (1979) has shown that ducklings learn to recognise their species-specific maternal calls when they are still in the egg. Moreover, by exposing eggs to different wavelengths of light just before hatching it is possible to visually imprint the embryos to light of a particular colour. After hatching, the embryos will approach and stand next to lights of the same colour to which they had seen exposed prior to hatching. Thus, during the last stages of incubation, the chicken embryo is already perceiving, learning and even behaving within the confines of its egg, and this prehatching learning could well have long-term consequences on its behaviour and survival.

In addition, the chicken brain is lateralised, such that the left hemisphere of the forebrain controls one set of behavioural functions and the right another (Rogers and Anson 1979; Rogers 1986), and direction of this lateralisation is determined by the orientation of the embryo in the egg (Rogers 1982; Zappia and Rogers 1983). During the last stages of incubation, when the visual pathways become functional, the embryo is positioned such that its left eye is occluded by its body and its right eye remains exposed to receive stimulation by light passing through the shell and membranes of the air sac. This lateralised input of light to the right eye stimulates the development of pathways from this eye to develop in advance of these connected to the left eye (Rogers and Sink 1988). After hatching, the right eye has superior learning ability on visual discrimination tasks (Mench and Andrew 1986; Gaston and Gaston 1984; Zappia and Rogers 1987) and the left eye controls attack and copulation behaviour (Rogers et al. 1985; Rogers 1986). Taking into account a wide range of results, Andrew (1988) has suggested that the left eye is concerned with topographical cues (the position of stimuli in space), whereas the right is concerned with the details of the stimulus itself.

Given that light before hatching can play such an important role in organising the lateralisation of brain function and that chicken embryos can form stable memories, consideration of the conditions of incubation, particularly over the last 3 days, may have applied significance to the poultry industry. Until now much emphasis has been placed on early posthatching behaviour and its effect on food intake and survival, but little or no attention has been paid to possible effects of the prehatching environment on later behaviour of the chicks.

Groups of chicks hatched from eggs incubated in darkness during the last 3 days of incubation consist of individuals which each have lateralisation of brain function, but there is no consistency between individuals in the direction of this lateralisation, and thus no group bias in lateralisation (Zappia and Rogers 1983). Half of the chicks hatched from eggs incubated in darkness learn better with the right eye and control attack and copulation with the left eye, and the other half are the other way around. We therefore hypothesised that, since groups of chicks hatched from eggs incubated in darkness have no consistent, or predictable, bias between individuals, they may be more unstable, or show less rigid social hierarchies (i.e. in contrast to groups of chicks hatched from eggs which have been exposed to light during the last 3 days of incubation and so have a consistent individual and group bias in lateralisation).

2. METHODS

Black Australorp x White Leghorn chicks were hatched from eggs which had either been incubated in darkness from day 17 on, or which had been exposed to

light (250 to 350 lux) over this period. After hatching all of the chicks were exposed to light, and they were organised into groups of eight birds. There were eight groups comprised of chicks hatched from eggs incubated in darkness (D groups) and eight comprised of chicks hatched from eggs which had received light exposure during the last 3 days of incubation (L groups).

From day 8 to 16 of posthatching life the social hierarchy was scored on the basis of competition for food (aggressive pecks were not used to score the 'peck order' as they are rarely given by chicks of this age). Each day the chicks were deprived of food for 3 or 4 hours, and then a small dish of food was placed in the corner of the cage, allowing access to the food by only 3 chicks at a time. Over a 10 min. scoring period the number of times each chick gained access to the food source was scored. Individuals were marked by differently coloured ring bands on the legs. In each group the chick which gained the most number of entries to the food dish (mean value per day over 9 days of testing) was considered to be in rank 1, at the top of the social hierarchy. The chick which gained the least number of entries to the food dish was placed in rank 8 at the bottom of the hierarchy. The other birds fell between these extreme ranks. Weights were also measured at several ages up to day 70 posthatching to investigate any possible relationship between position in the social hierarchy in early life and later weight gain.

A second series of experiments looked at competition between mixed groups comprised of both chicks hatched from eggs incubated in darkness and chicks hatched from light-exposed eggs. The mode of gaining access to the food dish was scored in these mixed competitive situations. Testing occurred from day 6 to 9 of life inclusive. When performing the behavioural tests, the experimenter was not aware of the prior incubation conditions of the chicks, as each was marked individually.

Competition between dark incubated and light-exposed chicks was also scored by housing chicks in a standard incubator and noting competition for putting the head through the holes to feed. Testing occurred on days 3,4,5 and 13 posthatching. The food troughs were removed for 3 hours prior to testing and replaced at testing. For a 10 min period, at one minute intervals, the number of heads protruding through the holes and belonging to D or L chicks was scored. The heads were identified by an ink mark around the comb.

3. RESULTS

The mean number of entries made to the food dish by chicks in the top ranks (1 and 2) did not differ significantly between the D (dark-incubated) and L (light-

Table 1. Entry to the food dish by the lowest ranking chicks[1]

	D groups		L groups	
Group	Rank 7	Rank 8	Rank 7	Rank 8
1	11.4 (1.4)	6.3 (1.0)	3.6 (1.3)	0.1 (0.1)
2	5.2 (1.8)	4.5 (1.4)	8.6 (0.9)	3.1 (1.5)
3	10.4 (1.3)	5.1 (1.2)	6.8 (1.2)	2.9 (1.0)
4	10.5 (1.3)	9.1 (1.2)	3.4 (1.5)	0.1 (0.1)
5	8.4 (1.3)	8.1 (2.1)	1.9 (1.0)	1.5 (0.7)
6	8.8 (1.4)	3.9 (1.1)	6.0 (2.2)	4.0 (1.4)
7	8.8 (2.4)	7.6 (1.9)	7.6 (1.3)	3.4 (1.1)
8	8.1 (2.7)	8.0 (1.8)	11.0 (1.2)	4.1 (2.0)

[1] The mean number of entries per day scored daily from day 8 to 16 posthatching (standard errors in brackets) is given for the chicks in ranks 7 and 8 of each group. The D groups are comprised of chicks hatched from eggs incubated in darkness, and the L groups from eggs which received light exposure. (from Rogers and Workman 1989).

exposed) groups. However, significant differences did emerge for chicks in ranks 7 and 8. As evident from Table 1, the mean entry scores for these lowest ranking birds were lower ($P < 0.001$) in the L groups than in the D groups.

In the L groups the lowest ranking chicks either never attempted to gain access to the dish or did so very rarely. In other words the hierarchy in the L groups was more stable and "more hierarchical". The entry scores for the lowest ranking chicks in the D groups were also more variable ($P < 0.01$; note larger standard errors for D groups in table 1) and low ranking birds in these groups obtained more access to the food over the measured period of time. Thus, one might expect fewer 'starve-outs' in the D groups.

In addition, by day 28 of life chicks scored as high ranking between days 8 and 16 were found to weigh significantly ($P < 0.001$) more than those scored at the bottom of the hierarchy. This difference between high and low ranking birds became larger with increasing age, and occurred independently of the sex of the chickens.

Table 2. Mean liveweight (g) of the chickens in the highest (Rank 1) and lowest (Rank 8) categories (Standard errors are given in brackets).

Age	Rank 1	Rank 8
Day 28	322 (12)	273 (17)
Day 55	905 (42)	785 (51)
Day 70	1270 (88)	1040 (42)

Thus, position in the social hierarchy as scored by competition for food in the second week of life provides a valid basis for predicting weight from day 28 onwards, irrespective of sex. Overall the D group chicks tended to weigh more than the L group chicks, but the difference was not significant.

In the second experiment it was found that D chicks adopt a different, and more successful, strategy of gaining access to the food dish. There are two extreme modes of access to the dish, one being by climbing on top of those already at the dish and pushing them away from the dish with the feet, the other achieved by crouching down and pushing in and up between the legs of those chicks already at the dish. The D chicks made a significantly higher percentage of their approaches to the dish by going over the top of the other chicks (70% of the approaches by D chicks were over the top, compared to 30% by the L chicks).

This competition between D and L chicks was also evident when the two types of chicks were raised in a standard brooder. On days 3,4,5 and 13 posthatching the percentage of D heads scored to be poking through the holes to feed was 86%, 67%, 63% and 61% respectively. Thus, the dark-incubated chicks are also more successful at gaining access to food presented in this manner, particularly in the first days after hatching.

4. DISCUSSION

As these experiments have shown, exposure to light during the last three days of incubation does indeed affect later social behaviour in terms of competition for food. Given that groups of chicks hatched from eggs exposed to light during incubation form more stable hierarchies, with the lowest ranking members being more reluctant to compete for food in early posthatching life, one might suggest that there is an advantage in incubating eggs in darkness. Chicks from eggs incubated in darkness adopt a more successful strategy for access to the food source and their lower ranking members are less likely to 'starve-out' by never competing for access

to food. In the long term, the dark-incubated chicks may also show a better mean overall weight gain, although this conclusion awaits further experimentation.

The results reported here are consistent with the original hypothesis that groups of chicks hatched from eggs exposed to light during the last stages of incubation would form more stable and rigid hierarchies as all individuals would be lateralised in the same direction, thus permitting consistency, or good predicability, bewteen all individuals in the group. The findings, however, do not prove this hypothesis. It could be argued that light exposure during this stage of development has a non-specific effect, such as a general effect on 'emotionality' or fear behaviour (see Jones 1989). Indeed, Dimond (1968) has shown that chicks hatched from eggs exposed to light during the last week of incubation are more fearful of a moving visual stimulus than are dark-incubated chicks. Other behaviours were also affected: the dark-incubated ones were found to imprint better than the light-exposed ones, and the light-exposed ones performed better in a task requiring them to go around a barrier to reach a group of their companions. Dimond also found that dark-incubated chicks direct twice as many of their pecks at other chicks in the group than do the light-exposed ones. This is another example of embryonic stimulation by light influencing early social behaviour, and the results may be consistent with those reported here, since higher levels of social pecking may indicate more instability in the social hierarchy. Once a social hierarchy has formed, pecking of other birds declines.

If, as predicted, the differences between the D and L groups do depend on the role played by light in determining the direction of lateralisation in the brain, as little as 2 hours of light on one of the days during the later stages of incubation may be sufficient to cause the effect (Rogers 1982). As little as 2 hours of continuous light is known to align the direction of lateralisation so that all individuals in the group are lateralised in the same direction. Light of varying intensity (flicking in an irregular pattern) may be more effective at even shorter durations. Illuminance measurements of the amount of light which penetrates the egg shell and membranes have been made by placing the air sac end of the egg over the photosensitive cell of an illuminance meter. For brown eggs some six percent of the light penetrates into the egg, whereas 11 percent penetrates white eggs. Thus, in these experiments as little as around 25 to 35 lux of light reaching the right eye of the embryo suffices to cause the observed effects. They are, in other words, extremely sensitive responses.

In the commercial situation no control is made over light exposure of the eggs, and those in the front of the incubator would receive more light exposure than those in the back. Thus, groups of commercially raised chicks are likely to be comprised of a mixture of light-exposed and dark-incubated chicks, and so may involve even more competition in which the dark incubated ones are much more successful while the light-exposed ones have an even higher risk of dying through starvation. Batch differences in survival rates may also be explained by these uncontrolled incubation procedures.

The differences between dark-incubated and light-exposed chicks in the strategy adopted to gain access to the food dish is difficult to explain precisely. Nevertheless, approaching the dish by climbing over the top of the other chicks may be a more successful strategy because it makes use of the feet by bringing them into contact with the eyes and heads of the other chicks. Such contact has aggressive components and this may be the reason why it makes the feeding chicks withdraw. As shown, when D and L chicks compete for access to food in a standard incubator by placing their heads through holes to reach the food trough, the D chicks are also more successful, particularly at the earlier stages of posthatching life. This result indicates that the findings reported here are not peculiar to laboratory housing conditions but extend to the commercial situation for raising chickens.

These experiments have dealt with visual exposure before hatching. Auditory exposure is likely to be of equal importance. Vince (1969, 1973) has shown that

auditory stimuli serve to synchronise the hatching of quail chicks; when clicking sounds are made by the more developed chicks, this speeds development of the rest of the clutch, and it can do so by up to 4 days. Vince and Toosey (1980) exposed chick embryos at day 20 of incubation to a foreign sound, and found that after hatching this sound was less disturbing to the exposed chicks. The auditory environment, first pre- and then post-hatching, is also important for social behaviour and survival. It would be worth investigating a possible role of auditory stimulation of the embryos on the later behaviour of the chicks. In incubators which have a loud noise produced by the fan there may be masking of the beak clapping and peeping sounds produced by the embryos, once their heads have entered the air sacs. This would be expected to desynchronise hatching to varying extents, and perhaps also to influence posthatching social responses to other chicks. Moreover, exposure of day 20 embryos to intermittent and novel stimuli (audible over the background, white noise of the fan) may interact with exposure to light. Vince and Toosey (1980) have shown that day 20 embryos respond to auditory stimuli by increasing the frequency and duration of beak clapping and opening of the eye. Hence, the retina of the right eye may receive more light exposure when the light exposure is coupled with auditory stimulation, and this, in turn, may mean shorter periods of light exposure are necessary to align the direction of lateralisation and have long-lasting consequences on social behaviour.

So far only white light has been tested for an effect on brain lateralisation and posthatching social behaviour. It is likely that some wavelengths will be more effective than others, and, if so, when the eggs need to be inspected, commerical hatcheries may be able to choose those wavelengths of light which do not influence brain lateralisation.

5. CONCLUSION

These experiments have shown conclusively that light exposure prior to hatching influences brain development and has consequences on social behaviour, in terms of competition for food, up to at least 16 days post-hatching and may have longer lasting effects on weight gain. It would seem that incubating eggs in darkness from day 17 on would be advantageous. With the proviso that the current methodology involves brief withdrawal of food, and therefore varies from many commercial practices, the results suggest that further research in this area is merited.

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GROWTH, FEED EFFICIENCY AND BODY COMPOSITION AS INFLUENCED BY VARIATION IN DIETARY PROTEIN:ENERGY RATIO IN CHICKENS SELECTED FOR DIFFERENT ASPECTS OF GROWTH AND BODY COMPOSITION

D.D.SACKEY

Although most lines of commercial broiler chickens around the world are now selected along with growth rate for improved feed efficiency and/or reduced fatness, there is limited information as to effects of such change in selection criteria on nutrient requirements of the growing birds. This study was designed to determine the effect of variation in dietary protein:energy ratio on growth, feed efficiency and abdominal fatness in five lines of chickens selected for increased (line F) or decreased (line L) abdominal fatness; increased 8-week liveweight (line W); increased 8-week liveweight combined with reduced abdominal fatness (line WL) or at random (line C).

The entire study included four isoenergetic (13.0 MJ ME/kg) diets ranging from 133 to 210 g crude protein (CP)/kg and both sexes. Growth rate and food intake were measured in 320 chickens housed in single cages from 21 to 49d and abdominal fat weight was measured at 49d. Due to sexing problems causing a marked sex imbalance in the groups allocated to the four diets and the requirement for further statistical analyses of the entire data set, the results presented here are restricted to the balanced comparison of 12 females from each line given either of two diets containing 153 or 210 g CP/kg. Results of this comparison are shown in the table.

Growth rate (g/d), FCR and abdominal fat (g/kg) in 12 pullets from each of the five lines given two isoenergetic (13.0 MJ ME/kg) diets containing 153 or 210g crude protein (CP)/kg

	Growth rate			FCR			Abdominal fat		
CP	153	210	Mean	153	210	Mean	153	210	Mean
Line									
F	28.6^{c}	30.8^{c}	29.7^{d}	2.69^{a}	2.46^{a}	2.58^{a}	33.9^{ab}	27.3^{a}	30.6^{a}
L	30.4^{bc}	34.6^{b}	32.5^{c}	2.59^{b}	2.32^{b}	2.46^{b}	27.5^{bc}	16.9^{c}	22.2^{b}
W	34.3^{a}	39.9^{a}	37.1^{a}	2.49^{c}	2.25^{b}	2.37^{c}	31.2^{abc}	25.6^{ab}	28.4^{a}
WL	32.7^{ab}	36.5^{b}	34.6^{b}	2.60^{ab}	2.29^{b}	2.45^{b}	26.1^{c}	19.9^{bc}	23.0^{b}
C	30.5^{bc}	31.4^{c}	31.0^{cd}	2.65^{ab}	2.41^{a}	2.53^{a}	37.7^{a}	21.6^{abc}	29.7^{a}
Mean	31.3	34.6		2.60	2.35		31.3	22.9	
$LSD_{0.05}$	2.7	2.9	2.0	0.10	0.09	0.07	7.1	5.9	4.6

Mean growth rate was higher and FCR and abdominal fat lower ($P<0.01$) in birds given the higher protein diet. There were significant differences between lines ($P<0.01$) for all three traits. The L line birds grew faster, converted food into body weight more efficiently and were substantially leaner than those in the F line. Birds from the W line grew somewhat faster and despite being considerably fatter, had a lower FCR than the WL line birds. Growth rate of the C line birds was intermediate to those in the F and L lines but feed efficiency and abdominal fatness was similar to the F line. Although there was some change in ranking between the lines on the two diets for each trait, the line X diet interaction was not significant ($P>0.05$) for any trait. If line differences in protein requirement exist, they are more likely to be revealed when the results of the entire data set including the four diets and both sexes are analysed. These will be presented at the meeting.

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EFFECTS OF OXIDATION ON THE QUALITY OF INGREDIENTS AND FEED FOR POULTRY

W. D. Shermer

Summary

Oxidation of poultry feed and feed ingredients can have a severe impact on the quality of the feed and the bird's performance. We have only scratched the surface trying to understand these complex interactions. Even low levels of oxidation can have deleterious effects on a bird's performance, as measured by body weight and feed conversion. The various quality control tests that can be used to determine the stability of feed or feed ingredients each have their merits and some of these can be used to measure the efficacy of antioxidants in feed ingredients.

I. Introduction

Oxidation has numerous deleterious effects on feed and feed ingredients including loss of metabolisable energy and destruction of fat soluble vitamins, xanthophylls, flavor and aroma components. Severe oxidation has been associated with encephalomalacia in chicks as well as steatitis in swine and cats and, systematically, may manifest itself as exudative diathesis, muscular dystrophy, necrotic tissue in various organs and poor fertility and hatchability. Deficiency syndromes such as encephalomalacia are the result of severe oxidation. Their symptoms are easily recognised and their cost is easily calculated. However, there is the possibility of a more subtle effect of oxidation - reduced feed efficiency. This will result in smaller birds and poorer feed conversion than otherwise might have been achieved with that same feed. Moreover, since there is nothing visually wrong with the flock, this effect can easily go undetected.

II.Oxidation and Broiler Growth

Despite all the academic studies and field experience, no data are available which define when the oxidation has advanced to a level to cause any of the problems mentioned above. In 1988, Waldroup and coworkers reported the results of a 7 week floor pen study designed to address part of this problem (Cabel et al. 1988). The objective of the test was to evaluate the effects of using unstabilised fats of varying levels of rancidity, in conjunction with various ethoxyquin levels, on overall broiler performance.

A 3 X 4 factorial arrangement of treatments was utilised.

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Ethoxyquin, at levels of 0, 62.5, and 125 mg/kg, was incorporated into diets formulated with fat which had previously been oxidised. Four peroxide levels were incorporated into the test: 0, 50, 100 and 170 meq of peroxide per kg of fat. These fats were incorporated into the diets at the 4% level and correspond to 0, 2, 4, and 7 meq of peroxides per kilogram of finished feed.

A total of 96 pens was used in the test allowing eight replicate pens for each ethoxyquin X peroxide level combination. There were 70 birds per pen, 35 males and 35 females, for a total of 6,720 birds. Body weight and feed conversion were measured at 21, 42, and 49 days.

These studies showed an inverse linear correlation of reduced body weight and poorer feed efficiency with increasing peroxide level. With unstabilised feed, the body weight fell an average of 15 g per meq of peroxide in the feed (Figure 1, Left Axis) and the feed conversion increased almost 2 points per meq of peroxide in the feed (Figure 2, Left Axis). The interaction of peroxides and ethoxyquin was also significant. In this test, when birds were fed diets containing 7 meq of peroxide per kg of feed, both the body weights of the birds (Figure 1, Right Axis) and the feed conversion (Figure 2, Right Axis) improved as ethoxyquin content increased and at the highest level of ethoxyquin content, 125 mg/kg, the body weight and feed conversion were almost equal to the unoxidised control (1.619 kg and 2.12 versus 1.635 kg and 2.10 respectively). The ethoxyquin was minimising the interaction of the existing peroxides with other feed nutrients and preventing the formation of additional peroxides.

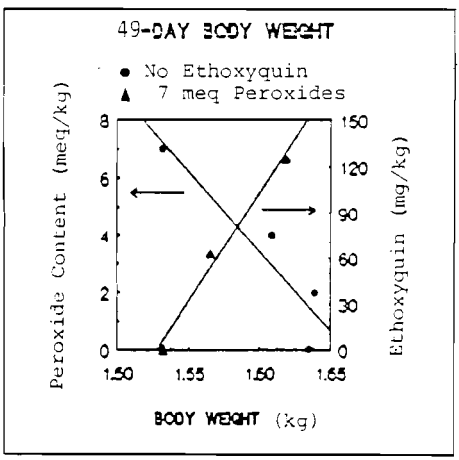

Figure 1. Body Weight As A Function of Peroxide Content (Left) and Ethoxyquin Content (Right).

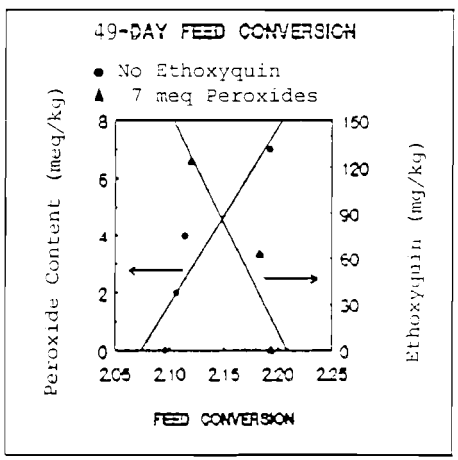

Figure 2. Feed Conversion As A Function of Peroxide Content (Left) and Ethoxyquin Content (Right).

Dr. O. W. Charles, University of Georgia, conducted a field survey of the oxidative status of stabilised and unstabilised broiler feed and feed ingredients from seven feed mills within that state. The purpose of the study was to determine if a correlation of feed oxidation with growth could be observed in the field. Three of these mills routinely, but not exclusively, used stabilised fats and by-product meal and the other four did not specify stabilised ingredients. Dr. Charles found that the levels of peroxides used in the Waldroup study were realistic and could be found under actual field conditions. Compared to broilers fed nonstabilised feed, broilers fed the stabilised feed had a 2.4% better energetic efficiency and 2% better livability (Charles et al. 1985).

III. The Chemistry of Oxidation

There is a strong similarity between the alkyl side chain of the structure of vitamin A and that of linoleic acid. It is in these highly unsaturated side chains that the oxidation reactions begin. The first step is the cleavage of a hydrogen atom from a carbon adjacent to one of the double bonds. This cleavage forms two free radicals. This reaction can occur at room temperature; but it can be increased very rapidly by the presence of metals such as copper and iron, higher temperatures, light, or the presence of other peroxides.

The free radical which is formed by the cleavage of the carbon-hydrogen bond can then react with oxygen to form a peroxide radical. The peroxide radical can then re-attack the starting material to form a second free radical plus the formation of a compound called a hydroperoxide. This is the chemical compound that is actually measured when a peroxide analysis is performed. The hydroperoxide can decompose to form two free radicals both of which are capable of re-attacking the starting material and forming another radical. This is the source of the accelerating rate of the oxidation reaction. One free radical becomes two. Two gives four, four will give eight, etc. The rate of this reaction also can be dramatically increased by the presence of metals, heat or light.

The oxidation reactions are interrupted when two free radicals react with each other to form a stable product or if they come into contact with an antioxidant. The nitrogen-hydrogen bond of ethoxyquin is the reactive portion of that molecule. It is the oxygen-hydrogen bond that is the reactive portion of BHA or BHT.

The structure of ethoxyquin allows it to act as an energy sink. The energy of the free radical that is formed by the cleavage of the nitrogen-hydrogen bond is delocalised over the entire complex of this compound. In simplest terms, this means that it does not have enough energy concentrated at one point to re-attack the starting material to create another free radical and continue the chain reaction.

Peroxides are not stable compounds themselves. They have two main reaction sequences by which they can be consumed. The first is a simple combination of free radicals to form a high molecular weight product. Since it is high in molecular weight, it will have

very low volatility and be essentially odorless. However, these compounds are often the cause of the off-taste in the feed. The second mode of reaction of these free radicals is to breakdown into smaller molecular weight products. These products include a wide variety of chemical compounds known as aldehydes, ketones, acids, alcohols, and esters. Since these are low molecular weight products they are usually very volatile and are the main source of the bad odors present in the feed.

IV. Analytical Procedures to Measure Quality

Many methods are used in an effort to monitor the quality of feed and feed ingredients. Those methods used relating to fats and by-product meals include titer, moisture by Karl Fischer and by Loss on Drying, Insolubles, Unsaponifiables, Saponification Number, % Fat, % Free Fatty Acids, and Fatty Acid Profile. Methods specific to oxidative stability include Initial Peroxide Value, 20-Hour Active Oxygen Method (AOM or Swift Method), Iodine Number and Thiobarbituric Acid Analysis (TBA).

Initial peroxide value determination is probably the most frequently used quality control test for fats and by-product meals. It is a very useful test but there are two points that need to be remembered when it is used. First of all, the peroxide determination gives you the value at only a single point in time. Secondly, the peroxides themselves are unstable intermediates. A typical graph of peroxide concentration as a function of time can be pictured as a common bell-shaped curve. Initially, the peroxide concentration increases very slowly but the rate increases more and more rapidly and passes through a period of a very rapid climb in the concentration of peroxide in that fat. The peroxides are unstable and proceed to form secondary reaction products. There is a point in time at which the rate of decomposition of peroxides equals the rate of formation. At this point, a plateau is formed on the plot of the peroxide concentration. As the raw materials are consumed, the rate of formation of peroxides begins to decrease whereas their decomposition to secondary products continues. This results in a decline in the peroxide concentration and it can actually proceed to the point of returning to the baseline. Because of this reaction profile it is recommended that people do both the initial peroxide determination as well as the 20-Hour AOM (the active oxygen determination) for peroxide value. The combination of these two values can enable you to more accurately predict where you are on that reaction profile.

Several accelerated stability tests have been developed to try to determine how long a feed or feed ingredient can be stored without turning rancid. The problem is to design a test that represents real world storage conditions but can produce a result within a short period of time. These two objectives conflict. The more quickly an answer is wanted, the more abnormal the test. Yet, a quick answer is necessary and these tests are accepted with these restrictions in mind. The 20-Hour AOM is the most commonly used method. A test tube containing 20 ml fat is placed in an oil bath controlled at 97.8°C and air is bubbled through the fat at a rate of 2.33 ml per tube per sec. After 20 hours, the peroxide content

of the fat is measured. The acceptable maximum is 20 meq peroxide per kg of fat.

The results of the 20-Hour AOM analysis can be correlated with the stability of a feed or feed ingredient. However, to accurately interrupt the results of this test, one has to keep in mind the limitations imposed on the analysis by the peroxide concentration curve. The more that is known about the history of the sample, the better the interpretation will be. Also, the results can be somewhat variable if the sample is an unstable ingredient or feed and if that sample is the portion of the peroxide curve where there is very rapid change in the peroxide concentration at the end of the 20-Hour AOM. In this part of the curve very small changes in time between analysis of samples can make some apparent very large differences in peroxide value. At this time, the absolute number is not important. It is more important to have learned that the sample is extremely unstable. This test is extremely sensitive to impurities present either in the air that is bubbled through the fat or to any residue left in the test tube from the previous analysis. There is one distinct advantage for this test, however. It can be used for the evaluation of relative effectiveness of different antioxidants.

The Iodine Number is another test that is frequently used to measure fat stability but it suffers some of the limitations of the Initial Peroxide Analysis. The Iodine Number measures the number of double bonds that are present in a fat. This then requires that something is known about the nature of the fat and the number of double bonds that should be present in a good quality sample of that fat. A good fish oil will have an iodine number as high as 90 or even 100. A good poultry fat may be 70 to 75, a pork lard may be down around 60 whereas a beef tallow could be as low as 40. The greater the extent of the oxidation, the lower the iodine number of that particular fat. Therefore, it is extremely important to know the nature of the fat that is being analysed as well as what a typical value should be. Like the peroxide value, the Iodine Number gives the status of an ingredient at a single point in time. Furthermore, high levels of peroxides may interfere with the Iodine Number analysis.

The Thiobarbituric Acid Analysis, the TBA test, is another method that has been used by some investigators to study the stability of feed or feed ingredients. However, it suffers from many limitations. This test measures the malonaldehyde concentration generated by the oxidation of a triglyceride. The malonaldehyde, however, is a secondary by-product of oxidation produced by the decomposition of the peroxides which are formed first. Therefore, the oxidation could potentially be well advanced before any malonaldehyde is actually produced. Secondly, malonaldehyde is only one by-product out of a wide variety of by-products which are produced by the decomposition of peroxides. Aldehydes are also unstable products. They can be further oxidised to form acids which are not analysed or detected by the TBA test. Therefore, a sample could give an erroneous low value for TBA and actually have undergone very extensive oxidation. Finally, this analysis is carried out with a UV spectrophotometer and there are numerous other materials which can absorb in the same wavelength

range as malonaldehyde and create some serious interferences in this analytical procedure. This method can be very useful in certain situations. For eample, the TBA analysis correlates well with the scorings from flavor panels of frozen food in shelf-life evaluations.

V. Feed Ingredient Stability

The 20-Hour AOM test has been used to measure the increased stabilisation of yellow grease through the addition of various antioxidants. The test was run at a commercial laboratory in Chicago, Illinois using yellow grease which was obtained locally. Samples of the various antioxidants were submitted to the lab and they prepared the samples, adding 500 mg/kg of an antioxidant to each sample, and ran the tests. The fats were held under the test conditions of the 20-Hour AOM test until the accepted limit of 20 meq per kg of fat was actually reached. The AOM stability of the unstabilised control was 4.5 hours, with BHT this was extended to 19 hours, Tenox R, 20 hours; and BHA, 190 hours. The lab ran out of the fat treated with ethoxyquin at 530 hours and it still had not reached 20 meq of peroxide.

A similar test with poultry fat was conducted using 750 mg/kg of each antioxidant. The control failed within 20 minutes indicating that it was a very unstable fat. The sample treated with BHT held up for 5 hours; and the BHA-treated sample lasted 78 hours before reaching the 20 meq peroxide limit. The ethoxyquin-treated fat still had not reached the limit after 640 hours.

A slightly different test was used with poultry by-product meal. Samples of poultry by-product meal were obtained, mixed with the various antioxidants and then stored in a temperature humidity cabinet at 45°C and 60% relative humidity. At 3, 6, and 15 weeks, samples of this by-product meal were then removed, the fat extracted and analysed for peroxide content. The peroxides rose rapidly in the unstabilised control. This was followed very closely by the sample that had been stabilised by 500 mg/kg of a typical formulation. BHT provided some stabilisation but by far the most stabilisation was provided by 500 mg/kg of ethoxyquin. Throughout the entire 15 weeks of this storage test, no increase in peroxide content was detected in the sample treated with ethoxyquin.

VI. Differential Thermal Analysis

While the standard methods of determining the stability of feeds have been useful, they have their limitations and new methods are needed. Instrumental methods, such as Differential Thermal Analysis (DTA), are available which can measure the heat evolved as some of these materials oxidise. Ethoxyquin is virtually the only antioxidant used in stabilising fishmeal. The results of this study demonstrate why this is the antioxidant of choice. Samples of Menhaden fishmeal were obtained every two weeks during the fishing season and sent to St. Louis for analysis. A DTA procedure developed by Monsanto was used in which it was possible to detect the heat generated as the fishmeal oxidised. With this

equipment a heat evolution of 1.25 kJ/kg/min was detected in the unstabilised control. The heat evolved is energy lost to the animal. When 100 mg/kg of ethoxyquin was added heat evolution was reduced by 85%.

Using a computer program to take the data generated by the DTA, it is possible to estimate how long it would take a pile of fishmeal to be in danger of catching fire. The untreated control could have caught fire in as short a time as three or four days. Treatment with 100 mg/kg of BHA extended that life time to about 20-25 days, 100 mg/kg of BHT to about 45 days, 100 mg/kg of ethoxyquin protected the fishmeal for as long as 125 days (Romoser 1979).

VII. Beta-Carotene Stability Test

Another sensitive test method for comparing the effectiveness of different antioxidants is the Beta-Carotene Stability Test. This procedure measures the stability of beta-carotene as the time required for one-half of the beta-carotene to decompose. Antioxidants can be added to the system and their effect on the beta-carotene half-life can be measured. Ethoxyquin addition increased the half-life from 60 minutes to 180 minutes, BHT to 84 minutes and BHA to 79 minutes (Shermer et al. 1983).

The beta-carotene method has also been used to measure the deleterious effects of contaminants such as copper and iron. Both of these metals are known to promote oxidation but copper has been reported to be the more reactive when present in fats. However, in the beta-carotene test, both metals have been found to be equally deleterious. Chelating agents are frequently added with antioxidants to neutralise the effects of the metals. Citric acid has been shown to be an effective chelator of iron in the stability test but a poor chelator for copper. The opposite effect was seen when sodium acid pyrophosphate was added.

VIII. Ethoxyquin and Vitamin Premix Stability

Reliable methods to study the stability of vitamin or vitamin/mineral premixes are more elementary. The use of storage stability tests under controlled temperature and humidity conditions is the most common method for these systems. The stability of the matrix is then determined by comparison of the analyses of one or more of the vitamins, as a function of time, with the initial value. Dr. Donald Parrish, Kansas State University, ran a test on the stability of a vitamin/mineral premix with and without an antioxidant using temperature and humidity as variables. He found that moisture was the most important factor during storage of the premix. Under dry conditions at 43°C, an average 10% loss of vitamin A was observed during one month storage, regardless of antioxidant treatment. This was probably due to the high levels of ethoxyquin already incorporated in the protective system for the vitamin developed by the manufacturer. However, in the presence of high humidity, the control lost 92% of the vitamin A in one month. The higher loss in the presence of high humidity is due to the hydrolysis of the vitamin A acetate to produce the free vitamin A. Vitamin A is naturally an alcohol and

is highly susceptible to oxidation in this form. When ethoxyquin was added to the premix, 47% of the Vitamin A remained after the one month storage at high humidity. (Parrish & Patterson 1987).

IX. Conclusions

Feed oxidation can produce a wide variety of problems which range from the very severe difficulties associated with deficiency syndromes like enchephalomalcia to the less obvious effects of reduced body weight and poorer feed efficiency. Regardless of severity, the bottom line is a loss of income to the grower. The use of an antioxidant such as ethoxyquin can help protect against these losses.

The oxidation of feed and/or feed ingredients typically begins in the highly unsaturated side chains of the fat or fat soluble components of that feed. Peroxides are the first product generated by the oxidation that takes place in the side chains but the peroxides themselves are unstable and will rapidly continue the oxidation process and produce secondary by-products which include polymers, acids, ketones, aldehydes and other similar compounds.

Each of the standard methods used for stability evaluation - Initial Peroxide Value, 20-Hour AOM, Iodine Number and TBA Analysis - have their advantages and disadvantages. Of these tests, the combination of the Initial Peroxide Value and the 20-Hour AOM test provide the most useful data to assess the current stability of samples and the projected shelf-life of those materials. New analytical methods such as DTA and the Beta-Carotene Stability Test can provide additional information. Using these methods, data from a variety of relative efficacy studies in fats, poultry by-product meal, and fishmeal confirm the efficacy and cost effectiveness of ethoxyquin.

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ENDOGENOUS AMINO ACID SECRETION IN CHICKENS FED GRADED LEVELS OF DIETARY FIBRE

P. Siriwan, W.L. Bryden and E.F. Annison

Intestinal endogenous protein secretion is variable and may be influenced by many factors including dry matter and protein intake, protein quality and crude fibre content of the diet (Sauer and Ozimek 1986). Most studies have been based either on the use of protein-free diets, or on the extrapolation to zero protein intake from measurements made on birds fed graded levels of protein. Both procedures yield values for endogenous secretion of questionable relevance to birds fed normal diets. In the present study we have examined the effects of dietary fibre level on endogenous secretion, expressed as values for individual amino acids after hydrolysis of ileal contents, using homoarginine as a marker. This technique overcomes many of the limitations associated with protein-free determination, or extrapolation to zero protein intake (Siriwan et al. 1987).

Male broilers (5 weeks old) were fed diets containing 20% casein and graded levels of dietary fibre (rice hulls; 0, 30, 60, 90, 120 g/kg) 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 of diet containing guanidinated casein. The diets and ileal contents were analysed for amino acids including homoarginine and acid insoluble ash. Endogenous amino acid values were calculated from homoarginine:amino acid values in guanidinated casein, and in ileal contents (Siriwan et al. 1987) and are presented in the table.

Endogenous amino acid concentrations (mg/kg DM intake) in ileal digesta of broiler chickens fed graded levels of dietary fibre

Amino Acid	Dietary fibre levels (g/kg)				
	0	30	60	90	120
Threonine	925	1033	1137	1387	1789
Valine	1122	1264	1321	1661	1959
Methionine	131	134	132	155	154
Isoleucine	1247	1256	1377	1305	1951
Leucine	529	554	687	729	1066
Phenylalanine	667	721	784	940	1264
Histidine	438	437	549	555	659
Lysine	567	625	641	688	755
Arginine	458	510	504	542	607

These data show that endogenous protein secretion into the gut is positively related ($P<0.05$) to the level of dietary fibre. It is likely that dietary fibre increases the secretion of enzymes into the gut and desquamation of the lining of the intestinal tract.

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PROGRESS TOWARDS THE DEVELOPMENT OF IMMUNOASSAYS TO DETECT ANTIBIOTIC RESIDUES

M.L.SMITH and J.A.SIMONS

Summary

Drug haptens were made for the sulphonamide and benzimidazole families, sulphamethazine, sulphamerazine and benzyl penicillin. Drug-protein conjugates were made using bovine serum albumin, hen egg albumin, bovine thyroglobulin and keyhole limpet hemocyanin. The conjugates were used to produce polyclonal antibodies in rabbits and sheep and monoclonal antibodies against sulphamethazine. Antibody titres were measured by enzyme-linked immunosorbent assay (ELISA). Antibodies were assessed for their ability to detect parent drugs using the indirect competitive ELISA. The test sensitivity for sulphamethazine and sulphamerazine is approaching the maximum residue limit (0.1ppm) for sulphonamides in animal tissues. Work is continuing to produce higher affinity antibodies to improve the test sensitivity for the sulphonamides, benzimidazoles and penicillins and to refine the procedures so that a test kit can be produced.

I. INTRODUCTION

Antibiotics* are widely used throughout the world in the meat, milk and egg producing industries. They are used at subtherapeutic levels to promote growth and general well-being and at therapeutic levels for the control of specific disease conditions. The use of these chemicals has profound benefits to producers and consumers, especially when used in the intensive industries.

However, in recent years, concern has been raised by public health authorities, the medical profession and consumer groups about the effects that antibiotics may ultimately have when they end up in the human food chain. These concerns include producing a reservoir of resistant organisms that may infect susceptible humans, allergic responses to antibiotics, especially penicillin, and other unknown effects of ingesting chemicals. These concerns require that there is restricted use of antibiotics in animals and that the use of these drugs is carefully monitored.

The regulatory authorities have addressed this issue in recent years by increasing the monitoring of chemical residues. There are a number of available methods. The microbiological inhibition test, which is based on the ability of a sample containing antibiotic to inhibit the growth of a standard bacterial culture, can be quite sensitive for some antibiotics. However, it is not able to identify a growth inhibitor without additional testing and is prone to false positive results. This test can be used as a screening test but in most cases results are impossible to achieve within a single working day.

Definitive antibiotic identification and quantitation require analytical chemical methods such as High Performance Liquid Chromatography (HPLC), gas chromatography, mass spectroscopy and thin layer chromatography. These procedures are unsuitable for large scale testing as extensive sample clean-up, a high level of technical expertise, expensive equipment and time are required to carry them out.

*" Antibiotics" in this communication includes the sulphonamides.

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Therefore, in order to determine the true extent of antibiotic residue contamination in foodstuffs, there is a great need for sensitive, specific and inexpensive tests that can be performed quickly and on a large scale. ELISAs were first applied to the detection of large molecules, but over recent years have been sucessfully applied to the detection of small molecules, namely drugs (see review by Newsome 1986). Commercial tests for drugs and hormones used in human medicine are available under the trade name EMIT (Syva Co. USA). In the EMIT assay the patient's specimen is allowed to react with excess antibody. Any antibody left unbound is free to react with, and inactivate, the drug-enzyme conjugate added in the next step. Therefore, with high drug concentrations, less antibody remains to inactivate the drug-enzyme and a colour change takes place when the substrate is added. Low drug concentrations leave most antibody free to react with, and inactivate, the drug-enzyme complex, so no colour change takes place. The test is read on the spectrophotometer.

Commercial immunoassays for antibiotics in animal serum, urine, tissues and feed have been produced overseas. These include the Charm Tests (Penicillin Assays, USA), radio-receptor assays for seven antibiotic families, used most widely to detect penicillin in milk (Charm and Chi 1982), and the E-Z Screen Test (Environmental Diagnostics, USA). The latter is a colorimetric test performed on a card containing antibody immobilised on filter paper-like material. This test is available for the antibiotics chloramphenicol, gentamycin, sulphadimethoxine, sulphamethazine and tylosin. IDEEX (USA) has recently produced the CITE test for sulphamethazine in milk and Idetek (USA) has the Lac Tek for sulphamethazine in milk and the Sulpha Test Kit and the Quick Test for sulphamethazine in pig serum, plasma, urine and feed.

The strong commercial interest in producing ELISAs for the antibiotics, coupled with the difficulty of the task, means there is little published literature on the subject. The detection of sulphamethazine in pig blood (Fleeker and Lovett 1985; Singh et al. 1989), pig urine and pig muscle (Dixon-Holland and Katz 1988) and milk (Dixon-Holland and Katz 1989) have been reported. The latter three authors describe procedures that are adaptable to large scale screening. Polyclonal and monoclonal antibodies to penicillin (in the form of its penicillinoyl derivative) have been made to investigate the role of penicillin derivatives in hypersensitivity reactions (deHaan et al. 1979,1985). These methods utilise protein linked onto penicillin through hydrolysis of the β-lactam ring. A method of producing conjugates without damaging the β-lactam ring results in improved sensitivity to the native penicillins (Kitagawa et al. 1978).

The number of anthelmintic drugs available in Australia has increased steadily since the introduction of thiabendazole in 1962 (for a review of the structure, use and metabolism of these drugs see Arundel 1985). The benzimidazole family (albendazole, fenbendazole, mebendazole, parbendazole, oxfendazole and thiabendazole) is part of this group. When ingested, these drugs form a wide variety of metabolites and the binding of these metabolites to animal tissues increases the risk of human toxicity. Analytical chemical procedures are the only means of identifying these drugs or their metabolites at present. The need for increased surveillance and improved detection methods has been identified (Committee on Feeds, Fertilisers, and Related Materials: Recommendations for Official Methods 1986; Ministry of Agriculture, Fisheries and Food 1987). A radioimmunoassay to detect mebendazole and flubendazole for use in pharmacokinetic studies has been reported (Michiels et al. 1982).

ELISAs have great potential for residue detection. They are very specific and do not require the same level of sample clean-up as do the analytical procedures. In addition, they are cost effective, can provide results quickly, can handle a large number of samples and lend themselves to automation. Using enzyme-immunoassay technology we are attempting to produce commercial tests for on-site-use that can be applied to blood, urine, tissues or eggs, for some of the commonly used antibiotics and anthelmintics in Australia.

2. EXPERIMENTAL AND RESULTS

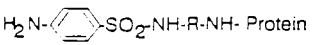
The criteria used in selecting the tests included the potential for residues in tissues and the similarity in chemical structure of a number of drugs within specific antibiotic and anthelmintic families. On this basis, four groups were selected, namely the penicillins, tetracyclines, sulphonamides and the benzimidazoles. The strategy involved in developing these tests is outlined below. So far,most work has concentrated on the penicillins, sulphonamides and benzimidazoles. Most results have been produced for the sulphonamides so unless indicated, the work presented will refer to this group.

(a) Preparation of drug or drug hapten conjugates

This is an essential first step in any ELISA for small molecules. Since these drugs are of low molecular weight, they must first be conjugated to a large carrier molecule (protein) in order to provoke an immunological response when injected into experimental animals. The antidrug antibodies produced are an essential component of the ELISA. The structures of the drugs and the linking chemistries used so far are shown in Figure 1.

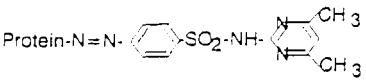
Fig. 1. Drug and hapten protein conjugates

Sulphonamide Hapten:

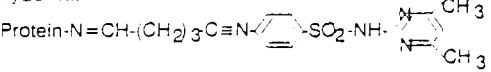


Sulphamethazine:

Diazo link

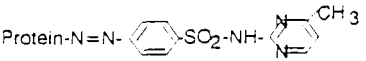


Gluteraldehyde link

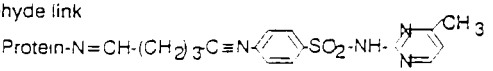


Sulphamerazine:

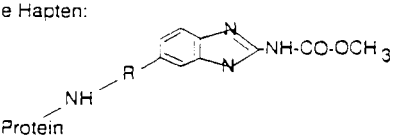
Diazo link



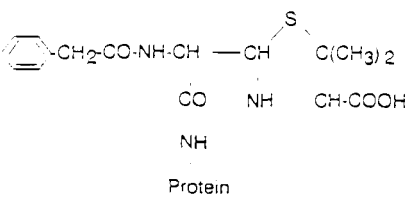
Gluteraldehyde link



Benzimidazole Hapten:



Benzyl Penicillin:



Many drugs do not contain suitable functional groups for protein conjugation, so these groups must be either incorporated into the drug or a similar reactive molecule synthesised to produce a hapten that is capable of reacting with a carrier protein. The initial aim was to produce a universal hapten that would generate antibodies capable of detecting the complete, or near complete, family of sulphonamides and benzimidazoles. To achieve this the protein was linked onto the variable region of the molecule, leaving the conserved region of the molecule intact and exposed for antibody production. The universal sulphonamide and benzimidazole haptens were synthesised (Institute of Drug Technology Australia Ltd, Boronia, Victoria) and provided a reactive group for protein binding. Sulphonamide-protein conjugates were made using the water-soluble carbodiimide reaction (Tijssen 1985) and benzimidazole protein conjugates were made using the mixed anhydride method (Patent:3 981 863, USA). The universal sulphonamide hapten did not provide very useful antibodies, so specific antibodies were also produced against the most widely used sulphonamide, sulphamethazine and the closely related drug, sulphamerazine. Two different conjugation methods were employed, diazotisation (Fleeker and Lovett 1985) and the gluteraldehyde procedure (Dixon-Holland and Katz 1988), as these methods produce linker arms with different chemical structures and also bind to different sites on the protein. Gluteraldehyde binds through lysine and diazotisation binds through tyrosine and tryptophan. In this way, cross-reactions between linker groups could be avoided in the ELISA. A benzylpenicillin protein conjugate was made by hydrolysis of the β-lactam ring (deHaan et al. 1979). Modifications of the above methods were made where appropriate to account for the different drug characteristics. The proteins used for conjugation included bovine serum albumin, hen egg albumin, bovine thyroglobulin and keyhole limpet hemocyanin (all supplied by Sigma Chemical Co., St Louis). Reaction products were purified by dialysis or Sephadex G-25 column chromatography and the molar conjugation ratios were determined using the protein concentration in the sample and the hapten extinction coefficient or the TNBS colour development test (Snyder and Sobocinski 1975). Conjugates were stored lyophilised at 4^{o}.

(b) Production of polyclonal antibodies

Polyclonal antibodies were produced in rabbits and sheep. Briefly, drug-protein conjugates were reconstituted to 1mg/ml with sterile deionised water and emulsified 1:2 in Freunds Complete Adjuvant (dose 1) or Freunds Incomplete Adjuvant (subsequent doses). Rabbits were injected intramuscularly with 0.5 ml in each hind leg. Sheep received 1ml intramuscularly into each hind quarter. Dosing was repeated every three to four weeks. Tests bleeds were carried out prior to the first injection and at varying intervals thereafter.

(c) Production of monoclonal antibodies

Monoclonal antibodies have been produced against sulphamethazine. Inbred Balb/c mice received 4 doses of sulphamethazine-bovine thyroglobulin, 100μl/dose, intraperitoneally in adjuvant (see (b)) over a three month period. Mice from this group to be used for a fusion were then injected each day for four days with 100μl of sulphamethazine-bovine thyroglobulin in NaCl as above and on the fifth day the spleen was removed and fused with NS1 myeloma cells. Hybridoma culture was done following standard procedures. Antibody titres were measured by ELISA. Two successful fusions so far have produced 7 monoclonal antibodies of isotype IgG1 (2) and IgM (5). Further fusions are planned and the characterising of the monoclonals is still in progress.

(d) Measurement of antibody titre

Antibodies were measured by ELISA. Moderate to high antibody titres (1:5120-1:$\geq$40960) were obtained for all the sulphonamide, sulphamethazine, sulphamerazine and benzyl penicillin conjugates in rabbits and sheep. The benzimidazole conjugates were less immunogenic and produced only low titres in rabbits and sheep (1:80-1:5120). To perform the test, the drug was bound to the solid phase (microtitre tray), (Nunc Immunoplate I) via its protein conjugate (using a different protein to that used to produce the antibody). Antiserum was added, titrated in two fold dilutions and incubated. Antibody bound to the immobilised antigen was detected using a commercial anti-species antibody conjugated to horseradish peroxidase followed by ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) substrate. The presence of antibody was indicated by a colour change from colourless to green. The starting dilution for serum was 1:320 and for hybridoma supernatants was 1:2. Tests were read on the automatic plate reader at wavelength 414nm.

Concentrations of reagents used were determined by checkerboard titration. The titre where 50% of the antibody was bound to the immobilised antigen was taken as the dilution to use in the indirect competitive ELISA for drug measurement.

(e) Drug measurement

The presence of drug was measured in the indirect competitive ELISA. This is a inverse test: lack of a colour change indicates the presence of drug and a colour change indicates the absence of drug. In this strategy, drug-protein was bound to the microtitre tray and allowed to react with a mixture of specific antibody (at a concentration determined as outlined in (d)) and free drug (standard or unknown sample). This resulted in competition between the free and bound drug for the limited amount of antibody. Antibody not bound to the free drug was available to react with the drug on the microtitre tray. The amount of bound antibody was measured by reacting it with an antispecies antibody coupled to horseradish peroxidase, followed by ABTS substrate. High concentrations of free drug bound most, or all, of the specific antibody, leaving little or none available to bind the drug on the microtitre tray. Therefore, less colour development took place when antispecies antibody-enzyme and substrate were added. Conversely, when no drug was present in the sample, all the antibody was available to bind to the drug on the microtitre tray and a colour change was produced when antispecies antibody-enzyme was added.

This aspect of our work is still very much in the development phase. Early tests of polyclonal antibodies to the universal sulphonamide hapten produced in both rabbits and sheep in the indirect competitive assay did not detect maximum residue levels of a range of sulphonamides and indicated that the antibodies produced were of low affinity. Specific sulphamethazine and sulphamerazine antibodies were tried and this, coupled with some modifications to the format of the assay, lead to an improvement in the test sensitivity to sulphamethazine and sulphamerazine down to levels approaching the maximum residue limit of sulphonamides in animal tissues (0.1ppm). Other changes included decreasing the concentration of immobilised antigen and increasing the amount of antispecies antibody-enzyme and avoiding the use of the same linker chemistries for antigen immobilisation and antibody production. However, further improvements are required to obtain an objective optical assessment at 0.1 ppm because the final test format will rely on an optical measurement rather than one using a spectrophotometer.

None of the monoclonal antibodies produced so far have improved the sensitivity of the assay over that of the polyclonal antibodies, but the search for a better monoclonal antibody will continue since there are advantages in having a uniform antibody to use in a commercial test.

Work is proceeding on several fronts to improve the assay sensitivity. High-affinity and low-affinity polyclonal antibodies have been separated by affinity chromatography and are being assessed in the indirect competitive ELISA; Fab fragments (the antigen-binding region of the IgG molecule) of specific antisera have also been produced and may be incorporated into the indirect competitive ELISA or used for sample concentration. Also, to produce a faster one-step test rather than the current two-step format, conjugates of horseradish peroxidase to sulphamethazine and sulphamethazine antibody have been made.

(f) Antibody specificity

This has been investigated to only a limited extent, as most effort up till now has been concentrated on improving assay sensitivity. It appears that, with polyclonal and monoclonal antibodies, small changes in molecular structure produce marked differences in sensitivities among the sulphonamides. For example, in the indirect competitive assay, antibody to sulphamethazine detects sulphamethazine itself at 0.39μg/ml, sulphamerazine at 6.25μg/ml and sulphadiazine at more than 25μg/ml. Sulphadiazine is similar to sulphamerazine except that it has no methyl groups on the pyrimidine ring.

CONCLUSION

This work is still in the developmental phase. The usefulness of the polyclonal and monoclonal antibodies produced so far has been limited by their low affinity in the indirect competitive ELISA. Modifications to the ELISA have improved the sensitivity to sulphamethazine

and sulphamerazine. Higher sensitivity levels are required for an optical test. The search for alternative methods of drug protein conjugation, the production of monoclonal antibodies and the development of one-step competitive immunoassays should help to achieve this.

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TOXICITY OF CYCLOPIAZONIC ACID IN MATURE MALE CHICKENS

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Cyclopiazonic acid (CPA) is a toxic indole-tetramic acid produced by species of Penicillium and Aspergillus (Cole 1984). Studies in animals have shown that the principal target organs of this mycotoxin are the liver, kidney and digestive tract (Cole 1986). In laying hens CPA reduced egg production and egg shell quality and caused high mortality at dosage levels above 5 mg/kg body weight (Suksupath et al. 1989). Van Rensburg (1984) has demonstrated that this toxin caused degenerative changes in the epithelial cells of the epididymis and vas deferens of the rat but studies on the influence of CPA on reproductive function in other species have not been reported. The present study was conducted to determine the effect of CPA in mature male chickens.

Commercial layer breeder roosters were allocated to three groups of six, placed in individual wire cages and fed a commercial layer ration. The experiment consisted of three periods; a pretreatment period of two weeks, followed by four weeks of dosing with CPA and a final two week recovery period. Daily doses of either 0, 2.0 or 4.0 mg CPA/kg liveweight were administered orally in gelatine capsules. During all three experimental periods, bird weight and feed consumption were recorded weekly and semen was also collected weekly.

Four roosters dosed with 4.0 mg CPA died within six days of the commencement of dosing but the two remaining roosters on this dosing regime and those on 2 mg appeared clinically normal. Average body weight and food consumption were decreased only during the first seven days of dosing. CPA caused a decrease in semen volume, sperm concentration and increased the number of abnormal spermatozoa, especially bent-tail and crooked-neck spermatozoa during the first seven days of dosing. There was no effect on sperm motility or live:dead sperm ratio.

In rats the male is much more susceptible to CPA than the female (Van Rensburg 1984) but a comparison of the present results and our previous study (Suksupath et al. 1989) suggest that there is no sex difference in CPA tolerance in the domestic fowl. Moreover, in the chicken, many of the effects on male reproductive function appear to be transient.

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REDUCED DIETARY PROTEIN LEVELS FOR BROILERS

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A 23% protein, corn-soybean meal diet was stepwise reduced in level of dietary protein while keeping the ratio of corn to soybean meal constant. As essential amino acids (EAA) in turn met NRC minimum requirement levels their adequacy was tested using the following mode. A 23% protein, corn-soybean meal diet meeting the minimum NRC EAA requirement values served as a positive control. Corn-soybean meal diets with the EAA in question at minimum requirement levels (but supplemented with other EAA to meet minimum requirement levels as level of dietary protein was continually reduced), served as negative controls. Three further test diets consisted of the negative control plus a 10% supplement of the particular amino acid in question, and non essential nitrogen added to both low-protein test diets to equal levels of the 23% protein control diet.

All EAA with the exception of tryptophan proved to be adequate at the NRC reported values. When dietary protein dropped below the 18% level a response, especially in feed:gain ratio was noted with non essential amino acid supplementation.

Histidine was the last EAA to just meet the NRC minimum requirement and this was with a level of 14% crude protein. Performance equalled that of the 23% protein control diet for all test diets. A further experiment demonstrated that with low-protein diets, where EAA met minimum requirement values, birds eat to satisfy their protein requirements.

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INDUCED PAUSE IN EGG PRODUCTION USING A SYNTHETIC HYPOTHALAMIC PEPTIDE

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A technique often used to extend the laying phase of hens at 60 to 80 weeks of age is to induce moulting by severe restriction of nutrients for 7 to 10 days. This is followed by increased egg production when feeding is resumed. Induced moulting causes a decrease in the secretion of luteinizing hormone (LH) from the pituitary and reduces the sensitivity of the pituitary gonadotrophs to gonadotrophin-releasing hormone (GnRH) leading to follicular atresia, followed by a regeneration of the reproductive tract once feeding is resumed (Tanabe et al. 1982). Chronic treatment of hens with an agonist of GnRH may result in endocrine events which are similar to induced moulting (Bahr 1987). Our aim was to determine if treatment of laying hens with a GnRH agonist would induce a pause in egg production.

Sixty four hens at 70 weeks of age were allotted at random to equal groups (n=16) consisting of: 1. Daily subcutaneous (s.c.) injections of saline for 7 days; 2. Offered whole oats for 7 days (i.e. nutrient restriction); 3. Daily s.c. injections of 100ug/kg of a GnRH agonist (D-Trp^6-Pro^9 N-ethyl amide GnRH) for 7 days; 4. Administered 4 prototype slow-release biocompatible pellets each containing 120ug of the agonist (s.c.). Daily egg production was measured for each bird before and 6 weeks after the commencement of treatment. Blood samples taken at weekly intervals from 4 birds selected randomly from each treatment before and for 6 weeks after treatment were assayed for LH.

Effect of the treatments on mean (±se) weekly egg production (EP;%) and luteinizing hormone (LH;ng/ml) of the hens

Treatment		Weeks after start of treatment[1]				
		Pretreat	1	2	4	6
1	EP	82±0.2^U	80±3.0^U	74±0.5^U	80±2.0^U	83±2.0^U
	LH	5.6±0.7^X	5.5±1.7^X	3.9±0.4^X	4.6±0.4^X	3.8±0.2^X
2	EP	86±0.3^U	39±1.0^V	3±2.0^W	86±2.0^U	88±2.0^U
	LH	6.8±1.2^X	1.8±0.3^Y	5.3±0.8^X	4.9±0.4^X	3.9±0.3^X
3	EP	84±2.0^U	45±3.0^W	66±4.0^V	71±1.0^V	80±4.0^U
	LH	6.1±0.4^X	3.9±1.2^{XYZ}	3.5±1.0^{YZ}	4.7±0.5^{XY}	4.0±0.6^Y
4	EP	84±1.0^U	45±5.0^V	72±5.0^U	72±3.0^U	77±3.0^U
	LH	5.3±0.7^X	4.9±0.7^X	4.0±0.6^X	5.7±0.8^X	3.8±0.2^X

[1] Within groups: ^{U,V,W} differ for EP; ^{X,Y,Z} differ for LH at P<0.05

Treatment with GnRH agonist reduced egg production and LH levels in the hens but not to the same extent as in those birds offered oats (Table). Nevertheless, these data suggest that treatment with a GnRH agonist may offer an alternative to induced moulting to extend the laying phase of hens.

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NEW ENZYME PREPARATION FOR ENHANCING POULTRY FEED UTILISATION

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Summary

An enzyme complex produced by fermentation of Trichoderma viride was developed to improve the nutritive value of poultry feeds. As the complex contains cellulose, B-glucanase, xylanase, pectinase and amylase the inclusion of high fibre cereals such as barley, oats and rye in feed of broiler chickens became practical without loss in performance.

The optimal inclusion rate of the standardized enzyme complex in broiler diets based on either barley or oats was determined to be in the range of 100 to 200 ppm.

The addition of the enzyme complex to broiler feeds significantly improves the nutrient retention and metabolizable energy of the diet by making the otherwise non-utilizable hemicelluloses available to the animals as additional energy. Thus, the growth performance, feed conversion and production efficiency of broiler chickens can be positively influenced. In addition, a better litter quality and a lower incidence of breast blisters in the birds could be observed.

Overall the potency of the new enzyme preparation as a feed additive for broiler chickens was documented by the results of a series of short-term experiments confirmed by large-scale efficacy studies.

I. INTRODUCTION

Cereals represent the basis of all rations for poultry and are, therefore, the major least cost energy sources for broiler diets. However, the differences in nutritional value between cereal grains have been recognized for decades. Especially barley has been considered less palatable, higher in fibre and lower in metabolizable energy and protein. Its inclusion in the diet causes wet, sticky excreta and results in a retarded growth rate compared to maize or wheat. This effect is caused mainly by the presence of mixed linked beta-1,3:1,4-D-glucans, which are major non-starch polysaccharides occurring in the cell walls of endosperm. They are not digestible by poultry and act as anti-nutritive factors increasing the viscosity of the intestinal digesta and impairing the utilization of other nutrients.

The current scientific literature, however, indicates that the feeding value of barley can be enhanced by the addition of specific enzymes from a bacterial or fungal source.

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The problems associated with inclusion of barley in poultry diets and its improvement by supplemental enzymes have been reviewed by HESSELMAN (1983, 1989), BROZ and FRIGG (1986 a), CAMPBELL et al. (1986), and NEWMAN (1986). Significant improvements in performance of broiler chickens were also obtained when beta-glucanase preparations were added to diets based on oats (SAETERBY 1984; BROZ and FRIGG 1986; EDNEY et al. 1989; CAMPBELL et al. 1987). The results of recent experiments with broilers finished to market weight (ELWINGER and SAETERBY 1986, 1987; JEROCH et al. 1988) demonstrated the beneficial effects of beta-glucanase addition to practical broiler diets based on barley or oats, STEVENS et al. (1988) showed that hulless barley, when supplemented with beta-glucanase enzyme source, can replace wheat as a major cereal grain in diets for turkeys.

In the present paper a review of scientific data on a new standardized enzyme preparation derived from trichoderma viride (ROXAZYME G) is given.

II. DOSE RESPONSE STUDIES

The recommended level of enzyme supplementation is dependent on the type and amount of feed ingredients to be degraded.

Two short-term experiments were conducted by BROZ and FRIGG (1990) to determine the dose response in broiler chickens fed all mash diets based on either barley 700g/kg or oats 660g/kg and supplemented with graded levels of the enzyme complex up to 800mg/kg. The results received with the barley diet (Table 1) indicated clear correlation between increasing enzyme dosage and the growth performance of birds. Table 1. Effects of Trichoderma viride enzyme complex on performance of broiler chickens receiving a low-energy diet based on barley.

Dietary enzyme level (mg/kg)[1]	Weight gain days 8-25 (g)	(%)	Feed intake days 8-25 (g)	(%)	Feed conversion days 8-25 (g:g)	(%)
0	440.4B	100.0	823.3B	100.0	1.89A	100.0
25	493.3A	112.0	897.5A	107.8	1.82B	96.3
50	486.8A	110.5	886.0A	106.5	1.82B	96.3
100	489.2A	111.1	886.7A	106.5	1.81B	95.8
200	502.7A	114.1	898.9A	108.0	1.79B	94.7
400	514.5A	116.8	916.2A	110.1	1.78B	94.2
800	500.9A	113.7	894.6A	107.5	1.79B	94.7

[1] Each treatment was allotted to 8 replicate groups
A, B Means without a common letter are significantly different ($P<0.05$)

The results of both experiments demonstrated that the nutritive value of low-energy diets containing either barley or oats can be considerably improved by the enzyme complex. The dose-related response could be observed to 400mg/kg. However, no significant differences among the inclusion levels of 100-400mg/kg were found. Magnitude of the relative improvements was quite comparable to the effects exhibited in similar trials by purified beta-glucanases (HESSELMAN et al. 1981, 1982; BROZ and FRIGG 1986a; NEWMAN and NEWMAN 1987). Thus, in agreement with previous reports (WHITE et al. 1981; BROZ and FRIGG 1986b), this fungal enzyme complex derived from Trichoderma viride was confirmed in vivo as a very potent source of endo-1,3:1,4-beta-glucanase activity.

Another titration experiment was carried out by WIEDMER and VOELKER (1989) with 5000 broiler chickens fed a pelleted diet based on barley 600g/kg over a period of 43 days. Data from this study, presented in Table 2, indicated an overall positive effect of enzyme supplementation on productivity of broiler chickens. In addition, an improved consistency of excreta could be observed which resulted in a better litter quality and a lower incidence of breast blisters in the birds. The addition of enzymes improved both carcass yield and quality.

Table 2. Effect of enzyme supplementation on performance of broiler chickens fed a pelleted barley-based diet.

Indices		Control	Enzyme Treatment (mg/kg) 100	200	400
Body weight (g)	day 21	671	688	675	676
	day 42	1836	1869	1859	1842
Feed intake (g)	day 0-21	1003 A	991 AB	992 AB	965 B
	day 0-42	3903 Aa	3712 B	3809 ABb	3672 B
Mortality (%)		4.2	4.6	3.8	4.2
Feed/gain ratio	day 0-21	1.575 AB	1.519 CDa	1.551 BDb	1.506 C
	day 0-42	2.168 Aa	2.025 B	2.088 b	2.031 B
Production Index[1]		189 a	205 b	199 ab	202 b
Carcass yield (%)		65.2	67.4	66.7	67.2
First quality carcass (%)		73	78	85	88

A,B,C,D = means without a common letter significantly different (P<0.01)
a,b = means without a common letter are significantly different (P<0.05)

[1] Production Index = $\frac{\text{average daily gain (g) x survival rate (\%)}}{10 \text{ x feed/gain ratio}}$

III. BALANCE STUDIES

In two balance studies the influence of the enzyme complex on retention of nutrients and energy utilisation was investigated.

In one study (BROZ and FRIGG 1990)enzyme supplementation of oats diet significantly increased the retention of both dry and organic matter (Table 3). The content of N-corrected metabolizable energy in the diet was significantly elevated by 0.43 MJ/kg DM (+4.2%).

Table 3. Influence of Trichoderma viride enzyme complex on the retention of nutrients and utilisation of energy from an oat diet.

Parameters	Dietary treatment		Effect of enzyme[1]
	Control	Enzyme (400mg/kg	
Dry matter retention (% of intake)	51.24	52.89	*
Organic matter retention (% of intake)	53.17	55.11	*
Nitrogen retention (% of intake)	47.71	45.68	NS
Metabolizable energy classical (KJ/kg DM)	10882	11282	*
Metabolizable energy N-corrected (KJ/kg DM)	10205	10633	*

[1] Significance indicated as * $P<0.05$, NS + not significant

In another study (BROZ 1987), in which a rye-based diet was used, the enzyme complex at 200mg/kg increased significantly the retention of dry matter (+8.7%) and nitrogen (+9.9%), while N-corrected metabolizable energy was elevated by 0.88 MJ/kg DM (+7.4%). Similar effects of enzymes on nutrient utilisation of chick diets containing barley were reported by HIJIKURO (1983), CLASSEN et al. (1985), BROZ and FRIGG (1986a) and ROTTER et al, (1989).

IV. PELLETING STABILITY

To be used as a feed additive under the practical conditions of the modern compound feed industry an enzyme product has to be resistant against high temperature during pelleting.

In order to assess in vivo possible interaction between the efficacy of the enzymes and pelleting conditions (85°C) a short-term growth assay was conducted (BROZ 1989). The T. viride enzyme complex was added at 200mg/kg to a low-energy barley diet which was fed to broiler chickens in either mash or pelleted form. Both experimental factors, i.e. pelleting or enzyme addition resulted in significant effects on chick performance (Table 4).

Table 4. Effects of T. viride enzyme complex on performance of broiler chickens receiving a barley-based diet either as mash or pellets.

		Mean weight gain days 7-21(g)			Mean feed conversion days 7-21(g:g)		
Diet form	Enzyme:	-	+	mean	-	+	mean
Mash		373.7	395.5	384.6	1.81	1.73	1.77
Pellets		516.7	539.7	528.2	1.71	1.63	1.67
Mean		445.2	467.6		1.76	1.68	

[1] Main effects of both factors were significant (P<0.001); no interaction was found.

In spite of the form of the diet the actual improvement in weight gain and feed conversion due to enzyme supplementation was similar. This shows that the pelleting stability of the new product is satifactory.

V. EFFICACY STUDIES

To confirm the efficacy of the enzyme complex under practical conditions of broiler production, trials were carried out in Switzerland, Germany, Canada and United Kingdom.

The results of these trials are summarized in Table 5. The diets used contained higher proportions of home-grown cereal grains like barley, wheat and rye and were supplemented with the enzyme product at 100, 150 or 200mg/kg. The addition of enzyme to barley-based diets resulted overall in an increase in the final liveweight of the birds (+2.2 - +6.7%) as well as in improved feed conversion (+2.4 - +6.2%). Most of these differences in comparision with the respective control groups were statistically significant. These effects in barley diets seem to be independent of the provenance of the cereal.

In the wheat-based rations the response to the enzyme supplementation appears to be dependent on the origin, variety or actual quality of the cereal. However, these experimental data indicate that it is even possible to enhance the nutritive value of wheat, which has been generally accepted to be of higher nutritive quality than barley.

Rye has been known to create problems when incorporated in chicken diets. The present data contribute to the findings of earlier research work (BROZ 1987) that its poor feeding value can be improved by the given enzyme product.

Table 5. Summary of efficacy studies carried out in different countries under local production conditions.

Type of diet (country)	Enzyme (mg/kg)	No.of animals	Duration (days)	Final weight (g)	(%)	Feed conversion (g:g)	%
Barley	0	216	42	2019	100.0	1.92	100.0
400/600g/kg[1]	100	216	42	2060	102.2	1.86	96.9
(Canada)	200	216	42	2128	105.4	1.83	95.3
Barley	0	800	38	1561	100.0	2.10	100.0
500g/kg	100	800	38	1641	105.1	1.98	94.3
(Germany)	200	800	38	1666	106.7	1.97	93.8
Barley	0	160	42	1766	100.0	2.06	100.0
630g/kg	200	160	42	1822	103.2	2.01	97.6
(Switzerland)							
Wheat	0	160	42	1848	100.0	1.90	100.0
670g/kg	200	160	42	1876	101.5	1.89	99.5
(Switzerland)							
Wheat	0	1400	42	2025	100.0	1.88	100.0
650/680g/kg[1]	200	1400	42	2129	105.1	1.84	97.9
(UK)							
Wheat/Barley	0	800	40	1720	100.0	2.03	100.0
350/200g/kg	100	800	40	1787	103.9	1.93	95.1
(Germany)	150	800	40	1746	101.5	1.94	95.6
	200	800	40	1779	103.4	1.93	95.1
Rye/maize	0	160	42	2055	100.0	1.99	100.0
200/380g/kg	200	160	42	2107	102.5	1.93	97.0
(Germany)							

[1] Starter/grower diet

Sources of data: BROZ (1989), SCHOLTYSSEK et al. (1989); unpublished data from ROCHE Research File.

Further to the reported trials other well-controlled investigations were initiated in Sweden, Spain, Canada and also in Australia. The results, available only in form of preliminary reports, in general confirm the benefical effects of the enzyme product.

VI. GENERAL DISCUSSION

In order to maximize the ulilisation of feed ingredients numerous research workers have through the past decades evaluated the possibility of applying enzymes to poultry diets (JENSEN et al. 1957; ANDERSON et al. 1961; CAMPBELL et al. 1986, 1987; ROTTER et al. 1989; BROZ and FRIGG 1986a, 1986b, 1990).

Based on an ample body of scientific data, which resulted from this research work, the possibility of developing a tailor-made fungal enzyme preparation as a feed additive for broiler production occurred.

The standardised enzyme complex (ROXAZYME G) produced by fermentation of a strain of Trichoderma viride, has the potency to imporve the nutritive value of broiler diets based on either barley, oats, rye or possibly wheat. By applying the new enzyme complex it is feasible to overcome the adverse effects of viscous non-starch polysaccharides, such as beta-D-glucans in barley and oats as well as pentosans in wheat and rye, which interfere with the digestion and absorption of nutrients from the gut.

These facts should lead to an increased flexibility in the feedmill industry and poultry production by allowing the use of alternative ingredients in the formulation of compound feed.

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FARM SURVEY ON FEATHER COVER

R.C. WOOLFORD, P.C. GLATZ and R.J. HUGHES

The feather cover of a laying hen is influenced by its age, cage design and shape (Tauson 1984; Hill and Hunt 1978) and stocking density (Adams et al. 1978). Information, however, on the factors affecting feather cover of commercial strains under Australian conditions is scant. The present survey was undertaken to ascertain the effects of age, level and quality of beak trimming, light intensity, cage condition and stocking density of 4 strains of hens in 27 flocks housed on 9 commercial farms in South Australia. Approximately 500 hens of each strain were scored for feather cover using the method of Charles (1977). The scores were 1=complete cover, 2=complete but showing signs of wear, 3=loss of many outer feathers, 4=some areas completely devoid of cover, and 5=near complete loss of feathers. Beaklength was scored 1 to 3 representing short, medium and long beaks, respectively. Quality of trimming and cage condition were scored 1 to 3 representing poor, average and good, respectively. Light intensity and numbers of hens per cage were recorded, also.

Data were analysed using a stepwise regression procedure to determine (a) which of the independent variables were significantly related to feather cover and (b) the relative strengths of those relationships. Strain and farm were not considered in the preliminary analysis. Covariance analysis was done on individual strains with age as a factor.

Age was shown to have the closest relationship with feather cover although it only accounted for 28% of the variation in the preliminary analysis. Length of beak was also significant, but accounted for only an additional 2% of the variation in feather cover. When individual strains were considered separately, age remained the major influence, with light intensity, cage density, beak length and beak condition becoming important. Apart from age, these other effects differed widely between strains in significance and order of importance.

This work shows that age is the key factor influencing feather cover but there remained a considerable amount of unaccounted variation. Hens with grown out beaks had poorer feather cover compared with those trimmed to normal commercial standards.

We conclude that egg producers can improve feather cover of their flocks and reduce feed costs by ensuring that correct beak trimming is practiced, light intensity is kept low, and birds are not overcrowded.

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AVIAN LEUKOSIS VIRUS AND SELECTION ON OVIPOSITION INTERVAL

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We started testing for avian leukosis virus (ALV) infection in 1983. Contrary to the published results (Gavora et al. 1980), the level of ALV infection was higher in our selection lines than in their randombred control lines (Yoo and Sheldon 1987). To investigate this further, we continued the test in one of the selection lines for another three generations.

An Australorp line (AS) had been selected for shorter oviposition interval since 1961, but with a mild secondary selection on egg number imposed from 1967. The interval was measured within clutches under continuous light for 5 weeks. A randombred control line (AC) has been kept since its derivation from AS in 1965. The birds were reared in battery brooders in groups of 50 for 4 weeks and then grown in colony cages in groups of 10 until caged individually at 18 weeks of age.

The ALV group-specific (gs) antigen shedders were detected by the ELISA test for egg albumen (Ignjatovic and Bagust 1982). Two eggs were sampled per hen, but when the test results differed, though this was infrequent, a third egg was sampled for confirmation.

The percentage of gs-antigen shedders was 13.1, 15.1, 14.7, 16.7 and 16.3 in 1984-1988 in the AS line (sample sizes ranged 332-473), compared to 6.1 (1984) and 6.6 (1988) in the AC line (sample sizes were 229 and 152, respectively). This was opposite to what has been found in lines selected for higher egg production (Gavora et al. 1980), noting that the selection for shorter oviposition interval is an efficient indirect selection for higher egg production (Sheldon et al. 1984).

We compared gs-antigen shedders and non-shedders of the AS line in 1984-88 for age at first egg (AFE), hen-day rate of egg production from first egg to about 43 weeks of age (RL) and within-clutch oviposition interval under continuous light (Table). As there was no significant interaction between year of hatch and gs-antigen status, the results were combined.

Age at 1st egg, rate of lay and oviposition interval for gs-antigen shedders and non-shedders (mean ± SE)

Characters	Shedders	Non-shedders	Difference
AFE (day)	177.21 ± 0.43	176.67 ± 0.19	0.54^{NS}
RL(%)	90.23 0.49	92.09 0.20	-1.86**
Interval (hr)	21.40 0.06	21.64 0.03	-0.24**

NS Not significant ** Significant at $P<0.01$.

Compared to the non-shedders, the shedders were slower in sexual maturity (though not significant) and laid fewer eggs at shorter intervals (both significant). This was opposite to a normal association of lower egg production with longer oviposition interval. Although the observed difference in oviposition interval explains why the shedders would have been favoured by selection in the AS line, this contradiction still remains to be explained. The difference in rate of egg production was smaller than, but consistent with, those reported earlier (Gavora et al. 1980).

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STRAIN RESPONSES IN EGG SHELL QUALITY TO SALINE DRINKING WATER

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We have conducted a number of studies in Australia over the past three years with two strains of commercial laying hens in which we have shown that egg shell quality declines when these hens are given drinking water containing between 0.2 and 2 g sodium chloride (NaCl)/l. Both these strains were obtained from the same commercial breeding organisation and the possibility exists that similarities in the genetic background of these strains may have influenced the observed responses in egg shell quality.

The present study was carried out to compare the shell quality responses of these two strains with those of four other strains recently introduced to the Australian layer industry. These were a tinted and a coloured egg layer strain from each of two other breeding organisations. Two replicates of 46 hens of each strain were given free access to town water containing 2 g NaCl/l from 42 to 45 weeks of age. Similar numbers of hens received town water as controls and all hens received a proprietary layer mash ad libitum. The results, shown in the Table, were analysed by factorial analysis of variance.

Responses of six strains of laying hens to saline drinking water

Breeding Organisation	Strain	Egg shell defects (/100 eggs)		Egg production (eggs/100 bd)		Water intake (ml/d)		Food intake (g/d)	
		Cont	NaCl	Cont	NaCl	Cont	NaCl	Cont	NaCl
A	Tinted	3.7	15.8	73	76	263	260	141	142
	Coloured	6.5	15.8	77	77	254	258	137	142
B	Tinted	4.2	11.0	75	76	231	216	125	122
	Coloured	4.0	10.3	76	73	220	232	126	129
C	Tinted	4.4	10.0	80	80	228	201	122	120
	Coloured	4.6	11.1	75	74	218	218	122	121
SEM (treatment)		0.20***		0.55		2.76		0.94	
SEM (strain)		0.34***		0.95**		4.79***		1.63***	

** P<0.01, *** P<0.001

No significant (treatment x strain) effects were observed. There were significant strain effects for all measured parameters. The only significant effect of water treatment was the increased incidence of egg shell defects, measured as soft-shelled, cracked and broken eggs from hens receiving the NaCl. All strains showed this increase with the two strains which we have used in our previous studies giving the highest incidence of shell defects. Since all six strains responded similarly to the saline drinking water the problem we have identified in our previous studies does not appear to be related to the specific genetic background of the hens.

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REDUCED HATCHABILITY OF EGGS FROM HENS RECEIVING SALINE DRINKING WATER

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Recent studies at Camden have shown that the presence of sodium chloride (NaCl) in drinking water, at concentrations between 0.2 and 2 g NaCl/l, reduces the shell thickness of eggs from some hens and increases the overall incidence of egg shell defects in commercial layer flocks. These responses occur by way of increases in the numbers of soft-shelled, cracked and broken eggs. Approximately one-third of all shell defects occur as thin cracks which are difficult to detect by normal candling procedures (Balnave and Yoselewitz 1988). Underground water sources in Australia often contain relatively high concentrations of NaCl so that it is probable that many of the eggs produced by breeder flocks will have thin shells or defective shells containing thin cracks. If so it is likely that overall hatchability will be less than optimal. The present study examined this possibility.

Fifty hens from a multi-strain, table-egg flock which had received drinking water containing 2 g NaCl/l for 6 weeks, and which were laying eggs with defective shells, were used in the present study. Forty-four hens which had received town water were used as controls. Hens were kept in individual cages and maintained on these water treatments. They were inseminated every 7 d with mixed semen collected from cockerels of a table-egg strain maintained on town water. Eggs were collected and stored at 12°C over a 7 d period before being visually checked to detect those with defective shells. These were removed and the remaining eggs were incubated in a Multiplo electric incubator. Eggs were candled at 7 d and 18 d of incubation to detect infertile eggs and embryonic deaths. They were transferred to a separate incubator at 18 d for hatching. Hatchabilities were compared using eggs from 6 consecutive hatches.

Results showed that hens receiving the NaCl intake in the drinking water laid significantly fewer eggs (73.0 VS 80.0%, $P<0.05$), produced significantly more unsettable eggs with defective shells (19.8 VS 3.8%, $P<0.01$) and had a significantly lower hatchability of fertile eggs (73.4 VS 86.9%, $P<0.01$). Percentage fertility was unaffected. Deaths to 18 d (11.0 VS 6.5%, $P<0.05$) and dead-in-shell at 21 d (9.1 VS 4.0%, $P<0.01$) were also significantly greater in hens receiving the NaCl supplement in the drinking water.

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