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THERMAL MANIPULATION DURING THE PERINATAL PERIOD – DOES IT IMPROVE THERMOTOLERANCE AND PERFORMANCE OF BROILER CHICKENS?

S. YAHAV[1]

Summary

Thermal manipulation during a chick's embryogenesis is based on the following hypotheses: a. it is possible to induce long-lasting physiological memory based on epigenetic adaptation during embryogenesis; b. a definition of long-lasting memory is: an alteration in the hypothalamic threshold response to changes in the environment; c. during sensitive periods in embryogenesis, thermal manipulation involving exposure to high temperatures for specified periods, will elicit improved thermotolerance during the birds' life span. It is well documented that thermal manipulation during the 1st week post-hatch results in improved lifetime thermotolerance.

Thermal manipulation during embryogenesis has been shown to achieve improvement in thermotolerance up to 10 days of age. However, recent studies have shown that broiler chickens do not exhibit any thermoregulatory advantage during thermal challenge at later ages. These results raise the following questions. a. Could it be that it is not possible to induce long-lasting thermoregulatory memory by thermal manipulation during embryogenesis? and b. Could fine tuning of thermal manipulation result in long term improvement in thermotolerance? Further research is currently underway, aiming to shed light on these questions, and there is evidence that fine tuning might lead to achievement of the targeted goal.

Abbreviation list: T_a = ambient temperature; T_b = core body temperature; T_3 = triiodothyronine; T_4 = thyroxine.

I. INTRODUCTION

Recent decades have seen significant development in genetic selection of meat-type poultry (i.e., broilers and turkeys); this has led to rapid growth, with increased feed efficiency and metabolic rate, and has thus provided the poultry industry with large, rapidly growing birds (Havenstein *et al.*, 2003). Such development necessitates parallel increases in the size and enhancement in the efficiency of functioning of the cardiovascular and respiratory systems. However, inferior development of such major systems has led to a relatively low capability to balance energy expenditure and body water balance under extreme environmental conditions. Thus, acute exposure of chickens to extreme conditions (i.e., hot or cold spells) has resulted in major economic losses (morbidity, mortality and development of metabolic disorders). It is estimated that the global mean surface temperature increased by 0.8 and 1.7°C during the 19th and 20th centuries respectively (U.S. National Climatic Center, 2001). Scientists expect that the average global surface temperature will rise by 0.6-2.5°C during the next 50 years. This situation, in which growth rate (accompanied by increased metabolic heat production) increases from year to year and the future projected increase in global surface temperature, demands an efficient means of economically improving thermotolerance in broiler chickens.

Birds are homeotherms and, therefore, are able to maintain their body temperature within a narrow range. An increase in body temperature above the regulated range, as a result

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of exposure to environmental conditions and/or excessive metabolic heat production, may lead to a cascade of irreversible thermoregulatory events that could be lethal for the bird. To sustain thermal tolerance and avoid the deleterious consequences of thermal stresses, three direct responses are elicited: the rapid thermal shock response (Parsell and Lindquist, 1994), acclimation (Horowitz, 2002; Yahav *et al.*, 1997b), and thermal manipulations based on epigenetic adaptation during the perinatal period (Nichelmann *et al.*, 1999; Tzschentke *et al*., 2001). The last strategy is based on the assumption that environmental factors, especially the ambient temperature (T_a), have a strong influence on the determination of the "set-point" for physiological control systems during "critical developmental phases" (first described as 'determination rule'; Dörner, 1974). Thermosensitive neurons located in the preoptic anterior hypothalamus (PO/AH) integrate afferent temperature signals from different areas of the body to elicit adequate thermoregulatory responses via the control of physiological, endocrinological, and behavioral responses, and so to keep the core body temperature (T_b) relatively constant (Boulant, 1996). The thermoregulatory response is mediated, to some extent, by the level of metabolism induced/permitted by the thyroid hormones (thyroxine – T_4, and triiodothyronine – T_3) (McNabb and King, 1993) and the hydration status of the animal which is mediated by arginine vasotocin (AVT) (Saito and Grossmann, 1998). Changing the sensitivity of the warm- and/or cold-sensitive neurons located in the PO/AH may change the threshold for heat production and/or heat loss of an animal. Heat acclimation in broiler chickens is mediated by reduced metabolic rate, blood volume expansion (Yahav *et al*., 1997b), and reduced T_b (Yahav *et al*., 1995). This process requires from 4 to 7 day to be completed in broilers (Yahav, 2000), and is rendered rather impracticable by the early marketing age of broilers, coupled with: (a) the necessity to keep the environmental temperature controlled up to the age of 21 days for brooding; (b) the deleterious effect of heat acclimation on broiler chickens; and (c) the enormous cost of temperature-controlled poultry houses. Epigenetic adaptation, which has been defined as a lifelong adaptation that occurs during prenatal (embryogenesis) or early post-hatching ontogeny, within critical developmental phases that affect gene expression (Nichelmann and Tzschentke, 2002; Tzschentke and Basta, 2002; Tzschentke *et al*., 2004), seems to be a suitable means of reaching the goal of improved thermotolerance acquisition in broilers. During early development most functional systems evolve from an open-loop system without feed-back into a closed control system with feed-back ("transformation rule", Dörner, 1974). Thermal manipulations during the critical phases of this development process may induce alterations in the thermoregulatory control system.

II. POST-HATCHING THERMAL MANIPULATIONS

Broiler chicks complete their brain and body temperature regulation at 10 days posthatch (Arad and Itsaki-Glucklish, 1991). During this period, body and brain temperatures are regulated at a lower level than in adult chickens. Subsequently, as age increases the difference between body and brain temperatures increases linearly and significantly. The epigenetic response has been successfully modulated by early-age thermal manipulation of post-hatching chicks, by exploiting the incomplete maturation of the thermoregulatory system. Thermal manipulation, involving exposure to 37-38°C at 60-80% relative humidity for 24 h in 3-day-old broilers, was found to improve acquisition of thermotolerance. The improvement achieved was manifested in the ability of the thermally manipulated chicks to efficiently reduce heat production during exposure to acute thermal challenge at market age (Yahav and Hurwitz, 1996). This was accompanied by: a. an alteration in sensible heat loss through convection and radiation (Yahav *et al*., 2005; Table 1); b. a significant reduction in the stress level of the thermally manipulated chickens, as indicated by their plasma

corticosterone concentration (Fig. 1); and c. pronounced increases in the 27-, 70- and 90-kDa heat-shock proteins (HSPs) in the heart muscle and lung tissue of the thermally non-manipulated chickens, compared with those of the manipulated ones, during thermal challenge (Yahav *et al.,* 1997a). It has been suggested that the induction of HSP was correlated with body temperature, and that the HSP response was not part of the long-term mechanism elicited by the thermal manipulation at an early age.

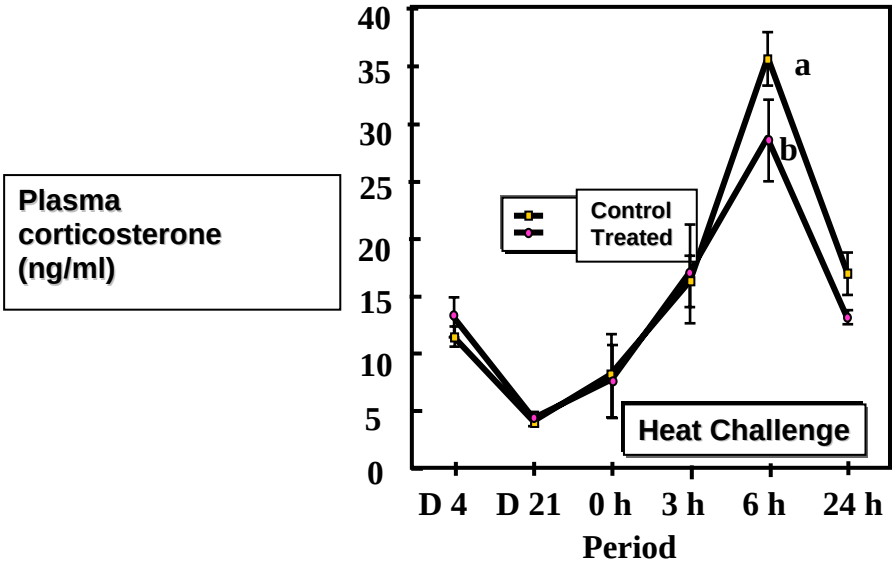

Figure 1: Plasma corticosterone concentration of thermal manipulated chicks at 3 days of age and during thermal challenge (0 to 6 h) at 42 days of age.

The reduction in heat production, coupled with an increase in sensible heat loss enabled a relatively slow development of hyperthermia and thus dramatically reduced mortality. Thermal manipulation at 3 days of age also induced compensatory growth, leading to improvement of performance and muscle growth, because of the proliferation of skeletal muscle satellite cells during the manipulation (Halevy *et al*., 2001).

Table 1. The effect of thermal manipulation at 3 days of age on sensible heat loss by broiler chickens during a thermal challenge of 35°C for 6 h at 42 days of age (Yahav *et al.*, 2005).

Treatments	*Sensible heat loss (watts)*		
	Radiation	*Convection**	*Total heat loss*
Control	1.723±0.181[b]	3.109±0.279	4.833±0.435
Thermally manipulated	2.382±0.195[a]	3.532±0.302	5.913±0.470

Values designated by different letters differ significantly ($P \leq 0.03$). n = 8.
*Air velocity during the thermal challenge was 0.25 m/s.

The effect of thermal manipulation on the PO/AH in the chicks was studied with reference to two genes: the R-Ras3 and brain-derived neurotrophic factor (BDNH). The R-Ras3 belongs to the small GTP-binding protein subfamily; it is activated by multiple extracellular stimuli. The Ras subfamily controls a variety of cellular events that culminate in

gene transcription. Ras plays an essential role in cell proliferation, differentiation and survival, and it is also significant in modulating synaptic functions. The BDNF binds specifically to the TrK-B receptor and initiates tyrosine phosphorylation which activates the phosphotyrosine-binding site. This initiates the internal cellular pathway of RAS, and results in transduction of genes involved in neuronal growth and maintenance.

In the chicks' PO/AH, significant increases in expression of the R-Ras3 (Labunskay and Meiri, 2006) and brain-derived neurotrophic factor (BDNH) genes (Katz and Meiri, 2006) were detected during thermal manipulation, which suggests that these genes are involved in thermal manipulation. However, uniform post-hatch temperature manipulation is difficult to achieve, whereas the use of such manipulations during incubation would probably be more efficient and uniform**.**

III. PRE-HATCHING THERMAL MANIPULATIONS

The hypotheses underlying these studies are that: a. during embryogenesis, it is possible to induce long-lasting physiological memory based on epigenetic adaptation; b. a reasonable definition of long-lasting memory is an alteration in the hypothalamic threshold response to changes in the environment and c. during sensitive periods in embryogenesis, thermal manipulation involving exposure to high temperatures for specified periods, will elicit improved thermotolerance during the birds' life span.

In poultry, control elements of the thermoregulatory system can function, but the efficiency of the system is low, possibly as a result of the uniform temperature used in commercial incubation. Endothermic reactions during the late stages of incubation may have a delayed, rather than an immediate influence on the efficiency of thermoregulation (Nichelmann and Tzschentke, 2002). Furthermore, the embryo is surrounded by the chorioallantoic membrane (CAM), which retains the extra-embryonic fluids and protects against deleterious effects on embryo hydration.

In contrast to the uniform temperature of commercial incubation, in nature, incubation conditions are non-uniform, as a result of searching for food, escape from predators, and non-uniform nest insulation. This may be one of the reasons why birds in the wild are better able to cope with extreme environmental temperatures.

It was previously reported that exposing embryos to high or low temperatures during incubation improved their capacity to adapt to hot or cold environments, respectively, in the post-hatch phase (Nichelmann *et al*., 1994; Tzschentke and Basta, 2002; Moraes *et al.*, 2003; Yahav *et al.*, 2004). Three critical parameters have to be considered in the approach to thermal manipulations during chick embryogenesis: a. the critical phase; b. the temperature level; c. duration of exposure.

Determination of the critical phase during embryogenesis, for application of thermal manipulation to improve acquisition of thermotolerance, was based on the hypothesis that the "set point" or "response threshold" of controlling systems can be altered most efficiently during the development/maturation of the hypothalamus-hypophysis-thyroid axis (thermoregulation) and/or the hypothalamus-hypophysis-adrenal axis (stress).

Until mid-incubation the thyroid gland possesses only limited ability to synthesize hormones. This period is characterized by the synthesis of monoiodotyrosine on E8 (*i.e.* 8^{th} day of embryogenesis), of diiodotyrosine on E9, and by synthesis of T_4 and thyroid stimulating hormone (TSH) on E10. The linkage of the hypothalamic-pituitary-thyroid axis is formed between E10.5 and E11.5. Levels of T_3 start increasing on E12 and increase significantly prior to hatching, in preparation for their role in the final maturation of many tissues and in the physiological integration of hatching. Therefore, application of thermal manipulations during the sensitive period of development of the axis (Reynes *et al.*, 2003)

might affect the heat production threshold "set point". Epple *et al*. (1997) suggested the embryo to be susceptible to stress. Therefore, increasing the incubation T_a during and/or after the hypothalamic-hypophyseal-adrenal axis has been activated (Wise and Frye, 1975) might affect the stress response of the post-hatch chick.

In recent experiments (Yahav *et al*., 2004; Collin *et al.*, 2006) the thermal manipulation of chick embryos, at 39.5°C for 3 h, on days 8 to 10 or 16 to 18 improved hatchability in the E16-E18 chicks, but did not affect the body weight of the hatched chicks. Also, it significantly reduced their metabolic rate, as indicated by body temperature (T_b) (Table 2). A parallel experiment conducted by Tona *et al*. (unpublished data) revealed a significant decline in the measured pre-hatching oxygen consumption of the thermally manipulated embryos, which proved that the reduction in metabolic rate was a result of the thermal manipulations.

Table 2. The effect of thermal manipulation during embryogenesis on hatchability, BW and T_b of chicks at hatch (according to Yahav *et al.*, 2004).

	Control	*TM E8-E10*	*TM E16-E18*
Hatchability	92.5[b]	89.89 [b]	97.9[a]
BW	46.8±0.30	47.25±0.29	46.99±0.29
T_b	38.12±0.09[a]	37.75±0.09[b]	37.72±0.09[b]

Within rows, values designated by different letters, differ significantly ($P \leq 0.05$). (TM – thermal manipulation; E - day of embryogenesis).

Challenging the chicks (41°C for 6 h on day 3 of age) revealed significantly improved thermotolerance in birds thermally manipulated during embryogenesis. The improved thermotolerance was indicated by a significantly lower metabolic rate, and coincided with non-significant and significant declines in the stress levels in the E8-E10 and E16-E18 embryos, respectively (Table 3).

Table 3. The effects of thermal challenge to a chick at 3 days of age on T_b, plasma thyroid hormones and corticosterone concentrations (according to Yahav *et al*., 2004).

	Control	*TM E8-E10*	*TM E16-E18*
T_b (^{0}C)	44.04±0.16[a]	42.78±0.25[b]	42.66±0.19[b]
T_4 (ng/ml)	4.53±0.42	3.53±0.48	4.84±0.54
T_3 (ng/ml)*	1.94±0.17	1.25±0.20	1.19±0.22
Corticosterone (ng/ml)	34.3±3.4[a]	23.8±3.8[ab]	18.6±3.7[b]

Within rows, values designated by different letters differ significantly ($P \leq 0.05$); *($P \leq 0.074$). (TM – thermal manipulation; E - day of embryogenesis).

In other experiments, a prolonged thermal manipulation (38.5°C) was applied to layer-strain chicken embryos from E18 until the end of incubation. On the last day of incubation the thermally manipulated embryos showed a significantly higher level of heat production than the controls (Loh *et al*., 2004). Similar effects were found in Muscovy duck embryos that experienced thermal manipulation from E29 until hatch. Prolonged exposure of Muscovy duck embryos to warm (38.5°C) or cold (34.5°C) conditions induced changes in the thermosensitivity of PO/AH neurons (Loh *et al*., 2004), which persisted until 10 days post-hatch (Tzschentke and Basta, 2002). Furthermore, during the first 10 days post-hatch Muscovy ducklings and turkeys that had been exposed to thermal manipulations during embryogenesis exhibited changes in heat production and in their preferred ambient

temperatures (Nichelmann *et al*., 1994). Janke and Tzschentke (2006) found that layer embryos that had been exposed to high or low incubation temperatures (38.5 or 34.5°C, respectively) on E18, and were heat stressed on E20 differed in their expression of c-Fos in the hypothalamus on the last day of incubation.

Most reported studies have demonstrated an improvement in thermotolerance during the first 10 days post-hatch. However, chicks that had been exposed to thermal manipulation during embryogenesis and then raised to marketing age, did not exhibit a long-lasting improvement in thermotolerance. Although thermally treated chicks had significantly lower T_b immediately post-hatch than untreated chicks, the difference persisted only until 4 or 5 weeks of age, after which it diminished. Furthermore, heat challenge at 42 days of age of thermally manipulated chickens during embryogenesis did not reveal any thermal advantages in these chickens (Collin *et al*., 2006; Tona et al., unpublished data). Piestone *et al.* (unpublished data) found that adopting E7 to E16 as the "critical phase" for thermal manipulation of chick embryos significantly enhanced thermotolerance but also caused teratogenic effects which were found to depend on the duration of exposure.

These conflicting results raised the questions of whether long-lasting thermal memory can be imparted by thermal manipulation during embryogenesis, and whether it is only a question of correct choice of the critical period. In mammals, it seems that for different control systems, as well as for specific functions of a system, *e.g*., as exhibited in the development of the mammalian visual system (Harwerth *et al*., 1986), several different, partially overlapping "critical phases" were found. Furthermore, species-specific differences have to be taken into consideration.

There is accumulating evidence that the epigenetic adaptation approach, and its association with changes in the environment in mammals and avian species, with emphasis on tuning the level and duration of stress to coincide with the "critical phase", will elicit an efficient epigenetic adaptation. This complex issue needs to be intensively studied to shed light on epigenetic adaptation in domestic poultry.

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GUT HEALTH, OSMOREGULATION AND RESILIENCE TO HEAT STRESS IN POULTRY

P.B. CRONJE[1]

Summary

The gut health of poultry is important not only because of its effects on productivity, but because impaired gut integrity can increase susceptibility to enteric pathogen invasion. Osmotic stress and heat stress can both damage the lining of the gut. Finely ground and pelleted diets of high digestibility exert considerable osmotic stress on the lining of the gut, which may exceed the ability of the cell to maintain water homeostasis and ionic balance. Many studies have shown that the ability of the gut to withstand osmotic stress can be improved by supplementation with betaine, an organic osmolyte, and that this results in decreased susceptibility to enteric pathogens. During heat stress, blood is diverted to the periphery at the expense of the gut, the integrity of which can be compromised by reactive oxygen species generated by an insufficient supply of nutrients and oxygen. If heat stress is sustained, the ability of the glutathione cycle to prevent reactive oxygen species from destroying cell membranes may be overwhelmed. Supplementation with selenium yeast has been shown to increase the activity of the glutathione cycle in heat stressed poultry, which may prevent heat-induced damage to the lining of the gut.

I. INTRODUCTION

The gut health of livestock species has received scant attention in the past, largely because the threat of pathogens that might have entered the body through an impaired gut lining was negated by the routine use of antibiotic growth promoters. That poultry industries in countries in which antibiotic growth promoters have been banned are now experiencing an increase in health problems and infections associated with enteric pathogens indicates that the gut health of poultry reared in modern production systems is far from optimal. Impaired gut health is economically significant for the industry not only because it affects the productivity of birds, but also because human outbreaks of food borne illnesses associated with poultry products affect the market for poultry products. Chicken consumption has been identified as a major risk factor for human *Campylobacter* infections in the USA, New Zealand and the UK, and consumption of egg products has been identified as a major risk factor for human *Salmonella* infections (see review by Doyle and Erickson, 2006).. Although pathogenic organisms are always present in the gut, beneficial gut bacteria and the gut immune system of the host normally restrict the numbers of pathogens present. High numbers of gut pathogens result when these defence mechanisms are impaired. This communication discusses two factors that can impair gut integrity: osmotic stress and heat stress.

II. CAUSES OF IMPAIRED GUT HEALTH

(a) Osmotic stress

Osmosis refers to the diffusion of water through semi-permeable membranes from solutions that have low concentrations of solutes to those that have high concentrations of solutes. The difference in osmolar concentration between two solutions determines the

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osmotic pressure exerted on the membrane separating them. Whereas the osmolarity of the interior of the cells lining the gut and that of the blood supply to the gut is relatively constant, the osmolarity of the contents of the gut varies substantially, increasing after a meal as the number of particles in solution (and hence the osmolar concentration) is increased by the action of digestive enzymes on food and decreasing as food particles are absorbed. During digestion, water in the spaces between the cells lining the gut is forced into the lumen of the gut by osmotic pressure and is replaced by water from the capillaries supplying blood to the gut. Water is thus drawn into the gut from the blood system shortly after a meal. As nutrients are absorbed, the osmolarity of digesta decreases and the osmolarity within and between the cells lining the gut increases because of the presence of the absorbed molecules. Consequently, water follows the nutrients back through the gut lining and into the blood. In poultry, the daily flow of water to and from the gut is double the mass of feed consumed (Hoerr, 1998).

The osmolarity of chicken plasma is about 300 mOsmol but the osmolarity of the gut in the fed bird varies from about 900 mOsmol in the duodenum to about 700 mOsmol in the caecum (Klasing *et al*., 2002), which exerts substantial osmotic pressure on the cells lining the gut and on the tight junctions that bind them together to form a barrier against entry of pathogens. Diets containing high concentrations of feedstuffs that have been processed by grinding or steam flaking to increase the rate and extent of hydrolysis of nutrients by digestive enzymes can increase the osmotic pressure of water moving from the blood into the digesta to such an extent that the junctions between the cells lining the gut give way, permitting microorganisms and toxins to enter the body. Pelleted and finely ground diets have been associated with gut lesions in pigs (Eiseman and Argenzio, 1999) and ruminants (Owens *et al*., 1998). Impairment of the integrity of the gut lining of poultry would explain why pelleting is associated with a high incidence of *Salmonella* in the gut of broilers (Huang *et al*., 2006) and why the incidence of mortality from necrotic enteritis is greater in poultry fed finely ground feed than in those fed coarsely ground feed (Branton *et al*., 1987). It is also likely that high osmotic pressure contributes to problems associated with indigestible non-starch polysaccharides present in barley, rye and wheat diets.

(b) Heat stress

In recent years, medical researchers have adopted a new paradigm to explain the aetiology of heat stroke in humans. Heat stroke has been redefined as a systemic inflammatory response caused by damage to the lining of the gut, which precipitates multi-organ dysfunction (Bouchama and Knochel, 2002).

One of the first responses of animals to a heat load is to increase the supply of blood to the periphery so that heat can be dissipated by radiation, convection and conduction. If insufficient heat is dissipated by this route, active cooling mechanisms are invoked. In birds, blood flow to the mouth and upper part of the throat is increased and heat is dissipated by panting and gular flutter. Redirection of blood to the periphery, throat and mouth is accomplished by dilation of the blood vessels at these sites. The blood supply to the gut decreases simultaneously to prevent blood pressure from decreasing. If exposure to high environmental temperatures is sustained, the reduced supply of oxygen and nutrients to the gut results in cell damage. Eventually, the gut barrier becomes compromised to such an extent that endotoxin, a component of bacteria in the gut, enters the body. The presence of endotoxin induces an exaggerated inflammatory response from the gut immune system, involving secretion of high levels of tumour necrosis factor and interleukin-1. These cytokines result in several harmful effects, including leakage of blood from capillaries, blood clotting and cell death, which precipitate multiple organ failure and death.

Evidence for the involvement of gut integrity in heat stress and its applicability to livestock species was reviewed by Cronje (2005), who concluded that available evidence on the pathology of heat stress in livestock species is consistent with this paradigm. The potentially additive nature of gut damage induced by osmotic stress and heat stress suggests that strategies to strengthen the immune system of the gut would not only increase productivity but also decrease susceptibility to pathogen invasion and heat stress.

III. IMPROVEMENT OF GUT HEALTH

(a) Protection against osmotic stress

Post-prandial osmotic pressure not only induces movement of water from the fluid surrounding the cells of the gut into the lumen of the digestive tract, but also exerts similar pressure on water inside the cells lining the gut. Loss of intracellular water causes cells to shrink, which decreases nutrient absorption and cell membrane transport and impairs important intracellular processes such as the metabolism of amino acids, ammonia, carbohydrates and fatty acids (Häussinger 1996). In extreme cases, the cells shrink to such an extent that they pull away from each other, destroying the tight junctions that bind them together and allowing pathogens and toxins entry to the body. Cells adapt to normal variations in external osmolarity by transporting ions into or out of the cell to reduce osmotic pressure. This is accomplished by ion pumps, the most important of which is the Na^+/K^+ pump. Although the efflux of water from gut cells can be prevented by increasing the concentration of ions within the cell, the extent to which Na^+ ions can be imported into the cells is limited because high Na^+ concentrations inhibit the uptake of nutrients into the cell, which exacerbates the problem by increasing the osmolarity of the digesta still further. The extent to which ions other than Na^+ can be imported into the cell is also limited because charged particles such as potassium, magnesium, and phosphate inhibit the action of enzymes necessary for the metabolism of nutrients within the cell.

Because of the limited ability of ion pumps to cope with extreme variations in osmotic pressure, organisms such as marine invertebrates, plants that grow in saline environments and bacteria effectively reduce osmotic pressure by transferring organic osmolytes into and out of their cells. These organisms use organic osmolytes because they can be imported into cells at high concentrations without impairing nutrient absorption or metabolism. Betaine is an osmolyte that occurs naturally in plants such as sugarbeet and is readily absorbed by the gut, liver and kidney cells of poultry (Kettunen *et al*. 2001a, b). Because the transport of betaine into the cell is coupled to the diffusion of Na^+ and Cl^- across the cell wall (Burg, 1995), the uptake of betaine increases when the osmolarity of the fluid outside the cell increases, making it an ideal supplement for improving the resilience of gut cells against osmotic stress. In *in vitro* trials, osmolyte supplementation was shown to increase the resilience of cells to osmotic stress by 42% (Moeckel *et al*., 2002). Kettunen *et al*. (2001b) showed that betaine supplementation of chickens decreased the movement of water out of intestinal cells when osmotic pressure was increased. Betaine supplementation also improves the morphology of the cells lining the small intestine of poultry and stabilizes the structure of the gut mucosa (Kettunen *et al*. 2001c). Several studies have shown that dietary betaine supplementation increases nutrient absorption in poultry (reviewed by Eklund *et al*., 2005), which suggests that the osmotic challenge presented by modern production diets exceeds the ability of the ion pumps of gut cells to maintain normal function. This concept is supported by the results of Moeckel *et al*. (2002), who showed that betaine reduces the activity of the Na^+/K^+ pump by 64% and that of the Ca^{++} pump by 73%. Reduction of ion-pump activity of this magnitude would have a beneficial effect on energy expenditure, as ion pumps consume considerable

amounts of energy. In pigs, maintenance energy requirements were reduced by 5.5% by betaine supplementation (Schrama *et al*., 2003).

The beneficial effects of supplementation with a compound whose main effect is to alleviate osmotic pressure in the gut constitutes compelling support for the hypothesis that modern poultry diets exert excessive osmotic pressure on the gut of poultry and thus compromise gut integrity. Furthermore, the practical benefits of improving gut health using betaine supplementation are reflected in studies showing that it attenuates the decrease in gut villus height associated with coccidiosis, enhances the destruction of coccidia by the immune cells of the gut, reduces the invasiveness of coccidia and decreases the incidence of coccidia-related gut lesions, (Augustine *et al*. 1997; Augustine and Danforth 1999; Kettunen *et al*. 2001c; Klasing *et al*. 2002).

(b) Protection against heat stress

During heat stress, deprivation of oxygen and energy generates reactive oxygen species within gut cells (Hall *et al*., 1999). These destructive molecules attack cell membranes, initiating chain-reactions that quickly impair cell structure and membrane integrity. As reactive oxygen species also impair the ability of ion pumps to maintain cellular ion homeostasis, betaine supplementation could also help alleviate susceptibility to the adverse consequences of heat stress. The cell has two main defence mechanisms against reactive oxygen species: glutathione (GSH) and its selenium-containing enzymes, which destroy reactive oxygen species, and heat shock proteins, which repair proteins that have been damaged by reactive oxygen species.

GSH is the most important antioxidant in the body and is essential for normal intestinal function. GSH not only protects gut cells against reactive oxygen species, but is also required for activation of heat shock proteins during heat stress (Rokutan *et al*., 1996). In order to provide sufficient protection, the GSH cycle requires an adequate supply of selenium and sulfur. It would appear that normal dietary selenium intakes are inadequate for heat stressed poultry because supplementation of broiler chickens with selenium yeast has been shown to increase the activity of the glutathione cycle during heat stress (Mahmoud and Edens, 2003, 2005). It has also been proposed that sulfur containing amino acids such as methionine may exert beneficial influences on the health and survival of gut cells through their effects on glutathione (Shoveller *et a*l., 2005). Selenomethionine, an organic product derived from selenium-enriched yeast, has been shown to protect cells against oxidative stress (Wan *et al*., 2006) and may confer greater resilience against damage to the gut and against heat stress than selenium or methionine alone.

IV. CONCLUSIONS

The extent of the adverse effect of modern poultry production systems on gut health and poultry productivity is emerging in countries in which antibiotic supplementation has been banned. Available evidence indicates that nutritionally induced osmotic stress and heat stress both contribute to impairment of gut integrity. However, it is unrealistic to expect that attempts to improve gut health by altering the form and composition of diets or by increasing expenditure on cooling systems would be adopted by the poultry industry. An alternative and more readily acceptable strategy is to increase the resilience of the gut against both these stresses by supplementing the diet with compounds that complement and enhance the natural defence mechanisms of the gut.

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THE CRUCIAL ROLE OF VENTILATION IN PERFORMANCE AND THERMOREGULATION OF THE DOMESTIC FOWL

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Summary

Recent decades have seen significant progress in the genetic selection of fast-growing meat-type broiler chickens and turkeys. However, fast growth has been accompanied by inferior development of the visceral systems, which contributes to difficulties in coping with heat stress. This situation, in which growth rate and, therefore, heat production increase from year to year, demands an efficient and economical means to improve the acquisition of thermotolerance by fowls in hot climates. It is important to understand the physical aspects of excess heat dissipation by poultry, in relation to the improvement of thermotolerance. This paper focuses on air velocity as a principal parameter that dramatically affects sensible heat loss and its contribution to the ability of acclimated poultry to maintain a favorable energy balance efficiently under hot conditions. The studies reviewed in this paper demonstrated that: a. air velocity plays a major role in broiler energy balance at high ambient temperatures; b. the optimal air velocity for maximizing growth performance varies with the ambient temperature and at ambient temperatures below 30°C, even quite low air velocities can cause chilling, which adversely affects broiler performance; c. air velocity affects turkey performance; d. genetic selection has improved growth performance, but to some extent at the expense of the broilers' ability to maintain a favorable energy balance.

I. INTRODUCTION

Recent decades have seen significant progress in the genetic selection of fast-growing meat-type broiler chickens and turkeys, as documented by Havenstein *et al*. (1994, 2003a). However, fast growth has been accompanied by inferior development of the visceral systems, such as the cardiovascular and respiratory systems (Havenstein *et al*., 2003b), which contributes to the difficulties of broilers in coping with heat stress.

Birds are homeotherms, *i.e.*, they are able to maintain their body temperature (T_b) within a narrow range. An increase in T_b above the regulated range, as a result of exposure to heat stress and/or metabolic heat production from rapid growth, may initiate an irreversible chain of thermoregulatory events that can be lethal. Acclimation is one of the mechanisms that enable homeotherms to withstand the environment successfully; it has been defined as a physiological response that reduces strain or enhances the resistance to stresses caused by environmental factors applied experimentally (acclimation) or naturally (acclimatization) (IUPS Thermal Commission, 2001). During acclimation the thermoregulatory response threshold for heat production and/or heat dissipation is altered (Yahav, 2000).

In birds, heat is dissipated through respiratory-evaporative mechanisms (Richards, 1968, 1970, 1976; Seymour, 1972; Marder and Arad, 1989), a passive evaporative cutaneous mechanism (Webster and King, 1987; Ophir *et al*., 2002) and sensible heat loss via radiation and convection. Evaporative heat loss via panting is associated with body water content and, therefore, dehydration will reduce heat loss via this pathway. An increase in sensible heat loss may, however, contribute to better thermotolerance acquisition at high ambient temperature (T_a). The difference between the surface and ambient temperatures is the driving force for

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sensible heat loss, and the adoption of thermal-imaging radiometry technology in the biological sciences has enabled determination of the contribution of sensible heat loss to body energy balance.

II. SENSIBLE HEAT LOSS

The driving force for sensible heat loss by convection and radiation is the difference between the surface and ambient temperatures. The convective heat flux, q_c, depends on the temperature difference between the body and the air, ΔT, the area of contact, A, and the heat transfer coefficient, h. Thus,

$$q_c = hA\Delta T$$

in which the average heat transfer coefficient, h, depends on the geometry of the body, the physical properties of the air, and the flow regime. The major difficulty in calculating q_c stems from the strong dependence of h on the flow regime. The radiative heat flux is estimated as:

$$q_r = \varepsilon_1 \sigma A_1 (T_1^4 - T_2^4)$$

where subscript r stands for radiation, indices 1 and 2 represent, respectively, the body surface and the environment, ε (= 0.96) is the emissivity of a biological tissue, σ is the Stefan-Boltzmann constant (= 5.669×10^{-8} W m^{-2} K^{-4}), A is the surface area and T (or K) is the absolute temperature. To calculate the heat transfer from the fowl, each part of the surface is represented by a corresponding geometrical shape. For each part, radiative and convective heat transfers are estimated by using available and specially developed heat transfer relationships (Yahav *et al.*, 2005).

The adoption of thermal-imaging radiometry in the poultry sciences (Yahav *et al.*, 1998, 2004, 2005) has enabled accurate measurement of the body surface temperature and, hence, determinations of the contribution of sensible heat loss to body energy balance.

It has been assumed that sensible heat loss does not play an important role in the domestic fowl when T_a is above the upper limit of the thermoneutral zone (for review see Hillman *et al.*, 1985). This assumption was based on: a. the small temperature differences between the body surface (T_s) and T_a; and b. the reasoning that since only limited areas are unfeathered in fully feathered birds (*i.e.*, legs, head, wattle and comb), convection at high ambient temperatures could be neglected (Tzschentke *et al.*, 1996). However, the present study demonstrated the importance of convection at high levels of T_a.

III. SENSIBLE HEAT LOSS – EFFECT ON BROILER AND TURKEY PERFORMANCE

Air velocity dramatically affects the body weight of broiler chickens exposed to different environments. Exposing broilers to 35°C and air velocities ranging from 0.8 to 3.0 m/s yielded a bell-shaped response curve of body weight vs air velocity, with a maximum response at 2.0 m/s (Table 1). Broilers exposed to air velocity of 2.0 m/s exhibited significantly higher body weight and feed intake than those exposed to lower air velocities (0.8 and 1.5 m/s) (Yahav *et al.*, 2004). It seems, however, that, maximal performance across a range of high ambient temperatures will be achieved at different air velocities. Exposing 3- to 5-week-old broilers to 30°C and air velocities ranging from 0.8 to 2.5 m/s resulted in maximal body weight at 2.5 m/s (Table 1). This coincided with significantly higher feed

intake and the highest feed efficiency (Yahav *et al*., 2005). Exposing broilers to 25°C resulted in maximal body weight at the lowest air velocity, 0.8 m/s.

Table 1. The effects of air velocity on body weight of broiler chickens at the ages of 7, 5 and 6 weeks (wk), following exposure to 35, 30 and 25°C, respectively, and to 60% relative humidity. n= 4 replicates each of 15 birds.

Variables	*Air velocity (m/s)*			
	0.8	*1.5*	*2.0*	*3.0*
Body weight (g); age - 7 wk; at 35°C	1878±54^{c}	2071±91^{b}	2308±39^{a}	2164±61^{ab}
Variables	Air velocity (m/s)			
	0.8	1.5	2.0	2.5
Body weight (g); age - 5 wk; at 30°C	1749±24^{b}	1777±22^{ab}	1789±21^{ab}	1845±21^{a}
Body weight (g) age - 6 wk; at 25°C	2507±29^{a}	2407±33^{b}	2489±31^{ab}	2417±28^{b}

Within rows, values designated by different letters differ significantly ($P \leq 0.05$).

These results suggest that there is an ambient temperature turning point in the response of broiler chicken performance to ventilation. This turning point is around 30°C, below which increased air velocity probably caused chilling, which led to increased energy expenditure on maintenance, and consequently reduced growth performance. It can further be speculated that at low ambient temperature, even a low air velocity may negatively affect the ability of the broilers to balance their energy losses.

One of the open questions raised during the research was: would turkeys of similar ages to those of the tested broilers (*i.e*., up to 6 weeks) respond in a similar manner to different air velocities? Male turkeys at the age of 3 weeks were exposed gradually (during one week) to an ambient temperature of 35°C (at 50% RH) and air velocities of 0.8, 1.5, 2.0 and 3.0 m/s. They were then raised under those conditions up to 6 weeks of age. Table 2 summarizes the effects of the environmental conditions on turkeys.

Table 2. The effects of different air velocities on performance parameters and body temperature of male turkeys exposed to constant 35°C, and 50% RH from 3 to 6 weeks of age.

Variables	*Air velocity (m/s)*			
	0.8	*1.5*	*2.0*	*3.0*
Body weight (g) 42 days	2556±35^{b}	2666±38^{a}	2692±36^{a}	2612±40^{ab}
Feed intake (g) 21-42 days	2856±41^{b}	2948±61^{ab}	3043±81^{a}	2873±27^{ab}
Feed efficiency (g /g) 21-42 days	0.621±0.008	0.635±0.002	0.625±0.00	0.633±0.006
Body temperature (°C)	41.49±0.06^{a}	41.23±0.09^{b}	40.64±0.09^{c}	41.22±0.07^{b}

Within rows, values designated by different letters, differ significantly ($P \leq 0.05$).
n = 4 replicates, each of 15 birds for performance parameters; n=10 for body temperature.

Air velocity affected the performance of turkeys aged 3 to 6 weeks: it was demonstrated that 2.0 m/s was the optimal air velocity for attaining maximum growth rate. It coincided with a similar trend in feed intake and with significantly lower T_b. These results were similar to those obtained for broiler chickens.

IV. SENSIBLE HEAT LOSS – EFFECT ON BROILER'S THERMOREGULATION

In a previous study in which broilers were subjected to 35°C, heat losses by radiation and convection were calculated (Yahav *et al*., 2004). In this study, although heat loss by radiation did not differ among treatments, heat loss by convection increased significantly and linearly with increasing air velocity (Table 3).

Table 3. Heat loss (in watts) by radiation (Qr), convection (Qc), total heat loss (Qt), and Qt as a percentage of energy expended for maintenance (Qt %) in broiler chickens exposed to high ambient temperature (35°C) and 60% relative humidity. (According to Yahav *et al*., 2005).

Variables	*Air velocity (m/s)*			
	0.8	*1.5*	*2.0*	*3.0*
Qr (watt)	1.05±0.09	1.11±0.22	1.33±0.13^{a}	1.32±0.11
Qc (watt)	2.32±0.16^{d}	3.26±0.41^{c}	4.37±0.30^{b}	5.52±0.29^{a}
Qt (watt)	3.37±0.43^{b}	4.37±0.46^{b}	5.69±0.43^{a}	6.83±0.42^{a}
Qt %	29.1±3.74^{b}	29.1±4.44^{b}	36.8±2.22^{ab}	44.7±4.75^{a}

Within rows, values designated by different letters differ significantly ($P \leq 0.05$; n = 8).

These results demonstrated for the first time that sensible heat loss can reach an average of 45% of the energy expended on maintenance; it therefore plays a major role in the broiler's energy balance.

The effort to control energy balance diverts a large proportion of energy towards maintenance in broilers exposed to an air velocity optimal for growth performance (Figure 1). It was expected, however, that thermally unbalanced broilers, such as those exposed to low air velocities of 0.5 to 1.0 m s^{-1}, which exhibited a T_b of 43.9°C (Yahav *et al*., 2004) would expend even more energy in controlling their body temperature, especially when energy consumption was lower under these conditions (Yahav *et al.,* 2001, 2004). However, the present findings suggest an opposite response, namely a decline in the amount of energy directed to maintenance, in chickens that exhibited difficulties in controlling body temperature (Figure 1).

It can be concluded that air velocity plays a major role in the performance of broilers and turkeys. The optimal air velocity for achieving maximal growth performance differs with ambient temperature, and possibly with the birds' age, and has a turning point (in chickens) at an ambient temperature below 30°C, where chilling affects the broiler.

The main conclusion is that, under each and every set of environmental conditions, a specific fine tuning of ventilation must be determined. It will be based on the age of the chicken, the density of the flock and the ambient conditions: including such factors as T_a, RH, ammonia and dust.

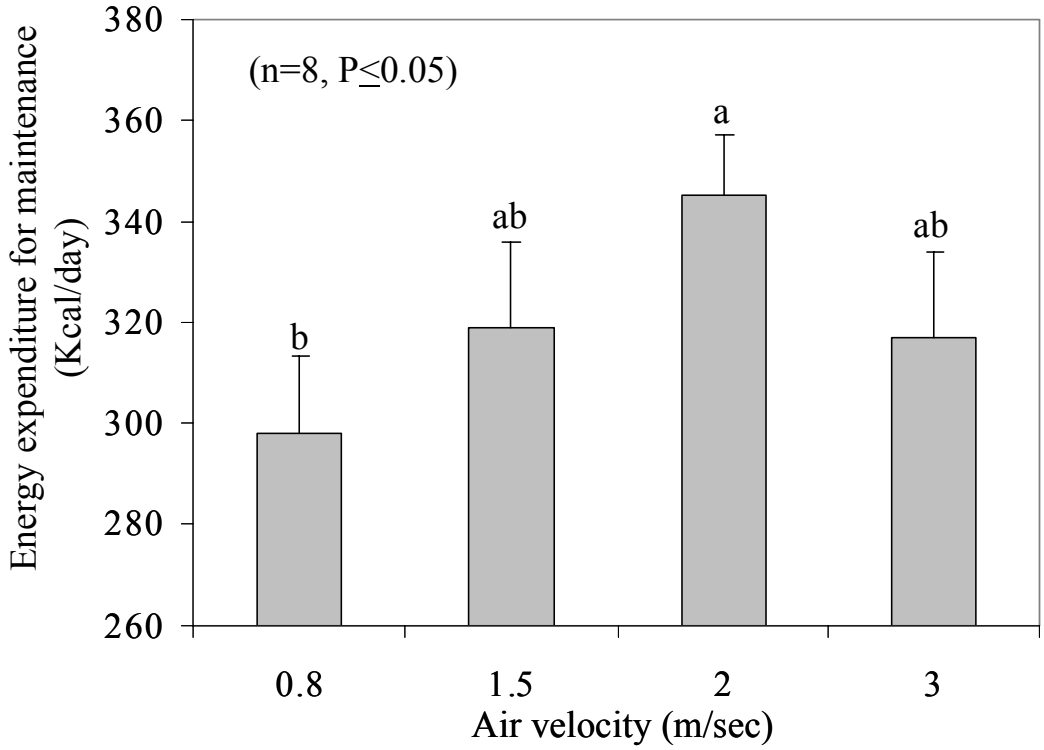

Figure 1: The effect of air velocity on energy expended for maintenance in broiler chickens exposed to 35°C and 60% RH. Columns with different letters differ significantly (P≤0.05). (Yahav *et al*., 2004, 2005).

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HOW TO USE A HAND-HELD CARBON DIOXIDE MONITOR TO EVALUATE SUMMER VENTILATION IN POULTRY HOUSES

C. BENNETT[1]

Summary

Minimising temperature gain, or the difference between the temperature inside and outside of a poultry house, is the first objective of any poultry ventilation system in hot weather. Carbon dioxide measurements are a rapid method of evaluating the temperature gain and airflow in poultry houses. They are easier to conduct and appear to be less variable than direct measurements of temperature gain. In a study of 46 commercial poultry houses in Manitoba, a strong linear relationship (r^2 = 97%) was observed between house carbon dioxide level and temperature gain. House carbon dioxide levels under 700 ppm were associated with a temperature gain of 1.5°C or less. Service people and farm managers can use hand held carbon dioxide monitors to provide quick estimates of airflow and heat buildup in poultry houses.

I. INTRODUCTION

During hot weather, minimising poultry house temperature gain is the major goal of any ventilation system. In a well run system, the air temperature inside the house will be only 1°C warmer than outside the house (temperature gain of 1°C). In a poorly designed or operated system, temperature gain could be as high as 3°C. While the difference between a good and poor system may appear to be small, the impact on the number of hours of heat stress experienced by the birds can be significant. For example, under typical summer conditions on the Canadian Prairies where the day time maximum temperature averages 26°C in July, a 1°C reduction in temperature gain can reduce the hours of heat stress experienced by the birds by 50%.

While managing temperature gain is a primary objective, it is surprisingly difficult to accurately determine if temperature gain is being kept at 1°C during hot weather. One of the problems with accurately estimating temperature gain is the need to simultaneously measure temperature both inside and outside the house to within a few fractions of a degree. Temperature outside the house is particularly difficult to measure because it fluctuates throughout the day, changes with shifting wind direction, and can be noticeably different on the sunny and shady sides of the house. As an example, the air temperature on the sunny side of the house can be 2°C warmer than on the shady side of the house. All of these variables can cause fluctuations in outside temperature that are greater than the size of temperature gain that you are trying to measure. Variation in air temperature within the house can further complicate the attempt to accurately measuring temperature gain.

For service people and farm managers who do not have the time to conduct the many temperature measurements needed to gauge temperature gain, house carbon dioxide measurements may offer a good alternative. Carbon dioxide is a by-product of respiration and related to heat production by the birds; plus it builds up in the house when airflow is inadequate to remove the heat produced by the flock. As part of a study of commercial poultry houses, carbon dioxide monitoring was evaluated as method of estimating temperature gain and airflow.

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II. METHODS

Air temperature, carbon dioxide level, and airflow were measured in 46 poultry houses (nine broiler, 26 layer, eight breeder, one pullet and two turkey houses). All measurements were taken when the stoves were turned off and the birds provided the only source of heat in the houses. For cool weather conditions, (temperature gain over 15°C), all measurements were done in layer houses with manure belts or gutters where the manure could be removed regularly to prevent ammonia buildup.

Outside air temperature was measured with a digital probe thermometer at the outside face of the inlet or eaves where the air first entered the house. The thermometer was positioned to keep it out of direct sunlight. During each farm visit, outside temperature was measured on two or more occasions at each of three or more locations. The same thermometer was used to measure temperature inside the house. In broiler houses, temperature was measured at six or more locations at bird height moving in a diagonal path across the house. In layer houses, the measurements were done at a minimum of two heights at six locations in a diagonal across the house. It was not unusual to conduct measurements at a dozen locations in layer houses.

Airflow through the houses was estimated based on the airspeed of the fan exhaust and the area of the fan opening. Fan airspeed was estimated based on nine measurements with a Kestrel vane anemometer. The method of determining fan airflow has been explained previously (Bennett, 2004).

Carbon dioxide was measured using an ACR Falcon 206 hand-held carbon dioxide meter (ACR Systems Inc., 210 – 12960 84 Avenue, Surrey, B.C. Canada). Inside broiler houses, carbon dioxide was measured at the same locations as air temperature. Inside layer houses, measurements were taken at two or more heights at six or more locations. Temperature was measured at all locations where carbon dioxide was measured but in large layer houses, temperature was measured in more locations than carbon dioxide. The carbon dioxide outside the house was measured at one to four locations.

III. RESULTS AND DISCUSSION

Carbon dioxide levels were relatively uniform between different locations in the poultry houses. Contrary to popular belief, the carbon dioxide levels were not higher near the floor compared to several feet above it. The carbon dioxide produced by the chickens was warm and tended to rise and mix in with the general airflow in the house. The highest carbon dioxide levels were instead found in areas of the house which were not in the direct path of the fresh air as it entered the house. Measuring the average carbon dioxide level inside the house appeared to be fairly easy.

In Figure 1, data for carbon dioxide and temperature gain in the 46 poultry houses are displayed. No supplemental heat was provided in the houses at the time of these measurements. A strong linear relationship between carbon dioxide level and temperature gain was observed:

$$\text{Temperature Gain (°C)} = -6.4542 + 0.0108 \times CO_2 \text{ (ppm)}, \quad r^2 = 97\%.$$

The steady increase in carbon dioxide as temperature gain increased was expected due to the low airflows needed to trap bird heat in the cool months when a large temperature gain was needed.

The strong linear trend was partly a reflection of the very wide spread in temperature gain observed over the study. Looking at the cases where temperature gain was under 3.5°C,

considerable variation was observed – in part due to the challenge of estimating the temperature gain. Still, within this subset of the data, lower temperature gain was associated with lower carbon dioxide levels. For the 15 houses with a temperature gain of 1.5°C or less, the carbon dioxide levels averaged 682 ppm whereas in the houses with a temperature gain of 1.5°C to 3.5°C, the average carbon dioxide level was 865 ppm. The increase is in basic agreement with the above regression equation which predicts a temperature gain of 1.5°C at a carbon dioxide level of 737 ppm. A reasonable objective for hot weather ventilation would be to keep the carbon dioxide levels below 700 ppm.

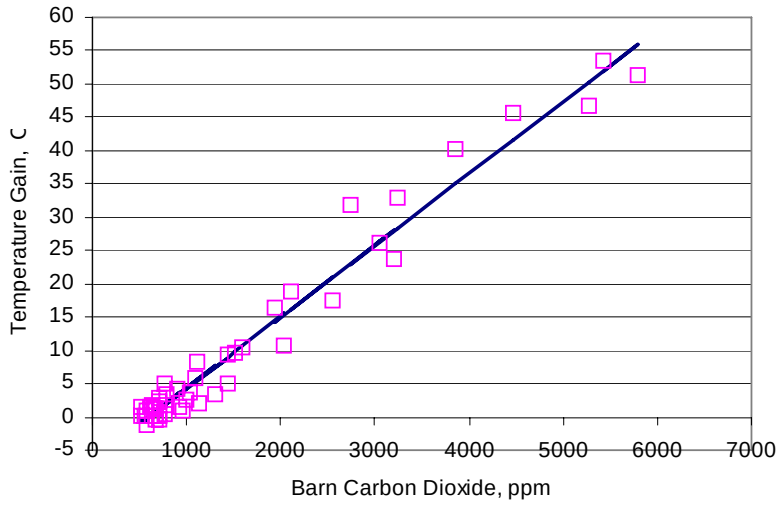

Figure 1.Temperature gain (°C) increases linearly with house carbon dioxide level (ppm)

In addition to predicting temperature gain, the carbon dioxide levels provided an estimate of airflow in the houses. In Figure 2, the relationship between carbon dioxide and airflow relative to live body mass is displayed. As airflow increased above the minimum winter ventilation rate, (approximately 0.15 litres/s/kg of live weight), carbon dioxide levels dropped dramatically. At flow rates over 1.5 litres/s/kg, however, further drops in carbon dioxide level were difficult to discern. Increasing fan capacity over 1.5 litres/s/kg did not appear to measurably reduce the buildup of carbon dioxide (and thus body heat) in the house. If fan capacities over 1.5 litres/s/kg are built into a house, the benefits will most likely come from increased airspeed instead of reduced temperature gain.

In poultry houses with misting cooling systems, carbon dioxide levels can be used to separate the effect of airflow and mist cooling on the temperature inside the house. For example, a poultry house with 950 ppm carbon dioxide would be expected to have a temperature gain of 3°C in the absence of mist cooling. If operating the misters drops the house temperature to 2°C below the outside temperature at the same time that 950 ppm of carbon dioxide is observed, the total amount of cooling is 5°C. The mist system should be given “credit” for eliminating the expected temperature gain as well as the drop below outside temperature that it produces.

In interpreting the data, it is interesting to note that the air immediately outside the houses had more than the 330 to 370 ppm of carbon dioxide normally expected in fresh air. Instead, in most cases, the air entering the houses contained 400 to 450 ppm of carbon dioxide. The elevated carbon dioxide reflected the large amounts of the gas exhausted by the house fans into the immediate surroundings.

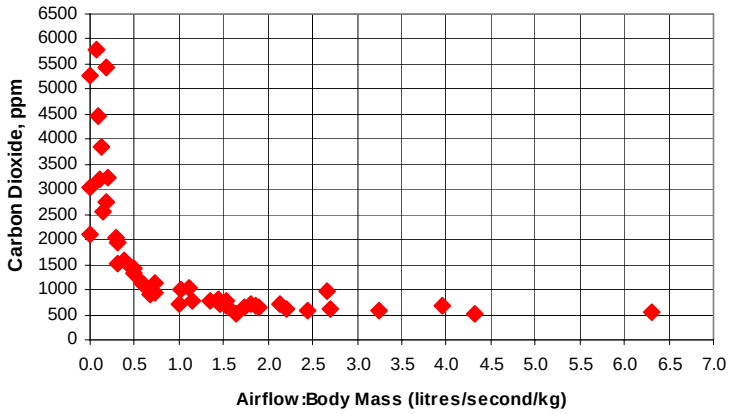

Figure 2. Relationship between house carbon dioxide level (ppm) and house airflow relative to bird body mass (litres/second/kg of live weight)

IV. CONCLUSIONS

A hand-held carbon dioxide monitor can be used to rapidly provide service people and farm managers an estimate of temperature gain and airflow in their poultry houses. A digital carbon dioxide monitor costs $850 Cdn. and requires little maintenance. Average carbon dioxide levels can be easily estimated in the time required to walk the length of the house. The relationship between temperature gain is strong and linear. House carbon dioxide levels below 700 ppm are indicative of a temperature gain of 1.5°C or less and are a sign of good airflow during hot weather. Carbon dioxide testing is a simple and practical method for evaluating summer ventilation in poultry houses.

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MAINTAINING ELECTROLYTE AND WATER BALANCE TO ALLEVIATE HEAT STRESS IN BROILER CHICKENS

M. A. M. SAYED[1] and T. A. SCOTT[1]

Summary

Under heat stress conditions, maintaining water and electrolyte balances are considered important factors affecting broiler chickens survivability and productivity. When exposed to high temperature, birds increase their respiratory rates (to dissipate heat by evaporation) resulting in higher losses of CO_2, and consequent increased blood pH. The acid-base balance is further disrupted by the increased electrolyte excretion through urine and faeces. Electrolyte supplementation to the diet has been shown to restore the acid-base balance and to improve bird's performance. Heat distressed broilers lose more water through urine and panting which decrease the heat dissipation capacity by evaporation and increase the osmotic stress on body cells. Organic osmolytes such as betaine have been shown to protect the cells from high osmotic pressure and to control intracellular water, suggesting their use in maintaining water balance under heat stress conditions.

I. INTRODUCTION

As ambient temperature increases, birds start to pant to lose heat by evaporation. Evaporative heat loss through panting is the most important mechanism to control body temperature under heat stress. However, panting is accompanied with increases in respiratory rates. The increased respiratory rate causes higher losses of CO_2 that result in increased blood pH and disruption of acid-base balance (Toyomizu *et al.,* 2005). When this balance is altered towards alkalosis or acidosis, metabolic pathways are diverted to homeostatic regulation rather than used for supporting growth (Mongin, 1981).

Moreover, under heat stress, birds lose more water (through panting and urine) than they do in their thermal comfort zone. A decrease in body water results in a reduced ability to dissipate heat via evaporation and/or through increased peripheral blood flow. As a consequence, birds increase water consumption to compensate for water loss and to increase the heat dissipation capacity. However, water retention is reduced due to the increased electrolyte excretion in urine and faeces (Belay *et al*., 1992; Belay and Teeter, 1996). Reductions in intracellular water adds further stress to the bird and understanding how this can be minimised will facilitate identification of effective dietary and management treatments.

II. SUPPLEMENTING AND BALANCING ELECTROLYTES

Electrolytes provided in diets are of great importance in maintaining acid-base balance, osmotic pressure and electrical potential of cell membranes; and are also essential for intracellular-extracellular homeostasis (Borges *et al*., 2003). Among these electrolytes, the monovalent ions (Na, K, and Cl) are the key minerals involved in acid-base balance of the body fluids (Mongin, 1981), because they have a higher permeability and have greater absorption than divalent ions (Ca and Mg) (Borges *et al*., 2004).

Hyperventilation during heat stress results in increased CO_2 loss and respiratory alkalosis develops (Toyomizu *et al*., 2005). Under such conditions, the kidney attempts to

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correct the acid-base balance by renal exchange of bicarbonate with Cl (Mongin, 1981). As a consequence Cl concentrations increase in plasma (Belay and Teeter, 1996). Bicarbonates are negatively charged ions that are coupled by positively charged ions such as K and Na and both are excreted in urine (Remus, 2002). During heat stress, Belay *et al.,* (1992) and Belay and Teeter (1996) reported increased K and Na excretion in urine and faeces, but decreased Cl excretion. As Na and K are alkalogenic ions, their loss can lead to body fluid acidification. The changes in systemic pH in response to heat stress are therefore complex involving an initial respiratory response phase which can produce a systemic alkaloidosis and then a compensatory phenomena involving homeostatic mechanisms that can produce systemic acidosis. Predicting the length and duration of these phenomena and how they interact with diet, and management remains problematical. These changes in acid-base balance are responsible (along with decreased feed intake) for growth retardation and poor performance under heat stress (Arad *et al*., 1983; Teeter *et al*., 1985).

It was observed, under heat stress, that supplementing diets with 0.3 or 1.0% Ammonium Chloride (NH_4Cl) significantly improved broiler weight gains by 9.5 and 25%, respectively and decreased blood pH. Also, adding 0.5 % sodium bicarbonate increased body weight gains by 9 %. Moreover, it was observed that both ammonium chloride and sodium bicarbonate had synergetic effect on broiler performance (Teeter *et al*., 1985). The use of ammonium chloride helps to reduce blood pH, therefore it is recommended to be supplemented in diets when birds experience panting-induced alkalosis. However, care must be taken as if it is supplemented in excess it may cause acidosis. Therefore, it is recommended that sodium bicarbonate be used in combination with ammonium chloride to adjust blood pH and to remove any acidification may develop. Bicarbonate can combine with excess acids to form carbonic acid that converts to carbon dioxide and water by the action of the enzyme carbonic anhydrase, restoring blood pH from acidosis (if developed). Similar results have been reported by (Naseem *et al*., 2005; Ahmad *et al*., 2006).

Smith and Teeter (1989) reported that NaCl, K_2SO_4 and KCl were all able to alleviate the adverse effects of heat stress when supplemented in drinking water, but they attributed their effects to increased water consumption which facilitates heat dissipation and reduces body temperature. It is also necessary to keep in mind the concentration and balance of electrolytes included in the diet as well as in the drinking water. Mongin (1981) reported that when dietary electrolyte balance (K + Na – Cl) is higher or lower than 250 m eq/ kg diet, alkalosis or acidosis develops resulting in growth depression. Similarly, Borges *et al*. (2004) observed that a dietary electrolyte balance of 240 m eq/ kg diet increased N, Na and K retention and water consumption and was more favourable under thermoneutral and heat stress temperatures than diets with dietary electrolyte balance of 140 or 340 m eq/ kg diet. When electrolytes are supplemented in the water, care must be taken as water intake is significantly greater than feed intake, (up to three times as much during high temperatures, Zhou *et al*., 1999; Tanveer *et al*., 2005).

III. MAINTAINING WATER BALANCE

To maintain homeostasis, water intake plus that formed by oxidative metabolism should equal water lost by evaporation and through urine and faeces. However, birds exposed to high ambient temperature lose more water in urine (>60 %) than those maintained in the thermoneutral zone (Belay and Teeter, 1993). As water deficit develops, extracellular fluid levels decrease causing a fall in circulating blood volume and pressure and an increase in plasma osmolality. A reduced blood volume stimulates the juxtaglomerular cells of the kidney to release renin that in turn stimulates thirst and drinking behaviour. In addition, there is evidence that certain cells in the hypothalamus are sensitive to changes in plasma

osmolality that stimulate the pituitary gland to secret arginine vasopressin (i.e antidiuretic hormone) which acts on nephrons in the kidney for increased water reabsorption and decreased urine output (Reece, 2004). At this point, if the amount of water lost is not completely compensated, dehydration and increased body temperature will occur. To overcome this problem, birds consume markedly more water (Zhou *et al*., 1999; Tanveer *et al*., 2005), causing plasma expansion, reduced plasma osmolality and whole blood viscosity (Yahav, 1999; Zhou *et al*., 1999). Plasma expansion under high temperatures facilitates heat dissipation by peripheral blood and evaporation. However, this expansion leads to a lowering of arginine vasopressin concentrations in blood resulting in a rise in urine flow. Although, birds consume more water to overcome these consequences, water retention is reduced due to increased electrolyte excretion (Belay *et al*., 1992) and due to continuous loss of water through panting.

Cells must accumulate ions and osmolytes to maintain intracellular water against the extracellular osmotic gradient. The osmotic pressure of intestinal fluid is hypertonic to plasma (650 mOsm in the jejunum versus 300 mOsm in the plasma; Mongin, 1976). To withstand this osmotic pressure, intestinal epithelia controls water and ion transport via ion pumps and water channels (Rao, 2004). However, these ion pumps (i.e., K, Na, Ca and Mg ATPases) use adenosine triphosphate as an energy source to operate (Moeckel *et al*., 2002), which means energy would be diverted from growth to regulate osmotic pressure. Under hyper tonicity conditions, cells respond quickly by accumulating inorganic ions to prevent water flux. However, these ions can perturb the structure of macromolecules such as proteins and enzymes. Therefore, cells increase synthesis of either organic osmolytes (e.g. betaine, sorbitol, inositol) or transporters for these osmolytes (ie., betaine aminobutyric acid transporter) and replace the inorganic ions with the organic osmolytes (Alfieri *et al*., 2002). Organic osmolytes have been shown to inhibit cell membrane ion ATPases activities (Moeckel *et al*., 2002) and protect the cells from hypertonic conditions (Kettunen *et al*., 2001; Alfieri *et al*., 2002). Extreme hypertonicity can induce apoptosis in various types of cells. Alfieri *et al.* (2002) reported that cultured pig arterial endothelial cells incubated in hypertonic media stopped proliferating and had clear apoptotic morphology. However when betaine was present in the medium, it restored growth and normal morphology.

The mechanism by which betaine exerts its effect as an osmoprotectant is still not clear. However it was suggested that betaine may affect the lipid membrane bilayer that can modify the activity of enzymes responsible for active transport of monovalent ions through ion pumps that indirectly affect water movement. Another suggestion is that betaine accumulation inside the cells may change the surrounding tonicity (Kettunen *et al*., 2001).

Moreover, betaine supplementation has been shown to be advantageous in controlling and minimising coccidial invasion. Augustine *et al.* (1997) reported that betaine and/or salinomycin supplementation significantly decreased the number of sporozoites of *E. tenella* and *E. acervulina* in broiler chicks. The effect was more pronounced when betaine was used in combination with salinomycin. It is not clear how betaine exerts its effect in limiting parasite invasion, however, it could be due to restoring intestinal epithelial water balance that enhance the resistance against coccidial proliferation (Klasing *et al*., 2002). Many reports indicate the beneficial effects of supplementing betaine or other organic osmolytes especially under certain conditions such as dehydration, diarrhoea as it reduces water loss in spite of the osmotic pressure.

Few studies have been conducted to investigate the effect of betaine supplementation on maintaining water balance during heat stress challenge, particularly to examine the effect of betaine in combination with electrolytes in diets and/or water. Therefore, we hypothesise that betaine along with electrolyte supplementation could assist broilers during heat stress. Electrolyte supplementation should be considered as a protective management practice during

heat stress as it helps the bird to regulate acid-base balance and stimulates water intake, thereby acting as a water sink to reduce body temperature. Dietary electrolyte balance (K+Na–Cl) should be maintained within the recommended range of 230 to 240 m eq / kg diet to avoid acid-base alteration. The use of betaine would help in maintaining body water balance and reducing the reliance of body cells on ion pumps to regulate their water balance, requiring more energy expenditure.

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SELECTION FOR GROWTH PERFORMANCE UNDER HIGH TEMPERATURES IN JAPANESE QUAIL

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Although a significant proportion of commercial flocks globally are expected to perform under high temperature conditions for much of the time, most of the genetic selection of the nucleus lines from which commercial strains of poultry used throughout the world have been derived, has been undertaken in temperate environments in Europe and North America. In this study Japanese quail (*Coturnix coturnix japonica*) were selected for increased 14 to 28-day weight gain under one of two environmental temperatures (25 or 32^{O}C) and given either high (250 g/kg) or low (170 g/kg) protein, isoenergetic (13.0 MJ ME/kg) diets. The lines were designated as follows: WH (warm, high protein), WL (warm, low protein), NH (normal, high protein) and NL (normal, low protein). Dietary protein was included as a selection environment variable due to the critical role that protein turnover plays in metabolic heat production. All lines were constituted from matings between 12 males and 36 females; chicks were pedigree hatched to sire and dam and wingbanded at hatch. Two weekly hatches were used to generate progeny to be selected each generation in each line subjected to one of the four environments. In each line, breeders were selected from a total of approximately 120 males and 120 females. A randomly selected control line was maintained; birds from this line were allocated to each cage in both temperature environments and on both diets.

Each generation, a third hatch was used to determine direct and correlated response in the lines in all four temperature X dietary environments. Individual 14 to 28 d growth rate and food intake were recorded in single-bird cages with individual feeders. There were approximately 20 and 14 birds from each line given each of the two diets under the normal (25^{O}C) and high (32^{O}C) temperature regimes respectively. Data on direct and correlated responses in 14-28 weight gain after five generations of selection are presented in table 1.

Table 1. Sex mean 14-28 d weight gain (±SEM) in the five lines given either high or low protein diets under the two temperature regimes, after five generations of selection.

		Line				
Diet	Temperature	Control	NH	NL	WH	WL
High	Warm	78.4± 3.3	92.1± 2.6	91.7± 3.8	97.3± 2.5	85.4± 1.7
	Normal	94.0± 1.9	108.4± 1.8	107.4± 1.7	111.2± 2.2	99.4± 2.1
Low	Warm	75.6± 1.7	86.1± 3.7	77.2± 2.5	81.6± 2.6	79.0± 3.3
	Normal	82.0± 1.7	97.7± 2.4	88.2± 2.8	88.9± 2.9	91.2± 1.2

There was evidence of response in all selected lines in all environments, although there was considerable variation in this across the four temperature X dietary regimes. Although not significant, there was an indication of better performance on the high protein diet in the WH than the NH line birds, particularly under the high temperature regime. Under no temperature X diet regime did the lines selected on the low protein diet outperform their high protein diet-selected counterparts, suggesting that there is little merit in selection based on performance on a low protein diet, irrespective of what dietary regime the progeny are likely to be grown under. Results to six generation of selection will be presented at APSS.

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CHOICE BEHAVIOUR OF LAYING HENS: EFFECTS OF DEPRIVATION OF FEED AND DUSTBATH SUBSTRATE

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Summary

It is proposed that choice behaviour in a Y maze reflects the intensity of the need for a resource in a Y maze, and thus can be used as a tool to measure hen welfare. When given a choice between feed or nothing in a Y maze, birds chose feed, on average, on 85% of choices. When given a choice between dustbath substrate (sawdust) or nothing, birds did not choose sawdust on more than 50% of choices (chance level). Birds that were deprived of feed did not show any increase in the proportion of choices for feed, however they moved faster through the Y maze, possibly indicating an increase in intensity of need for the feed resource. However, when deprived of sawdust, birds neither changed the proportion of their choices nor moved faster in the Y maze, indicating a low intensity in need for sawdust in these birds.

I. INTRODUCTION

As shown by Arnold *et al*. (2006), laying hens are able to make choices in a Y maze to gain access to a desired resource. The research team has proposed that choice behaviour reflects the intensity of the need for a resource in a Y maze, and thus can be used as a predictive, quantitative tool to measure animal welfare. Therefore the current study was aimed at measuring the impact of deprivation of a particular resource on preference and motivation in a Y maze. Two experiments were conducted; one in which birds were deprived of feed and then given feed vs. nothing choices in a Y maze, and the other in which birds were deprived of dustbath substrate (sawdust) and then given sawdust vs. nothing choices in the maze.

Dustbathing is a natural behaviour in birds and when deprived of dustbathing opportunity, hens have been found to increase the duration of their dustbathing session when subsequently allowed (Vestergaard, 1982), indicating an increase in motivation to dustbath after a period of deprivation. Further, birds deprived of dustbath substrate have shown increases in corticosterone concentrations, a sign of physiological stress (Vestergaard *et al.*, 1997), suggesting that the increase in motivation to dustbath may be accompanied by a degree of stress.

The feeding behaviour of laying hens has been found to have a distinct diurnal pattern and previous research has found that feed deprivation increases exploratory behaviour (Nicol and Guilford, 1991) and decreases the time taken to move through a Y maze (Petherick *et al.*, 1993) when feed is available in the maze.

Thus, it was hypothesised that deprivation would lead to an increase in the percentage of choices for feed and sawdust in the Y maze and an increase in speed of movement through the maze.

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II. MATERIALS AND METHODS

A purpose-designed Y maze apparatus with metal mesh walls, floor and removable roof was constructed and placed within a climate-controlled poultry facility alongside the cage system. Twenty birds (Hyline Brown Strain) aged 28 weeks were housed in furnished cages (each containing a nestbox, perch and dustbath) and were individually moved into the Y maze for experimental sessions. Each bird was placed in the Y maze and introduced to the maze 4 times each per day over 3 days to familiarise them with the maze environment. There were 2 parts to the experiment. In Part A, half of the birds were trained, over 4 trials per day for 2 days, to associate one arm of the Y maze with access to feed and the other with nothing, while the other half were trained to associate one maze arm with sawdust and the other with nothing. Testing was then conducted over 12 tests per bird across 3 days where birds were free to choose either maze arm during each test. During the test phase, half of the birds that were trained with the feed resource were deprived of access to feed for 4 h prior to testing and half of the birds that were trained with the sawdust resource were deprived of access to sawdust for approximately 22 h prior to testing. Testing was then conducted over 12 tests per bird across 3 days. In Part B, the treatments were reversed and birds were re-trained to associate one arm of the Y maze with access to the resource not previously tested (feed or sawdust) and the other with nothing and all birds were re-tested as in Part A.

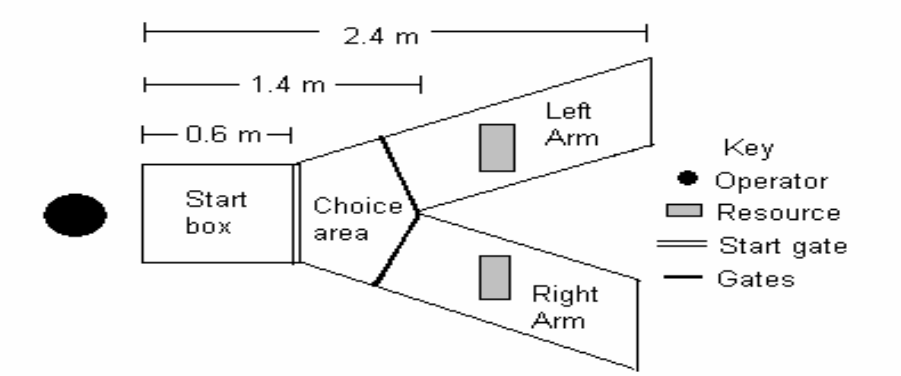

Figure 1. Diagram of Y maze

III. RESULTS

An Analysis of Variance (ANOVA) was used to compare proportion of choices for the resource between treatment (deprived or control) and resource type (feed or sawdust). Results showed that deprivation of feed or sawdust did not affect the choice behaviour of birds for either resource ($p=0.536$ and $p=0.089$ respectively). However, the hens chose the resource arm significantly more often when feed was the resource presented in the Y maze than when sawdust was the resource (Fig. 2).

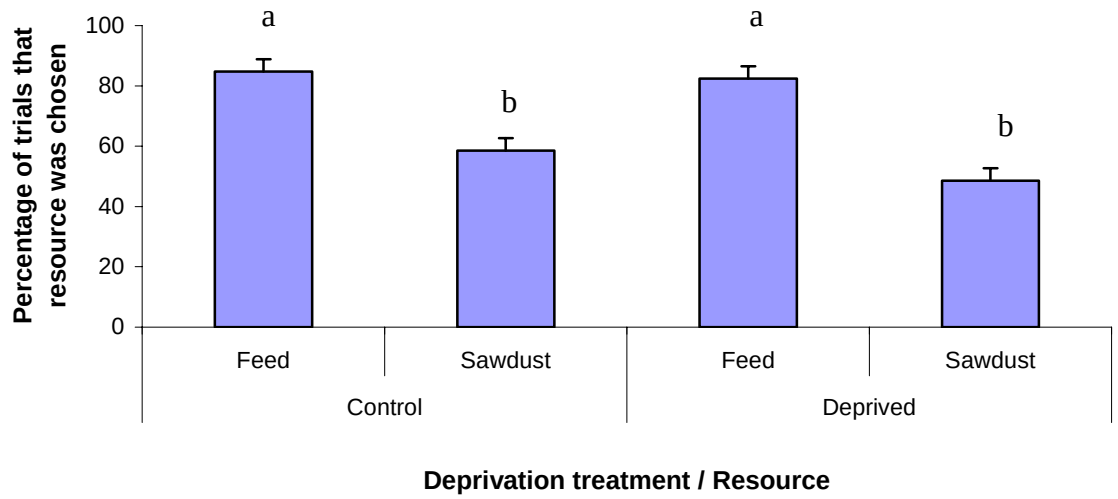

Figure 2. The average proportion of preference test trials in which the resource arm was chosen (different letters indicate significant differences, $p<0.01$).

An ANOVA was conducted to compare time taken to move out of the startbox during feed trials between treatments (deprived or control) and across trial number each day (1,2,3 or 4). Hens that were deprived of feed for four hours moved signficantly faster ($p<0.05$) into the Y maze from the starting box than hens that were not deprived (Fig 3). Further, regardless of deprivation treatment, hens took increasingly longer ($p<0.001$) to enter the Y maze over each of the four trials in each day. The same analysis on sawdust trials showed that deprivation of sawdust had no effect on time taken to enter the Y maze ($p=0.228$), however there was a tendency ($p=0.053$) indicating an increase in time to enter the maze as trial number increased.

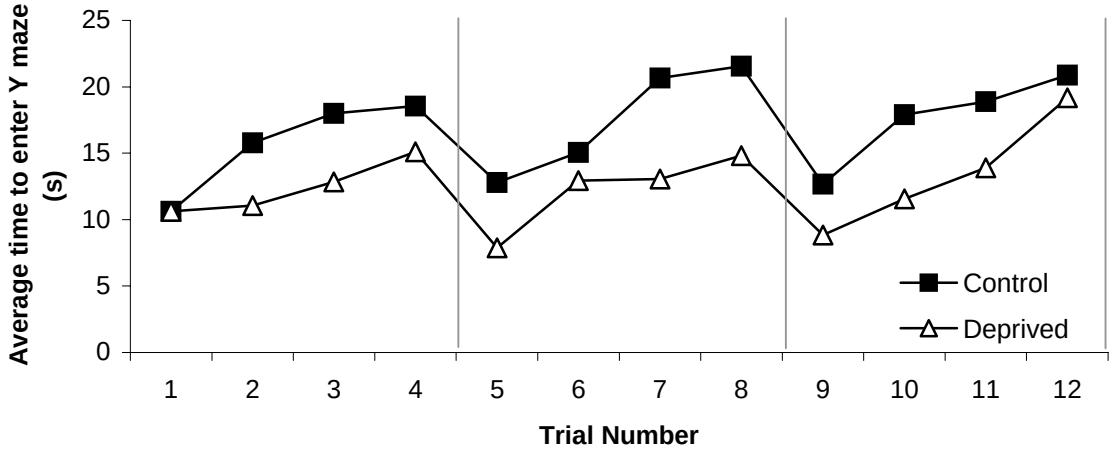

Figure 3. Average time to enter the Y maze for feed choice trials. Vertical lines indicate end of each day (day 1; trials 1-4, day 2; trials 5-8, day 3; trials 9-12).

IV. DISCUSSION

Results indicate that hens deprived of feed for 4 h did not show an increased preference for feed compared to those that were not deprived, perhaps because preference for feed was already relatively high (an average of 85% of choices were for feed). The reasons

why animals often do not always choose a valued resource in preference tests has been discussed by various researchers and it seems likely that the minority choice option is sometimes chosen because it holds some adaptive significance for the animal (Duncan, 1978). Occasionally choosing the least preferred option may arise in case the situation has meaningfully changed since the last visit (Arnold, 2005). However, feed-deprived birds did move faster through the Y maze, suggesting that they were more motivated to obtain access to feed. Possibly a deprivation period of greater than 4 h may have led to an increase in the already high preference for the feed in the Y maze. Alternatively, the provision of 4 choices per day may have reduced motivation to choose the feed resource each time as there were 4 opportunities per session to obtain feed, possibly allowing birds greater flexibility to satisfying both feed and exploratory motivations.

Deprivation of sawdust did not appear to affect either preference for sawdust in the Y maze or speed of movement in the Y maze. In fact birds appeared to have no preference to choose sawdust in the Y maze at a level any greater than chance, suggesting that this was not an important resource to these birds even when deprived for 22 hours. It is possible that this lack of preference for sawdust may have been due to a lack of experience with dustbath substrates during rearing. These birds were reared in cages with wire floors and therefore had never been exposed to a dustbath substrate of any sort prior to this experiment. In conclusion, this experiment has shown that laying hens show a markedly greater preference for feed than for sawdust and has provided some evidence that deprivation of feed causes increases in speed of movement in a Y maze test, although birds do not necessarily choose to access the resource any more often. However, when deprived of a resource of little value, (in this case, sawdust) these behavioural changes in the Y maze do not occur.

ACKNOWLEDGEMENTS

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THE CHOICE BEHAVIOUR OF LAYING HENS FOR FEED AND DUSTBATHING

N.A. ARNOLD[1], C. RALPH[1], J.C. PETHERICK[2] and P.H. HEMSWORTH[1]

Measuring the preferences of hens for various resources that could be included in their environment (e.g cage-mates, dustbath substrate, space) can be used as an indirect, but compelling (due to high acceptibility by consumers), method of assessing bird welfare. Within a larger research program, the results of preference tests will be ultimately tested against the hypothesis that preferences align with biological requirements; that birds will choose resources that assist with maintenance of homeostasis. A simple method of measuring preferences is using a Y-shaped maze allowing a choice between two alternatives. In the current experiment, a purpose built Y maze was trialed to measure the preferences of 20 birds housed in furnished cages (containing a nestbox, perch and dustbath; birds were housed in these cages for 2 months prior to the experiment). First, each bird was familiarised to the maze over 12 exposures (120 secs each). Then, in Part A, half the birds were trained to associate one maze arm with feed and the other with nothing (resource randomly assigned to arm), while the other half of the birds were trained to associate one arm with sawdust (dust-bathing substrate) and the other with nothing. Testing was then conducted over 12 tests per bird where birds were free to choose either arm, and the resource was visible from the start of the maze. In Part B, the treatments were reversed and birds were re-trained to associate one maze arm (opposite of that used in Part A) with the resource not previously tested (feed or sawdust) and the other with nothing and the birds were re-tested.

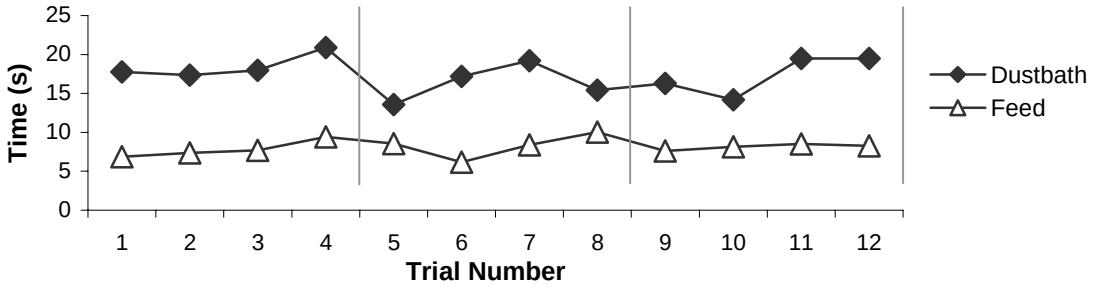

Figure 1. Average time to enter the Y maze for feed choice trials. Vertical lines indicate end of each day (day 1; trials 1-4, day 2; trials 5-8, day 3; trials 9-12).

Birds chose feed significantly more often than sawdust (85% vs. 48%, $P<0.001$), and the choice proportions were not affected by order of training (i.e. trained with feed first or trained with sawdust first, $P=0.322$). When birds were presented with feed vs nothing, they moved faster through the maze than when presented with sawdust vs nothing ($P<0.01$, Fig. 1). These results are likely due to a combination of high motivation for feed (an important resource for all animals but also a selected trait in laying hens) and little or no motivation for access to sawdust. This is perhaps partially a result of rearing experience; these birds were reared in cages with no experience of dust-bath substrates. Overall, the results of this experiment have demonstrated that laying hens can, and will, make choices in a Y maze that are consistent, both within and between birds, and intuitively sensible.

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HUMAN CONTACT AND FEAR RESPONSES IN LAYING HENS

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Summary

Human-animal interactions were studied at nine Australian farms and nine US farms. Measures of hen fear of humans, human activity in the laying shed, productivity records and shed parameters were collected at each shed. Significant differences between countries were found for shed parameters, human behaviours and bird behaviours, and after adjusting for country differences, significant correlations were found between some of these human and animal variables. While the sample size is small and caution is required in interpreting the results, these relationships support the hypothesis that a sequential relationship exists between human behaviour, bird behaviour and productivity, and that the opportunity exists to improve hen welfare and productivity by manipulating this relationship.

I. INTRODUCTION

Extensive studies in the livestock industries have shown marked between-farm variation in the fear responses of farm animals, including poultry, to humans (Hemsworth and Coleman, 1998). Furthermore, Barnett *et al.* (1992) found a negative inter-farm correlation between fear of humans and the productivity of the laying hens. Such negative correlations indicate that high levels of fear of humans may be an important factor limiting the productivity and welfare of livestock.

Studies in the dairy and pig industries have shown significant sequential relationships between the stockperson's attitudes and behaviour towards animals and the fear responses of animals toward humans (see Hemsworth and Coleman, 1998). The existence of sequential relationships between human and animal variables in the livestock industries indicates that the opportunity exists to modify stockperson attitudes and behaviour in order to improve livestock welfare and productivity, and such opportunities may also exist in the poultry industries.

Since the work by Barnett *et al.* in 1992, new strains of laying hens have been introduced to the Australian industry, and the use of fully-enclosed environmentally-controlled sheds has increased. Due to the observed behavioural differences in hen strains (Fraisse and Cockrem, 2006), and the more intensely controlled environment of the fully enclosed laying sheds, the present impact of human-bird relationships is unknown. The aim of the present study was to examine human-bird relationships with the current strains of laying hen in the modern commercial environment.

II. MATERIALS AND METHODS

Between April 2005 and May 2006, field work was conducted at ten Victorian farms, two NSW farms and 9 US farms, providing a total of 29 study sheds. The Australian farms used the Hyline Brown and ISA Brown strains of hen, with one farm using an Ingham Hisex strain. The US farms used the Hyline White 36 strain, with one farm using a Lohmann strain. All hens were aged between 26 and 106 weeks of age. The preliminary results for the

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relationships between human behaviour and hen fearfulness at eighteen of these sheds are discussed.

The amount of avoidance behaviour that an animal displays to humans is considered indicative of the level of fear of humans that the animal is experiencing (Hemsworth and Coleman, 1998). The level of fear of humans in laying hens in the present study was assessed by measuring the withdrawal response of birds to an unfamiliar human using several behavioural tests (adapted from Cransberg *et al.*, 2000; Hemsworth *et al.*, 1993). The primary test used was The Approaching Human Test, and was conducted at every 10^{th} cage along the aisle resulting in a total of 1233 focal cages from the US and 598 focal cages from Australia. This discrepancy in cage numbers is due to the large variation between Australia and the US in shed size. The Australian sheds housed between 1300 to 29000 birds whilst the US sheds housed between 40500 and 183500 birds. During the Approaching Human Test the observer moved directly in front of the focal cage for 5 s and then stepped away for another 5 s. This procedure was repeated twice over a 20-s period. During each 5-s period, the experimenter counted the number of birds with their heads extended through the front of the cage, the maximum number of birds that moved their head into the front 5 cm of the cage, and made a point count of the number of heads still at the cage front at the end of each 5-s period.

This test resulted in a large number of variables, and a Principal Component Analysis was used to reduce the number of variables studied to a manageable number by identifying a small number of components or groups of variables that were statistically interrelated. The two resulting variables accounted for 81 % of the variance in the avoidance response. These data were converted to scores and labelled as the variables 'FORWARD SCORE' and 'HEADS OUT SCORE'. A high score for either was considered to indicate a low fear of humans. Shed averages of these scores were used in analysis.

Stockperson activity in the shed was observed over a 2-d period, and the total amount of time that stockpeople spent in different areas of the shed and the total number of human interactions with birds (tactile, close visual and auditory contact) were recorded. From these observations, the following behaviour variables were calculated: presence in the aisle without cage approach ('AISLE PRESENCE', frequency/m of aisle length), close approach to birds ('CLOSE APPROACH', frequency/m of aisle length), hand in cages ('HAND IN CAGE', frequency/m of aisle length), total number of husbandry activities that occurred in the aisles ('HUSBANDRY ACTIVITIES', frequency/m of aisle length) and total time in shed (TIME IN SHED, secs) . The duration of noise created by stockpeople using various equipment was recorded ('NOISE', frequency/m of aisle length). Frequency of the above behaviours was recorded using a 5-s bout criteria interval. The attitudes of the stockpeople toward the laying hens and their work were also assessed through the use of an attitudinal assessment questionnaire, however these data are not presented here.

Shed parameters such as aisle length, cage width, number of birds in the shed, space allowance and light levels were also collected. Productivity records were obtained where possible, however records for Australian farms, particularly for feed conversion efficiency, were not always available. The productivity variables included were peak hen day production ('PHDP'), cumulative percent mortality at 29 wks of age ('MORTALITY'), peak feed conversion efficiency in kg of feed per kg of eggs ('PEAK FCE') and FCE at PHDP ('FCEp').

III. RESULTS

Substantial variation in shed size and bird behaviour was observed between Australia and the USA. An independent-samples t-test was conducted to compare the variables collected in each country (SPSS Version 14.0), and significant differences were found for

shed parameters, human behaviours and bird behaviours. These country differences were adjusted for by subtracting the mean country value from individual variables for each farm prior to analysis.

Correlation analyses were conducted using the Pearson correlation. There were no significant relationships between shed parameters and any of the bird behaviour (fear of humans) variables and few significant relationships between bird behaviour and productivity variables. The correlations between the main human behaviour and bird behaviour variables are presented in Table 1, and the correlations between the main human behaviour and shed variables are presented in Table 2.

Table 1. Correlations between the main bird behaviour, human behaviour and productivity variables

	Adjusted Forward Score		Adjusted Heads Out Score	
	r	P-value	r	P-value
NOISE	-0.63	0.007	-0.51	0.036
AISLE PRESENCE	0.45	NS	0.71	0.001
CLOSE APPROACH	0.45	NS	0.60	0.011
HAND IN CAGE	0.48	0.05	0.51	0.038
FCEp	0.26	NS	0.81	0.003

Table 2. Correlations between the main human behaviour and productivity variables

	PHDP		Mortality		Peak FCE		FCEp	
	r	P-value	r	P-value	r	P-value	r	P-value
TIME IN SHED	0.08	NS	-0.57	0.034	-0.63	0.052	-0.51	NS
HUSBANDRY ACTIVITIES	0.09	NS	-0.56	0.039	-0.66	0.038	-0.45	NS
NOISE	0.75	0.002	-0.15	NS	0.11	NS	0.43	NS
AISLE PRESENCE	0.11	NS	-0.21	NS	0.02	NS	-0.73	0.016

IV. DISCUSSION

While the sample size is limited, there are some consistent findings that support the hypothesis that human interactions may affect bird welfare and productivity.

Fear is generally considered an undesirable emotional state of suffering in both humans and animals (Jones and Waddington, 1992) and one of the key recommendations proposed to the United Kingdom Parliament by the Brambell Committee in 1965 (Anonymous, 1965) was that intensively-housed livestock should be free from fear. In this study a number of human behaviours were associated with fear. The duration of noise in the sheds was correlated with bird fear, with increasing duration of noise associated with increased fear. The majority of noise observed in the sheds was related to the use of air hoses and leaf blowers used for cleaning and indeed Campo *et al*. (2005) found that 1 h of exposure to a 90dB noise stimulus resulted in an increased stress (heterophil to lymphocyte ratio) and fear (tonic immobility duration) response in laying hens. In contrast to the behavioural data, the duration of noise in the shed was positively correlated with peak HDP. Whilst loud noise may be aversive, a clean shed may improve HDP.

Increased frequency of some stockperson behaviours, such as presence in the aisle, close approach and hands in cages, was correlated with reduced fear. Placing a hand in the cage would have been considered by many to be an intuitively aversive procedure for the hens.

While few fear variables were correlated with productivity, a number of human variables corresponding to the amount of human contact, such as time in shed, presence in the aisle and husbandry activities, were related to productivity and mortality. Increased human contact was associated with better peak FCE and lower mortality. These correlations may reflect increased opportunity for stockpeople to identify and address production problems.

In contrast, increased presence in the aisle was associated with poorer FCE at peak HDP. Explanations for this correlation are not obvious.

While the number of farms studied is small, the observed relationships between human behaviour, bird behaviour and productivity provide limited support for the hypothesis of a sequential relationship between human behaviour, hen fearfulness and productivity in the egg industry. However, it should be noted that few fear variables were associated with productivity. This work provides a basis for further research on the effects of human behaviour on bird welfare and productivity, and methods for reducing fear of humans in laying hens. The human behaviour and fear relationships found in the Australian and US industries are an important finding since the need to reduce fear to improve welfare is becoming increasingly recognised.

ACKNOWLEDGEMENTS

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CONSISTENT SITE SELECTION FOR EGG LAYING IN CAGES WITH A NEST BOX

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Summary

Individual egg-laying patterns were studied in 56 Hy-Line Brown hens housed in groups of 2, 4 or 8 hens in cages measuring 1.2 m wide by 0.5 m deep with a nest box. Hens showed a consistent choice in egg-laying site. From the eleventh to the fortieth egg, 66% of hens consistently (at least 80% of their eggs) laid in the nest box and 27% of hens consistently laid on the wire floor in an area equivalent in size to the nest box. The findings raise a number of questions that will be investigated in the future, such as what the physical and social factors were that influenced the establishment of a nest box as the preferred egg-laying site, and for those hens that laid on the floor, even though a nest box was present, the size (area) of the preferred egg-laying location and the features of the location.

I. INTRODUCTION

A key issue in the debate on the welfare of laying hens in cages is the importance of the nest box (NB). While no differences were found due to the presence of furniture, including a NB, on physiological parameters of welfare (Guesdon *et al*., 2004; Barnett *et al*., 2005), other studies have equated reduced welfare with lack of a suitable nest site and the consequent inability to perform nesting behaviour or the performance of frustration behaviours such as pacing (see Cooper and Albentosa, 2003; Weeks and Nicol, 2006). The latter authors also concluded that hens place a high value on access to a 'suitable' (discrete and enclosed) nest site for egg-laying. However, while the motivation of hens to access such a nest site increases as the time of egg-laying approaches (Cooper and Appleby, 1995; Freire *et al*., 1996), some hens choose not to lay in the NB. For example, the incidence of floor eggs in cages with a NB has been reported to range from 10-57% (Wall *et al*., 2002; Guesdon and Faure, 2004; Cronin *et al*., 2005). We are studying the importance of the NB and pre-laying behaviour for the welfare of hens in cages. Measuring the consistency of egg-laying site, defined as repeated laying in one particular location, is likely to improve our understanding of the importance of the site to the hen. This paper presents preliminary data on consistency of egg-laying site by hens in cages with a NB.

II. MATERIALS AND METHODS

Hy-Line Brown hens were introduced at 16 weeks of age to commercial cages measuring 1.2 m wide, 0.5 m deep and 0.45 m high at the rear of the cage. Each cage contained a NB, located at the right side, measuring 0.24 m wide, 0.5 m deep and 0.27 m high at the front. The floor was 'astro turf' (0.37 m x 0.22 m x 15 mm thick). The cages were located in two adjacent controlled climate rooms and the experiment had 2 replicates in time. In each replicate there were three observation cages per room (within a bank of 10 cages) with a group of 2, 4 and 8 birds. Thus there was a total of 56 hens in the experiment. Day length was increased each week by 30 min until the hens were exposed to 16 h light per day at 22 weeks of age and feed and water were available *ad libitum*.

A continuous video record was made of all birds from the day of introduction until

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38 weeks of age. Video cameras with built-in infra-red (IR) lights were positioned above and below cages as well as inside the NB to provide vision of each hen at all times. At introduction to the cages spiral leg bands were placed on the hens. Leg bands were either white or black and were applied so that each bird within a cage had a unique combination of bands. In addition, birds were marked on the head and back feathers with carbon-based ink (Cronin and Desnoyers, 2005). This combination of markings enabled individual identification of hens under IR light on the video record. From 16-25 weeks of age, the date, time and location of every egg laid by the hens were recorded. From 26-38 weeks of age, oviposition data were sampled on 3 days per week (Tues-Thurs). Analysis of variance was used to examine differences due to group size and replicate on age of hens at first egg and live weight (adjusted for bird age covariate) (GenStat 8.1, Lawes Agricultural Trust) and the experimental unit was the cage of birds.

The consistency of egg-laying site was analysed from each hen's records for her first 40 eggs by first identifying where each egg was laid (NB or cage floor). For the floor eggs, oviposition location was quantified according to 4 areas across the width of the cage, equivalent to the area of the NB. Data were examined in two ways using: 1) histograms of 10-egg cohorts to determine the number of hens that used the NB for oviposition and the number of eggs they laid in the NB within the cohort, and 2) Pearson's goodness of fit statistic. For the latter, the expected number of counts for each of the 5 cage locations was calculated as the number of eggs laid times the average (over birds) proportion of eggs laid in the location. The Pearson's goodness of fit statistic is an index of the consistency of laying in particular locations, allowing for the possibility there may be different preferences.

III. RESULTS

Age at first egg was affected by the batch of birds (cage means were 138 vs 128 days, respectively for replicates 1 and 2; sed 2.5, $P<0.01$). There was no effect of group size and no interaction. The pooled mean values (SD, minimum and maximum) for replicates 1 and 2 were 137.9 (5.01, 127, 146) and 128.3 (7.97, 113, 147) days. Mean live weight of birds at 115 days of age tended ($P=0.098$) to be lower in replicate 1 than 2 (adjusted for age: 1.51 vs 1.66 kg, respectively; sed 0.079). By 173 days there was no difference in mean live weight (adjusted for age: 2.00 vs 2.12 kg; sed 0.109; $P=0.31$).

As indicated in Figure 1, 44.6% of first eggs were laid in the NB. While the proportion of NB eggs increased between egg 1 to 7, from egg 8 to 40 the proportion of NB eggs was relatively static, fluctuating around a mean of 70.1% (Std Dev 2.26). The consistency of NB use for oviposition is first described by histograms showing the number of hens that laid from 0-10 eggs per 10-egg cohort in the NB (Figure 2). For the first 10 eggs laid, there was a broad distribution of eggs laid per hen in the NB. Thereafter, there is evidence of consistency of NB use. For example, if consistency was defined as '100% eggs laid in the same site', then the number of hens that laid all eggs in the NB was 15, 28, 33 and 31 hens, respectively, for the 10-egg quartiles. In comparison there were 8, 10, 12 and 11 hens, respectively, that laid zero eggs in the NB in the 10-egg quartiles. If the definition was less strict, eg. at least 80% of eggs laid in the NB, then 31, 37, 38 and 37 hens, respectively, laid 8-10 eggs in the NB across the quartiles. The number of hens that laid 0-2 eggs in the NB was 15, 16, 14 and 14, respectively. Of the remaining hens (inconsistent NB layers that laid 3-7 eggs in the NB), the number of hens per quartile of ten eggs was 10, 3, 4 and 5, respectively.

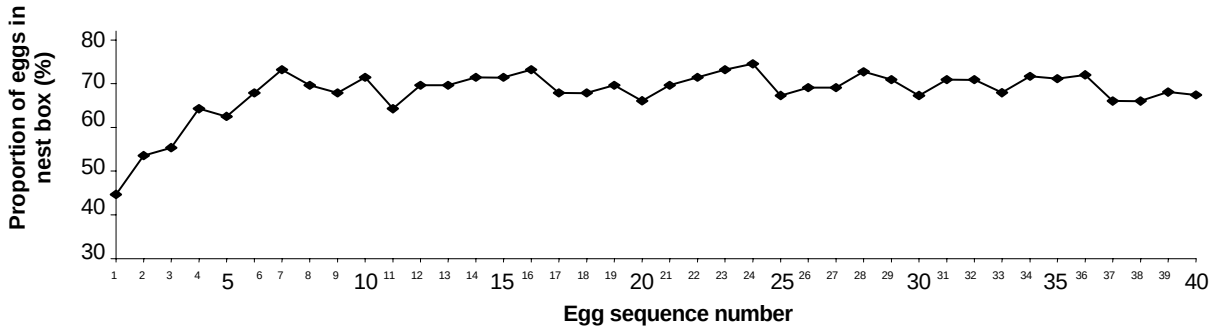

Figure 1. The proportion of eggs laid in the NB over the first 40 recorded eggs per hen.

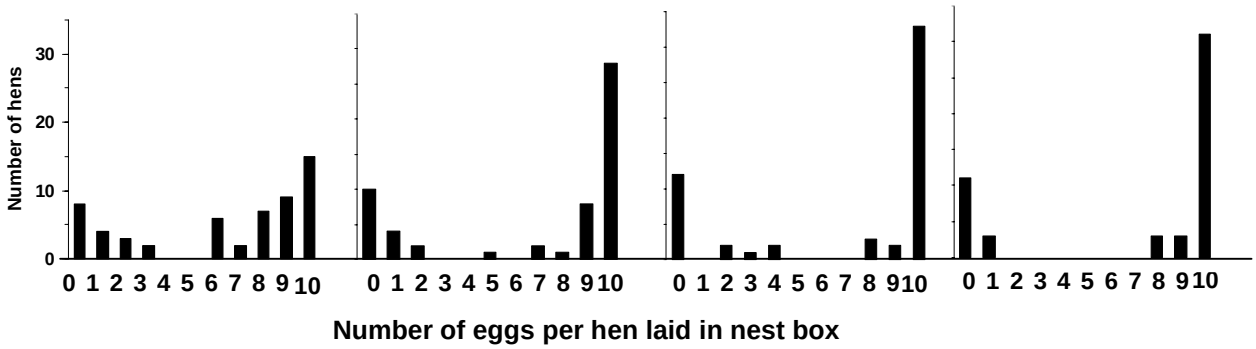

Figure 2. Histograms of the number of hens laying in the NB, from zero to 10 eggs, for the first (far left histogram) to fourth (far right histogram) quartile of 10 eggs laid; N=56 hens.

Pearson's goodness of fit statistic scores were calculated for each hen (10 eggs per hen per quartile). The mean scores across the quartiles were 22.8, 29.1, 30.2 and 28.8, respectively. For comparison, the Chi-squared values for $\nu = 4$ are 11.07 (P=0.05) and 18.47 (P=0.001). The score was lower than the P=0.05 significance level for only 16 of the 56 hens in the first quartile of eggs, then 10 of the 56 hens thereafter. The proportion of hens with scores greater than the P=0.001 significance level was 57% for the first quartile, and about 70% of hens thereafter. The Pearson's goodness of fit score also calculates consistency of use of non-NB locations. Based on the earlier definitions of consistency, only one hen was found to lay all her eggs in a quartile (the fourth) in the one location outside the NB on the wire floor. For the less-strict definition of at least 80% of eggs laid in one location, there was a total of 8 hens that consistently laid in the one location. These hens all laid at the far left of the cage on the opposite side to the NB.

IV. DISCUSSION

Preliminary analysis showed that most hens laid at a consistent site. Consistency of site selection seemed to develop over the period in which the first 10 eggs were laid. Using our two definitions of consistency, that hens laid 100% and 80%, respectively, of eggs in a single location, 54% and 66% of hens were consistent NB-layers from their eleventh egg onwards. For the remaining hens, 3.8% (1 of 26 hens) would be defined as 100%-consistent and 15.8% (3 of 19 hens) as 80%-consistent floor layers, using a single location outside the NB on the wire floor. Similar findings were reported by Cooper and Appleby (1996), who studied NB use by 20 birds housed individually in cages from point of lay for 6 weeks. While the latter authors found that the incidence of floor eggs was 25% in the first week of lay, this

proportion declined to 5% by week 6 and consistency of NB use correspondingly increased, such that in weeks 2-6, 70% of the hens laid all eggs in the NB. Further, 30% of hens were classified by Cooper and Appleby (1996) as consistent floor layers. In our experiment the apparent lower consistency of laying on the cage wire floor may be due to the arbitrary sub-division of the wire floor into 4 equal areas (of equivalent size to the NB). This aspect needs to be further examined (see question 2 below).

The observed difference of 10 days in age at first egg between the two replicates was due to hens being lighter in the first replicate. While we have not determined if age at first egg has an influence on consistency of egg-laying site, this could be determined from further analysis of the data. Further study of the digital video records will provide a wealth of data, including information on the following questions:
1) For hens that either consistently lay in a NB or on the cage floor, are some hens forced into a less preferred location? The future examination of social dominance hierarchies within cages may provide relevant information. 2) Is the percentage consistency of cage floor layers affected by the arbitrary definition of the 'egg laying site'? This will be evaluated by using a 12-location grid compared to the current 4-locations. Future examination of the behaviour data around the period in which the first 10 eggs are laid may provide additional information on the development of consistent egg-laying site selection for both NB- and floor-layers. 3) For hens that lay on the floor, what is the area of the preferred egg-laying site and the features of the site (eg. near a wall, away from the feeder, etc)? Are the sites of egg laying similar or different in size to a NB?

ACKNOWLEDGEMENTS

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ENVIRONMENTAL ENRICHMENT STRATEGIES FOR IMPROVED WELFARE OF EXPERIMENTAL LAYER CHICKENS HOUSED IN ISOLATORS

K.G. RENZ[1] and S. W. WALKDEN-BROWN[1]

Summary

The aim of this study was to reduce feather pecking in layer chickens kept in isolators for research purposes by inclusion of bunches of string and/or a sand box in a 49 day experiment in non beak trimmed SPF White Leghorn chickens. Inclusion of string from day 0 significantly reduced the incidence of feather pecking and skin injury and significantly improved ($P<0.001$) feather coverage and feather condition score. Inclusion of a sand box from day 14 onwards did not significantly affect these variables although there was a trend for improvement in each case, particularly when combined with the presence of string. The inclusion of a sand box at day 0 warrants further investigation. We have shown that simple environmental enrichment within isolators can lead to a significant reduction in feather pecking behaviour and now routinely include string in our experiments.

I. INTRODUCTION

There are two hypotheses to explain the origin and development of feather pecking. According to Blokhuis and Arkes (1984), feather pecking evolves as redirected ground pecking. However, Vestergaard et al. (1993) consider feather pecking to be the result of an abnormal development of the perceptual mechanism responsible for detection of substrates for dust-bathing. Feather pecking has potentially catastrophic implications for the birds' welfare for several reasons: i) they may suffer pain when they are pecked (Gentle, 1986); ii) the occurrence of pecking-related feather loss increases susceptibility to injury; iii) feather pecking and the removal of feathers can cause bleeding from deeper damage to the skin or follicles and may thereby lead to further cannibalism and the painful death of target birds. Birds which are kept in isolators for research purposes are typically maintained in a relatively bare environment optimised for disinfection rather than bird welfare. They are usually also handled intensively. These birds are therefore at risk of developing harmful behaviours including feather pecking. Remedial measures like beak trimming and reduced light intensity are often associated with welfare problems themselves. For example, beak trimming can cause chronic pain (Gentle, 1986) and keeping the birds under low light intensities not only impoverishes the visual environment but it can also result in malnutrition and in the development of eye abnormalities.

In our isolator facility at the University of New England, we have found that feather pecking can be a welfare issue in commercial layer and specific pathogen free (SPF) White Leghorn chickens whereas it is rarely a problem in broiler chickens. This experiment was therefore designed to test the effect of two forms of environmental enrichment on feather cover and the incidence of feather pecking in SPF White Leghorn chickens.

II. MATERIALS AND METHODS

The experiment was conducted as a 2x2 factorial design with 6 replicates using 24 identical isolators over a time period of 7 weeks. The two factors in the experiment were the

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presence or absence of 2 bunches of sterile yellow polypropylene hay baling twine (string) or the presence or absence of a sand bathing box. Each isolator contained 19-20 chickens which were challenged by a variety of Marek's disease virus (MDV) isolates as part of a separate study with a different MDV isolate for each isolator. Bunches of string were suspended from the top of the isolator to approximately 2.5 cm off the floor. Sand boxes were 47x37x15cm cat litter boxes with in-turned top edges to limit ejection of material by scratching. Each contained 2kg sterile washed river sand. Chickens were maintained at 12D:12L photoperiod with moderate light dimming. Lights were turned up to full strength during working and inspection periods. The 470 experimental chickens were SPF White Leghorn chickens (SPAFAS Australia bird, Ex CSIRO WLH line). They were not beak trimmed. Chickens had access to the string bunches throughout the experiment but access to the sand boxes was provided from 2 weeks of age when the chickens were big enough to get in and out the box. The incidence of feather pecking in each isolator was estimated weekly throughout the experiment. Average feather coverage was also estimated for each isolator with 100% representing complete feather cover and 0% no feather cover. Mean isolator feather condition score and skin injury score was estimated using the scoring system of Bilcik and Keeling (1999). Feather condition was scored from 0-5 with 5 being almost totally denuded, and skin injury was scored from 0-4 with 4 representing a wound larger than 2cm in diameter. Score and percentage data were not normally distributed and not amenable to transformation so were coded as ordinal and subjected to ordinal logistic analysis using JMP IN 5.1 (SAS Institute, NC, USA). The effects of string, sand box, day of sampling (chicken age) and their interactions were fitted, with non-significant interactions removed from the final model. Because data are not normally distributed, standard errors are not provided with means.

III. RESULTS

The incidence of feather peck throughout the experiment is shown in Figure 1. It was significantly affected by string ($P<0.001$) and chicken age ($P=0.003$) but not sand box ($P=0.170$) with no significant interaction between these effects, although there was a trend towards a interaction between the effects of sand box and string ($P=0.18$, Figure 1A,). Chickens with string in their isolators had a lower incidence of feather peck than those without string (2.02 and 7.16% respectively, $P<0.001$). The effect of age was manifest mainly as a sharp increase in incidence between weeks 2 and 3 of age as shown in Figure 1B. The trend towards interaction between the effects of sand box and string suggests that the effect of string may be greater when a sand box is present.

Feather coverage was significantly affected by String ($P<0.001$) and chicken age ($P<0.001$) but not sand box ($P=0.616$) with no significant interaction between these effects (Figure 2A). Chickens with string in their isolators had a higher feather coverage score than those without string (97.0 and 89.9% respectively, $P<0.001$). The effect of age was manifest mainly as a sharp decrease in feather coverage between weeks 2 and 3 of age as shown in Figure 2B.

Skin injury score was significantly affected by String ($P<0.001$) and chicken age ($P<0.001$) but not sand box ($P=0.350$) with no significant interaction between these effects although there was a trend towards an interaction between the effects of sand box and string ($P=0.196$). Chickens with string in their isolators had a lower mean skin injury score than those without string (0.33 and 1.1 respectively, $P<0.001$). The effect of age was manifest as a small increase in incidence between weeks 1 and 2 of age, followed by a large increase between weeks 2 and 3 of age with no further increase beyond this. The trend towards interaction between the effects of sand box and string is such to suggest that the effect of string may be greater when a sand box is present.

Feather condition score was significantly affected by String (P<0.001) and chicken age (P<0.001) but not sand box (P=0.547) with no significant interaction between these effects. Chickens with string in their isolators had a lower mean feather condition score than those without string (0.54 and 1.45 respectively, P<0.001). The overall effect of age was manifest as a small increase from 0 to 0.29 between weeks 1 and 2 of age, followed by a large increase to 1.54 in week 3 followed by a gradual decline thereafter to a value of 0.58 at the end of week 7.

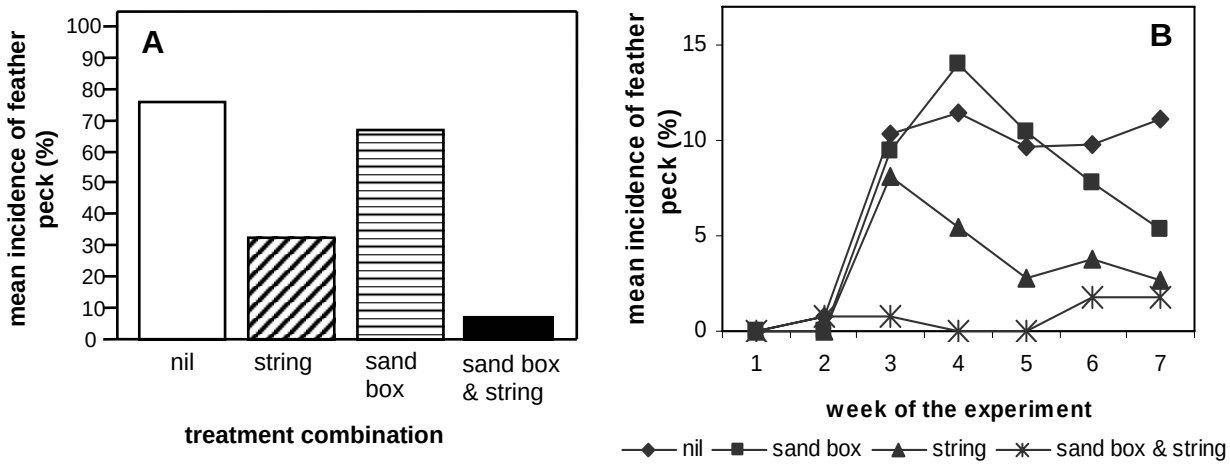

Figure 1. Mean incidence of feather pecking by treatment (A) and by treatment and week of the experiment (B).

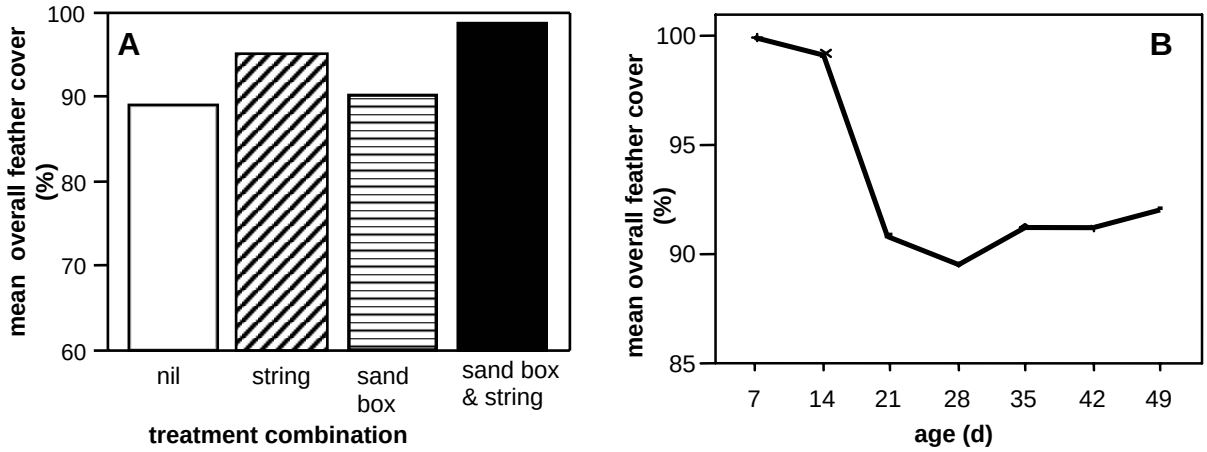

Figure 2. Mean feather coverage score by treatment (A) and by chicken age for all treatment groups (B).

IV. DISCUSSION

This study has clearly demonstrated the benefits of inclusion of string in isolators in terms of limiting feather pecking and skin damage and improving feather cover and condition. The effects of including a sand box were less clear with no significant effects on these variables although they trended towards more favourable in each case. For every variable, the most favourable outcome was observed for the isolators in which the sand box and string were both included. Sand boxes were not introduced until day 14 of the

experiment, just as feather-pecking activity was escalating. This may have contributed to the lack of significant efficacy. Providing access to a shallower sand box for the initial two weeks is something worth investigating in the future. The larger sand box was too high for chick access during the early period and this is why access was delayed until day 14. The introduction of sand boxes is unlikely to have been causally associated with the increase in feather pecking observed at the time of introduction as the increase was also observed in isolators without sand boxes. It is more likely that the timing was associated with behavioural changes and the changeover from chick down to proper feathers during this period. Infection with MDV is a confounding factor in the design and could possibly have affected the incidence of feather pecking as a reaction of the birds suffering from the infection. However, only 2 birds died or were euthanised with evidence of severe pecking injury, one of which showed atrophy of the thymus and bursa typical for infection with MDV. The use of 6 replicates (isolators) for each enrichment treatment combination, with random allocation of MDV isolates to isolators also mitigates against a systematic effect of MDV isolate. Furthermore, the peak of MDV-induced lesions was observed on day 38 post challenge, considerably later than the major rise in feather pecking activity between days 14 and 21, and during a period where there was no increase in severity of feather pecking lesions. Several birds in all isolators regardless of environmental enrichment had minor feather damage which may have been due to sharp edges on the galvanized steel feeder boxes. This may have acted as a trigger for feather pecking, especially in the isolators with no environmental enrichment. During the last 3 weeks of the experiment, there was no major increase of feather pecking. Instead, feathers of pecked birds started to regrow and skin injuries started healing as well leading to improvement of the overall feather coverage as shown in Figure 2B. The findings of this study are consistent with those of Jones (2002) that bunches of string attracted continuous interest from the chickens and reduced feather pecking. The results of the study support the hypothesis of feather pecking as redirected ground pecking as described by Blokhuis and Arkes (1984) rather than an abnormal behaviour due to lack of substrates for dust-bathing (Vestergaard et al., 1993) as sand boxes did not reduce feather pecking to the same extent as string bunches.

The results of this experiment clearly demonstrate that simple environmental enrichment such as the hanging of bunches of string in isolators can significantly reduce feather pecking in chickens housed in isolators. Further research into the use of sandboxes is indicated, ensuring that access is provided from the time of placement in the isolators.

ACKNOWLEDGEMENT

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GROWTH PERFORMANCE AND ITS PREDICTION IN TWO COMMERCIAL STRAINS OF MEAT DUCKS

G.C. HAY[1] and T.A. SCOTT[2]

Summary

A study was made of the growth performance and body composition of two strains of meat ducks presently used by an Australian duck producer (Pepe's Pty Ltd). 432 day-old ducklings from each of two strains of Pekin ducks (Cherry Valley (CV; UK) and Grimaud Frères (GF; France)) were grown to 41 d to develop a growth curve whilst determining water and feed intake, feed efficiency, metabolisability of dietary energy and carcass composition. For proprietary reasons, the strains have been coded and are referred to here as A and B. All birds exceeded the suggested optimum market liveweight at 42 days of 2.85kg, with a mean of 3.3 kg at 41 days. Strain B birds were heavier (13.5%) at market age, consumed more feed, yet had similar mean feed conversion ratio (FCR) and breast muscle yields to strain A. The AME study showed no significant difference in metabolisability of dietary energy between the strains and sexes. A simple growth model determined a close relationship between seven day weight and 41 day weight across the four sex X strain groups.

I. INTRODUCTION

In 2005 Australia produced approximately five million ducks, equal to 10000 tonnes of duck meat (FAOSTAT, 2006). This is less than 0.5 % of world production, yet the industry provides direct employment for over 400 individuals (Holdings, 2001). Due to changing cultural demographics, the demand for duck meat in Australia is increasing. The industry must meet increasing consumer demands by providing birds of an appropriate size and conformation, with methods that are both environmentally and welfare friendly. The Cherry Valley (CV;UK) and Grimaud Frères (GF; France) strains (based on white Pekin) have the genetic potential to attain an average (male and female) market weight of 3.3 and 3.5kg at 47 and 49 days, respectively, with a FCR of 2.35 and 2.40, respectively for the two strains (Cherry Valley Farms, 2006; Grimaud Frères, 2006). Breast muscle is the most popular portion of meat, being one of the most edible cuts of the duck carcass. However, breast muscle yield at slaughter only ranges from 15 to 20 % of the carcass (Holdings, 2001) and is influenced by age at marketing.

Producers and processors would benefit from a growth model that allowed accurate prediction of weight for age for the different available strains. Mazanowski *et al*. (2003) suggested that the optimum slaughter age of ducks is between seven and eight weeks of age, when meat deposition is levelling off and the carcass still has a relatively low level of fat. However, at this age body weight of the bird exceeds that required by the Australian market of about 2.85 kg (Pepe's Pty Ltd, pers. comm., 2006).

II. MATERIALS AND METHODS

A total of 864 day old ducklings (216 for each strain (CV vs GF) and sex) were obtained from Pepe's Commercial Duck Hatchery, Kenthurst. Each treatment was randomly assigned with six pen replicates each of 36 birds and 24 floor pens in total. Each pen had

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wood shavings litter, a water line with four nipple drinkers and two hanging feeders. Supplemental heat was provided by electric brooders in each pen and the height of the brooders was adjusted to accommodate duck comfort. Water and feed were provided *ad libitum* and intake of both was measured weekly. A starter diet (220 g CP/kg, 2900 ME/kg) was given to all birds from 0 to 14 d followed by a grower diet (190 g CP/kg, 3050 ME/kg) from 14 to 41 d. At seven days of age, all birds were wing banded and birds were individually weighed weekly. The project was approved by the University of Sydney Animal Ethics Committee.

One bird per pen was selected and humanely killed for carcass analysis each week. The measurements for each bird included body weight, skinless breast (bone in), drum and thigh (skinless), liver, empty gizzard, and length of intestinal segments (upper and lower small intestine and total length of caeca). All carcass components were expressed relative to body weight for statistical analysis.

At two weeks of age, four birds per pen were transferred to 24 bioassay cages and given the grower diet with 1% added Celite™, an acid insoluble ash marker. After a one week adjustment period, excreta samples were collected. Excreta was frozen, freeze-dried, finely ground and then the gross energy and acid insoluble ash content determined and used to calculate AME (MJ/kg diet).

III. RESULTS

It was hypothesised that males would have faster growth rates than females and that strain A and B ducks would have similar growth rates, but potentially different breast meat yields. At completion of the trial, all birds exceeded the suggested target market weight of 2.85 kg at 42 days of age, with an overall mean body weight of 3.27 kg at 41 days. The average weekly growth of each sex within strain is presented in Figure 1. While males (3.37 kg) were heavier than females (3.16 kg), they had a lower mean proportion of breast muscle in their bodies (12.4 and 13.6 % respectively). Strain B birds were heavier (by 13.5%) at market age, consumed more feed (by 15.3%), had smaller gut proportions, but had similar feed conversion ratios (2.36) and breast muscle yields to strain A birds (13.3%). Tables 1.1 and 1.2 present the means for FCR and breast muscle for the strain X sex groups. The study also demonstrated that Pekin ducks drink large quantities of water, with all birds having a water to feed ratio of 3.3:1. There was no significant difference in AME between the four strain X sex groups.

A simple prediction equation for growth (Figure 2) was developed and shows a significant relationship between seven day body weight and 41 day body weight. Further research will be required to develop growth curves for the respective strains X sex groups relative to season, diet and management.

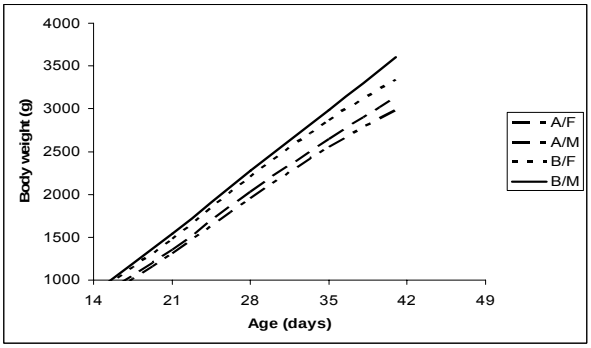

Figure 1. Average weekly growth of two strains of commercial Pekin duck, and two sexes from hatch (0 days) to market (41 days). (A and B denote strains, M and F denote sexes).

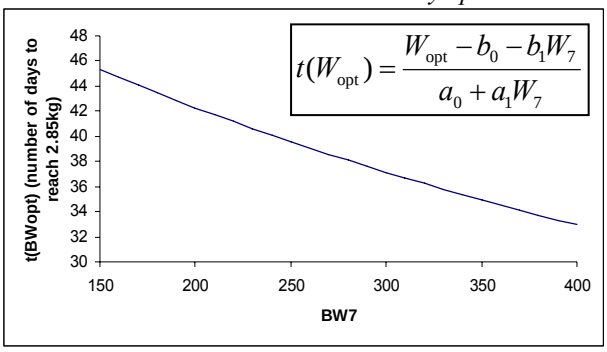

Figure 2. Predictor model of two strains of commercial Pekin duck, and two sexes.

Table 1. The effect of strain and sex on FCR (± SE) in commercial Pekin ducks from hatch to 41 d

	FCR		
Treatment interaction	0 - 14 d (starter)	14- 41 d (grower)	0 – 41 d (total)
Number of observations	24	24	24
Strain x Sex	NS	NS	NS
A x ♀	1.31±0.02	4.27±0.34	2.43±0.03
A x ♂	1.26±0.01	3.79±0.13	2.29±0.01
B x ♀	1.27±0.01	4.11±0.32	2.44±0.02
B x ♂	1.28±0.03	3.66±0.30	2.27±0.04

Table 2. The effect of strain and sex on weekly mean breast weight percentage (± SE) in commercial Pekin ducks from 7 to 41 d of age.

	Average Relative Breast Weight (%)					
Treatment interaction	7 d	14 d	21 d	28 d	35 d	41 d
Number of observations	24	24	24	24	24	24
Total	3.56±0.08	4.07±0.09	5.76±0.13	7.90±0.13	11.3±0.3	13.0±0.2
Strain x Sex	NS	NS	NS	*	NS	NS
A x ♀	3.60±0.12	3.70±0.17	5.66±0.22	7.43±0.12b	11.9±0.8	13.6±0.4
A x ♂	3.63±0.13	3.94±0.15	5.60±0.16	7.48±0.31b	10.5±0.7	11.8±0.5
B x ♀	3.74±0.13	4.38±0.16	6.20±0.28	8.77±0.29a	12.4±0.6	13.7±0.5
B x ♂	3.29±0.21	4.27±0.24	5.57±0.34	7.93±0.25a	10.2±0.5	13.0±0.4

* = P<0.05

IV. CONCLUSIONS

The success of the duck industry is dependant on the capacity to efficiently produce ducks of a given size and conformation at slaughter. Pepe's market the birds specifically at 2.85kg in order to meet restaurant preparation and cooking requirements. Any deviation from this weight will either reduce profit or value of the meat sold. In the present study, the mean body weights of the four strain X sex groups were greater than the suggested optimum market weight at the predefined market age of 42d. The smallest birds (strain A females) had

a market weight of 2.98 kg and the largest birds (strain B males) had a market weight of 3.61 kg. Thus none of the birds met market specifications.

It is evident that there is significant variation in the growth rates of ducks of different strains and sexes. Farhat and Chavez (2000) have identified that a problem with marketing birds at six weeks of age, is an insufficient yield of lean breast muscle. Females had a smaller body size but higher proportion of breast muscle in their bodies than males. It is accepted that females "mature" earlier, and are expected to have a higher relative breast yield at younger ages; however, females also lay down more fat earlier. This is important as it indicates there may be a benefit to growing ducks in a separated sex program. The birds in this study were grown in a separated sex program, and Farhat and Chavez, (2000) found that significantly greater body weights can be achieved when the sexes were grown separately, than when grown mixed. Furthermore, a greater degree of uniformity would be expected in separate sex-reared ducks and this has a significant effect on processing efficiency (i.e. equipment set for the average size may not efficiently de-feather the smaller birds and may cause damage to the larger birds). Duck producers tend to grow ducks in a mixed sex program as it is quite labour intensive to sex ducklings, due to their lack of sex linked phenotypes. Ducks must be sexed by vent sexing, which may result in some mortality and reduced adaptation to the housing environment during the first day after placement.

The simple growth prediction model formulated from the results of this study requires to be further developed to take into account the effects of a range of other factors (e.g. temperature, diet, strain, sex etc) which impact on growth performance and body conformation. The present disparity between optimum market weight of the available strains and the suggested market requirement for a 2.85 kg bird at 42 days, suggests a need to produce a smaller, lean and more "compact" bird.

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This work was kindly provided with resources by Pepe's Pty Ltd. We also acknowledge the help of the poultry staff at the University of Sydney, Camden. This study was part of a 4th year honours thesis.

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THE EFFECT OF SEASON AND HEN AGE ON EGG PRODUCTION AND FERTILITY OF COMMERCIAL PHEASANTS IN AUSTRALIA

I.A. MALECKI[1] AND G.B. MARTIN[1]

A commercial flock of the ring neck pheasant (*Phasianus colchicus*) was studied during the 2005/2006 breeding season to determine the effect of season and hen age on egg production and fertility. The flock was kept in a shed receiving natural daylight. The shed consisted of 12 pens each 30 m^2 in floor area. Six pens were each stocked with 60 1 yo birds and the other six with 60 2 yo birds; the sex ratio in each pen was 1M:5F. A pheasant breeder diet containing 12 MJ ME and 180 g CP/kg and water were provided *ad libitum* in open-type feeders and drinkers. The daily rate of lay per hen was calculated from daily egg records per pen. Fertility was estimated from 5% of fresh eggs collected daily from each pen over three two-week periods in early November, mid December and mid January. Eggs were stored at 8°C for up to 14 days, and then opened to determine morphology of the germinal disc. When the germinal disc did not contain a blastoderm the egg was deemed unfertilised.

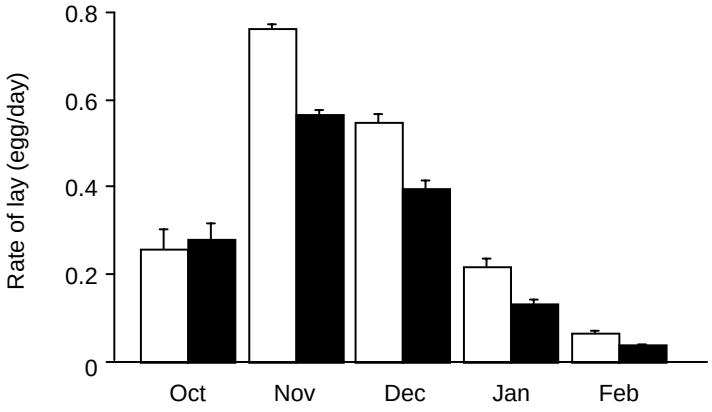

Figure 1. The mean (± sem) daily rate of lay of commercial pheasants (open bars - 1 yo old hens; closed bars - 2 yo hens).

The rate of lay of 1 yo and 2 yo hens was affected by month ($P<0.001$), age ($P<0.001$), and month x age interactions ($P<0.001$). The laying of 1 yo and 2 yo hens had a similar seasonal pattern starting in mid October (spring), reaching a peak in November (spring), then gradually declining in December and terminating in early February (summer). 1 yo hens had a higher ($P<0.01$) rate of lay than 2 yo birds in November, December and January, but there was no difference between the two in October and February. Fertility was not affected by female age ($P>0.05$) but showed a seasonal decline ($P<0.01$) from 96 ± 2% in November to 88 ± 2% in January. Pheasant production is thus constrained by a short breeding season and influenced by a highly seasonal pattern of egg production and fertility. Whilst fertility does not appear to be affected by hen age, 2 yo breeders are not as productive as 1 yo birds. Rate of lay and fertility of 1 yo hens in this study appear similar to those in commercial pheasant flocks in Northern Europe (Deeming and Wadland 2002; Kuznicka et al. 2005).

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WATER REQUIREMENTS FOR EARTHWORMS *(Eisenia andrei)* GROWN EXCLUSIVELY ON BROILER LITTER

J.R. TURNELL[1], G. HINCH[2] and R.D. FAULKNER[1]

Summary

The use of vermiculture to value-add to broiler litter (BL) has been seen as problematic due to the high levels of uric acid and salts in poultry manure. Results from this experiment indicate that these issues can be overcome with frequent watering over the first 8 days of the system's operation. The conversion of BL into vermicast was maximised with twice daily applications of 100 and 200ml of water. These frequent water applications also led to average total mortality being lower and healthier earthworms.

I. INTRODUCTION

Vermis is Latin for worm; therefore vermiculture is the intensive production of earthworms. Vermiculture systems world wide most commonly use two earthworm species, *Eisenia andrei* and *Eisenia fetida* (Dominguez *et al.* 2005, Elvira *et al.* 1996), and there are a wide variety of systems used. Vermiculture in Australia is receiving increased attention due to its potential to value-add to organic wastes such as animal manures while providing an alternative odour free disposal option (Edwards and Steele 1997). Vermicast is highly regarded as a plant growth promoter (Arancon *et al.* 2003) and soil improver (Ndegwa and Thompson 2000) and has also been shown to reduce pollution effects of animal waste on the environment due to the stabilisation of nutrients (Tripathi and Bhardwaj 2003). Vermiculture based bio-integrated waste management systems, specially developed for the poultry industry could produce vermicast and protein (vermimeal) at relatively low costs.

The Earthworm's ability to achieve homeostasis of body fluids in a terrestrial environment to some extent explains their evolutionary success (Wallwork 1983). Maintaining the right balance between water and salts has been shown to be critical to earthworm survival in wastes with high electrical conductivity (EC) such as broiler litter (Turnell *et al.* 2006). Ammonium (NH_4^+) concentrations in substrates above 0.5g/kg have been shown to kill earthworms, unless the substrate has been either washed or pre-composted (Edwards 1995). This poses a challenge to the use of BL as a substrate due to high NH_4^+ concentrations in BL (Edwards *et al.* 1985). The importance of NH_4^+ was illustrated in a vermiculture study using cow manure, where effective performance was only achieved when using urine free manure (Elvira *et al.* 1996).

Poultry wastes including BL, dead birds, hatchery waste and bird processing sludge are all potential food sources for earthworms. The purpose of the present study was to determine if earthworms could be exclusively grown on BL and the effect of water application rate of BL conversion into vermicast.

II. MATERIALS AND METHODS

Small containers were used as the basis for a mini replicated vermiculture experiment. BL was collected at shed cleanout (~40 days old) chilled and transported to the University of New England (UNE). The BL (15% moisture content) was then homogenised

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without removing the feathers and 900g was loaded into each 4800cm^3 experimental container. Mature adult earthworms (*Eisenia andrei*) were supplied from a commercial vermiculturalist, and by using light retraction methods, were separated from their bedding ready for loading into each container. All containers were then covered with a dense black polypropylene lid to reduce light, leaving a small gap (1.5 cm) to allow for gas transfer.

Two experiments lasting 23 days with 5 replicates of each treatment group were performed. Experiment 1 used two volume applications of water (100 and 200ml) applied at 0.5, 1, 2, 3, 4 or 5-day intervals as well as one treatment of 50ml only applied every 0.5 days (12 hours). Experiment 2 used two water volumes (100 and 200ml) at daily intervals with watering ceasing on either day 8, 13 or 23 of the experiment.

Physical attributes of the earthworms were measured including mortality, dispersion and retraction. A dispersion score from 1 to 4 was assigned based on behavioural patterns, with 1 representing highly dispersed earthworms on the surface and 4 indicating earthworms bunched together. Retraction rate was measured by timing how long it took for earthworms to retract away from light with immobile and dead earthworms excluded from this measure. A retraction time greater than 60 s indicated that the earthworms were avoiding the substrate. After earthworm retraction had occurred the dead earthworms left behind were counted to give daily mortality, and then very lightly covered with moist coconut husk (<0.25g/container) which prevented these earthworms from being counted again at a later assessment. On day 23 all containers were destructively sampled in order to determine the proportion of BL converted into vermicast. For the duration of the experiment the substrate was at field capacity, therefore substrate moisture content was not collected.

III. RESULTS

(a) Experiment 1

The conversion of BL was effected by the experimental factors (watering interval and volume, Figure 1). However there was a treatment effect whereby 100ml/0.5d achieved greater BL conversion (P<0.001) than 100ml/1d, this relationship did not hold for the 200ml/0.5d and 200ml/1d. The 50ml/0.5d treatment suggests that less BL will be converted over 23 days than by either 100 or 200ml applied once or twice a day.

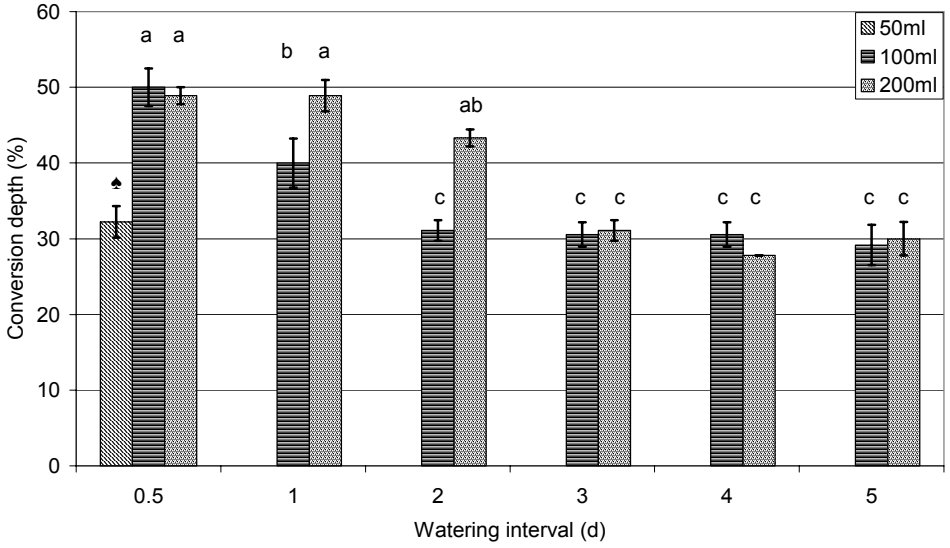

Figure 1. Mean depth of converted BL over 23 days as a response to watering interval and volume (±SE)
♠ 50ml applied twice daily representing a low volume/small interval treatment. Different superscripts show significant differences between treatments (α=0.05) ^chi-square transformation

Both experimental factors had an effect on percentage earthworm mortality, and a treatment effect was apparent, where 100ml/3d had higher mortality ($P<0.05$) than 200ml/3d. In comparison there was a trend indicating that 100ml/0.5d would result in lower mortality than 200ml/0.5d (Figure 2).

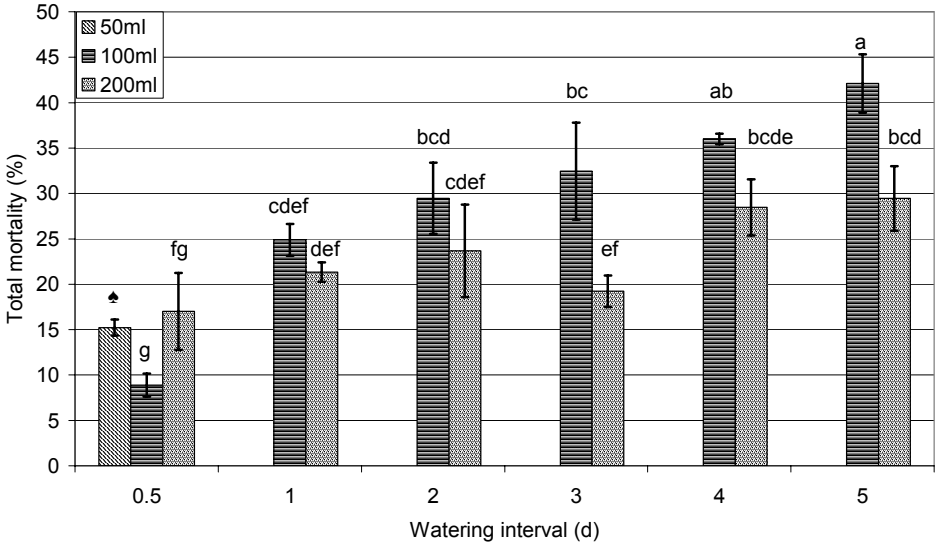

Figure 2. Mean total percentage mortality over 23 days as a response to watering interval and volume (±SE)
♠ 50ml applied twice daily representing a low volume/small interval treatment
Different superscripts show significant differences between treatments ($\alpha=0.05$)

As watering interval increased earthworm dispersion scores and retraction rates also increased ($P<0.001$), however water volume had no significant effect (Table 1).

Table 1. The effect of watering interval on earthworm dispersion and retraction

	Watering interval (Int.)					
	0.5	1	2	3	4	5
Dispersion (score)^	1.33 ^{d}	1.35 ^{d}	1.53 ^{c}	1.60 ^{bc}	1.89 ^{a}	1.77 ^{ab}
Retraction (sec)°	7.88 ^{c}	9.57 ^{c}	15.6 ^{b}	16.5 ^{b}	23.4 ^{a}	24.0 ^{a}

Different superscripts show significant differences between treatments ($\alpha=0.05$)
^Chi-square transformation °square root transformation

(b) Experiment 2

Ceasing watering on day 8 resulted in less BL being converted ($P<0.01$), greater dispersion scores ($P<0.01$) and greater retraction rates ($P<0.01$) when compared to ceasing watering on either day 13 or day 23. There was a water volume effect on conversion rate ($P<0.05$); however this factor had no effect on either dispersion or retraction (Table 2).

Table 2. The effect of ceasing watering at day 8 and 13 on conversion depth, earthworm dispersion and retraction

	Day water stopped		
	23	13	8
Conversion depth (%)	44.4 [a]	42.2 [a]	36.7 [b]
Dispersion (score)^^	1.4 [b]	1.4 [b]	1.5 [a]
Retraction (sec)~	9.6 [b]	9.0 [b]	14.7 [a]

Different superscripts show significant differences between treatments (α=0.05)
^^Rank transformation ~Log transformation

Increasing water volume from 100 to 200ml decreased earthworm mortality ($P<0.001$) from 26 to 20 %, while the experimental factor (day stopped) had no effect on mortality. Higher earthworm mortality was encountered at the start of the experiment when using 100ml water applications daily (Figure 3). When ceasing watering on day 8, earthworm mortality remained low for both 100 and 200ml volumes for the duration of the experiment.

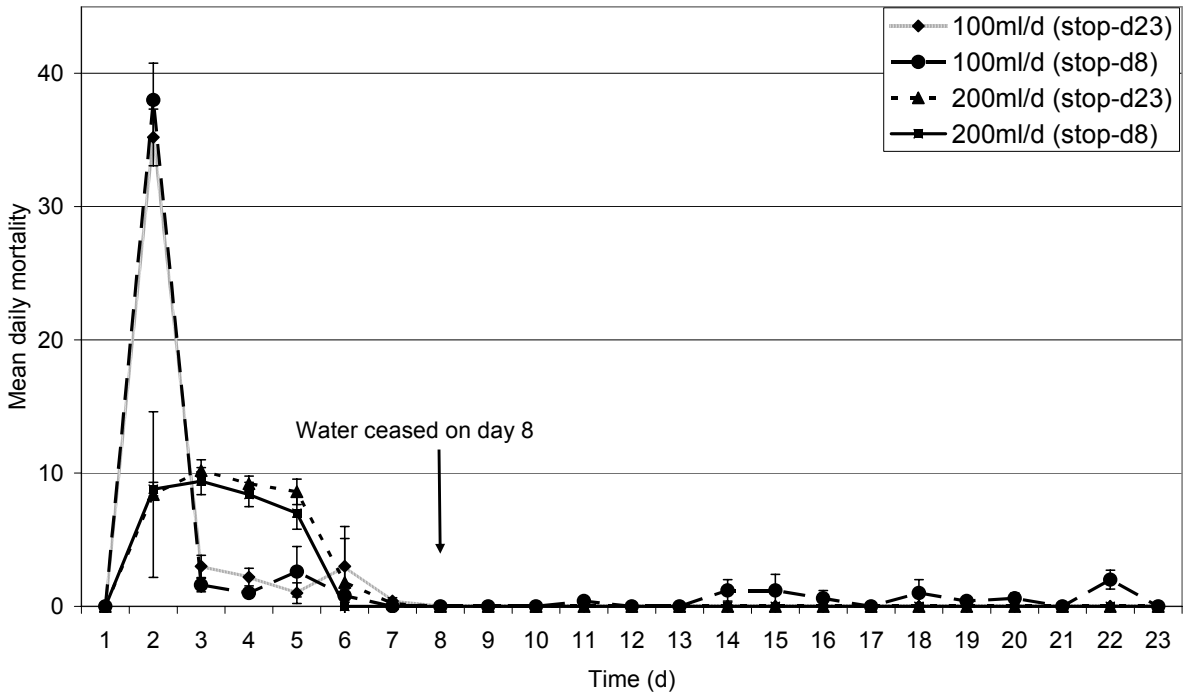

Figure 3. The mean daily mortality for treatments receiving 100 and 200ml volumes daily where watering was ceased on day 8 and 23 (±SE)

IV. DISCUSSION

The results of this experiment indicated that BL could be used as a food source for earthworms *(Eisenia andrei)* which has previously been considered difficult due to the high N concentration in poultry manures (Edwards *et al.* 1985). The use of specially developed vermiculture techniques and regular 100 and 200ml water applications enabled the conversion of fresh BL into vermicast. Twice daily applications of 50ml of water resulted in relatively low conversion, indicating that water volume was important when trying to maximise BL conversion rates (Figure 1). This was a concern as earthworm mortality was relatively low for this treatment (Figure 2) and did not indicate that poor BL conversion was taking place.

A regular watering interval also resulted in lower earthworm mortality, especially when combined with the larger water volume. Interestingly the 200ml/3d application resulted in lower mortality than 100ml/3d water application, suggesting that if watering events are extended beyond daily watering then larger water volumes will be required (Figure 2). Generally the higher water volume resulted in lower mortality. An exception was the 200ml/0.5d treatment which was higher, and could have been due to the drainage rate of the containers not being great enough to remove the salty leachate away from the earthworms. Earthworm dispersion scores and retraction rates were lower for shorter watering intervals, indicating lower stress on earthworms in a BL substrate (Table 1).

By ceasing watering on day 8 and 13 it was expected that conversion rates and earthworm health would suffer, however the response was less than expected (Table 2). This suggests that the most critical time for the process was the first 8 days, since ceasing watering on day 13 resulted in no less conversion than treatments continuing to receive daily water until completion on day 23. Interestingly, earthworm mortality was not affected by the day water was ceased but was highly affected by the water volume (Table 3). This can be explained by the higher mortality experienced by 100ml treatments on day 2, where the majority of earthworm deaths occurred, which overshadowed mortality for the rest of the experiment (Figure 3). This again highlights how sensitive these systems are in the first few days.

V. CONCLUSION

With regular initial water applications BL can be used as the sole food source for *Eisenia andrei* without having to first process the litter. By applying initial large volumes of water earthworm mortality and stress were reduced, and the rate of BL conversion was increased. This represents an alternative value adding disposal option for the poultry industry as BL can be rapidly converted into an odour free saleable vermicast and vermimeal.

ACKNOWLEDGEMENTS

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A MICROBIAL ENZYME IMPROVES PERFORMANCE AND DIGESTIBILITY OF NUTRIENTS IN BROILER BREEDERS FED CORN-SOYBEAN MEAL DIETS

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Summary

The effects of supplementing corn-soybean meal based broiler breeder diets with a multi-enzyme preparation were examined in a total of 576 Arbor Acres breeding hens (28-week old) randomly divided into four treatments: Positive control (PC, standard diet as used in the commercial breeder farm); PC + Enzyme; Negative control (NC, reducing ME by 0.29 MJ/kg and lysine by 0.07 percentage units); and NC + Enzyme. The enzyme, included at 500 g/t diet, contained endo-1,4-β-xylanase 2,200 visco units/g and endo-1,3(4)-β-glucanase 200 AGL units/g plus other auxiliary activities (Rovabio™ Excel AP 10%) from single fermentation of a non-GMO fungus *Penicillium funiculosum.* The trial was conducted from 29 to 45 weeks of age. Results showed that enzyme supplementation improved ($P<0.05$) laying rate, egg mass and feed conversion, with pronounced improvements observed at peak and after peak production. Enzyme supplementation increased ($P<0.05$) fertility rate at week 36, the proportion of quality chicks at week 45 and the digestibility of dry matter, gross energy, neutral detergent fibre and acid detergent fibre. It is concluded that dietary supplementation with the enzyme enhanced performance of broiler breeders, through improvements in digestibility of dietary nutrients.

I. INTRODUCTION

Non-starch polysaccharides (NSPs) are present in all feed ingredients of plant origin and impair the access and function of the endogenous enzymes present in the gastrointestinal tract. They thus exert a negative impact on the digestion and absorption of nutrients linked to the cell walls (Choct and Annison 1992). Numerous studies have shown that supplementation with appropriate microbial enzymes can eliminate the anti-nutritional effects of NSPs and improve the utilisation of dietary energy and amino acids, resulting in improved performance in both broilers and layers (e.g. Geraert *et al*., 2003). However, information on the efficacy of enzymes in diets for breeding hens is scanty. In recent years, feed ingredients of animal origin have been phased out in broiler breeder diets and microbial enzymes may have an important role to play. This study investigated a microbial enzyme preparation (Rovabio™ Excel AP 10%) fermented from a non-GMO fungus *Penicillium funiculosum*. The product contains mainly endo-β-1,4-xylanase (2,200 visco units/g (equivalent to 140 AXC units /g) and endo-1,3(4)-β-glucanase (200 AGL units/g) with about 19 enzyme activities, presumably efficacious towards a broad range of plant feed ingredients for monogastric animal species.

II. MATERIAL AND METHODS

576 Arbor Acres breeding hens were randomly allocated into four treatments and eight replicates per treatment, in a 2 × 2 factorial design: standard ME (10.127 MJ/kg, Lys 0.87%) or ME reduced diet (ME 9.887 MJ/kg and Lys 0.80%), without or with supplementation of Rovabio. The diets were based on corn and soybean meal (Table 1), and were offered in mash form. The birds were housed in cages with free access to water but food intake was restricted

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to follow commercial practice for broiler breeder flocks. The trial lasted 18 weeks from 28 to 46 weeks of age.

Measurements included a) laying performance, i.e. number of eggs, egg weight, feed intake, feed conversion, mortality, number of hatchable eggs, b) reproductive performance was examined in weeks 38 and 45, respectively, at which time eggs were collected from each treatment and hatched in order to determine fertility, hatchability and the proportion of quality chicks, and c) digestibility of nutrients. A digestibility trial was conducted during week 35, using chromic oxide as the marker and total excreta collection following the procedures of Bourdillon *et al*. (1990). Data were analysed by ANOVA and means were separated using the LSD procedure.

Table 1. Composition and calculated nutrients of the basal diets (g/kg)

Ingredients	Positive	Negative
Corn	630.0	646.0
Soybean meal	248.0	245.0
Soybean oil	19.0	6.0
Calcium carbonate	14.0	14.0
Limestone	76.0	76.0
Monocalcium phosphate	3.0	3.0
Premix[1]	10.0	10.0
Nutrients [2]		
ME, MJ/kg	11.92 (11.02)	11.63 (10.77)
Crude protein	155.0 (158.2)	155.0 (156.8)
Calcium	40.0 (42.4)	40.0 (43.7)
Total phosphorus	6.0 (6.1)	6.0 (5.9)
Available phosphorus	3.8	3.8
Lysine	8.6 (8.7)	8.6 (8.0)
Methionine	2.7 (2.6)	2.7 (2.8)
Met+Cys	5.4 (5.7)	5.4 (5.5)

[1]The vitamin and mineral premix provided per kg diet: Vit. A 12000 IU, D3 1500 IU, E 25 IU, K3 1.0 mg; B1 5.5mg, B2 5.0mg, B3 16mg, B6 8.0mg, Biotin 0.3mg, choline chloride 500mg, folic acid 1.8mg, B12 0.008mg, Fe 90mg, Cu 20mg, I 0.45mg, Mn 80mg, Zn 80mg, Se 0.2mg, DL- methionine 1.50g. [2] Data in brackets are the determined values.

III. RESULTS AND DISCUSSION

Laying performance

The results are shown in Table 2. During weeks 29 to 37, enzyme supplementation of the standard diet improved ($P<0.05$) laying rate by 1.4 percentage units (pcu), egg mass by 2.3 g/hen/day ($P<0.05$) and feed conversion by 16 FCR points ($P<0.05$), while in the reformulated diet, enzyme addition increased ($P<0.05$) laying rate by 2.0 pcu, egg mass by 2.2 g/hen/d, and feed conversion by 16 FCR points. No differences ($P>0.05$) were observed in feed intake due mainly to restricted feeding. During weeks 38-46, enzyme supplementation numerically increased laying rate, egg mass and feed conversion ($P>0.05$). Comparing the two control diets, hens fed on standard basal diets displayed numerically better feed conversion ratio than those fed on reformulated basal diet, reflecting lower levels of dietary ME and lysine, but the differences were only observed during the first period and not significant ($P>0.05$).

Figure 1 shows production curves indicating pronounced positive effects of enzyme addition at and after peak production for both positive and negative control diets.

Table 2. Effect of the enzyme supplementation on performance of broiler breeders (Mean ± SD)

	Standard diet		Reformulated diet	
	Control	+ Enzyme	Control	+ Enzyme
29-37 week age				
Laying rate (%)	79.7±1.5[a]	81.1±0.4[b]	79.5±2.1[a]	81.5±0.5[b]
Egg weight (g)	61.4±2.3	61.7±2.9	61.1±2.7	61.3±2.2
Egg mass (g/hen/d)	48.2±1.2[bc]	50.5±2.5[a]	47.8±1.4[c]	50.0±1.3[ab]
Feed intake (g/hen/d)	174.6±0.0	174.5±0.4	174.5±0.4	174.6±0.0
Feed conversion ratio	3.62±0.09[ab]	3.46±0.18[c]	3.66±0.11[a]	3.50±0.09[bc]
Hatchable egg (%)	90.4±1.8	87.7±3.7	88.3±2.6	87.6±1.6
38-46 week age				
Laying rate (%)	72.7±3.0	76.0±3.0	74.3±3.8	74.9±2.7
Egg weight (g)	63.4±1.9	63.3±2.2	62.9±2.1	63.1±1.9
Egg mass (g/hen/d)	46.7±2.0	48.1±2.0	46.8±2.0	47.7±2.0
Feed intake (g/hen/d)	164.9±0.4	165.0±0.0	164.8±0.4	164.6±0.5
Feed conversion ratio	3.53±0.17	3.43±0.14	3.52±0.14	3.46±0.17
Hatchable egg (%)	94.7±1.6[a]	93.5±1.8[ab]	94.0±2.7[ab]	92.0±2.6[b]

Values in the same row not bearing the same superscripts differ significantly (P<0.05)

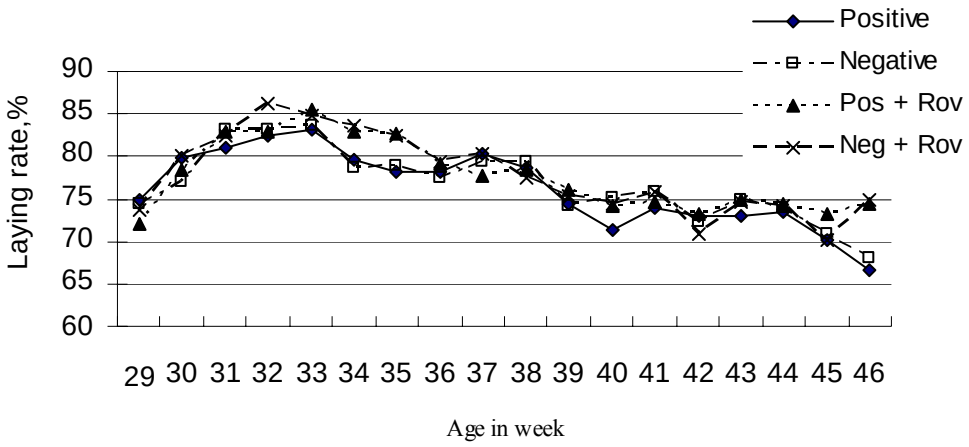

Figure 1. Effect of adding Rovabio on laying performance

Fertility and hatchability

The results are shown in Table 3. Enzyme supplementation of the standard diet increased (P<0.05) fertility rate at week 36. When the enzyme was incorporated into the reformulated diet, improvements were recorded in the proportion of good quality chicks at week 45. No improvements (P>0.05) were observed in hatchability at either 36 or 45 weeks.

Table 3. Effect of enzyme supplementation on reproductive performance (Mean ± SD)

	Standard diet		Reformulated diet	
	Control	+ Enzyme	Control	+ Enzyme
Week 36				
Fertility (%)	85.6±2.7^{b}	90.0±4.7^{a}	92.5±3.5^{a}	93.9±4.7^{a}
Hatchability (%)	94.7±1.4	94.5±2.0	95.0±1.6	95.1±1.8
Good quality chicks, %	93.8±2.9^{b}	95.4±1.9^{ab}	95.1±2.1^{ab}	96.4±2.3^{a}
Week 45				
Fertility (%)	91.3±3.7	92.0±3.2	91.7±1.2	94.2±1.3
Hatchability (%)	91.4±1.6^{b}	91.2±1.3^{b}	92.0±2.0^{ab}	94.1±1.8^{a}
Good quality chicks, %	93.6±2.0^{ab}	96.4±1.6^{a}	92.9±0.7^{b}	96.3±2.6^{a}

Values in the same row not bearing the same superscripts differ significantly (P<0.05)

Digestibility of nutrients

As shown in Table 4, enzyme supplementation significantly increased the digestibility of dry matter and gross energy by 2-3 pcu, equivalent to ME 0.20-0.30 MJ/kg or 70-100 kcal/kg (P<0.05). Apparent digestibility coefficients of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were significantly increased (P<0.05). Enzyme supplementation increased (P<0.05) apparent digestibility of lysine, methionine, and threonine, with an overall increase by 2.7% and 5.4%, respectively, for the standard and the reformulated diets.

Table 4. Effect of enzyme supplementation on digestibility of nutrients (mean ± SD)

	Standard diet		Reformulated diet	
	Control	+ Enzyme	Control	+ Enzyme
Dry matter, %	65.2±1.3^{b}	67.1±1.9^{c}	63.3±0.9^{a}	66.6±2.0^{bc}
Gross energy, %	71.5±1.5^{a}	73.4±1.4^{b}	70.8±1.2^{a}	73.6±2.4^{b}
NDF, %	39.4±3.4^{a}	44.4±2.2^{b}	40.4±4.7^{a}	47.7±4.5^{b}
ADF, %	36.1±4.9^{a}	43.0±2.0^{c}	37.6±4.3^{ab}	41.0±3.8^{bc}
Phosphorus, %	24.6±4.0	24.0±6.7	20.6±5.1	23.1±2.1
Lysine, %	87.2±3.1^{b}	89.0±1.7^{ab}	86.9±2.4^{b}	89.8±1.7^{a}
Methionine, %	24.6±4.0	24.0±6.7	20.6±5.1	23.1±2.1
Total amino acids, %	81.3±2.4^{a}	84.0±1.9^{b}	79.3±2.0^{a}	84.8±2.0^{b}

Values in the same row not bearing the same superscript differ significantly (P<0.05)

The results of this study demonstrated the benefits of supplementing corn-soybean meal based broiler breeder diets with a microbial derived enzyme product. The addition of Rovabio improved both laying and reproductive performance of breeding hens and this was attributed to improved digestion and utilization of dietary nutrients. Economic savings were achieved through enzyme supplementation of the reformulated diet.

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ENZYME COMPLEX CONTAINING NSP-ENZYMES AND PHYTASE IMPROVES THE PERFORMANCE OF BROILERS FED ON CORN-BASED DIETS

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Summary

The benefits of a multi-enzyme preparation containing carbohydrolase and phytase activities on the performance of broilers offered corn/soybean meal-based diets were investigated. Ross broiler chickens were fed either phosphorus reduced (AP), AP and energy (ME) reduced-, or AP, ME and protein (CP)-reduced diets, without and with the enzyme complex. Supplementation of the reformulated diets with the multi-enzyme complex improved weight gain of birds, and weight gain values were comparable to those observed in the positive control group receiving a diet formulated to meet requirements for AP, ME and CP. A marked improvement in weight gain (5.6%) was observed when birds were fed on the reduced AP, ME and CP diet supplemented with the multi-enzyme complex. Additionally, birds offered enzyme-supplemented diets had feed conversion ratios comparable to those of birds fed the positive control. The supplementation of the enzyme preparation to reformulated corn/soybean meal-based diets improved the productive performance of broilers between 1 and 42 days of age.

I. INTRODUCTION

The efficacy of exogenous enzymes in improving the performance of poultry has been well documented; however, a variety of responses to combinations of enzymes such as glycanases and phytases have been reported (Ravindran *et al*., 1999; Zyla *et al*., 1999; Wu *et al*., 2004; Cowieson and Adeola, 2005). Rovabio™ Max is a multi-enzyme preparation produced from the fermentation of *Penicillium funiculosum*. It contains a minimum of 20 compatible enzyme activities with relevance to a broad range of plant feed ingredients in monogastric diets (Maisonnier-Grenier *et al.*, 2004).

II. MATERIALS AND METHODS

To evaluate the efficacy of this multi-enzyme preparation on the performance of broilers given corn/soybean meal-based diets, 2,240 male day-old Ross broiler chicks were allocated to 56 pens (40 chickens per pen) and subjected to a total of seven treatments. A positive control treatment (T1) consisted of a diet formulated to meet the requirements of the birds for available phosphorus (AP), energy (ME) and crude protein (CP). Three of the treatments consisted of an AP-only reduced diet (T2, with a reduction of 1 g/kg in AP), or an AP and energy reduced diet (T4, with reductions of 1g/kg and 0.25 MJ/kg, respectively, in AP and ME) or an AP, energy and protein reduced diet (T6, with reductions of 1 g/kg, 0.25 MJ/kg and 4 g/kg respectively in AP, ME and CP). Treatments 3, 5 and 7 were treatments 2, 4 and 6 respectively plus the enzyme (Rovabio™ Max) included at a rate to provide 1,100 visco units (equivalent to 70 AXC) of endo-β-1,4-xylanase, 100 AGL units of endo-1,3(4)-β-glucanase, and 350 RPU of 3-phytase per kg of diet. The body weights and feed intakes of each pen of birds were recorded at 0, 21 and 42 days of age. Data from the birds on the

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different dietary treatments were analyzed as a completely randomized block design. Mean values for feed intakes, weight gains, and feed conversion ratios were subjected to analysis of variance and the standard errors of the means were tested for significance, using STATVIEW (Abacus Concepts, 1996).

Table 1. Ingredient and nutrient compositon of the starter / grower experimental diets.

Ingredient (%)	Positive control (T1)	Negative control 1 (T2)	Negative control 2 (T4)	Negative control 3 (T6)
Corn	59.0 / 64.5	59.3 / 64.9	60.4 / 66.2	61.5 / 67.4
Soybean meal	35.5 / 27.1	35.4 / 27.0	35.2 / 26.8	34.1 / 25.7
Soybean oil	1.2 / 0.4	1.1 / 0.4	0.2 / 0.3	0.1 / 0.3
Limestone	0.8 / 0.7	1.0 / 0.9	1.0 / 0.9	1.0 / 0.9
Dicalcium phosphate	1.5 / 1.2	1.0 / 0.8	1.0 / 0.8	1.0 / 0.8
Sodium chloride	0.32 / 0.30	0.32 / 0.30	0.35 / 0.29	0.35 / 0.29
DL-Methionine	0.22 / 0.19	0.21 / 0.19	0.21 / 0.19	0.22 / 0.20
L-Lysine hydrochloride	0.06 / 0.10	0.06 / 0.10	0.06 / 0.10	0.10 / 0.14
Vitamin/mineral premix	0.5 / 0.5	0.5 / 0.5	0.5 / 0.5	0.5 / 0.5
Calculated Analyses				
Crude protein (%)	22.0 / 18.4	22.0 / 18.4	22.0 / 18.4	21.6 / 18.0
ME (MJ/kg)	12.30 / 13.38	12.30 / 13.38	12.08 / 13.12	12.08 / 13.12
Calcium (%)	0.85 / 0.75	0.85 / 0.75	0.85 / 0.75	0.85 / 0.75
Available phosphorus (%)	0.44 / 0.38	0.34 / 0.28	0.34 / 0.28	0.34 / 0.28
Met + Cys (%)	0.90 / 0.79	0.90 / 0.79	0.90 / 0.79	0.90 / 0.79
Methionine (%)	0.57 / 0.50	0.57 / 0.50	0.57 / 0.50	0.57 / 0.51
Lysine (%)	1.20 / 1.00	1.20 / 1.00	1.20 / 1.00	1.20 / 1.00
Threonine (%)	0.84 / 0.71	0.84 / 0.71	0.84 / 0.71	0.84 / 0.69

Premix provides the following per kilogram of diet: vitamin A, 16,800 IU; cholecalciferol, 7,000 IU; vitamin E, 70 IU mg; menadione, 4.2 mg; thiamine, 2.8 mg; riboflavine, 9.8 mg; pyridoxine, 7 mg; cyanocobalamin 0.02 mg; folic acid, 1.4 mg; pantothenic acid 21 mg; niacin, 70 mg; biotin, 0.28 mg; Co, 0.7 mg; Cu, 14 mg; Fe, 112 mg; I, 1.4 mg; Mn, 140 mg; Se, 0.28 mg; Zn, 112 mg.

III. RESULTS AND DISCUSSION

Supplementation of the reformulated diets with the enzyme complex (T3, T5, and T7) improved the weight gain of the birds. As shown in Table 2 and Figure 1, the weight gains of birds fed on the diets supplemented with the enzyme complex were similar to the birds fed on the positive control diet (T1). There was a marked improvement in weight gain (5.6%) due to enzyme supplementation in the birds given the restricted AP, ME and CP diet (T7 cf. T6). As well as having the best rates of growth, birds on the enzyme-supplemented diets (T3, T5, and T7) had feed conversion ratios comparable to those of birds fed on the positive control diet (T1), as shown in Table 1 and Figure 2. Although no significant difference was observed between T2 and T3, or between T6 and T7, birds given the restricted AP and ME diet supplemented with the enzyme preparation (T5) and the restricted AP, ME and CP diet supplemented with the enzyme preparation (T7) had the best feed conversion ratios,

particularly during the growing/finishing period (21-42 days) of the trial (Table 2) when feed intakes are at their highest and most costly.

Table 2. Effect of dietary treatments on growth performance of broiler chickens

Treatment		Added Enzyme	FI (g/bird)		BW gain (g/bird)		FCR (g:g)	
			1-21 d	21-42 d	1-21 d	21-42 d	1-21 d	21-42 d
1	PC	no	1170 [a]	3537	794 [a]	1917 [ab]	1.478	1.845 [a]
2	NC1	no	1096 [b]	3431	749 [c]	1847 [bc]	1.463	1.860 [a]
3	NC1	yes	1166 [a]	3517	782 [ab]	1907 [ab]	1.492	1.848 [a]
4	NC2	no	1165 [a]	3557	753 [bc]	1840 [bc]	1.548	1.936 [b]
5	NC2	yes	1156 [a]	3501	773 [abc]	1895 [abc]	1.495	1.849 [a]
6	NC3	no	1142 [ab]	3485	751 [bc]	1827 [c]	1.523	1.907 [ab]
7	NC3	yes	1179 [a]	3610	764 [abc]	1958 [a]	1.545	1.845 [a]
SEM[1]			18	45	11	28	0.025	0.027

[a-c] Means within the same column with no common superscript differ significantly (P<0.05)
[1] Pooled standard error of means

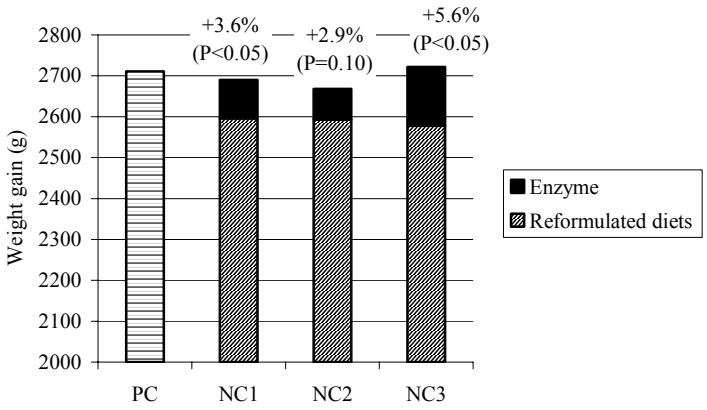

Figure 1. Effect of the addition of the multi-enzyme complex on the weight gain (g) of broilers from 1 to 42 days of age.

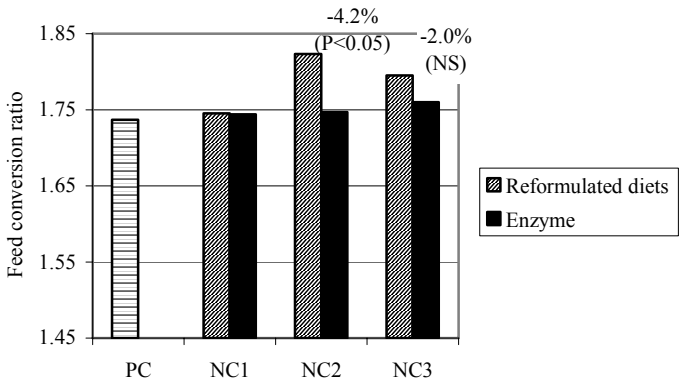

Figure 2. Effect of the addition of the multi-enzyme complex on the feed conversion of broilers from 1 to 42 days of age.

The results observed in this study showed that phosphorus (AP) was the first-limiting nutrient for growth in broiler chickens. Body weight gain was significantly decreased in birds given the restricted AP diet, and a minor additional impairment in performance was observed when birds were given diets also marginally deficient in ME and CP. Although xylanase can improve the digestibility of AP (Kim *et al*., 2005), it was not effective in improving performance of broiler chickens fed an AP deficient diet (Cowieson and Adeola, 2005). A combination of carbohydrolases and phytase is required to restore growth performance. The activity of one type of feed enzyme is facilitated by the other in a reciprocal fashion. Such combination also improves response in nutrient digestibility and mineral retention, suggesting a synergistic effect of both types of enzymes (Ravindran *et al*., 1999; Juanpere *et al*., 2005).

The supplementation of the multi-enzyme complex to reformulated corn/soybean meal-based diets in the present study improved the productive performance of broilers between 1 and 42 days of age. The results suggest that full dietary reformulation is required for better expression of a multi-enzyme complex containing NSP-enzymes and phytase.

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EFFECTS OF PHYTASE AND XYLANASE ADDITION TO CORN AND WHEAT-BASED DIETS ON BROILER PERFORMANCE

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Summary

An experiment was conducted to evaluate the effect on broiler performance of adding phytase and xylanase, alone or in combination, to diets based on corn, wheat, soybean meal and rapeseed meal. All experimental diets, including a normal phosphorus commercial diet (NP, 4.5 g AvP/kg for day 0 to 21 and 4.0 g AvP/kg for day 22 to 35), low phosphorus diet (LP, Reduced AvP of NP by 1.5 g/kg for both starter and finisher diets), LP diet with phytase (LP+P), xylanase (LP+X) and their combination (LP+P+X) respectively, were offered to broilers as mash from day 1 to 35. Feed intake and body weight gain were measured weekly. Growth rate and feed efficiency were depressed in the birds given the LP diet, but performance was improved to the level of birds given the NP commercial diet when the LP diet was supplemented with 500 FTU/kg phytase. The addition of xylanase to the LP diet had no effect on performance (P>0.05). The supplementation of xylanase to the LP+P diet, however, tended to produce an improved feed conversion compared with the LP diet supplemented with phytase alone.

I. INTRODUCTION

It has been generally recognized for some time that arabinoxylan is a key anti-nutritional factor in poultry diets compounded from wheat and its byproducts. Arabinoxylan has a high water holding capacity, which can increase the viscosity of digesta and reduce nutrient digestibility, consequently depressing growth performance. Annison (1993) reported that wheat contains 6.2 to 7.0% and corn contains 4.1% to 4.5% arabinoxylan. Due to the lack of endogenous fibre-digesting enzymes in poultry, the digestion of dietary fibre mainly occurs in the hindgut through bacterial fermentation, with the digestibility of arabinoxylan being related to the maturity of birds. More importantly, the existence of arabinoxylan in the diet can restrict other nutrients being digested and absorbed by birds, resulting in depressed growth performance. The addition of exogenous enzymes to wheat- and corn-based diets increases digestibility of nutrients in broilers (Pen et al. 2003). It may also provide additional dietary energy as well as short chain fatty acids and oligosaccharides (Iji 1999).

Phytate is another anti-nutritional factor commonly found in many plant-based ingredients in poultry diets and which can substantially reduce phosphorus availability. Typically only about 30% of dietary phosphorus can be utilized by birds with the rest being excreted into the environment. Apart from the effect of phytate on the availability of phosphorus, phytate also has the capability to bind with protein, reducing the availability of amino acids. Many published research studies have shown that addition of exogenous phytase not only improves the bioavailability of phytate phosphorus, but also improves the digestibility of energy and amino acids (Ravindran et al, 1999).

The aim of the present study was to investigate the effect of phytase and xylanase on broiler performance in birds given diets based on corn, wheat and rapeseed meal.

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II. MATERIALS AND METHODS

2000 day-old male AA broiler chicks were assigned to 40 floor pens in a completely randomized block design with 50 birds per pen. There were five dietary treatments, each allocated to 8 pens. The enzymes used in this study were supplied by Danisco Animal Nutrition with a phytase activity (Phyzyme® XP) of 5000 FTU/g and a xylanase activity of 8000 U/g.

The five treatments were;

- Diet 1, normal phosphorus level (NP), dietary available phosphorus for day 0 to 21 was 4.5 g/kg and 4.0 g/kg for day 22 to 35;
- Diet 2, Reduced available P of diet 1 by 1.5 g/kg for starter and finisher diets (LP);
- Diet 3, Diet 2 + 500 FTU/kg phytase (LP+P);
- Diet 4, Diet 2+2000 U/kg xylanase (LP+X);
- Diet 5, Diet 2+ 500 FTU/kg phytase and 2000 U/kg xylanase (LP+P+X).

Diets were fed *ad libitum* as mash and water was available at all times. The ingredient and nutrient composition of the normal and low phosphorus diets are shown in Table 1.

Birds were weighed at 1, 7, 14, 21, 28 and 35 day of age, and feed intake was measured at the end of each week. At day 1, 7, 14, 21, 28, 35, one bird from each replicate pen was killed and the tibia was collected for ash measurements. At the end of the feeding trial, birds were processed at a slaughtering house and carcass yield was calculated. Data were analyzed with ANOVA (SAS) to assess the main effects of diet.

III. RESULTS AND DISCUSSIONS

The available P in the negative control was 1.5 g/kg lower than in the positive control. The reduction in available P is higher than the recommendation by Danisco Animal Nutrition for Phyzyme XP application in broilers, but this ensured that available P was the first limiting nutrient for broilers in these diets. Broilers given the negative control diet had a significantly lower average daily gain, feed intake and poorer FCR over the 35 day period than those given the positive control diet ($P<0.05$), although carcass yield was not affected ($P>0.05$, table 2). Adding phytase to the low phosphorus diet improved body weight gain, feed intake and FCR to the level of the positive control (Table 2), and increased tibia ash content ($P<0.05$, Table 3). These results are consistent with reports by Watson (2006) and Cheng (1999).

Xylanase supplementation of the low phosphorus diet did not improve bird performance although FCR tended to be further improved (1.90 *vs* 1.85) when xylanase was added in combination with phytase. Adding phytase and xylanase in combination to the LP diet produced no further improvement in tibia ash. Growth rate and FCR in birds given the LP diet were limited by low phosphorus availability. Phosphorus was the first limiting nutrient, but apparent metabolisable energy was not limiting in the LP diet compared with the positive control; thus any potential effects of the xylanase on energy or amino acid availability were not reflected in growth or FCR.

Table 1. Ingredient and nutrient composition (g/kg) of the normal and low phosphorus diets

	Day 1-21		Day 22-35	
Ingredients	NP	LP	NP	LP
Corn	413	425	454	466
Wheat	200	200	200	200
Corn oil	7.9	4.0	20.0	16.1
Soybean meal, 48%	242	240	191	189
Rapeseed meal, 38%	80.0	80.0	80.0	80.0
Corn gluten meal, 55%	20.0	20.0	20.0	20.0
Salt	3.1	3.1	3.4	3.4
Limestone	9.4	12.1	8.7	11.4
Diacalcium phosphorus	15.8	6.9	13.3	4.5
Choline chloride 50%	1.00	1.00	0.80	0.80
Lysine-HCl 78.8%	1.14	1.18	1.87	1.91
Methionine 99%	1.13	1.12	1.12	1.11
Threonine98.5%	0.24	0.25	0.47	0.47
Mould inhibitor	1.00	1.00	1.00	1.00
Antioxidant, 33%	0.40	0.40	0.40	0.40
Vitamin premix,0.2%[1]	2.00	2.00	2.00	2.00
Mineral premix,0.2%[2]	2.00	2.00	2.00	2.00
Total	1000	1000	1000	1000
Nutrients				
Protein	220	220	200	200
ME, MJ/kg	12.0	12.0	12.55	12.55
Calcium	9.00	8.00	8.00	7.00
Total phosphorus	7.10	5.60	6.50	5.00
Available phosphorus	4.50	3.00	4.00	2.50
Na	1.80	1.80	1.90	1.90
Cl	2.80	2.80	3.10	3.10
Salt	3.00	3.00	3.30	3.30
Digestible lysine	10.20	10.20	9.50	9.50
Digestible methionine	4.30	4.30	4.10	4.10
Digestible threonine	7.00	7.00	6.50	6.50

[1] Vitamin premix (supplied per kg feed): V_A 12000IU,V_{D3} 3500IU,V_E 50mg, V_{K3} 4mg,V_{B1} 3mg,V_{B2} 10mg, V_{B6} 6mg,V_{B12} 0.025mg, Biotin 0.2mg, Niacin 50mg, Pantothenate 12mg, Folic Acid 1mg

[2] Trace mineral premix (supplied per kg feed): Cu 10mg, Fe 100mg, Mn 120mg, Zn 100mg, Se 0.3mg, I 0.8mg.

Table 2. Effects of phytase and xylanase on growth performance of broilers given the five diets for 35 days

Treatment	NP	LP	LP+P	LP+ X	LP+P+X	SEM	P value
ADFI,g	84.34[a]	81.99[b]	85.07[a]	81.63[b]	81.42[b]	0.755	0.002
ADG,g	44.33[a]	41.56[b]	44.76[a]	41.35[b]	44.11[a]	0.730	0.0001
FCR	1.90[a]	1.97[b]	1.90[a]	1.97[b]	1.85[a]	0.023	0.0005
Carcass yield	67.52[ab]	67.56[ab]	68.49[a]	66.38[b]	67.47[ab]	0.335	0.011

*Mean values bearing different superscript letters within a row are significantly different (P<0.05); ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio

Table 3. Effects of phytase and xylanase on tibia ash (%)

Treatment	NP	LP	LP+P	LP+ X	LP+P+X	SEM	P value
Day 7	32.40	32.34	34.47	34.08	33.83	0.442	0.127
Day 14	36.03[a]	30.93[b]	34.89[a]	34.22[a]	34.92[a]	0.867	0.001
Day 21	37.42[a]	33.61[b]	37.39[a]	32.73[b]	34.99[ab]	0.959	0.006
Day 28	39.84[a]	34.43[b]	38.33[a]	34.84[b]	37.92[a]	1.047	0.0001
Day 35	37.26[a]	33.30[b]	35.03[ab]	33.27[b]	36.49[a]	0.812	0.001

*Mean values bearing different superscript letters within a row are significantly different (P<0.05)

IV. CONCLUSION

The inclusion of 500 FTU/kg phytase (Phyzyme® XP) to a diet with low available phosphorus (3.0 g/kg) significantly improved daily gain, feed intake and feed utilization efficiency of broilers during the 35-day growth period. Adding xylanase alone to the low phosphorus diet did not improve bird performance, probably because P availability was the first limiting factor in the diet, and the potential effects of xylanase on energy utilization and amino acid digestion were not reflected in growth performance.

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VARIATION IN BROILER PERFORMANCE DUE TO WHEAT SOURCE AND ENZYME SUPPLEMENTATION

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Summary

A broiler chick bioassay was used to establish the feeding value (nutrient level, availability and intake) of 34 wheat-based diets (75% inclusion) without or with feed xylanase and phytase. As demonstrated in previous studies, high variation was found in voluntary feed intake and this was significantly correlated with lower growth and poor feed conversion. In the present study we measured the relative immunoglobulin (IgA, IgG and IgM) of blood plasma from chicks fed each dietary treatment. There were no significant differences in these immune parameters between treatments, except for a reduction in plasma IgA with enzyme inclusion. However, there were significant negative correlations between the immune parameters and growth and feed intake, signifying faster growing birds may have expended less energy on immune response and this may be due to grain source and/or enzyme supplementation.

I. INTRODUCTION

Defining the value of feed grains for poultry, in particular for rapidly growing broiler chicks, is constantly evolving. Based on many bioassay measurements, Scott (2005) indicated that "feed value" should include not only nutrient level and availability, but also nutrient intake and retention as saleable meat. This is based on significant differences in voluntary intake that can negatively impact growth and feed conversion (Scott, 2005). There are also significant impacts of cereal source on dressing yield (van der Klis and Jansman, 2002). It is also our concern that antigenic challenge due to protein profile of some cereal grains may significantly increase maintenance costs. Van der Klis and Jansman (2002) indicated that a high level of immune system stimulation reduced feed intake and daily gain with up to a 40% reduction in daily protein accretion. The present study measures the feed value and includes an estimation of immune response to sources of wheat-based diets fed without and with exogenous enzymes.

II. MATERIALS AND METHODS

The data on 34 wheat samples presented herein was part of a bioassay evaluation of the feed value of cereal grains (Scott, 2006). The grain samples were incorporated (750 g/kg) in a common basal diet (Scott, 2006) formulated to meet or exceed the nutrient requirements (NRC, 1994) for starting broiler chicks. The diet was then split and one portion supplemented with commercial xylanase and phytase (0.5 g/kg diet Avizyme 1302 and Phyzyme, respectively; Danisco Animal Nutrition, UK; Feedworks Pty Ltd, Australia), producing 68 test diets. All diets contained 10g/kg Celite™, an acid insoluble ash marker, for the determination of AME.

The 68 test diets were each fed *ad libitum* to one cage of six male broilers (Cobb 500 provided by Baiada Poultry Pty Limited, Cordina Hatchery, NSW) from 4 to 17 d of age. This was repeated four times in separate bioassay series ensuring that all diets were handled in a similar manner (i.e. fed to identical sources of chicks in each series). Variations between

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series, presumably due to differences in chicks and/or environment, were estimated and it was deemed unnecessary to correct for these differences. At 16 d of age, a 6-hr excreta collection was conducted, excreta (free of feathers and feed contaminants) was freeze-dried, ground and used in analysis of AME (MJ/kg diet; Scott, 2006). At 17 d of age each pen of birds was weighed, feed intake determined and FCR (corrected for mortality) calculated. Blood was collected from two birds per pen by cardiac puncture and then birds were humanely killed by cervical dislocation, the two middle toes of each bird were collected and used to determine toe ash as an estimate of bone mineral deposition. Total plasma IgM, IgG and IgA titres were determined using a sandwich ELISA (Muir *et al.,* 2002). The project was pre-approved by the Animal Ethics Committee of Sydney University.

Statistically the main effects of wheat source and enzyme supplementation (and their interaction) were based on the respective values for four groups of broilers. The SAS general linear models procedure was used and significant differences between mean values determined with Duncan Multiple Range Test; references to significance in the text are based on a $P<0.05$ value. Pearson correlation coefficients were calculated between respective measurements for each pen of birds fed the various diet combinations.

III. RESULTS AND DISCUSSION

The emphasis of our study was to evaluate the relationships between physiological parameters and variation in production parameters, notably growth, feed intake and FCR. As presented in Table 1, there were major differences in performance of individual cages of male broilers fed wheat-based diets with or without enzyme. Overall average body weight for individual cages of six male broilers at 17 d ranged from 346 to 673 g, this difference ((maximum – minimum)/minimum x 100) is 95% or nearly a doubling in weight from the lightest to the heaviest. The variation was in part explained by significant differences in wheat source and enzyme use, but no significant interaction was observed.

Table 1. The variation in male broiler (4 birds per treatment) performance, AME, intake, digesta viscosity and plasma measurements of immunoglobulins (expressed as % hyperimmune; IgG, IgM, and IgA) when fed 34 different sources of wheat with or without xylanase and phytase (Range and % difference reflect individual pens)

	Wheat	Enzyme	Wht*Enz	Range	Mean±StdE	%Diff
Body wt 17d (g)	**	**	NS	346 - 673	507±3.3	95
Feed intake g/b/d	*	**	NS	28.4 - 59.1	41.7±0.26	108
FCR 4-17d	**	**	**	1.13 - 1.93	1.41±0.01	71
AME diet MJ	**	**	**	6.2 - 14.9	11.6±0.07	140
AME intake MJ/b/d	**	**	**	0.22 - 0.78	0.48±0.001	255
Digesta viscosity	**	**	**	2.5 - 58.8	13.2±0.96	2252
Plasma IgG	NS	NS	NS	23 - 145	76.1±1.43	537
Plasma IgM	NS	NS	NS	35 - 142	91.0±1.60	307
Plasma IgA	NS	**	NS	16 - 130	64.8±1.75	718

There were also significantly large differences (108%) in feed intake between pens of birds. Both main effects (wheat source and enzyme supplementation) were significant, with no interaction indicating that enzymes had a consistent effect between wheat sources. Previous studies (Scott, 2005) highlighted similar large significant differences in feed intake between sources of wheat measured using broiler bioassays.

Both wheat source and enzyme supplementation had significant effects on FCR. There was a highly significant interaction between wheat source and enzyme

supplementation for FCR. The average, across enzyme treatments, for FCR of wheat sources varied from 1.29 (n=8) to 1.55, a difference of 21%; again, similar to those reported for other studies using wheat and enzyme supplemented diets as summarised by Scott (2005).

The variation between mean values for FCR (as well as body weight and feed intake) reflects the degree of variation reported for individual groups of birds. The large variation between groups of birds for the various production parameters is of concern; it not only reflects diet differences, but also individual pen variation due to differences in source of birds, micro-environment, management and presumably health conditions (i.e. immune challenge). Similar differences were observed for production parameters for control birds fed a broiler starter using six replicate pens of birds within each series (data not shown).

The retention of energy (AME, Table 1) was significantly affected by both main effects (wheat source and enzyme); the two-way interaction was also significant. Similar treatment effects were observed for AME intake (MJ/bird/d); however variability in AME intake (255%) was significantly greater than AME, reflecting the added variation of intake.

The extremely large variation in digesta viscosity (>2000%, Table 1) is a direct effect of the capacity of enzymes to reduce the water holding capacity of digesta containing high levels of soluble non-starch polysaccharides (NSP). The main effects (wheat source and enzyme) as well as the two-way interaction were significant; this is expected since the type of wheat source (and its level of soluble NSP) will impact the change in digesta viscosity with enzyme supplementation.

There were no significant effects of wheat source and/or enzyme supplementation on toe ash, which averaged 14.3±0.8 % across treatments. Similarly, there were no significant treatment effects for plasma IgG and IgM. Broilers given diets with and without enzymes had IgA plasma levels of 61.6 $\pm$ 2.51 and 68.0 $\pm$ 2.40, respectively ($P<0.05$; data not shown). The difference in level of relative immunoglobulin values, averaged from two blood samples per cage, was high (537, 307 and 718%, respectively, for IgG, IgM and IgA).

Our interest in making these measurements for broilers fed the different sources of grains was to identify the contribution of physiochemical characteristics of grain source (e.g. soluble NSP, etc) and the birds' immunological competence and/or challenge. Thereby, explaining variation in performance parameters for each diet. Hence, we estimated Pearson correlation coefficients for the traits described in Table 1 and these are presented in Table 2.

Table 2. The correlations (r^2) between performance parameters, AME and AME intake, digesta viscosity and plasma measurements of immunoglobulins (IgG, IgM, and IgA) when fed one of 34 different sources of wheat with or without xylanase and phytase.

	Feed Intake	FCR	AME diet	AME Intake	Digesta Viscosity	% hyperimmune		
						IgG	IgM	IgA
Body wt 17d	0.75**	-0.70**	0.34**	0.71**	-0.34**	-0.39**	-0.48**	-0.46**
Feed intake g/b/d		-0.40**	0.15*	0.76**	-0.26**	-0.28**	-0.30**	-0.31**
FCR 4-17d			-0.37**	-0.51**	0.31**	0.30**	0.36**	0.34**
AME diet MJ				0.75**	-0.21**	-0.26**	-0.32**	-0.34**
AME intake MJ/b/d					-0.30**	-0.36**	-0.41**	-0.42**
Digesta viscosity						0.03	-0.01	0.13
Plasma IgG							0.83**	0.71**
Plasma IgM								0.81**

In Table 2, as reported previously (Scott 2005, 2006), there are significant high positive correlations between body weight and feed intake ($r^2=0.75$) for the wheat-based diets with and without enzyme. Also as reported previously, the rate of growth has a significant negative ($r^2=-0.70$) relationship with FCR, indicating that faster growing birds will experience better utilisation of diet (or alternatively, these birds are consuming sufficient

amounts of a diet to satisfy maintenance requirements as well as a higher growth). In some of the previous studies, we have noted a significant negative correlation between feed intake and FCR; this is explained, as described above by supporting both maintenance and higher growth. This strengthens our concern that there are limitations to intake of some sources of wheat and these factors can significantly negatively impact growth and feed efficiency.

There were no significant correlations between any of the parameters and toe ash; hence these values have not been included. This may relate to more than adequate levels of supplemental vitamins and minerals in the basal diet which may have negated any effect that wheat source or enzyme supplementation would have on bone mineralisation. Previous studies have shown positive effects of phytase supplementation on toe ash measurements (Silversides *et al.*, 2004), but these diets were formulated to have low available phosphorus.

Of note were the moderate significant negative correlations between performance parameters (growth and feed intake) and the immunoglobulin levels of the plasma; there were likewise positive correlations between FCR and immunoglobulin level. It could be hypothesised that faster growing birds experienced less "challenge" (related to diet source and/or environment) and hence had lower immunoglobulin response; alternatively, it could also indicate that faster growing birds have a lower immunoglobulin response and utilise nutrients devoted to this to achieve higher growth. Cook (2001) indicated that genetic lines of meat ducks selected for high performance had significantly lower immunoreactivity and antibody response (29 to 79% less, respectively) than in unselected control birds. Our study used the same source of broilers in each series, and hence genetic variability would have been minimal. The significant negative relationship between immunoglobulin levels and growth (and feed intake) could be diet related. The lack of dietary response is in part due to the large variation between birds and series; it was noted that there was a significant increase in immunoglobulin value with each successive series of birds measured. This latter observation may be a factor to a build up of challenge as the series were conducted in three-week cycles, with only a four-day pause between a series ending and new chicks being placed.

Although our results are not conclusive, they indicate that it may be important to establish the antigenic response to specific cereal grains and how this may or may not be alleviated by use of supplements, such as enzymes, to reduce negative gut challenges and minimise immune stimulation.

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EFFICACY OF PHYTASE SUPPLEMENTATION OF LOW PHOSPHORUS CORN-SOYBEAN MEAL BASED DIETS FED TO BROILERS

A. KUMAR[1], J.G. DINGLE[1] and J. SANDS[2]

Summary

A study was conducted to demonstrate the effects of two sources of phytase at different levels of inclusion in low phosphorus corn and soybean meal based diets fed to male broilers. Two basal diets, standard P and the low P diet supplemented with phytase A at four levels and phytase B at two levels were formulated and fed as starter diets from 0 to 21 d and as finisher diets from 21 to 42 d. Mean body weight of broilers at 21 d was not significantly affected by level of phosphorus in the starter diet. However, phytase supplementation of the low P diet significantly improved mean body weight and feed conversion ratio. Forty two day mean body weight, weight gain and feed intake were significantly decreased on the low phosphorus diet, but this was for the most part corrected by the different levels of phytase supplementation of the low P diet**.**

I. INTRODUCTION

About 60-70% of the total phosphorus in cereal grains and vegetable protein meals is in the form of phytate phosphorus, which is not efficiently utilised by chickens (Perney *et al*., 1993). Phytate not only binds phosphorus but also exhibits other antinutritional properties in the feed by forming complex bonds with amino acids and minerals (Vohra et al., 1965; Oberleas, 1973 Caldwell, 1992; Camovale et al., 1998) which reduce nutrient digestibility and availability. It has been shown that phytase supplementation of the feed improves the phytate phosphorus utilisation in chickens (Edwards, 1993; Broz et al.,1994; Sebastian et al., 1996a; Kies et al., 2001) This study was undertaken to compare the effects of adding two sources of phytase enzyme at different levels to low phosphorus corn-soybean meal diets on the production performance of broilers compared with their performance when fed standard phosphorus diets without a phytase supplement.

II. METHODOLOGY

In this trial 2400 Ross male broilers were selected at one day of age according to average weight and allocated fifty to each of 48 deep litter floor pens. There were eight experimental diets and each diet was offered to six pens (a total of 300 chickens per diet). The broiler starter diets were offered from one to three weeks and the finisher diets from three to six weeks. There were two basal diets at each stage, a standard P and a low P diet. Each basal diet contained maize and soybean meal as major ingredients to provide 12.76 MJ ME, 215g CP and 12.3g lysine/kg in the starter diet and 13.03 MJ ME, 195g CP and 11.0g lysine/kg in the finisher diet. The major difference between these two basal diets was in total and non-phytate P content.

The standard P diets had available P contents of 4.0 and 2.8 g/kg in the starter and finisher stages, respectively, whereas the low P diets had available P contents of 3.2 and 2.0 g/kg in the starter and finisher stages, respectively. To test the efficacy of the phytase

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enzymes, the low P diets were supplemented with four levels of Phytase A* and two levels of Phytase B.** The treatments were as follows: 1. Standard P, no phytase, 2. Low P, no phytase, 3. Low P + 250U phytase A /kg, 4. Low P + 500U phytase A /kg, 5. Low P + 750U phytase A /kg, 6. Low P + 1000U phytase A /kg, 7. Low P + 500U phytase B /kg and 8. Low P + 1000U phytase B /kg. All birds and resudue feed were weighed in each pen on days 21 and 42, and when mortality occurred, time of death and the weight of the dead bird were recorded to make adjustment in the calculation of feed conversion ratio (FCR). Group data were analysed statistically using the SAS (SAS Inc., 2000) programme to determine the significance of difference among the treatments for the measured parameters.

III. RESULTS

The mean body weight, feed intake, FCR and mortality of chickens fed the phytase supplemented and unsupplemented corn soybean meal based diets are presented in Table 1.

0-21 days performance:

The chickens fed the low P diet supplemented with 750U of phytase A were significantly heavier than those fed the non-supplemented low P diet. However the mean body weights of the other groups of phytase supplemented birds were not significantly different from the groups fed either the standard or the low P basal diets. The feed intakes of the groups fed the low P diet supplemented with 500 or 750U of phytase A were significantly less than the feed intake of the group fed the normal P basal diet, but the intakes of the other groups did not differ significantly. The feed conversion ratio was significantly better for the group fed the low P diet supplemented with 750U phytase A than for all the other groups except those fed the low P diets supplemented with 500U or 1000U of phytase A. Broiler performance was not significantly different between the groups fed the diets supplemented with phytase A or phytase B. Mortality level was not significantly different among the groups fed the phytase supplemented or unsupplemented diets.

0-42 days performance:

The birds fed the standard P diets were significantly heavier (by 4.3%) at 42 d than those fed the low P basal diets. There was no significant difference in mean body weight between the groups fed the standard P diet and all the phytase supplemented low P diets except for the group that was supplemented with 750U of phytase A, which was not significantly different from the group fed the low P diet. The two groups fed the low P diet supplemented with phytase B were significantly heavier than those fed the unsupplemented low P diet. Similar improvements in broiler performance after phytase supplementation were reported by Edwards, (1993); Broz et al., (1994); Sebastian et al., (1996a); Kies et al., (2001) and Huang et al., (2003). Birds fed diets supplemented with phytase A at 250, 750 and 1000U/kg of feed ate significantly less feed than those fed the positive control diet. Feed conversion ratio was not significantly affected by the source of phytase enzyme or its level of inclusion in diets.

*marketed by Danisco Animal Nutrition, UK. ** marketed by BASF, Germany

Table 1. The effect of phytase supplementation of low phosphorus corn-soybean meal based diets on body weight, weight gain and food intake (g/bird), FCR and mortality (%) in broilers at 21 and 42 days of age.

Diets	0-21 Days					0-42 Days				
	Body weight	Weight gain	Feed intake	FCR	Mortality	Body weight	Weight gain	Feed intake	FCR	Mortality
Standard P Diet	799 ^{abc}	759^{abc}	1392^{a}	1.724^{ab}	3.00^{ab}	2688^{a}	2648^{a}	4927^{a}	1.873	5.0^{a}
Low P Diet (Low P)	777^{bc}	739^{bc}	1343^{abc}	1.725^{ab}	2.0^{ab}	2571^{c}	2532^{c}	4727^{c}	1.879	2.3^{ab}
Low P + 250 U Phytase A / kg	803^{ab}	764^{ab}	1315^{bc}	1.628^{bc}	2.66^{ab}	2635^{abc}	2596^{abc}	4783^{bc}	1.861	3.0^{ab}
Low P + 500 U Phytase A / kg	770^{c}	731^{c}	1374^{ab}	1.783^{a}	1.66^{ab}	2628^{abc}	2589^{abc}	4846^{abc}	1.894	3.6^{ab}
Low P + 750 U Phytase A / kg	810^{a}	770^{a}	1272^{c}	1.566^{c}	3.33^{ab}	2605^{bc}	2565^{bc}	4770^{bc}	1.885	4.3^{ab}
Low P + 1000 U Phytase A / kg	807^{ab}	767^{ab}	1356^{ab}	1.666^{bc}	4.00^{a}	2631^{abc}	2591^{abc}	4781^{bc}	1.877	4.3^{ab}
Low P + 500 U Phytase B / kg	802^{ab}	762^{ab}	1357^{ab}	1.683^{ab}	1.00^{b}	2654^{ab}	2614^{ab}	4825^{abc}	1.859	2.0^{b}
Low P + 1000 U Phytase B / kg	801^{abc}	762^{ab}	1346^{ab}	1.673^{b}	2.33^{ab}	2654^{ab}	2615^{ab}	4859^{ab}	1.870	3.0^{ab}
$LSD_{0.05}$	31	31	71	0.102	2.42	74	74	129	0.055	2.9

The highest mortality of 5% was recorded in the group fed the normal P diet which was significantly greater than in the group given phytase B at 500 U/kg (2.0%) Most of the deaths in the trial were attributed to Sudden Death Syndrome. The results show that the performance of broilers fed low phosphorus corn-soybean meal based diets can be significantly improved by phytase A or phytase B supplementation. The present data indicates that phytase A or phytase B can replace 41% and 54% DCP in starter and finisher diets, respectively, without significantly affecting broiler performance.

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BROILER PERFORMANCE RESPONSE TO (LYSO-)PHOSPHOLIPID INCLUSION IN WHEAT BASED DIETS WITH ADDED TALLOW

R.R. CARTER[1] and R. PEREZ-MALDONADO[2]

The young bird's capacity to digest and absorb dietary fats is limited. Lipid digesting enzyme activity is low and bile secretion is considered to be the rate limiting factor in fat utilization in the first weeks after hatching (Nitsan *et al.,* 1991; Krogdal and Sell, 1989). Lysoforte® Booster Dry (LBD) is a source of phospholipids and lyso-phospholipids which has been shown to increase weaner pig performance (Carter and Henman, 2003).

Day old chicks (Ross 308) were used with four replicate cages per sex, and three LBD treatments (0, 0.5 or 1.0 g/kg) in a randomised block layout. The crumbled starter diets (0-21 d, 12.6 MJ ME/kg; 53 g total fat/kg) contained 32 g tallow/kg, and the pelleted finisher diets (21-42 d, 13.0 MJ ME/kg; 68 d total fat/kg) contained 48 g tallow/kg. All diets contained a xylanase based enzyme (Kemzyme®). Feed intake and weight gain were measured on a cage basis at days 21 and 42. Excreta were collected from each cage, mixed for each treatment and a single sub-sample taken for fat analysis. Broiler performance data (not excreta fat data) were analysed as three dietary treatment regimes x two sexes. The effect of Lysoforte Booster Dry on performance and excreta fat of male and female birds at 21 and 42 days of age is shown in the table.

Table Feed intake (g), weight gain (g), FCR and excreta fat (g/kg DM) at 21 and 42 d in male (M) and female (F) broiler chickens given Lysoforte Booster Dry at 0, 0.5 and 1.0 g/kg of feed

Lysoforte Booster Dry	Day 0-21 Feed intake			Day 0-21 Weight gain			Day 0-21 FCR			Day 21 excreta fat	
	M	F	Ave	M	F	Ave	M	F	Ave	M	F
0	1134	1000	1117	886	839	862	1.281	1.311	1.296[a]	66.4	79.9
0.5	1101	1077	1089	883	838	860	1.246	1.286	1.266[b]	45.7	41.7
1.0	1151	1070	1110	911	836	873	1.266	1.284	1.275[b]	49.2	38.3
LSD (P<0.05)	44.99	44.99	31.68	44.57	44.57	30.62	0.030	0.030	0.021		
Lysoforte Booster Dry	Day 0-42 Feed intake			Day 0-42 Weight gain			Day 0-42 FCR			Day 42 excreta fat	
	M	F	Ave	M	F	Ave	M	F	Ave	M	F
0	4906	4476	4691	3044	2628	2836	1.608	1.703	1.656[a]	66.1	53.6
0.5	4830	4424	4627	3077	2648	2863	1.567	1.669	1.618[b]	56.9	55.3
1.0	5013	4423	4718	3175	2648	2911	1.591	1.670	1.630[b]	57.7	58.9
LSD (P<0.05)	219.3	219.3	161	118.5	118.5	89.1	0.034	0.034	0.024		

[a,b:] values within a column with different superscripts are significantly different at P<0.05

Lysoforte Booster Dry significantly improved feed conversion efficiency at both inclusion levels (P<0.05). This improved efficiency appeared to be associated with reduced excreta fat concentrations in males and females at day 21 and in males at day 42.

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EFFECT OF DIFFERENT SOURCES OF PHYTASE SUPPLEMENTATION ON THE PERFORMANCE AND EGG QUALITY OF LAYING HENS

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Summary

Ninety hens were divided into six groups as a 2×3 factorial design and fed diets containing wheat bran (WB) at two levels of zero and five percent and the enzyme phytase at three levels of 0, 150 and 300 FTU/kg. Egg weight, egg production, feed intake and feed conversion ratio (FCR) were determined. Eggs were collected on two consecutive days at fortnightly intervals to measure egg size and egg component weights. Shell thickness was measured. Egg production, egg weight, FCR and feed intake were not affected by WB. Egg production, egg weight and feed intake were significantly higher in phytase-supplemented groups than unsupplemented groups. FCR differed significantly between dietary treatments as phytase supplementation significantly decreased FCR. Inclusion of WB to the diets had no effect on egg size and albumen weight. Phytase supplementation did not affect yolk weight, although albumen and shell weight were significantly affected.

I. INTRODUCTION

The major ingredients used in poultry diets are of plant origin. About two thirds of the phosphorus (P) in these feedstuffs is as phytate-bound P which is not available for utilization by poultry (Schwarz, 1994). Therefore, inorganic P supplementation is necessary to support optimal performance which significantly increases the cost of formulation. Improving the availability of phytate-bound P would reduce the necessity to include feed phosphates in the diets. This would result in a lower P excretion and reduce P-linked environmental pollution.

Exogenous dietary phytase improves performance and phytate-bound P utilization in hen (Boling *et al.*, 2000). Phytase is also found naturally in plant seeds (Cavalcanti and Behnke, 2004). As compared to other cereals, a higher concentration of endogenous phytase is found in the bran of both rye and wheat (Greiner and Egli, 2003). High-endogenous-phytase cereals and their by products can enhance phosphorus utilization by hens (Cavalcanti and Behnke, 2004). This study was conducted to determine the effect of endogenous and exogenous (microbial) supplementation on egg quality and production in layer hens.

II. MATERIALS AND METHODS

Ninety 46 wk-old white Leghorn laying hens were housed in 30 cages at the research center of Bu-Ali Sina University, Iran. Hens were weighed individually and divided into six groups of five replicates (cages). Groups were arranged in a factorial of 2×3 with wheat bran (WB) in two levels of zero and five percent and phytase (NataphosV®) in 3 levels of 0, 150 and 300 phytase units (FTU kg^{-1}) and fed as part of isocaloric and isoprotein diets containing 12.13 MJ ME, 165 g CP, 35 g Ca and 1.2 g AvP/kg.

Hen-day egg production and food intake were measured over an 8-week experimental period and feed conversion ratio (FCR) was calculated per cage. Eggs from each replicate were collected on two consecutive days every fortnight. Each egg was weighed then broken

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out onto a flat surface. The yolk was separated from the albumen and weighed. Shell thickness was measured. Egg shells were washed, dried and weighed. Albumen weight was calculated. Data were analyzed by ANOVA using the general linear models procedure of SAS software (SAS, 1997). Comparison between means was done using Duncan Multiple Range Test method. Significance was assumed at $P<0.05$.

III. RESULTS

Egg production, egg weight, feed intake and feed conversion ratio (FCR) were not affected by the inclusion of WB (Table 1). Egg production and feed intake were significantly higher in phytase supplemented groups than the unsupplemented group. FCR differed significantly between dietary treatments as phytase supplementation significantly decreased FCR. There were no significant interactions between WB and phytase supplementation for all the parameters.

Inclusion of WB in the diets had no effect on egg size, albumen weight shell weight or shell thickness (Table 2). Yolk weight was significantly lower in the group given 50 g WB/kg ($P<0.02$). There was a significant effect of phytase supplementation on albumen weight, shell weight and shell thickness, but egg weight was unaffected by phytase supplementation (Table 2). Albumen weight was significantly higher in the group given 300 FTU/kg phytase. Phytase supplementation generally improved shell weight and shell thickness. There was no significant interaction between WB level and phytase supplementation.

Table 1. Effect of endogenous and exogenous phytase on laying hen performance

Parameters	Wheat bran (WB)		Phytase (Ph)			P value			MSE
	0	5	0	150	300	WB	Ph	WB×Ph	
Egg Production (%)	69.13 [a]	73.90 [a]	60.27 [b]	75.83 [a]	78.45 [a]	0.0816	0.0001	0.1043	51.69
Egg weight (g)	57.05 [a]	57.27 [a]	56.11 [a]	57.44 [a]	57.91 [a]	0.7525	0.1116	0.7731	3.63
Feed intake (g/hen/day)	88.64 [a]	92.14 [a]	82.60 [b]	92.76 [a]	93.21 [a]	0.0916	0.0036	0.0564	28.57
FCR	2.33 [a]	2.22 [a]	2.62 [a]	2.14 [b]	2.06 [b]	0.3009	0.0005	0.0983	0.083

[a-c] Means within a main effect within a row with different superscripts are significantly ($P<0.05$) different using Duncan's multiple range test.

IV. DISCUSSION

Although WB is one of the best sources of endogenous phytase it did not affect egg production and other measurements of hen performance (Table1). The amount of active phytase present in cereals will vary depending on the cultivar, age and storage conditions (Cavalcanti and Behnke, 2004). Supplementation of diets with exogenous phytase caused significant increase in egg production, feed intake and feed utilization efficiency. The significant increase in egg production observed in the present study is in the agreement with the investigation on the effect of microbial phytase on egg production (Keshavarz, 2000). In the absence of supplemental phytase, feed intake was significantly decreased, in agreement with the results of Punna and Ronald (1999). The higher feed intake in the enzyme

Table 2. The effect of endogenous and exogenous phytase on egg size and egg component weights

Parameters	Wheat bran (WB)		Phytase (Ph)			P value			MSE
	0	5	0	150	300	WB	Ph	WB×Ph	
Egg Size (g)	57.75[a]	57.45[a]	56.73[a]	57.41[a]	58.30[a]	0.9429	0.0039	0.1655	17.325
Albumen Weight (g)	36.58[a]	36.44[a]	35.69[b]	36.36[ab]	37.14[a]	0.7004	0.0007	0.0613	11.19
Yolk Weight (g)	16.20[a]	15.97[b]	16.11[a]	15.98[a]	16.17[a]	0.0229	0.2108	0.5691	1.327
Shell Weight (g)	5.03[a]	5.02[a]	4.86[b]	5.09[a]	5.04[a]	0.9035	0.0016	0.6718	0.272
Shell Thickness (mm)	0.380[a]	0.377[a]	0.370[b]	0.383[a]	0.378[a]	0.2646	0.0249	0.0989	0.0011

[a-c] Means within a main effect within a row with different superscripts are significantly (P<0.05) different using Duncan's multiple range test.

supplemented groups is related to the higher egg production in these groups. FCR was significantly lower in the phytase supplemented groups. Liebert *et al.* (2005) found that microbial phytase significantly improved feed conversation efficiency whereas Boling *et al.* (2000) reported that FCR was not affected by phytase supplementation. Our results for egg production are in agreement with those of Boling *et al.* (2000) who reported that the addition of 100 FTU/kg phytase was adequate in a P-unsupplemented corn- soy bean meal-based diet.

Inclusion of WB to the diets had no effect on egg and egg component weights. Although Cavalcanti and Behnke (2004) reported that endogenous phytase resulting from inclusion of WB to a low available phosphorous (AvP) diet was able to improve P utilization, we did not see any effect of WB on layer hen performance. This might be due to the fact that, in the present study, AvP was similar for all the diets. Phytase supplementation significantly affected shell and albumen weight. Keshavarz (2000) also reported that phytase supplementation significantly increased egg size in laying hens.

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EFFECTS OF CHINA TEA (*CAMELLIA SINENSIS*) SUPPLEMENTATION OF DIETS ON CHOLESTEROL CONTENT OF BROILERS

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There is evidence that tea consumption reduces the risk of myocardial infarction in humans (Sesso *et al.,* 1999), and studies in hypercholesterolemic rats and mice have demonstrated a decrease in plasma cholesterol concentration following consumption of flavonoids or tea (Muramatsu *et al*., 1986; Matsumoto *et al*., 1998). Very little information, however, is available on the effect of supplementation of diets with tea leaves on plasma and tissue levels of cholesterol in poultry. The experiment reported here employed a completely randomised design with four replicates. Two hundred and forty 3-week-old male broilers (Ross 301) were randomly divided into 20 pens (1.5 x .8 m.) with 12 birds per pen. The experimental diets contained 200 g CP and 12.6 MJ ME/kg and tea leaves were supplemented at 0, 5, 10, 15 or 20 g/kg. Body weight and feed consumption data were collected each week per group. The birds were weighed at 49 days of age when the experiment terminated. Four birds per pen were sampled at random for carcass analysis and were individually weighed. Cholesterol concentration in the thigh muscle of each bird was determined using the technique developed by Will and Greenfield (1984).

There was no effect of inclusion of china tea leaves at any level on feed intake, weight gain, FCR, dressing percentage or abdominal fat pad proportion. However, as shown in the table, there was a significant decrease in thigh meat cholesterol concentration at supplementation levels of 10 and 15 g/kg.

Table. Effect (Mean ±SEM) of dietary supplementation with tea leaves on thigh meat cholesterol concentration (mg/100 g) of broilers.

	Dietary concentration of tea leaves (g/kg)				
	0	5	10	15	20
Cholesterol	85.5±2.5^{a}	78.5±0.5^{ab}	75.5±1.5^{b}	75.0±3.0^{b}	81.0±3.0^{ab}

Different superscripts indicate significant differences ($P<0.05$)

The result agrees with those obtained by Chan *et al.* (1994) who showed dietary supplementation with tea leaves (epicatechins) to interfere with fat absorption in hamsters.

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EFFECT OF CORTICOSTERONE ON THE IMMUNE RESPONSE OF BROILER CHICKENS

J.C. LOPEZ[1], R. MCFARLANE[1] and O. AMOAFO[1]

Summary

The effect of environmental stressors on the immune system of broilers was mimicked by the administration of corticosterone (CORT). Commercial broilers were randomly assigned to one of four treatment groups (n=50): group 1 [not challenged with infectious bronchitis virus (IBV) or injected with exogenous antigen but treated with corticosterone (CORT) from 23 days of age]; group 2 [challenged with IBV, injected with sheep red blood cells (SRBC) and phytohaemagglutinin (PHA) but not treated with CORT]; group 3 (challenged with IBV, injected with SRBC and PHA and treated with CORT from 23 days of age), and group 4 (challenged with IBV, injected with SRBC and PHA and treated with CORT from 26 days of age). The birds given CORT in the drinking water from five days before injection with SRBC (23 days of age), showed an enhancement in the humoral immune response, in comparison to the birds given CORT three days later (two days before challenge) or the birds that were untreated with CORT. Furthermore, the birds given CORT five days before IBV challenge, developed a higher cell mediated immune response as measured by the level of INFγ released by splenic lymphocytes, after *in vitro* stimulation with IBV. That is, the immunological effects of administering physiological amounts of CORT, which may reflect temperature extremes, are dependent on timing relative to exposure with environmental antigen.

I. INTRODUCTION

Extremes of ambient temperature are important stressors that confront poultry in many regions of the world and large economic losses can occur because of mortality and decreased production (Altan *et al*., 2000). Results from studies on the effect of temperature extremes on humoral and cell-mediated immune responses in chickens are inconsistent (Heller *et al.,* 1979, Subba Rao and Glick, 1970). We have recently shown that low temperatures (10°C) can cause elevated blood levels of CORT (unpublished data). It has been proposed that the time between the release of CORT and challenge is a factor that can change the immune response (towards inhibition or enhancement) (Dhabhar, 2002). The goal of this study was to assess the effect of administering physiological doses of CORT on humoral and cell-mediated immune responses in chickens, following infection with IBV and injection with SRBC and PHA.

II. MATERIALS AND METHODS

Two hundred, day-old broiler chickens (Cobb breed) were obtained from a commercial hatchery. At 23 days of age the birds were randomly assigned to one of four treatment groups (n=50): groups 1 and 3 [treated with CORT (14.7 ng/ml) in the drinking water from 23 days of age (five days before challenge)]; group 2 (not treated with CORT); and group 4 [treated with CORT in the drinking water (14.7 ng/ml) from 26 days of age (two days before challenge)].

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At 28 days of age all birds in groups 2, 3 and 4 were infected intranasally with 100µl of 10^6 EID_{50}/ml of IBV (NZ strain C - supplied by the National Centre for Disease Investigation, Upper Hutt, New Zealand), and injected with 0.5 ml of packed sheep red blood cells (50% v/v in PBS) into the breast muscle. Additionally, at 45 days of age, eight birds from groups 2, 3 and 4 were injected in the right wattle with 200 µg of PHA in 0.1ml of sterile, pyrogen-free, physiologic saline solution, while the left wattle was injected with saline as a control. The wattle thickness of each bird was measured with a dial micrometer 24h later, as described by Tella *et al.* (2002).

Antibody production against SRBC was measured using a micro haemagglutination test (1:2 dilution), as described by Wegmann and Smithies (1966) and results were expressed as the reciprocal of the highest dilution of serum showing specific agglutination with antigen.

Twelve birds from each of the four groups were slaughtered at 23, 28, 38, and 43 days of age in order to assess the levels of INFγ from splenocytes that had been separated on Ficoll-Paque™ Plus (Amersham Biosciences, Sweden), washed twice in RPMI 1640 medium (Sigma-Aldrich, USA), and resuspended in the same medium supplemented with 10% FCS. Cells, at a concentration of 1x 10^7/ml, were incubated at 39°C for 72 h with IBV antigen whole virus at 10 µg/ml, as per Lambrecht *et al.* (2000). Supernatants were collected after centrifugation at 3,000 rpm for 15 minutes and stored at -70°C. The levels of INFγ in the supernatant were estimated using a sandwich ELISA (Biosource International, USA).

The CORT levels were measured by radioimmunoassay (RIA), at the Institute of Veterinary, Animal and Biomedical Sciences, Massey University (Littin and Cockrem, 2001). Significance was assessed using an analysis of variance, and differences between the means were determined using the Least Significant Difference (Minitab Statistical Software).

III. RESULTS and DISCUSSION

By 28 days of age, the birds, from Group 1 and 3, that started the CORT treatment five days before challenge (23 days of age) had a significantly higher ($P<0.01$) level of plasma CORT than the birds not treated with CORT (group 2). At 36 days of age the birds from the groups treated with CORT (groups 1, 3 and 4) had significantly ($P<0.01$) higher levels of plasma CORT than the birds not treated with CORT (group 2) (Figure 1a). These CORT levels are similar to levels found in poultry housed at low (10°C) temperatures (unpublished data).

Eight days after injection with SRBC it was found that the chickens treated with CORT from 23 days of age (five days before challenge) had a significantly higher HA titre ($P<0.01$) than the birds not treated with CORT, which in turn developed a higher HA titre ($P<0.05$) than the birds treated with CORT from two days before challenge (26 days of age) (Figure 1b). Moreover, the birds that were given CORT in the drinking water five days before the challenge developed a higher cell mediated immune response, as measured by the level of INF-γ released by splenic lymphocytes after *in vitro* stimulation with IBV, in comparison to the birds exposed to CORT for two days before challenge (Figure 1c). In humans, exposure to cortisol for up to a week before a challenge with endotoxin enhances TNF-ά and IL-6 levels, whereas cortisol at the time of or after endotoxin suppresses the cytokine response (Barber *et al*., 1993). The skin reaction to PHA injected 24 hours previously (49 days of age) showed that the birds from the groups treated with CORT, either two or five days pre-challenge, had significantly smaller reactions than untreated birds. This *in vivo* test provides a general index of cell mediated immunity (Tella *et al*., 2002), as compared to the *in vitro*, antigen-specific (IBV) assay measuring INFγ production from T cells present in the spleen (Lambrecht *et al*., 2000).

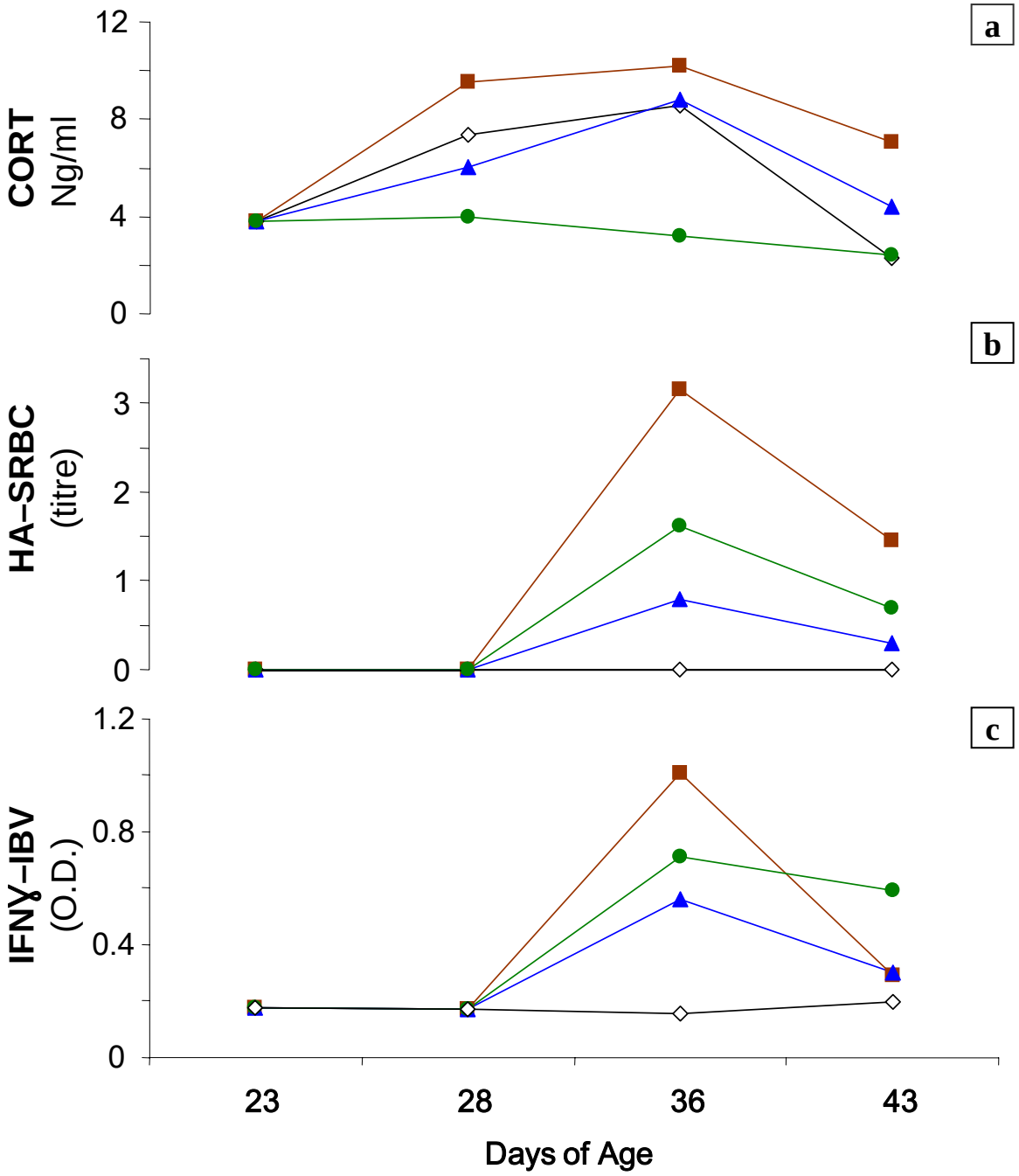

Figure 1. (a) Levels of CORT in plasma, (b) haemagglutinin titre to SRBC and (c) IFNγ release from cultured splenocytes stimulated with IBV *in vitro*.
From chickens treated with CORT from two days before challenge with SRBC and IBV (at 28 days of age) (▲), treated with CORT from five days before challenge with SRBC and IBV (■), treated with CORT from 23 days of age but without challenge with SRBC or IBV (◊) and birds not treated with CORT but challenged with SRBC and IBV (●)

It has been argued that the immunological actions of CORT are vested in the basal levels of CORT in blood at the moment of challenge and possibly the pre-exposure period, causing down-regulation of glucocorticoid receptors or alteration of their affinity (Armario *et*

al., 1994). In this experiment the transient immuno-enhancement was associated with the longer duration of steroid intake (five days) and immuno-suppression occurred with the shorter duration (two days). Plasma CORT levels did not differ at the time of challenge due to timing of administration. In an experiment described by Post *et al.* (2003) the immunosuppressive effects of the exogenous CORT were noted after three days of CORT treatment achieving very high plasma CORT concentrations (30-35 ng/ml) in comparison to the physiological levels reached in this experiment (9.5 ng/ml). Enhancement of the immune response may occur via an increase in cytokine receptor expression on several cell types (e.g., T cells) for IFNγ (Strickland *et al*., 1986), or alternatively, by leukocyte redistribution (Dhabhar, 2002).

The majority of the studies on corticosteroids have focused on the immunosuppressive actions of these stress hormones. In this experiment it was found that in chickens, as for other species, the time between the beginning of the CORT treatment and the moment of challenge is crucial in determining response. This is consistent with the view that corticosteroids can exert distinct, seemingly paradoxical, effects on cytokine expression, cytokine receptor expression and cytokine-regulated biological responses (Wiegers and Reul, 1998).

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ULTRASTRUCTURAL EXAMINATION OF HETEROPHILS OF CHICKENS EXPOSED TO CORTICOSTERONE

S. SHINI[1], W.I. MUIR[2], A. SHINI[1] and W.L. BRYDEN[1]

In chickens, the absolute number and relative proportion of blood leukocytes provide an important representation of the state of activation of the immune system. Moreover, morphological and cellular identification of leukocytes is an important tool in understanding the response of lymphoid and nonlymphoid tissue to microbial and non-microbial challenges. Previously, it has been indicated, that a variety of stressors, including exposure to corticosterone (CORT) challenges the leukocyte response and affects the H/L ratio. In fact, the H/L ratio itself represents a mathematical calculation of limited clinical value (Latimer and Bienzle, 2000). It has been also documented that heterophilia predominates mainly in the acute inflammatory response and in bacterial protection. Therefore, the role played by CORT in the regulation of the leukocyte cell response (i.e. their number, morphology and distribution) needs further investigation. This study examined ultrastructural alterations of leukocyte cells separated from peripheral blood and bone marrow in stressed chickens.

At 7 weeks of age, 120 layer hens were randomly assigned to 4 treatment groups: Group 1 was untreated. Group 2 was given 0.1% ethanol as their drinking water for 10 days and injected IV on day 1 with 0.5 ml of 0.85% saline solution. Group 3 was given CORT dissolved in ethanol via the drinking water to provide 20 mg CORT and 1 ml ethanol per litre water, for 10 days. Group 4 was injected IV on day 1 with 8mg/ kg BW lipopolysaccharide (LPS) from *Escherichia coli* dissolved in 0.5 ml sterile 0.85% saline solution. Blood samples were taken for haematology and CORT measurements on days 0, 1, and 10. Leukocytes were isolated from whole blood and bone marrow and prepared for transmission electron microscopy (TEM). Ultra-thin sections were viewed and photographed under TEM.

Distinct alterations in ultrastructures were detected in leukocytes from both CORT- and LPS-treated birds. Treatment with CORT caused changes in heterophil shape, characterized by flattening with extensive membrane ruffling and pseudopot formation, and an increase in density and granule size. LPS-treated birds demonstrated changes in heterophil shape and degranulation. Bone marrow isolated from CORT-treated birds showed an increased number of promyelocytes and myeolocytes, mainly heterophil myeolocytes. This increase was associated with the presence of band heterophils in the blood stream, indicating the recruitment of immature heterophils from the bone marrow due to CORT. Morphological changes were examined in lymphocytes, and thrombocytes in peripheral blood and bone marrow of CORT-treated birds. It was demonstrated that corticosterone and LPS both stimulate an increased release of heterophils into the circulation from bone marrow and movement from the marginated pool. Nevertheless, the question remains whether these cells can be readily activated, and, if so, is their function significantly enhanced? It is expected that their activation initiates a complex process promoting non-specific defence. However, the mechanism behind this response which involves the activation of the cytokine network and the neuroendocrine system is likely to differ in response to stress and bacterial infection. Further research will be carried out to investigate functional changes of leukocytes during stress and to identify and measure the cytokines responsible for this regulation.

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PERFORMANCE AND DIGESTIVE TRACT CHARACTERISTICS OF BROILERS AS INFLUENCED BY PARTICLE SIZE AND FEED FORM

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Summary

The effects of wheat particle size (fine, medium and coarse) and feed form (mash or pellet) on the performance and digestive tract characteristics of broiler chickens were investigated in a 3-week feeding trial. The birds fed pelleted diets grew faster ($P<0.05$) and were more efficient ($P<0.05$) compared to those fed mash diets. Pelleting evened out differences in particle size distribution between treatments and, as a result, wheat particle size had no effects ($P>0.05$) on the performance of broilers fed pelleted diets. In mash diets, coarse grinding of wheat improved ($P<0.05$) weight gain and feed efficiency compared to medium grinding. The relative weights of gizzard and caeca were lower ($P<0.05$) in birds fed the pelleted diets.

I. INTRODUCTON

To study the effect of grain particle size on performance, diet form (mash or pelleted) should also be considered. Interaction between particle size and feed form in broiler diets is known. Available evidence suggests that the effects of grain particle size are more critical in mash feeds, but less so in pelleted feeds (Nir *et al*., 1995; Svihus *et al*., 2004). The objectives of the present study were to investigate the influence of particle size and feed form on the performance, digestive tract characteristics and energy utilisation of broilers fed wheat-based diets.

II. MATERIALS AND METHODS

The experimental design was a 3 x 2 factorial arrangement of treatments evaluating three wheat particle sizes (fine, medium and coarse) and two feed forms (mash and pellet). The three particle sizes were achieved by grinding the whole wheat in the hammer mill to pass through 1, 3 and 7 mm sieves, respectively. Broiler starter diets, based on wheat and soybean meal, were formulated. After mixing, half of each of the three basal diets were cold-pelleted (70 ^{0}C). Each diet was fed to six pens of eight male broilers (Ross) each from day 1 to 21 post-hatching. The diets were offered *ad libitum* and water was freely available at all times. Body weights and feed intake were recorded at weekly intervals, and feed per gain, corrected for mortality, was calculated. From day 17 to 20 post-hatching, feed intake and total excreta output were measured quantitatively per pen for the determination of nitrogen-corrected apparent metabolisable energy (AME_n).

On day 21, two birds per replicate, closest to the average pen weight, were selected. The birds were weighed, euthanised and weights of digestive tract components were recorded. Particle size spectra of the mash and pelleted diets were determined using the wet sieving method as described by Lentle *et al.* (2006). Briefly, the feed samples were suspended in 50ml of water and left to stand for 30 min prior to sieving to ensure adequate hydration. The samples were then wet sieved in a set of Endocot sieves of size 2, 1, 0.5, 0.25, 0.106 and 0.075 mm. The masses of particles from each sieve were expressed as percent of total dry

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matter recovered including fines. The data were statistically analysed using the General Linear Models procedure of SAS (1997).

III. RESULTS AND DISCUSSION

The original experimental design for this study was a 2 x 3 factorial arrangement of treatments. The birds fed the fine mash diet, however, grew extremely poorly, reaching a body weight of only 96 g at two weeks of age as against 258 g in birds fed the medium mash diet. This group was removed from the study at two weeks for welfare reasons and excluded from final analysis. Severe impaction of the digestive tract was observed in birds fed the fine mash diet, which may have been caused by highly viscous digesta. High digesta viscosity has been reported in birds fed fine mash diets compared to those fed medium or coarse mash diets (Yasar, 2003).

The particle size distribution of mash and pelleted diets is shown in Figures 1 and 2, respectively. It can be seen that the differences in particle size distribution between the two feed forms were minimised by pelleting. During the pelleting process, the feed is passed through steam, which softens the particles and pressed through the die by the rolls in the pellet press, which appears to cause an extra grinding effect (Svihus *et al.*, 2004).

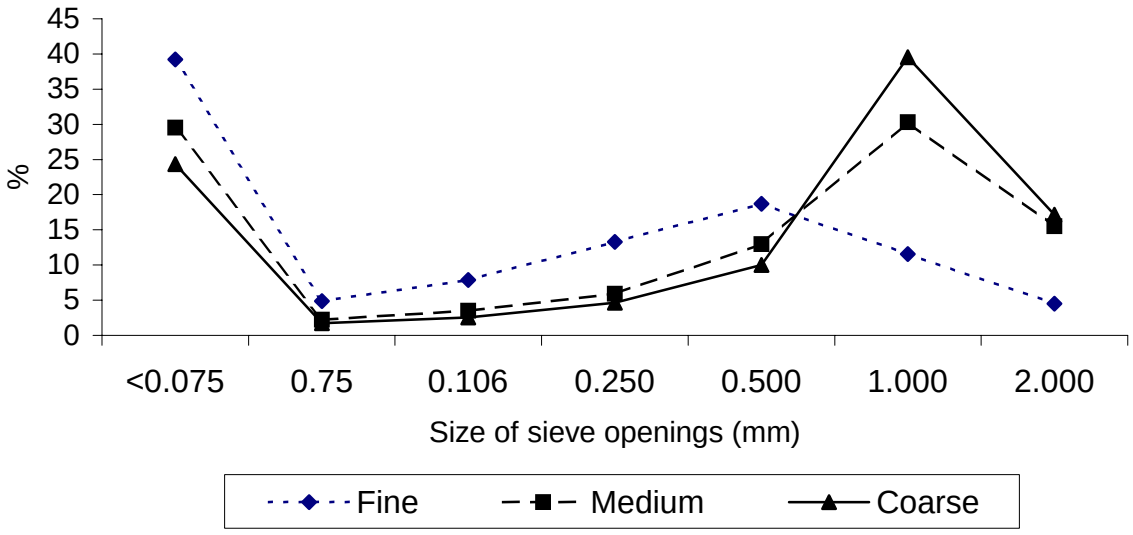

Figure 1. Particle size distribution of mash diets

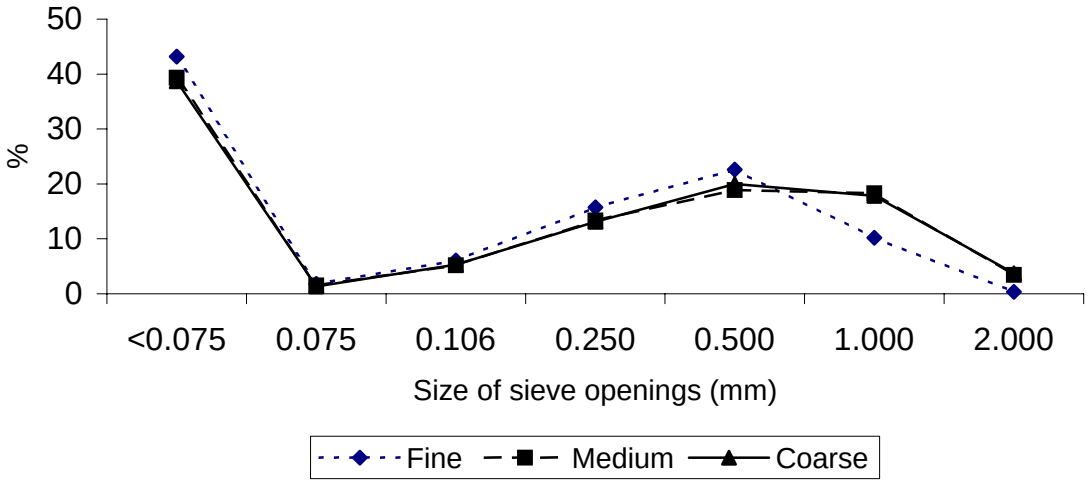

Figure 2**.** Particle size distribution of pelleted diets

Pelleting improved (P<0.05) the weight gain, feed intake and feed/gain compared to mash diets (Table 1). These results confirm the superiority of pelleted diets over mash diets in improving broiler performance. Bird performance was similar (P>0.05) between birds fed pelleted diets based on fine, medium and coarse grinds and this may be explained by effect of pelleting in evening out the particle size differences (Figure 2). Previous research conducted using pelleted diets have also reported no effect of particle size on broiler performance (Svihus *et al.*, 2004).

Table 1. Weight gain (g/bird), feed intake (g/bird), feed per gain (g/g) and AMEn (MJ/kg dry matter) of broilers as influenced by feed form and particle size

	Mash		Pellet			SEM
	Medium	Coarse	Fine	Medium	Coarse	
Gain	453[a]	539[b]	821[c]	834[c]	824[c]	16.10
Feed intake	777[a]	877[b]	1266[c]	1271[c]	1253[c]	20.74
Feed/gain	1.717[a]	1.629[b]	1.543[c]	1.525[c]	1.521[c]	0.018
AMEn	14.39[a]	14.23[ab]	13.69[c]	13.65[c]	13.85[bc]	0.14

[a,b,c] Means in a row not sharing a common superscript differ (P < 0.05).

In mash diets, coarse grinding improved (P<0.05) broiler performance compared to medium grinding (Table 1). This agrees with previous studies, which showed that broilers have preference for large particle size diets, which increased feed intake and growth rate (Nir *et al*., 1995). Increasing diet particle size in wheat-based diets has been shown by Yasar (2003) to lower digesta viscosity and this may explain, at least in part, the better performance of birds fed the coarse mash diet. In the present study, pelleting reduced the AMEn (P<0.05) compared to mash diets (Table 1). This finding may be related to the higher feed intake in the pellet treatments. The higher intake of pelleted diets has been suggested to increase the starch load in the gut and to lower the digestibility of starch (Svihus and Hetland, 2001).

The influence of treatments on the relative weights of digestive organs and digestive tract is shown in Table 2. Dietary treatments had no effect (P>0.05) on the relative weights of

digestive organs, except on the gizzard, pancreas and caeca. Mash feeding resulted in a larger ($P<0.05$) gizzard and caeca compared to those in birds fed pelleted diets.

Table 2. Relative weight of digestive tract components (g/kg body weight) of broilers as influenced by feed form and particle size

	Mash		Pellet			SEM
	Medium	Coarse	Fine	Medium	Coarse	
Crop	3.1	2.9	3.2	3.3	3.1	0.22
Proventriculus	5.8	5.1	4.95	5.24	5.5	0.05
Gizzard	22.0[a]	20.1[a]	9.4[b]	10.7[b]	11.3[b]	0.66
Pancreas	3.4[a]	3.1[ab]	3.1[ab]	2.8[b]	3.1[ab]	0.12
Liver	29.0	29.0	31.9	32.0	32.8	1.14
Small intestine	28.9	25.7	28.8	28.3	28.3	0.97
Caecum	2.3[a]	2.1[a]	1.6[b]	1.5[b]	1.7[b]	0.01

[a,b] Means in a row not sharing a common superscript differ ($P < 0.05$).

In conclusion, under the conditions of the present study, pelleting evened out the differences in particle size distribution between treatments and, presumably as a result, particle size had no effects on the performance of broilers fed pelleted diets. Although pelleting had a negative effect on AME values, broiler performance was superior in birds fed pelleted diets compared to those on mash diets. In mash diets, coarse grinding of wheat improved weight gain and feed efficiency compared to medium ground wheat.

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INFLUENCE OF PARTICLE SIZE ON THE PERFORMANCE, DIGESTA CHARACTERISTICS AND ENERGY UTILISATION OF BROILERS FED MAIZE AND WHEAT BASED DIETS

A.M. AMERAH[1], V. RAVINDRAN[1], R.G. LENTLE[1] and D.G. THOMAS[1]

Summary

The effects of grain type (wheat and maize) and particle size (fine and coarse) on the performance, digesta characteristics and nutrient utilisation of broilers during 1-21 days post-hatching were investigated. Coarse grinding improved ($P<0.05$) bird performance and increased ($P<0.05$) relative gizzard weights compared to those fed diets based on fine particles. Differences in particle size distribution still existed between diets after pelleting especially in the proportion of coarse particles (1 mm and over). However, grinding in the gizzard evened out differences in particle size between the diets, which resulted in no difference ($P>0.05$) in particle size distribution in the duodenal digesta of the birds fed diets based on fine and coarse particles. Grain particle size had no effect ($P>0.05$) on apparent metabolisable energy. The results of the present study suggest that coarse grinding is advantageous to broiler performance.

I. INTRODUCTON

Available evidence suggests that grain particle size is more critical in mash feeds, but less so in pelleted feeds (Nir *et al.,* 1995; Svihus *et al.*, 2004). However, pellets dissolve in the crop after consumption (Nir and Ptichi 2001) and the effects of particle size on broiler performance may be maintained even after pelleting.

Grain type is known to influence the particle size when milled under the same conditions, with grinding through the same screen size in a hammer mill yielding different particle size distributions. This suggests that during grinding using a hammer mill, different screen sizes may have to be used according to the grain type to obtain the desired particle size distribution. Nir *et al.* (1995) reported that grinding wheat with the same hammer or roller mill under the same conditions gave higher geometric mean diameter (GMD) compared to sorghum. In the study reported herein, the effects of grain type (wheat and maize) and particle size (fine and coarse) on broiler performance, digesta characteristics and energy utilisation were investigated.

II. MATERIALS AND METHODS

The experimental design was a 2 x 2 factorial testing two grains (wheat vs. maize) and two particle sizes (fine vs. coarse); all diets were fed in pelleted form. The two particle sizes were achieved by grinding the whole wheat and maize in the hammer mill to pass through 1 and 7 mm sieves. Broiler starter diets, based on wheat and soybean meal or maize and soybean meal, were formulated. Each diet was fed to six pens of eight male broilers (Ross) each from day 1 to 21 post-hatching. The diets were offered *ad libitum* and water was freely available at all times.

Body weights and feed intake were recorded at weekly intervals, and FCR, corrected for mortality, was calculated. From day 17 to 20 post-hatching, feed intake and total excreta

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output were measured quantitatively per pen for the determination of nitrogen-corrected apparent metabolisable energy (AME_n). On day 21, two birds per replicate, closest to the average pen weight, were selected. The birds were euthanised and digesta samples from the duodenum were obtained. Particle size spectra in the diet and duodenal digesta samples were determined using the wet sieving method as described by Lentle *et al.* (2006). Briefly, the diet and digesta samples were suspended in 50 ml of water and left to stand for 30 min prior to sieving to ensure adequate hydration. The samples were then wet sieved in a set of Endocot (London, U.K.) sieves of size 2, 1, 0.5, 0.25, 0.106 and 0.075 mm. The masses of particles from each sieve were expressed as percent of total dry matter recovered including fines. The data were statistically analysed using the General Linear Models procedure of SAS (1997). Particle size distributions were compared by discriminate analysis in SYSTAT (Wilkinson, 1990).

III. RESULTS AND DISCUSSION

Wet sieving of pelleted diets indicate that the differences in particle size distribution still existed between diets after pelleting especially in the proportion of coarse particles over 1mm (Figure 1).

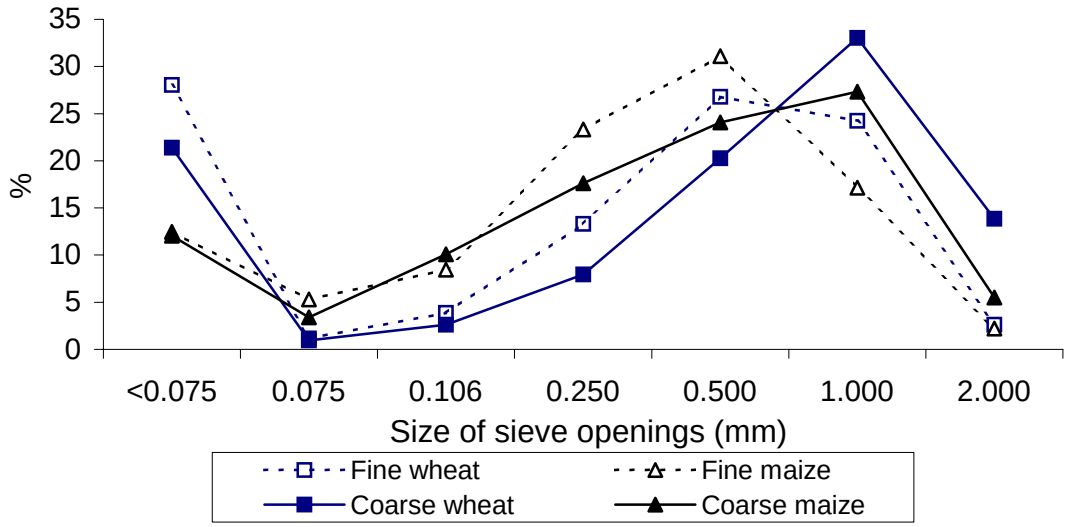

Figure 1. Particle size distribution of pelleted diets

The influence of dietary treatments on the performance of broilers is summarised in Table 1. Grain type tended (P=0.06) to influence weight gains, with birds on wheat-based diets having a greater gain than those on maize-based diets. There was, however, a tendency (P=0.07) for treatment interactions, with a particle size effect being evident only in maize-based diets. Feed intake was influenced (P<0.001) by grain type, with the intake of wheat-based diets being greater than those of maize-based diets. Particle size affected feed intake in wheat-based diets and not in maize-based diets, as indicated by a tendency (P=0.10) for treatment interaction. Birds fed maize-based diets had a better (P<0.001) feed efficiency than those fed wheat-based diets. FCR was lower on coarse than finely-ground grain diets.(P<0.001). No interactions (P > 0.05) were observed for FCR.

Table 1. Weight gain (g/bird), feed intake (g/bird), FCR (g/g), AME_n (MJ/kg DM) and relative gizzard weight (g/kg BW) of broilers as influenced by grain type (G) and particle size (PS)

Treatment	Wheat		Maize		SEM	Significance (P<0.05)		
	Fine	Coarse	Fine	Coarse		G	PS	G x PS
Weight gain	888	872	823	870	17	.06	NS	.07
Feed intake	1357	1262	1191	1173	22	.0001	.02	.10
FCR	1.528	1.467	1.448	1.360	.013	.0001	.0001	NS
AME_n	12.07	12.41	13.25	13.04	.13	.0001	NS	.06
Gizzard wt.	9.03[a]	10.08[a]	9.40[a]	12.63[b]	.44	.001	.0001	.01

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

For maize diets, previous research has shown that birds fed fine maize in pelleted form had better feed efficiency than those fed coarse particles (Lott *et al.*, 1992; Kilburn and Edwards, 2001). Lott *et al.* (1992) reported that fine maize (GMD, 716 microns) improved broiler performance compared to those fed coarse particles in (GMD, 1196 microns) in crumbled form. In contrast, coarsely ground maize resulted in better feed efficiency in the present study.

Published data on the effects of wheat particle size in pelleted diets on broiler performance have been contradictory. Peron *et al.* (2005) reported a tendency for better feed efficiency in birds given coarse wheat (GMD, 955 microns) compared to those fed fine ground wheat (GMD, 388 microns) and this enhancement was associated with a greater relative gizzard weight. Lentle *et al.* (2006), using three different wheat cultivars, also found that the diet with the higher relative proportion of coarser particles resulted in the best feed/gain in broiler chickens. These findings are similar to those observed in the present study. On the other hand, Svihus *et al.* (2004) reported that pelleting evened out differences in particle size distribution in the pelleted diets and no effect of wheat particle size on broiler performance was observed in their study. It would, therefore, appear that the effect of particle size in pelleted diet may depend on particle size distribution after pelleting, which in turn may depend on the hardness of the grain used.

In this study, grain particle size had no effect (P>0.05) on the AME values (Table 1). However, there was a tendency for treatment interaction (P=0.06) with coarse grinding improving the AME in wheat-based diets and not in maize-based diets. This finding is in disagreement with those of Svihus *et al.* (2004) who reported no effect of wheat particle size and of Kilburn and Edwards (2001) who found that fine grinding of maize improved true metabolisable energy values.

Relative gizzard weights were increased (P<0.05) by coarse particles (Table 1). However, the increases were greater in the maize-based diets than in the wheat-based diets as indicated by a significant (P<0.01) grain type x particle size interaction.

The discriminant analysis failed to show distinction (P>0.05) between particle size distributions of the duodenal digesta from birds fed fine and coarse particle sizes of wheat-based diet (Table 2). Similarly, the discriminate analysis failed to show significant difference (P>0.05) between particle size distributions of duodenal digesta from birds fed fine and coarse particle sizes of maize. These results suggest that the gizzard evened out the differences in particle size of the diet.

Table 2. Proportion of particle size classes (mean ± SE) in the duodenal digesta (on a dry weight basis) of broilers as influenced by grain type and particle size

	Wheat		Maize	
	Fine	Coarse	Fine	Coarse
<0.075mm	0.535±0.058	0.463±0.040	0.334±0.086	0.397±0.090
0.075-0.106mm	0.056±0.010	0.077±0.013	0.100±0.025	0.119±0.023
0.106-0.25mm	0.167±0.021	0.184±0.031	0.251±0.035	0.202±0.051
0.250-0.500mm	0.134±0.025	0.151±0.026	0.210±0.040	0.214±0.042
0.500-1.000 mm	0.094±0.048	0.067±0.014	0.089±0.015	0.078±0.014
1.000-2.000 mm	0.013±0.002	0.046±0.010	0.016±0.006	0.034±0.009

Under the conditions of the present study, grain particle size maintained its effect after pelleting; birds fed coarse particle size diets showed better feed efficiency than those given finely ground grain diets. The former group had larger gizzards, which is thought to be beneficial in terms of the motility, function and gut health (Ferket, 2000; Gabriel *et al*., 2003).

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BROILER PERFORMANCE IN AUSTRALIAN SORGHUM-BASED STARTER AND FINISHER DIETS (2005 HARVEST)

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Summary

Broiler performance was investigated in 0-21 and 21-42 day (d) old birds, fed sorghum-based crumbled starter and pelleted finisher diets respectively, compared to a wheat-based control diet. At 21 d, birds on the wheat diet had higher ($P<0.05$) live weight gain (LWG) than those fed sorghum-based diets except for sorghum variety K-based diet which was similar to wheat. The feed conversion ratio (FCR) was also more efficient for birds on the wheat diets during the same period. However, during day 21-42 period, LWG was comparable ($P>0.05$) for both wheat and sorghum diets, with FCR similar ($P>0.05$) for 43% of sorghum-based diets when compared to wheat diets. At 43 d all sorghum-based diets produced birds with similar fat pad value ($P>0.05$) and breast-meat yield to those given the wheat-based diet except for the sorghum variety A-based diet, where breast-meat yield was 1.6% lower ($P<0.05$). These results agree with earlier studies from the 2004 harvest

I. INTRODUCTION

In Australia, sorghum is an alternative grain to wheat for feeding poultry due to its availability, lower price and less competition with other grains. The nutritional value of sorghum is generally lower than wheat due to a lower crude protein content and amino acid digestibility. However, its apparent metabolisable energy (AME) is higher and is less variable than wheat. Where sorghum is used instead of wheat as the primary grain in broiler diets formulated to the same nutrient specifications, significant variability in breast-meat yield and poor FCR have been observed.

The inferior FCR and high carcass variability may be due to anti-nutritional factors (ANF) such as condensed tannins (CT) and other polyphenolic compounds still present in new sorghum varieties (Perez-Maldonado *et al*., 2006), limiting the availability and digestibility of essential nutrients. These CT and phenolic compounds can rapidly increase during adverse environmental conditions, such as drought (Perez-Maldonado 1994).

Other factors affecting performance may be associated with the sorghum prolamins proteins (Taylor *et al.,* 1984), designated *alpha*, *beta* and *gamma* kafirins (Shull *et al.,* 1991). *Beta* and *gamma* kafirin are rich in cysteine and are linked together with *alpha* kafirin by disulphide bonds into oligomers of peptides which can reduce protein and starch digestibility (Taylor 2005). The present study investigated production performance of 14 sorghum-based diets from the 2005 harvest compared to a wheat-based diet, offered to broiler birds as part of a larger study into the nutritional characterisation of Australian sorghums.

II. MATERIALS and METHODS

In this study, 14 sorghum-based treatment diets and one wheat-based diet (control) were offered to Arbor Acres (n=608) male broilers housed in 76 cages with five replicates

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per sorghum treatment and six replicates for the control diet. The experimental unit was a cage of eight birds in a completely randomised design. The study evaluated production performance parameters and carcass quality in two age groups, 0-21 d (starter phase) and 21-42 d (finisher phase). Birds were offered crumbled starter (12.0 MJ AME and 127 g total lysine/kg) and pelleted finisher diets (13.0 MJ AME and 110 g total lysine/kg) formulated to achieve maximum production. The control diet (starter-finisher) was formulated using wheat (637-698 g/kg), soybean meal (188-153 g/kg), meat and bone meal (55-39 g/kg), canola meal (50-30 g/kg), sunflower meal (30-20 g/kg) with a commercial xylanase enzyme added according to industry practice. The remaining sorghum treatments were formulated using sorghum (602-599 g/kg), soybean meal (256-234 g/kg), meat and bone meal (54-40 g/kg), canola meal (35-30 g/kg), sunflower meal (44-20 g/kg). All diets were supplemented with vitamins, minerals and amino acids. Feed and water were provided *ad libitum* with lighting and temperature in an environmentally controlled poultry house according to industry practice. Animal ethics approval was received prior to commencement of this study. On days 21 and 42, birds from each cage were group weighed. Feed intake (FI g/bird) was recorded for each treatment cage by weighing each feeder plus contents on days 0 (start of trial), 21 (end of starter period) and 42 (end of finisher period). Performance variables were: FI, live weight gain (LWG) and FCR. On day 43, three birds per cage were euthanised for evaluation of carcass quality, which included fat pad and breast-meat yield measurements, expressed as % of bird body weight. Data were analysed using ANOVA and significant ($P<0.05$) differences between treatment means were determined using the Least Significant Difference test.

III. RESULTS

The mean production performance, carcass evaluation values of broilers (0-21 and 21-42 d old) fed either sorghum-based diets or a wheat-based diet and CT values of sorghum grain, are presented in Table 1.

At 21 d, birds offered sorghum diets B, C, D, E, F, G, I, K and M exhibited similar ($P>0.05$) FI to birds offered the wheat diet. Birds at 21 d given the control diet had superior ($P<0.05$) LWG (944 g/bird) than those given the sorghum treatments (836-902 g/bird) other than sorghum diet K (931 g/bird). FCR was also lower for birds on the control diet (1.263) than for those given the sorghum diets.(1.296-1.355). However, during the finisher phase, there was no difference in LWG between the birds given the wheat or sorghum-based diets, but FCR on sorghum-based diets A, C, H, L, M and N (1.645 – 1.697) was similar to that of the birds on the wheat-based diet (1.655).

The carcass evaluation at 43 d revealed that all sorghums produced birds with similar fat pad value ($P>0.05$) and breast-meat yield to those on wheat-based diet except for those given sorghum diet A where breast-meat yield was 1.6% lower ($P<0.05$). CT analysis of all sorghum grains revealed that sorghum variety K, had the lowest CT content (0.73 g/kg)

Table 1. Mean Feed intake (FI), Live weight gain (LWG), Feed conversion Ratio (FCR), Fat pad and Breast-yield of broilers (0-21 and 21-42 d old) fed 14 sorghum-based diets (A-N) compared to a wheat-based (control) diet. Condensed Tannins (CT, g/kg grain).

Treatment	CT	FI (g/bird)			LWG (g/bird)			FCR (g:g)			Fat pad	Breast-yield
Age in Days	g/kg	0-21	22-42	0-42	0-21	22-42	0-42	0-21	22-42	0-42	(% BW)	(% BW)
Sorghum A	4.63	1117de	3610	4727^{cd}	845^{fg}	2127	2972^{d}	1.329^{abcd}	1.697^{de}	1.590^{de}	0.83	20.50^{d}
Sorghum B	5.14	1187^{ab}	3693	4880^{abc}	877^{cdef}	2121	2997^{cd}	1.355^{a}	1.742^{bc}	1.628^{ab}	0.83	22.00^{bc}
Sorghum C	4.02	1174^{abc}	3723	4897^{abc}	902^{bc}	2203	3105^{abc}	1.302^{de}	1.690^{de}	1.577^{ef}	0.90	22.10^{bc}
Sorghum D	3.14	1160^{abcd}	3622	4781^{abcd}	880^{cdef}	2134	3014^{bcd}	1.319^{cde}	1.712^{bcd}	1.595^{cde}	0.73	22.00^{bc}
Sorghum E	4.80	1189^{ab}	3714	4903^{abc}	899^{bc}	2076	2975^{d}	1.332^{abcd}	1.790^{a}	1.650^{a}	0.75	22.60^{ab}
Sorghum F	4.15	1164^{abcd}	3652	4816^{abcd}	861^{defg}	2157	3018^{bcd}	1.352^{ab}	1.703^{cd}	1.602^{bcde}	0.78	22.40^{abc}
Sorghum G	4.36	1200^{a}	3754	4954^{a}	896^{bcd}	2154	3050^{bcd}	1.340^{abc}	1.743^{bc}	1.624^{abc}	0.74	21.70^{bcd}
Sorghum H	4.40	1130^{cde}	3674	4804^{abcd}	856^{efg}	2170	3025^{bcd}	1.322^{bcde}	1.693^{de}	1.588^{de}	0.89	22.30^{abc}
Sorghum I	3.81	1161^{abcd}	3644	4805^{abcd}	883^{cde}	2134	3016^{bcd}	1.315^{cde}	1.750^{ab}	1.616^{bcd}	0.84	21.70^{bcd}
Sorghum J	4.98	1111^{e}	3701	4812^{abcd}	836^{g}	2185	3021^{bcd}	1.328^{abcd}	1.705^{cd}	1.598^{bcde}	0.88	21.80^{bcd}
Sorghum K	0.73	1205^{a}	3712	4916^{ab}	931^{ab}	2157	3088^{bc}	1.296^{e}	1.746^{abc}	1.607^{bcde}	0.83	22.70^{ab}
Sorghum L	4.59	1129^{cde}	3640	4770^{bcd}	853^{efg}	2188	3041^{bcd}	1.324^{abcde}	1.676^{def}	1.576^{ef}	0.83	22.00^{bc}
Sorghum M	6.00	1148^{bcde}	3724	4873^{abc}	849^{efg}	2200	3049^{bcd}	1.352^{ab}	1.693^{de}	1.598^{bcde}	0.80	21.60^{bcd}
Sorghum N	6.07	1138^{cde}	3525	4663^{d}	848^{efg}	2155	3003^{cd}	1.342^{abc}	1.645^{f}	1.557^{fg}	0.84	21.00^{cd}
Wheat(control)		1189^{ab}	3696	4890^{abc}	944^{a}	2258	3205^{a}	1.263^{f}	1.655^{ef}	1.537^{g}	1.00	22.10^{bc}
LSD (P=0.05)[1]		47	157	177	35	98	112	0.031	0.045	0.031	0.17	1.4
LSD (P=0.05)[2]		45	151	170	34	94	107	0.030	0.043	0.030	0.16	1.3

Different superscripts in columns indicate significantly (P<0.05) different means. [1] comparing wheat *vs.* sorghum. [2] comparing between sorghums

IV. DISCUSSION

In this study, reduced broiler performance was observed in birds given the sorghum-based diets only during the starter phase but not during the finisher phase. This is in agreement with previous studies using similar sorghum varieties from the 2004 harvest (Perez-Maldonado *et al.,* 2006, Robertson *et al.,* 2006), which have also shown better broiler performance on wheat than sorghum-based diets in the starter phase. Similarly, Cadogan *et al.,* (2005), in a study of the effect of feed enzymes in sorghum diets, showed a positive effect on LWG and FI in the starter phase. These studies suggest that differences between sorghum and wheat disappear during the finisher phase. However, because finisher period FCR in the present study in more than half of the sorghum-based diets was inferior ($P<0.05$) to that on the wheat diet, there is still opportunity for improving sorghum nutrient digestibility and availability to similar levels to those in the starter phase, which may improve the FCR in the finisher phase.

The inferior FCR observed in sorghum diets, especially in the starter phase, appears to be likely due to ANF such as CT and phytate-bound complexes which restrict nutrient availability. In this study, the 0-21 d performance of birds offered sorghum diet K, which had the lowest CT content (Table 1), was similar to those given the wheat-based diet, suggesting the importance of CT as an ANF. Arabinoxylan, another ANF found in sorghum, also impedes bird performance which may be improved by adding enzymes. This is supported by the positive effect reported on LWG and FI when adding phytase and or a multi-enzyme product containing xylanase, protease and amylase to sorghum diets (Cadogan *et al.,* 2005).

Despite the fact that all diets were formulated to contain similar metabolisable energy for the starter and finisher periods (12.5 and 13.0 MJ/kg respectively), the efficiency of energy utilization *per* kg of body weight during the starter phase was superior (~ 1.0 MJ) in birds given the wheat-based diets. However, during the finisher period this difference was reduced to only 0.2 MJ. Other reports have also shown a lower energy availability in birds given sorghum- than in those given wheat-based diets (Black *et al.,* 2005). Our studies on AME determination in sorghum (Perez-Maldonado and Rodrigues, 2006, unpublished) have shown a strong negative relationship ($R= -0.753$) between sorghum CT content and AME value. Therefore, future work will be aimed at reducing these ANF and treatments will be directed at young birds (0-21 d) where a greater response is expected.

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EFFECT OF BIRD AGE AND DIET PREPARATION ON THE APPARENT METABOLIZABLE ENERGY OF SORGHUM GRAIN - IMPLICATIONS FOR BROILER PERFORMANCE

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The apparent metabolisable energy (AME) determined in grains is usually carried out with birds of 22-28 days (d) old. Grain is normally hammer-milled and offered in mash diets. AME of sorghum is sometimes determined on diets prepared as whole grain cold pellet (WGCP) diets containing (g/kg): 804 sorghum, 155 casein, 11 limestone, 20 di-calcium phosphate, 3 salt, 5 vitamins and minerals, and 2 choline chloride. Total collection of excreta and measurement of food intake (FI), over 4d after a 3d adaptation period, are made on four replicate cages each of six broiler chickens (22-28 d old). The determined AME (MJ/kg DM) of the grain is then used to formulate broiler diets. In the present study the AME of three sorghums collected during 2004 and prepared as WGCP diets, as described above, were compared with AME determined on the same sorghums using 14-21d old birds. The effect of diet preparation was also investigated in diets as mash (M), cold pelleted (CP), hot pelleted (HP) and WGCP (Table 1). Broiler performance in 2004, using AME of 17 sorghums (2004 harvest) evaluated with birds 22-29 d, was compared with broiler performance in 2005, using AME of 14 sorghums (2005 harvest) evaluated with birds aged 14-21 d (Table 2). Birds within and between periods consumed diets formulated to contain similar AME, amino acids, vitamins, minerals and were housed under similar ambient parameters.

Table 1. Mean AME (MJ/Kg DM) values comparing method of preparation (mash (M), cold pellet (CP), hot pellet (HP) and whole grain cold pellet (WGCP)) and bird age comparison of three sorghum grains (SG) determined in 2004 and in 2005

	14-21 d, 2005					14-21 d, 2005	22-29 d, 2004	
	SG 1	SG 2	SG 3	Mean		WGCP	WGCP	Difference
M	14.71	14.88	14.92	14.84	SG 1	14.61	15.65	1.04
CP	14.40	14.54	15.03	14.66	SG 2	14.93	15.60	0.67
HP	14.90	14.76	14.88	14.85	SG 3	14.52	15.61	1.09
WGCP	14.61	14.93	14.52	14.69				
SEM	0.261	0.261	0.261	0.151	Mean	14.69	15.62	0.93

Table 2. Mean feed intake (FI), live-weight gain (LWG) and feed conversion ratio (FCR) on starter (0-21 d), finisher (21-42 d) and overall periods in diets formulated using sorghum AME values obtained in 2004 (birds 22-29 d) and in 2005 (birds 14-21 d)

	SG (n)	FI (g/d)			LWG (g/d)			FCR (g:g)		
SG 2004	17	1200	4044	5244	829	2245	3074	1.454	1.824	1.717
SEM		20.0	79.16	90.44	14.0	49.88	55.39	0.012	0.025	0.018
SG 2005	14	1158	3670	4828	872	2154	3026	1.329	1.713	1.600
SEM		16.64	55.74	62.76	12.53	34.78	39.47	0.011	0.016	0.011

The results in Table 1 indicate that method of preparation did not influence sorghum AME values ($P>0.05$). However, AME determined with older birds (22-29 d) overestimated its value by close to 0.9 MJ/Kg DM. This AME difference may explain differences in FI and FCR (Table 2) of birds given identically specified diets but using the AME values obtained with younger birds. Birds on energy adjusted sorghums (2005) exhibited similar LWG with substantially lower feed intake resulting in superior feed utilisation efficiency.

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THE ABSORPTION OF BIOPLEX-TRACE MINERALS

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Summary

An experiment was conducted to examine the effects of bioplex or inorganic Cu, Fe, Mn and Zn supplementation of a mineral-deficient broiler diet on bird performance on deep litter and on absorption site and digestibility. A deficiency of trace minerals severely depressed feed intake and growth rate, but did not adversely affect FCR. Supplemental organic (Bioplex) sources of trace minerals significantly ($P<0.01$) improved zinc absorption in the ileum.

I. INTRODUCTION

Copper, manganese and zinc are trace minerals which are essential for broiler chicken growth. The deficiency of these trace minerals significantly reduces feed intake of broiler birds (Bao et al., 2006) and therefore negatively impacts on broiler production. In commercial practice much higher safety margins in feed formulation are used to guarantee the bioavailability of these trace minerals. In previous research it was demonstrated that Bioplex trace minerals could meet modern broiler bird requirements more efficiently, and theoretically the Bioplex trace minerals may be able to be supplemented at a significantly lower level (Bao et al., 2005).

Copper, manganese and zinc are absorbed according to need depending on broiler growth rate and age. Surplus trace minerals do not contribute to bird growth and are excreted. Due to this pattern of excretion, the apparent absorption of Cu, Mn and Zn does not provide a suitable measurement of the bioavailability of trace minerals (Ammerman, 1995), and an excess of trace minerals always leads to underestimation of the potential trace mineral bioavailability (Underwood & Suttle, 1999). It is clear that regulation of trace mineral absorption is complicated and the mechanisms of trace mineral absorption have not been well defined to date.

Bioplex-trace minerals are organically-complexed with proteinates, which protect them from interactions that interfere with their bioavailability (Scott et al., 1982), and because trace minerals are normally believed to be absorbed in the duodenum, jejunum and also the ileum (Underwood & Suttle, 1999), it is necessary to evaluate trace mineral digestibility in the small intestine of birds so as to avoid excretion affects. In these experiments Celite was used as a marker to determine trace mineral digestibility in the jejunum and ileum.

II. MATERIALS AND METHODS

A total of 800 one-day old male Cobb broiler chicks were randomly allocated to 32 deep litter pens with 8 replicates of 25 birds per dietary treatment. For the first two weeks, the birds were given starter diets, followed by finisher diets for 3 weeks. During the finisher period, Celite was added to all diets as a marker for digestibility determinations using the acid-insoluble ash technique. The pelleted basal diet was formulated with sorghum and isolated soy protein, supplemented with vitamin/mineral premix, free of coper, manganese,

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iron and zinc (Control). The second dietary treatment was the control diet supplemented with Bioplex containing Cu, Fe, Mn and Zn at 4, 20, 40 and 30 mg/kg diet, respectively and termed, Organic. The third and fourth treatment diets were provided with inorganic sources (feed-grade sulphate) of these minerals, at two different levels (Cu: 4, 8 mg/kg diet, Fe: 20, 40 mg/kg, Mn, 40, 60 mg/kg diet, Zn: 30, 40 mg/kg diet) designated Inorganic and the NRC respectively. The Inorganic treatment diet was designed to achieve equivalency with the Organic Bioplex treatment.

Body weight and feed intake were recorded weekly. Body weight gain and FCR were determined weekly and corrected for mortality. At 35 days of age, two birds of average weight from each pen were killed to collect digesta from the jejunum and ileum for acid insoluble ash analysis (Choct & Annison, 1990) and trace mineral contents analysis by inductively coupled plasma emission spectroscopy (ICP) The digestibility of trace minerals was calculated from the levels of trace minerals and Celite in the diets and small intestine digesta.

The data were analyzed statistically using one-way ANOVA with STATGRAPHICS software and the significance of difference between means was determined by Duncan's multiple range test.

III. RESULTS AND DISCUSSION

a) Broiler performance

During the entire 35 day experimental period, there was no significant difference ($P>0.05$) in FCR between the control diet and the experimental diets but feed intake and growth rate of broilers given the control diet were severely depressed due to the deficiency of trace minerals (Table 1). These results differ from our previous cage trial, where the deficient control diet also resulted in depressed food utilisation efficiency (Bao et al, 2006). It is possible that the floor pen and litter conditions used in this experiment may have influenced the result through access to trace minerals in the droppings and litter.

Table 1. Effect of different diets on live performance for broilers (0-35 days)

Treatment	Feed intake (g/bird)	BWG (g/bird)	FCR (0-35 d) (g/g)
Control	1597.2 [b]	1020.4 [b]	1.565
Organic	3149.4 [a]	2012.5 [a]	1.565
Inorganic	3174.5 [a]	2037.4 [a]	1.540
NRC	3110.6 [a]	1950.4 [a]	1.599
Pooled SEM	64.5	40.9	0.037
P value	<0.001	<0.001	0.741

a, b means within the same column with unlike superscripts differ significantly ($P<0.05$).

b) Trace mineral digestibility in intestine

Zinc was mainly absorbed in the ileum (63%) and is consistent with the results reported in rat small intestine (Antonson et al., 1979). Copper was absorbed both in the jejunum and the ileum, but manganese was absorbed poorly in both these two segments of intestine (Fig.1). Manganese absorption appears to occur throughout the length of the small intestine and the level of manganese in the diet does not have a pronounced effect on the rate of absorption (Keen & Zidenberg-Cherr, 1996). However, in both the jejunum and ileum, there was no significant difference in copper absorption between inorganic and organic

sources, but zinc absorption in the ileum from Bioplex zinc was significantly improved (P<0.01) compared with inorganic zinc (Table 2.). Therefore, on the current control diet, the addition of 30 mg/kg of Zinc from Bioplex Zn may be sufficient to support optimal performance with improved absorption in the ileum. The results for iron absorption varied widely suggesting that this method may not be suitable for investigating iron absorption site and digestibility.

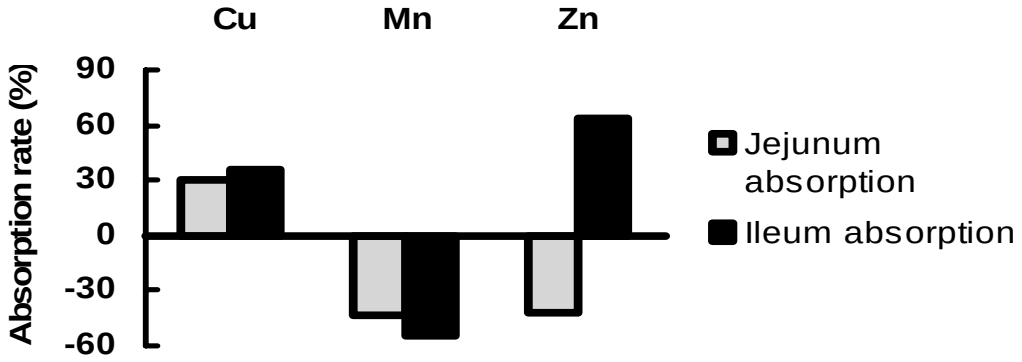

Figure 1. Trace mineral digestibility in the ileum and jejunum on the control diet

Table 2. Trace mineral digestibility in the ileum (%)

Treatment	Cu absorption	Mn absorption	Zn absorption
Control	36.4	-54.1	63.8 [a]
Organic	33.9	-26.8	63.7 [a]
Inorganic	28.3	-19.4	56.2 [b]
NRC	37.4	-41.0	55.2 [b]
Pooled SEM	1.7	16.5	3.6
P value	0.300	0.164	0.002

a, b means within the same column with unlike superscripts differ significantly (P<0.05).

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EFFECT OF DIFFERENT LEVELS AND SOURCES OF ZINC ON EGG QUALITY AND LAYER PERFORMANCE

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Summary

Eighty layer hens were assigned in a completely randomized design to four dietary treatments containing zinc sulphate or organic zinc as Albino-Zn in two levels of 25 or 50 ppm. Feed intake was expressed on a per hen basis. Daily egg collection was expressed on a hen-day basis. Eggs were weighed to calculate egg mass production. Feed conversion ratio was calculated as feed consumed per egg mass. All eggs produced on days 14, 28 and 42 were collected and used for egg quality parameters. Albumen height was measured and Haugh Unit index (HU) was calculated. The yolk and dried shell were weighed and albumen weight was calculated. There was no effect of zinc source or zinc level on egg production, egg weight or feed conversion ratio. However, feed intake was lower in the group receiving 50 mg/kg organic zinc. There were no significant treatment differences for weight of egg components or shell thickness, but albumen height and HU were higher in the groups receiving organic zinc at 25 or 50 mg/kg than in the un-supplemented group.

I. INTRODUCTION

Zinc is commonly supplemented in the diets for poultry and other livestock because many natural feed ingredients are marginally Zn-deficient. Zinc is an integral part of more than 300 enzyme systems that are involved in metabolism of energy, carbohydrates, nucleic acids and protein. Moreover, zinc lays a key role in the immune system, and transport and use of vitamin A (Cousins and Hempe, 1990). Zinc also functions in the formation of eggs. Zinc deficiency affects the quality of the epithelium due to the role of zinc in protein synthesis. Zinc also indirectly affects epithelial secretions, by affecting the structure of the epithelium or directly during the synthesis of egg shell membranes. Zinc plays a role in the magnum during the deposition of albumen and in the isthmus where egg shell membranes are produced. Further zinc is important in shell formation in the uterus (Zinpro, 2002). Organic complexes of zinc have been proposed to be a more available source of zinc for layer hens (Cheng and Guo, 2004) and may be metabolized differently than inorganic forms (Spears, 1989). The main objective of the present study was to investigate the effect of levels and forms of dietary zinc on laying hen performance and egg quality.

II. MATERIALS AND METHODS

A total of 80 Hi-Line 36 layer hens were weighed individually and assigned to 40 pens in a completely randomized design. There were 5 replicates of 2 pens and each pen contained 2 birds in each treatment. Hens were maintained on a 16 h light- 8 h dark schedule. Hens were 50 wk-old when given one of four dietary regimens for 6 weeks. The dietary treatments were: 1) basal diet (containing 29 mg kg^{-1} Zn) without additional zinc, 2) basal diet supplemented with 50 mg kg^{-1} inorganic zinc as $ZnSO_4$, 3) basal diet supplemented with 50 mg kg^{-1} organic zinc as Albino-Zn, 4) basal diet supplemented with 25 mg kg^{-1} organic

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zinc as Albino-Zn. The basal diet was formulated as per NRC (1994) recommendation to meet or exceed hen requirements except zinc (Table 1). Birds were allowed *ad libitum* feed and tap water that contained no detectable Zn. Feed consumption was recorded per replicate. Feed intake was expressed on a per hen basis. Daily egg production was recorded and expressed on a hen-day basis. Collected eggs from each replicate were separately weighed to calculate egg mass production. Feed conversion ratio (FCR) was calculated as feed consumed per unit egg mass. Also all eggs produced on days 14, 28 and 42 were collected and measured for egg quality parameters. Each egg was weighed separately then broken onto a flat surface. The thick albumen height was measured with a micrometer and the Haugh Unit Index (HU) also was calculated (Haugh, 1937). The yolk was separated from the albumen and weighed. Shell was washed and dried at 40°C for 24 h and then weighed. Albumen weight was calculated from the difference between egg weight and weight of yolk and shell. Before drying the shell, shell thickness was measured using a micrometer.

Data were analyzed by GLM Procedure of SAS software (SAS, 1997). Comparison between mean values was done using the Duncan Multiple Range Test method.

Table 1. Ingredient and nutrient composition (g/kg) of the basal diet

Ingredient							
Corn grain	Soybean meal	Soybean oil	Oyster shell	Common salt	Min. premix*	Vit. Premix**	DL-Methionine
625.5	238.6	31.1	80.7	3.2	2.5	2.5	0.1
Nutrient							
ME (MJ/ Kg)	Protein	Calcium	Aval. Phos.	Sodium	Methionine	Lysine	Met + Cys
12.34	165.0	35.0	4.5	1.5	3.0	9.2	5.81

* Supplied per kilogram of diet: Cu: 6 mg, Fe: 55 mg, Mn: 65 mg, Se: 0.2 mg and I: 1 mg.

** Supplied per kilogram of diet: vitamin A: 10500 IU, vitamin D_3: 2200 IU, vitamin E: 15 IU, vitamin K_3:1.0 mg, riboflavin: 5 mg, niacin: 39 mg, pantothenic acid: 10 mg, folic acid: 0.4 mg, thiamin: 4 mg, pyridoxine: 8 mg, biotin: 0.15 mg, vitamin B_{12} 0.08 mg.

III. RESULTS

The results on layer hen performance during the 42-day experimental period are presented in Table 2. Feed intake of the hens on the higher organic Zn diet was significantly ($P<0.05$) lower than that of other treatments. No significant difference was observed between the treatments for FCR, percentage rate of egg production or for egg mass production.

The effect of zinc supplementation on egg quality parameters is shown in Table 3. Zinc supplementation, regardless of source and level, had no effect on egg, albumen, yolk or shell weights or on shell thickness. Albumen height ($P<0.006$) and HU ($P<0.002$) were significantly higher in the groups receiving 25 or 50 mg/kg organic zinc than in the group given the basal diet.

Table 2. Mean values of hen's performance as affected by zinc source and level

Parameters	Treatment				P value	MSE
	Basal	Inorganic Zn 50 mg/kg	Organic Zn 50 mg/kg	Organic Zn 25 mg/kg		
Feed intake (g /hen/ day)	96.70 [a]	96.89 [a]	88.30 [b]	96.33 [a]	0.0020	11.25
FCR	2.208 [a]	2.216 [a]	2.172 [a]	2.193 [a]	0.9848	0.0376
Production (%)	70.60 [a]	73.75 [a]	66.46 [a]	71.40 [a]	0.1271	19.078
Egg mass (g /hen/ day)	43.43 [a]	42.32 [a]	38.84 [a]	43.13 [a]	0.1778	12.016

Table 3. Effect of different sources and levels of zinc on egg quality parameters

Parameters	Treatment				P value	MSE
	Basal	Inorganic Zn 50 mg/kg	Organic Zn 50 mg/kg	Organic Zn 25 mg/kg		
Egg Weight (g)	60.07 [a]	59.38 [a]	60.35 [a]	60.34 [a]	0.4511	19.8478
Albumen Weight (g)	37.85 [a]	37.28 [a]	38.48 [a]	38.14 [a]	0.1658	12.2908
Yolk Weight (g)	16.86 [a]	16.73 [a]	16.63 [a]	16.97 [a]	0.4123	1.9745
Shell Weight (g)	5.32 [a]	5.22 [a]	5.29 [a]	5.28 [a]	0.5706	0.2299
Shell Thickness (mm)	0.396 [a]	0.394 [a]	0.387 [a]	0.388 [a]	0.1814	0.0010
Albumen Height (mm)	7.20 [c]	7.25 [bc]	7.49 [ab]	7.57 [a]	0.0058	0.6722
HU	84.43 [b]	85.10 [ab]	86.09 [a]	86.61 [a]	0.0217	25.4244

IV. DISCUSSION

In the present study, zinc supplementation, regardless of its source had no effect on egg weight or production. Cheng and Guo (2004) also reported that different sources of zinc had no effect on egg production and egg weight. However, Khajarern *et al.* (2006) observed improvements in egg production, egg and egg shell quality for layers fed organic zinc. There was a significantly lower feed intake on the higher organic Zn diet in our study, suggesting the possibility of a problem with palatability.

Zinc deficiency lowers the rate of growth, feed efficiency and egg production (McDowell, 1992). However Stahl *et al.* (1986) found that supplementing different amounts of zinc to layer diets did not alter the performance parameters of laying hens. Carbonic anhydrase is a zinc dependent enzyme that plays a role in converting blood bicarbonate into calcium carbonate which is needed for egg shell formation (Keshavarz, 2001). The increased albumen quality in the present study, as measured by increased albumen height and HU is in agreement with the report of Sahin and Kucuk (2003) who reported that zinc supplementation positively affected HU. Apart from this effect, there were minimal differences in egg quality parameters between the treatments. This may be due to the level of zinc in the basal diet (29 mg/kg) which possibly was sufficient for normal activity of carbonic anhydrase. Alternatively, the feeding period of 42 days may have been too short to allow the effects of zinc deficiency on other egg quality parameters to be expressed.

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EFFECT OF DIFFERENT STORAGE CONDITIONS AND HEN AGE ON EGG QUALITY PARAMETERS

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Summary

To determine the effect of hen age and storage condition on egg quality, fresh eggs were sampled from two commercial flocks of White Leghorn hens at 28 and 68 weeks of age. Half of the sampled eggs from each flock were stored at 6ºC and the other half was stored at room temperature. Fifteen eggs were randomly sampled from each treatment following collection and at 5-day intervals to 20 days of storage. Measurements were made of egg and egg component weights and albumen quality and pH. Significant differences were observed for egg weight and egg component weights in relation to hen age. Albumen weight was affected by temperature. Egg and albumen weights significantly decreased as storage time increased, but weights of yolk and shell were not affected by storage time. Albumen height, HU and albumen pH were significantly affected by hen age, temperature and storage time.

I.INTRODUCTION

The hen's egg is a high quality food (Silversides and Budgell, 2004). The physical appearance of an egg is critical to consumer acceptance; if the product does not meet perceived expectations, consumer confidence diminishes. Furthermore high internal quality is important to egg product manufacturers because it allows for better separation of components without crossover contamination, especially when producing albumen products (Jones and Musgrove, 2005). Much of the cited literature on egg quality was conducted some years ago, and updated research is needed (Scott and Silversides, 2000).

Albumen quality is a standard measure of egg internal quality, usually measured as the height of the thick albumen or Haugh unit (HU) (Silversides and Scott, 2001). Albumen quality can also be estimated by albumen pH. Silversides and Villanueva (1994) reported that changes in albumen quality during storage are described equally well by albumen height and HU. However, Scott and Silversides (2000) showed that albumen height is biased by the hen age and they suggest using albumen pH as a measure of freshness because it lacked this bias. As egg weight increases, albumen height decreases even as the total amount of albumen increases (Silversides and Scott, 2001). The albumen height of all eggs is at a maximum when the egg is laid and decreases with increasing storage time and temperature. The yolk is comprised of approximately 50% water. Water moves from albumen into yolk but a decreased storage temperature slows down this rate of movement (Brake *et al.*, 1997).

The current study was undertaken to examine the effect of hen age, temperature and time of storage on quality factors of shell eggs.

II. MATERIAL AND METHODS

Eggs were sampled from two commercial flocks of White Leghorn hens at 28 and 68 wks of age in Hamedan, Iran. Hens were fed the same diet (12 MJ ME and 165 g CP/kg). Eggs were sampled from 27000 eggs from 28-week-old hens and 18000 eggs from 68-week-old hens. Half of the sampled eggs from each flock were stored at 6ºC and the other half was

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stored at room temperature (approximately 21ºC). A random sample of 15 eggs was taken from each treatment for measurement. Initial sampling was conducted one day after collection and at subsequent 5-day intervals to 20 days. At the time of sampling, each egg was weighed and then broken on to a flat surface. The thick albumen height and yolk height were measured and Haugh unit was calculated (Haugh, 1937). The yolk was separated from the albumen and weighed. The shell was washed, dried at 40ºC for 24 h and then weighed. Albumen weight was calculated from the difference between egg weight and weight of yolk and shell. Albumen was homogenized and pH measured with an HANA pH210 pH meter. For dry matter (DM) estimation of albumen or yolk, the collected albumen or yolks from three eggs were mixed and kept at 50ºC for 48 hours. Data were analyzed using factorial method (2×2×5) as a completely randomized design by SAS software (SAS, 1997). Age of hen, temperature and time of storage were the main effects. Mean values were compared using the Duncan Multiple Range Test.

III. RESULTS

Older hens laid significantly ($P<0.001$) larger eggs than younger hens, with more albumen, yolk and shell (table 1). No significant effect of age was observed on yolk DM % ($P>0.05$) but albumen DM was significantly lower in old hens ($P<0.001$). No significant differences were observed for egg, yolk and shell weights due to different storage temperatures ($P>0.05$) but albumen weight at the higher temperature was significantly lower than that of eggs stored at the lower temperature ($P<0.05$). Storage temperature had a significant effect on yolk DM percentage ($P<0.001$). Weights of eggs and albumen decreased significantly as storage time increased, but weights of yolk and shell were not affected by storage time ($P>0.05$). DM percentage of albumen increased with storage time ($P<0.05$) whereas yolk DM % decreased as the storage time increased ($P<0.001$).

Results of effect of layer age and storage conditions on egg freshness factors are presented in Table 2. Albumen height and HU ($P<0.001$) and albumen pH ($P<0.01$) as indexes of egg freshness were significantly affected by age of hens. Albumen height and HU were significantly lower in older hens and albumen pH was higher in these hens. Storage temperature had a significant effect on all the parameters ($P<0.001$). At the higher storage temperature, albumen weight, HU and yolk height were significantly lower than at the lower temperature, but albumen pH was greater at the higher temperature. There were significant effects of storage time on all “freshness” parameters ($P<0.001$). Albumen height and HU decreased significantly as storage time advanced. A reverse pattern was observed for albumen pH. Yolk height was significantly lower at 20 day storage time than at all other storage times ($P<0.001$).

Table 1. Effect of hen age and storage temperature and time on the weight and composition of eggs.

Parameters	Weight (g)				DM (%)	
	Egg	Albumen	Yolk	Shell	Albumen	Yolk
Hen Age						
Old	60.43 [a]	38.84 [a]	16.66 [a]	4.94 [a]	12.44 [b]	49.42 [a]
Young	51.02 [b]	33.40 [b]	13.00 [b]	4.61 [b]	14.11 [a]	49.60 [a]
Storage Temperature						
High	55.23 [a]	35.43 [b]	14.88 [a]	4.78 [a]	13.35 [a]	49.00 [b]
Low	56.10 [a]	36.61 [a]	14.67 [a]	4.77 [a]	13.16 [a]	50.02 [a]
Storage Time (d)						
1	57.36 [a]	37.81 [a]	14.57 [a]	4.84 [a]	12.74 [c]	50.85 [a]
5	56.07 [ab]	36.48 [ab]	14.57 [a]	4.75 [a]	12.87 [bc]	50.07 [ab]
10	54.06 [c]	34.67 [c]	14.72 [a]	4.68 [a]	13.55 [ab]	49.90 [b]
15	55.48 [bc]	35.45 [bc]	15.19 [a]	4.80 [a]	13.67 [a]	48.62 [c]
20	55.29 [bc]	35.67 [bc]	14.80 [a]	4.81 [a]	13.40 [abc]	48.23 [c]
P value						
Age	0.0001	0.0001	0.0001	0.0001	0.0001	0.3669
Temperature	0.1659	0.0132	0.789	0.763	0.4117	0.0002
Time	0.01	0.0001	0.1307	0.428	0.048	0.0001
Age×Temperature	0.6448	0.4725	0.975	0.5261	0.2411	0.2246
Age×Time	0.4683	0.1171	0.2375	0.042	0.32	0.7880
Temperature×Time	0.8575	0.6982	0.8973	0.891	0.4377	0.0181
Age×Temperature×Time	0.8683	0.6221	0.5857	0.9636	0.4719	0.4375
MSE	22.19	13.79	1.73	0.225	1.28	1.64

Means within a main effect with different superscripts differ significantly.

Table 2. The effect of hen age and time and temperature of storage on internal egg quality parameters.

Parameters	Albumen Height (mm)	HU	Albumen pH	Yolk Height (g)
Hen Age				
Old	5.39 [b]	70.38 [b]	8.68 [b]	15.04 [a]
Young	6.49 [a]	82.48 [a]	8.72 [a]	14.91 [a]
Storage Temperature				
High	5.48 [b]	73.25 [b]	8.83 [a]	14.46 [b]
Low	6.44 [a]	80.13 [a]	8.58 [b]	15.47 [a]
Storage Time (d)				
1	6.85 [a]	82.74 [a]	8.34 [d]	15.17 [a]
5	6.14 [b]	78.36 [b]	8.72 [c]	15.09 [a]
10	6.31 [b]	80.59 [ab]	8.79 [b]	14.88 [a]
15	5.78 [c]	75.42 [c]	8.83 [ab]	15.22 [a]
20	4.66 [d]	66.08 [b]	8.85 [a]	14.43 [b]
P value				
Age	0.0001	0.0001	0.0055	0.3261
Temperature	0.0001	0.0001	0.0001	0.0001
Time	0.0001	0.0001	0.0001	0.001
Age×Temperature	0.458	0.5968	0.5955	0.8047
Age×Time	0.9086	0.0021	0.027	0.3473
Temperature×Time	0.0001	0.0001	0.0001	0.0001
Age× Temperature×Time	0.405	0.0417	0.8313	0.0128
MSE	0.684	43.55	0.013	0.959

Means within a main effect with different superscripts differ significantly.

IV. DISCUSSION

Egg weight was higher in older hens with higher albumen, yolk and shell weights. Other researchers have also reported an increase in egg size with increased age of hens (Silversides and Budgell, 2004). Loss of moisture from eggs with increase in time and temperature of storage resulted in decreased egg weight, decreased albumen weight and increased albumen DM (Brake *et al.*, 1997)

Lower albumen height in older hens seen in this study was also reported by Silversides and Scott (2001) who found that albumen height decreased as hens aged. Similarly HU decreased in older hens. High temperature storage significantly decreased yolk and albumen height and HU. Keener *et al.* (2006) reported that HU values significantly decreased with increased temperature. Albumen height and HU also decreased significantly during storage. Jones and Musgrove (2005) and Silversides and Scott (2001) also reported a decline in albumen height and HU with increase in storage time. The increase in albumen pH with storage time was also in agreement with the findings of Silversides and Scott (2001).

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POULTRY BONE DISORDERS

M. PINES[1]

Summary

Bone growth is controlled by a precise mechanism and any deviation from normal bone growth will result in bone disorders that have the potential to present a major economic problem to the poultry industry. The enormous losses attributed to skeletal disorders in poultry are caused by increases in mortality and condemnations at the processing plant, and further downgrading caused by the trimming of breasts and legs. From the economic point of view, the major skeletal disorders are associated with the rapid increase in growth rate that has been achieved during the last few decades. Whether the disorders are direct effects of the growth rate or indirect effects that result from increased body weight and improper development of bone, muscles and/or tendons is still to be determined. The economic dilemma facing the industry lies in the balance between continued selection for rapid growth, on the one hand, and the losses caused by bone and other metabolic disorders, on the other. The regulation of bone growth and development appears to be complex, with various levels of interactions among the regulating agents. Multidisciplinary research at various levels, such as the genomic and proteomic approaches, cell culture methodology, genetic selection, nutritional manipulation, and environmental control will provide us with better understanding of the molecular mechanisms underlying bone disorders. Such understanding might enhance our knowledge and perhaps help in the design of rational strategies for the treatment of bone-related diseases

I. INTRODUCTION

The skeleton provides the physical support for and determines the shape of the body, and its mineral fraction serves as a calcium reservoir that is used for the maintenance of the extracellular Ca^{2+} concentration. Calcium concentration is of the utmost importance in the management of normal cellular function and of intracellular information processing. Bone growth is controlled by a precise mechanism, as can be inferred from the near-symmetry of the limbs and the hereditary element of adult height. Any deviation from normal bone growth will result in bone disorders that have the potential to present a major economic problem to the poultry industry (Sullivan, 1994). Several extensive reviews of various aspects of poultry bone disorders have been written during the last few years (Orth and Cook 1994; Julian 1998; Cook, 2000; Edwards, 2000; Rath *et al*., 2000; Pines *et al*., 2004). The aim of this presentation is to provide an introduction to the field, but by no means to cover all aspects of poultry bone disorders.

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II. BONE DEVELOPMENT

Bone development starts in the early stages of embryo development and continues throughout maturity. Even after adult stature is attained, bone development continues, for repair of fractures and for remodeling to meet various challenges. There are two types of ossification: intramembranous and endochondral.

a) Intramembranous bone formation

Intramembranous ossification involves the replacement of sheet-like connective tissue membranes with bony tissue. Bones formed in this manner are called intramembranous bones; they include certain flat bones of the skull and some of the irregular bones. During intramembranous formation mesenchymal cells migrate to the site of eventual bone formation. These cells condense, align and secrete an organic framework of extracellular matrix (ECM), to form the osteoid. The cells continue to proliferate during the entire osteogenic process, and all cells involved in bone formation retain this ability. Osteoblasts line the osteoid and begin to deposit bone ECM and the inorganic salt components that impart strength, some flexibility and the ability to hold a defined structure. The organic strands – the trabeculae – are mineralized and consecutive growth rings are added to them to increase the thickness of the lamella. The lamella is added in the course of cycles of osteoid secretion and mineralization-appositional growth. When multiple trabeculae within the developing bone contact one another a lattice structure forms. Regions of bones may become completely filled in with mineralized osteoid to form compact bone; if not completely filled in they form primary cancellous bone. Most bones are mixtures, comprising a compact outer layer and a cancellous interior.

b) Endochondral bone formation

Mesenchymal cells migrate to the site of eventual bone formation and become chondrocytes. The chondrocytes proliferate into a very dense mass of cells, devoid of blood vessels, and form cartilage in the shape of the forming bone. They then secrete ECM, which forces them apart, and they become encapsulated within the cartilage, which grows in a process called interstitial growth. The cartilage is surrounded by a layer of mesenchyme-derived connective tissue cells the perichondrium. These secrete ECM and add to the cartilage by adding more layers, so causing appositional growth. Blood vessels invade the cartilage, which becomes replaced with bone. The outer layer of mesenchyme cells, which support appositional growth over areas where cartilage has been replaced with bone, is now called the periosteum. As the cartilage is degraded, remaining strands of cartilage act as templates for osteoblasts to secrete additional ECM, which is subsequently calcified.

III. CALCIUM HOMEOSTASIS

In contrast to the concentration of cytosolic calcium, that of extracellular calcium remains almost constant and, under normal circumstances, varies by only a few percentage points throughout life. The near constancy of the extracellular calcium concentration is maintained by a complex homeostatic system, which primarily involves the secretion of parathyroid hormone (PTH) from the parathyroid glands (PGs), calcitonin (CT) from the C cells of the ultimobrachial glands, and vitamin D, and their effects on calcium metabolism at renal, skeletal and intestinal sites. Changes in the set point of the PGs elicit major changes in PTH secretion at any given concentration of extracellular calcium, and these changes, in turn, are closely related to the steady-state concentration of the serum calcium. The G-protein-

coupled calcium-sensing receptor (CaR) of the PGs is the major regulator of PTH secretion (Brown, 2000; Yarden *et al*., 2000). Vitamin D, either obtained from the diet or derived from sunlight, is a major regulator of calcium homeostasis. The first hydroxylation reaction, which takes place in the liver, produces 25-hydroxyvitamin D_3. This reaction is not tightly regulated and so an increase in the amount of dietary intake will immediately result in an increased amount of circulating 25-hydroxyvitamin D_3. The second hydroxylation reaction, at the α position of carbon 1, takes place in the proximal convoluted tubules of the kidney, and results in 1,25-dihydroxyvitamin D_3, which is the most biologically active form. PTH, thyroid hormones, oestrogens and calcitonin regulate this reaction. The 1,25-dihydroxyvitamin D_3 stimulates intestinal calcium absorption and resorption of calcium and phosphates from bone and kidneys in order to ensure the presence of enough minerals for bone mineralization (Norman, 1990). When the level of calcium in the blood decreases, the parathyroid glands are stimulated to secrete the PTH that will activate renal 1α-hydroxylase, which then catalyzes the second hydroxylation reaction of vitamin D_3 (Norman and Hurwitz, 1993).

IV. INHERITED AND RARE BONE DISORDERS

Nanomelia is a recessively inherited connective tissue disorder of chickens; it affects cartilage development and is characterized by chondrodystrophy and shortening of the long bones. It involves low aggrecan production and diminished aggrecan mRNA levels. Aggrecan mRNA is present in the nucleus of the nanomelic chondrocyte but its level is greatly reduced in the cytoplasmic compartment. A stop codon has been identified at codon 1513, which is located in the eighth repeat of the chondroitin sulfate 2 domain of the large tenth exon (Primorac *et al*., 1994).

Ametapodia is a mutation associated with abnormal limb development that appeared in a strain of Light Brown Leghorn chickens. The mutants are characterized by the complete absence of the tarsometatarsals, and severely hypoplastic development of the metacarpals is also present. The disease is inherited as an autosomal recessive and the affected chicks do not normally survive beyond 2-4 days of age. (Smyth *et al.,* 2000).

V. BONE DISORDERS OF THE SPINE

Scoliosis is characterized by a lateral deviation of the spine, with rotation of the vertebrae. The effect of intense light on melatonin secretion by the pineal gland is probably involved in the incidence of the disease (Nette *et al*., 2002). A genetic study of an inherited form of scoliosis in chickens revealed that the incidence of expression of a scoliotic parent line implicated three major autosomal, recessive genes (McCarrey *et al*., 1981).

Spondylolisthesis (kinky-back) is characterized by a ventral dislocation of the anterior end of the articulating fourth thoracic vertebra, with over-riding of the posterior end by the fifth, which causes pinching of the spinal cord. Dislocation may also occur between other cervical and thoracic vertebrae. Damage to the spinal cord causes leg weakness that is usually followed by partial posterior paralysis (Riddell and Howell, 1972; Wise, 1973; McNamee *et al.,* 1998). Incidence was greatest in chickens fed *ad libitum* and kept in batteries, and it varies between flocks (Khan *et al*., 1977).

VI. BONE DISORDERS DUE TO MYCOPLASMA INFECTION

Leg weakness characterized by chondrodystrophy of the hock joints, clear fluid in hock joint spaces, valgus deformities and shortening of the tarsometatarsal bones, and curled toes were observed in turkey poults infected with mycoplasma (Trampel and Goll, 1994).

Joint lesions and curved toes were observed in turkey embryos inoculated with mycoplasma, and scanning electron microscopy of the tarsometatarsal joints showed fissures in the cartilage (Lam *et al*., 2004).

VII. VITAMIN AND MINERAL DEFICIENCIES

There are numerous studies describing the involvement of vitamins, minerals and their interactions on skeletal disorders in poultry (Baker *et al*., 1998; Williams *et al*., 2000b; Jin *et al*., 2001; Zhang *et al*., 2003). For example broilers suffering from pyridoxine deficiency had tibias of reduced dry weight and cortical thickness. Histomorphometry revealed a disproportionately large eroded surface, reduction in the amount of osteoid tissue and in the width of mineralized trabeculae and in the number of secondary ossification centers, along with coarse trabeculation. The metaphyseal cartilage showed irregular trabeculae and a markedly reduced amount of collagen and, probably, impaired collagen cross-linking (Masse *et al.,* 1990, 1994). The effect of deficiencies of other minerals and vitamins such as manganese, biotin, and choline have also been studied (Stock and Latshaw, 1981; Liu *et al*., 1994).

In poultry, the involvement of vitamin D and its metabolites is by far the most studied factor in relation to bone disorder (Edwards, 2000). Decreased extracellular phosphate, which occurs in vitamin D deficiency, may play a key role in rickets. The involvement of vitamin D in longitudinal bone growth and, especially, on the growth plate at the end of the long bones has been studied (Ben-Bassat *et al*., 1999; Nilsson *et al*., 2005). In rickets the width of the hypertrophic zone of the growth plate is increased and mineralization is defective. The effects of vitamin D on the growth plate are mediated primarily through the vitamin D receptor, and are expressed in intestinal epithelial cells, which exhibit increased calcium and phosphate absorption from the intestinal lumen. Vitamin D metabolites, however, may also act directly on the growth plate. For example, injection of 24,25-dihydroxyvitamin D_3 directly into rachitic chick growth plates resulted in healing (Lidor *et al*., 1987). *In vitro*, 24,25-dihydroxyvitamin D_3 stimulated differentiation but partly inhibited proliferation in resting-zone cells, whereas 1,25-dihydroxyvitamin D_3 decreased proliferation in the resting and the proliferative zones (Boyan *et al*., 2002). The effects of vitamin D (Atencio *et al.,* 2005), 1α-hydroxycholecalciferol (Driver *et al*., 2005), and 25-hydroxycholecalciferol (Bar *et al*., 2003) on bone development and disorders have been evaluated. Addition of phytase in combination with 1α-hydroxycholecalciferol or 1,25-dihydroxyvitamin D_3 ameliorated leg problems and decreased the incidence of rickets, respectively (Mitchell and Edwards, 1996; Driver *et al*., 2005).

VIII. BONE AND RAPID GROWTH

From an economic point of view the major concern is the rapid increase in growth rate achieved by genetic selection (Havenstein *et al*., 2003), and its impact on bone development. Comparison between fast-growing and slower-growing strains revealed less mineralization, more porous cortical bone and increase in the Ca/P ratio in the former (Williams *et al.,* 2000a). The porosity was a result of rapid primary osteon formation at the periosteal surface, and incomplete infilling of the resultant canal with osteoblasts. These reductions in density and mineral content resulted in altered biomechanical properties, which caused high rates of bone breakage during catching, transport and handling at the processing plant. Use of feed restriction led to the suggestion that the rapid growth rate and not the genetic potential was responsible for the changes in the biochemical properties of the bones (Williams *et al*., 2004)

IX. TIBIAL DYSCHONDROPLASIA

Tibial dyschondroplasia (TD) is one of the most common skeletal abnormalities that result in deformed bones and lameness (Leach and Gay, 1987; Leach and Lilburn, 1992; Orth and Cook, 1994; Pines *et al*., 2004). The economic loss caused by TD, as manifested in the high rates of mortality and morbidity, is enormous: up to 30% of birds may be affected during an outbreak. Those with severe lesions are more susceptible to fractures during handling at the processing plant, with consequent economic loss. In addition, lameness associated with TD in broilers is a welfare issue. TD is a disease of rapidly growing birds, especially in broilers (Leach and Lilburn, 1992) and turkeys (Wyers *et al*., 1991), growing at their maximal genetic potential. Thus, genetic selection for growth rate has actually resulted in increased incidence of this skeletal disease. Various factors have been found to be involved in the aetiology of the disease. They include dietary (Sauveur, 1984; Thorp *et al*., 1993; Rennie *et al*., 1993), environmental (Riddell and Classen, 1992); and genetic (Leach and Lilburn, 1992; Wong-Valle *et al.*, 1993) factors.

TD is a disease of the growth plates at the end of the long bones. The growth plate is populated by chondrocytes, arranged in columns parallel to the long axis of the bone, and it comprises several zones arranged in succession from the proximal to the distal border: (a) the resting or reserve zone, containing stem cells; (b) the proliferative zone, with stacks of flattened cells (cell division occurs mostly in the longitudinal direction and leads to cell column formation); (c) the hypertrophic zone, containing hypertrophic chondrocytes; and (d) the degenerative zone, with a partially calcified matrix and invading capillaries. Each chondrocyte, once formed, remains in a spatially fixed location, in which it accomplishes all of its physiological functions, throughout its cellular life cycle.

Chondrocytes located in the various zones differ in their morphology, secretion of extracellular matrix components, activity of various enzymes, and expression of hormone receptors. Synthesis of collagen type II is characteristic of chondrocytes in the proliferative state whereas that of collagen type X is restricted to the hypertrophic state (Knopov *et al*., 1997). The alkaline phosphatase (AP) activity observed in the hypertrophic zone is of particular interest, since its appearance marks the onset of calcification. The receptor of parathyroid hormone (PTHR), the major hormone responsible for the minute-to-minute regulation of the extracellular calcium concentration, is expressed in the maturation zone (Ben-Bassat *et al*., 1999). Chondrocyte maturation is accompanied also by changes in the rate of proteoglycan synthesis, and synthesis of non-collagenous phosphorylated matrix proteins such as osteopontin (OPN) and bone sialoprotein (BSP) (Pines *et al*., 1999).

In comparison with the mammalian growth plate, the avian one contains longer columns of cells, which become randomly oriented. In the hypertrophic and calcified zones, cell columns are no longer apparent. More cells are found in each zone and the metaphyseal blood vessels penetrate more deeply, in the avian than in the mamalian growth plate (Leach and Gay, 1987; Pines and Hurwitz, 1991). The growth plate of a 4- to 7-week-old chicken contains approximately 200 cells per column as compared with 25 cells in that of the rat. The transition between zones was observed to be more orderly in Leghorns than in broilers (Reiland *et al*., 1978). A wider proliferative zone was found in a heavy than in a light turkey strain (LeBlanc *et al*., 1986). For many species of birds, the changes in the length of the proliferative zone correlate strongly with variations in growth rate (Kember *et al*., 1990). In chickens and turkeys, age-dependent growth rates were correlated with the width of the hypertrophic zone rather than with that of the proliferative zone (Hurwitz *et al*., 1992), suggesting that, as in mammals (Breur *et al*., 1991), cell hypertrophy is the main determinant of longitudinal growth in these species.

Similarly to TD, rickets is also a disease of the growth plate. In rickets the proliferative zone of the growth plate is enlarged, and there are additional chondrocytes in each column. It is of the utmost importance to distinguish between the two lesions when studying TD and designing strategies to prevent the disease. This is especially important since in some cases TD-afflicted birds have a rickets background. It is possible to distinguish between them visually, but determination of molecular markers such as PTHR gene expression is much more accurate. In TD, normal expression of PTHR is observed, whereas in rickets the receptor is down-regulated because of high parathyroid hormone levels, and no expression of the receptor is observed (Ben-Bassat *et al*., 1999).

TD is characterized by the appearance of a plug of unvascularized, unmineralized, opaque white cartilage, which dominates the proximal metaphysis of the tibiatarsus and, occasionally, the tarsometatarsus (Hargest *et al*., 1984). The various morphological and biochemical manifestations of the TD lesion, such as changes in carbonic anhydrase (Gay *et al*., 1985), AP activity, collagen type II and X production (Chen *et al*., 1993; Knopov *et al*., 1995), and OPN and BSP synthesis (Pines *et al*., 1999) suggest that TD-chondrocytes fail to undergo the complete differentiation that normally leads to cartilage vascularization and mineralization. In addition, OPN is probably involved in vascularization since its expression was demonstrated in the front of new blood vessels in the growth plate (Knopov *et al*., 1995). There are various tools for the study of TD; each has its advantages as well as limitations that should be considered.

Field TD - Many of the studies of TD were performed on samples taken from the field at the final stage of the disease when lameness was obvious and the lesions were enlarged. The percentage of afflicted birds in each flock varies widely and some of the features observed during a progressive stage of the disease are secondary and do not necessary reflects the early events responsible for the TD lesion. For example, reduction in matrix vesicle formation, which is important for mineralization in the TD-affected growth plate (Nie *et al*., 1995), may be due to necrosis resulting from energy depletion, impaired oxidative metabolism and a lack in tissue vascularization (Hargest *et al*., 1984).

Selected lines -The incidence of TD can be changed by genetic selection although to date no specific genetic defect has been identified. An experimental breeding program selecting for TD usually results in only 60-70% of the birds developing the disease. Use of lines selected for high and low incidence of TD makes it possible to study the time-dependent morphological, endocrinological and molecular events associated with chondrocyte differentiation (Ben-Bassat *et al*., 1999; Pines *et al*., 1999; Ling *et al*., 2000; Shen *et al*., 2004) and environmental and nutritional effects in relation to the development of TD (Mitchell *et al*., 1997a, b; Punna and Roland, 2001).

Induced TD -TD can be induced by a variety of protocols, all leading to the same phenotype, which suggests a down-stream common pathway. For example, TD can be induced by manipulation of nutritional factors such as the calcium-to-phosphorus ratio (Rennie *et al*., 1993), cysteine supplement (Bai and Cook, 1994), and by Fusarium-infected feedstuff (Chu *et al*., 1996). Various dithiocarbamates, such as thiram, are used to induce very high incidence of TD (Ben-Bassat *et al*., 1999; Rath *et al.*, 2004). This model has the advantage that following thiram removal spontaneous recovery can be studied, and induction of TD by thiram can be prevented by copper supplementation, as demonstrated by AP activity, and expression of collagen type II and X gene (Pines *et al*., 2004). It remains to be determined whether all the modes of TD induction lead to a result that completely resembles the field TD.

Organ cultures-The regulation of limb growth, and the signals involved in chondrocyte proliferation, maturation, and hypertrophy can be studied in organ cultures (Di Nino *et al*., 2001). Organ cultures taken from embryos of different ages can be used for

evaluating the local effect of various agents simultaneously, in a system where cellular interactions are intact.

Cell cultures -Isolated growth plate chondrocytes in culture form the most widely used system. Chondrocytes are easy to grow and can be manipulated in culture, by means of ascorbic acid, to change their phenotype from proliferative (collagen type II positive) to hypertrophic (collagen type X, OPN, AP positive) cells (Halevy *et al*., 1994; Barak-Shalom *et al*., 1995). Cells can be isolated from normal and TD-afflicted chicks (Farquharson *et al*., 1995) and can be fractionated into distinct populations by means of the Percoll density gradient (Farquharson *et al*., 1999). Various growth factors (Praul *et al*., 2002), growth factor receptors (Halevy *et al*., 1991, 1994) and hormone receptors (Monsonego *et al*., 1993, 1997; Ben-Bassat *et al*., 1999) have been studied by using these cells.

TD can be induced by nutritional manipulations or by toxic agents, as well as by selective breeding (Sauveur, 1984; Orth and Cook, 1994; Ben-Bassat *et al*., 1999). Thus, the various protocols that result in TD may initially act via distinct pathways, but downstream they probably share common pathway(s) that lead to the same phenotype. An association between TD and rickets was demonstrated by the finding that supplementation of high doses of vitamin D or its analogs could ameliorate TD in selected lines (Edwards, 2000; Whitehead *et al*., 2004; Atencio *et al*., 2005). The divergent selection of broilers for low or high TD altered the physiological response to nutritionally inadequate levels of dietary D_3 (Shirley *et al*., 2003). During recent years various strategies were used in attempts to understand the causes of TD. Aspects investigation included: the mechanisms involved in chondrocyte differentiation (Knopov *et al*., 1997; Pines *et al*., 1999; Farquharson *et al*., 2001); chondrocyte apoptosis (Praul *et al*., 1997; Ohyama et al., 1997; Rath *et al*., 1998); effects of hormones and their receptors (Ben-Bassat *et al*., 1999; Webster *et al*., 2003); and fingerprint techniques to compare gene expression in normal and TD chondrocytes (Jefferies *et al*., 2000).

X. CONCLUSION

The regulation of bone growth and development appears to be complex, with various levels of interactions among the regulating agents. A high degree of precision in the genetic control is essential, and deviation beyond a certain threshold would cause abnormal bone growth. Multidisciplinary research at various levels, such as the genomic and proteomic approaches, cell culture methodology, genetic selection, nutritional manipulation, and environmental approaches will provide us with better understanding of the molecular mechanisms underlying bone disorders. Such understanding might enhance our knowledge and perhaps help in the design of rational strategies for the treatment of bone-related diseases.

The enormous losses attributed to skeletal disorders in poultry are caused by increases in mortality and condemnations at the processing plant, and further downgrading caused by the trimming of breasts and legs. From the economic point of view, the major skeletal disorders are associated with the rapid increase in growth rate that has been achieved during the last few decades. Whether the disorders are direct effects of the rapid growth rate or indirect effects that result from increased body weight and improper development of bone, muscles and/or tendons is still to be determined. The economic dilemma facing the industry lies in the balance between continued selection for rapid growth, on the one hand, and the losses caused by bone and other metabolic disorders, on the other hand.

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CAUSES AND PREVENTION OF BONE FRACTURE

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Summary

Structural weakness and/or distortion lead to bone fracture in live birds and during processing, especially with automated systems for deboning or mechanical meat recovery. Genetic, nutritional and environmental factors influence both bone strength and deformity. Bone strength in end-of-lay hens has been found to be highly heritable and divergent selection on this basis has resulted in lines with markedly different bone strengths. A quantitative trait locus (QTL) for bone strength has been identified and it is possible that genetic markers for bone strength can be developed for use in breeding programmes. Calcium, phosphorus and vitamin D are important nutrients for bone strength, but recent studies have also highlighted the beneficial effects of n-3 fatty acids. Bone deformities, particularly broiler leg bone deformities also have a genetic component and the importance of including criteria of leg quality in broiler breeding programmes is paramount. Tibial dyschondroplasia (TD) can be selected against but it also has a strong nutritional component, with vitamin D especially important. Dietary supplementation with different vitamin D metabolites, as well as endogenous production of vitamin D by UV irradiation, has been shown to minimise TD. Exercise is also plays a part in minimising leg bone deformities and broiler house layout, lighting pattern and stocking density are all important factors affecting birds' locomotion.

I. INTRODUCTION

The problem of bone fractures in poultry is important for two reasons. Firstly, the pain and discomfort caused by fractures is a cause of poor welfare for the birds. Secondly, the presence in meat products of bone splinters from the fractures impairs the quality of poultry meat products. This is especially likely with automated systems for deboning or mechanical meat recovery. The problem is especially severe with spent laying hens where a survey by Gregory and Wilkins (1989) showed that 29 % of hens had one or more bone fractures during life and that 98% of carcasses had fractures after processing. There is more recent information to suggest that the problem is getting worse, with over 50% of hens showing some form of skeletal damage during life (Sandilands *et al*., 2005). This skeletal fragility of hens is making them largely worthless from the point of view of meat recovery. The problem of bone fractures also occurs with broilers. In the case of these birds, the problem is often associated with leg bone deformities which can contribute to fractures, particularly during processing, in two ways. Firstly, deformities may result in excessive loads being placed on bones when legs are forced into shackles. Secondly, deformed leg bones may impact with machinery during automatic deboning. Any weakness in bones will increase the chances of fracture during either process. This review discusses the various factors that can influence bone strength and deformity in poultry.

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II. BONE STRENGTH

Bone is a living tissue and its strength can be influenced by a number of variables. Genetic factors can determine the way a bone grows and subsequently remodels. Nutrition supplies the components from which bone is constructed. But bone is also a dynamic tissue and its growth and remodelling can respond to mechanical forces placed upon the bone. All of these factors come into play in relation to the major problem of bone strength in laying hens. In these birds, osteoporosis brings about a progressive loss of structural bone and bone strength over the reproductive period with resultant increased susceptibility to fracture.

(a) Genetics

Genetic effects on bone strength have been studied most extensively in relation to laying hen osteoporosis. Heritabilities of a range of bone traits have been measured and have been found to be particularly high for the 3-point breaking strengths of tibia (0.45) and humerus (0.30). A bone index (BI) was devised as a basis for selection for increased bone strength and resistance to osteoporosis: it contained functions for tibia and humerus strength, keel bone density and a negative function for bodyweight to prevent bone strength increasing in response to greater bodyweight. The heritability of the BI was 0.40

BI = 0.61 x tibia strength (N) + 0.37 x humerus strength (N) + 0.27 x keel radiographic density (mm Al eq.) – 0.35 x bodyweight (kg)

Divergent selection in a strain of White Leghorn hens using this BI resulted in the production of two lines, H (high BI) and L (low BI) lines. Divergence in bone strength, particularly tibia strength, was rapid and after 3 generations the lines differed by 25% for tibia strength, 13% for humerus strength and 19% for keel radiographic density at end of lay (Bishop *et al*., 2000). The divergence has continued and after 9 generations of selection there is now a 83% difference in tibia strength and 35% difference in humerus strength. The selection has been carried out on hens only with males in the breeding programme chosen on the basis of family values. However, males have also shown considerable effects of selection on bone traits. Interestingly, these changes have occurred without any corresponding changes in egg output or shell quality. Biological comparisons have shed some light on the mechanisms involved. The improvement in bone strength was attributable to a small increase in bone formation during the growing period and a large decrease in cortical bone resorption during the laying period in the H line. The latter observation could be explained by the decrease in numbers of osteoclast (bone resorbing) cells in the H line. There was also a small increase in the amount of medullary bone in long bones. Medullary bone is a labile source of calcium for shell formation and is not as strong as structural bone, but it does make a contribution to overall bone strength.

These results show that it is possible to select birds for improved bone strength. The BI selection procedure has involved retrospective selection on the basis of post mortem values. It is quite labour intensive and not particularly suited to application in commercial breeding programmes. A simpler, more predictive system based on genetic markers would be preferable. In an effort to understand the genetic basis of bone strength, an F_2 reciprocal cross was established between the H and L lines. The F_2 was used to detect and map quantitative trait loci (QTL) affecting the BI and the component traits of the index (Dunn *et al*., 2007). Phenotypic data from 372 hens in 32 families were analysed by within-family regression analyses using 136 microsatellite markers in 27 linkage groups. A significant QTL was found on chromosome 1, centred on 370 cM, with an F ratio of 9.29 which exceeded the P=0.05 F-

statistic threshold. The region also contained QTL for two of the components of the bone index, tibia and humerus breaking strength. The F-ratio for tibia breaking strength QTL was 13.2 which exceeded the P=0.01 F-statistic threshold and the humerus breaking strength QTL was just below the P=0.05 F-statistic threshold. After full genotyping there were no other locations detected that contained significant QTL at the P<0.05 level for any of the traits analysed. Putative QTL on chromosomes 3, 6 and 9 were not confirmed after genotyping of the whole population and the inclusion of more markers. The additive effects for tibia breaking strength represented 34% of the trait standard deviation or 7.6% of the phenotypic variance of the trait. This is the first significant QTL related to bone quality in poultry and is particularly important as it is directly relevant to commercial populations. The next steps in the project will be to identify single nucleotide polymorphic (SNP) marker(s) within the region of the QTL that would have predictive value across different genotypes. If suitable markers can be established for laying hens, it will be of considerable interest to determine whether they also have predictive applicability to other types of poultry such as broilers and turkeys.

Influences of genetic selection have also been seen in broilers. Continued selection for faster growth rate has been shown to result in greater cortical porosity and also an increased ratio of Ca:P in bone mineral (Williams *et al*., 2000a; 2000b). The implications of this for bone strength are as yet unclear but it will be advisable for broiler breeders to pay attention to possible changes in bone strength in the newer genotypes. These genetic changes are already having an implication for nutrition, as discussed next.

b) Nutrition

Bone is a composite material of 30% organic matrix and 70% hydroxyapatite mineral. Hydroxyapatite is composed mainly of calcium and phosphorus in the weight ratio of 2.15:1. Bone is therefore highly dependent on the dietary supply of constituent nutrients, particularly Ca and P. The retentions of Ca and P from the diet are linked, but can be independently regulated to a small extent. Thus nutritionists endeavour to supply these nutrients in the diet in the approximate proportions of 2.15:1, cf NRC (1994) broiler starter requirement of 10g Ca, 4.5g available P/kg. Ca and P absorption and homeostasis are strongly regulated by vitamin D and requirements for this nutrient increase substantially as dietary Ca/P deviates from the ideal. Vitamin D is discussed more fully in a later section. The vitamin can help the bird adjust its absorption and retention of Ca and P within limits, but any substantial supply of Ca and/or P below the requirement or outwith the ideal ratio will result in rickets, characterised by soft bones and growth plate abnormalities.

The genetic changes in bone composition referred to above involved increases in the bone Ca:P ratio to up to 2.5:1 at about 10 days of age. Evidence that this may be changing the requirements of young broilers has come from a factorial study involving different dietary Ca and available P concentrations (Williams *et al*., 2000b). Optimum growth plate morphology was observed when the diet contained 11 to 12g Ca and 4.5g available P/kg. It may thus be advisable to increase the Ca content of broiler starter diets above the amount recommended by NRC (1994).

Other vitamins and minerals have effects on bone growth and composition. However, under current conditions of commercial nutrition, these nutrients do not seem to be associated with any particular practical problems.

Bone development is influenced by a wide range of metabolic regulatory factors. Prostaglandins are important regulatory factors that are derived transiently from polyunsaturated fatty acids (PUFA) of the n-6 and n-3 series. They influence many metabolic pathways, including bone formation and development. Prostaglandins derived from n-6 fatty

acids have been shown to have some inhibitory effects on bone development, while prostaglandins derived from the n-3 series can have more beneficial effects of stimulating osteoblast function and bone formation, as demonstrated in cell culture studies (Chang *et al*., 1998). Broiler diets, especially starter diets, are usually rich in n-6 fatty acids and providing a better dietary n-3/n-6 fatty acid balance in the diet may thus benefit bone development in birds. Studies have shown that feeding diets containing PUFA from fish oil can improve tibial strength in quail (Lui *et al*., 2003a; b).

A recent study has confirmed an effect of dietary n-3/n-6 ration on bone strength in broilers (McCormack *et al*., 2007). Experimental diets comprised a basal diet based mainly on wheat and soybean meal with natural oil content of 28g/kg and containing normal concentrations of other nutrients from which isoenergetic and isonitrogenous experimental diets of different n-3/n-6 balances were obtained by adding different combinations of supplemental maize oil (MO) and salmon oil (SO). These oils were chosen because MO is rich in n-6 fatty acids, mainly linoleic acid, and has a low content of n-3 fatty acids whilst SO is a rich source of n-3 fatty acids, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The results are shown in Table 1. There were significant improvements in liveweight in the diets containing SO in experiments 2 ($P<0.05$) and 3 ($P<0.01$). Feed-to-gain ratios were improved by SO addition in all experiments though the differences did not attain statistical significance ($P>0.05$). Three point breaking strengths of the tibia showed significant ($P<0.001$) increases with SO inclusion in all experiments. These treatment differences were also significant ($P<0.01$) after covariate analysis for liveweight. Tibia ash % was increased by SO inclusion in experiments 2 ($P<0.001$) and 3 ($P<0.01$). The morphometric analyses in experiment 2 did not reveal any dietary effects on cortical structure or bending stress. The results indicated that diets with a low ratio of n-3/n-6 fatty acids resulted in sub-optimal performance and also gave poorer bone strength. The improvement in bone strength was partly a response to greater liveweight but was also directly attributable to effects of dietary fatty acids as confirmed by covariate analysis for liveweight. Increasing the n-3/n-6 ratio above 0.04 by provision of long chain PUFAs present in SO resulted in progressive improvements in performance and bone strength. It is concluded that addition of SO to diets of low n-3 fatty acid content has a beneficial effect on bone strength and performance in young broilers. The results do not give any indication of the relative merits of other n-3 fatty acids such as linolenic acid. However, it seems probable that diets lacking a source of n-3 fatty acids will give suboptimal bone strength.

c) Environment

Environment, particularly the opportunity it gives a bird for exercise, can have a strong effect on bone strength. Thus housing systems that allow birds the opportunity to fly are particularly effective in increasing bone strength. However, this may not reduce fracture incidence in the husbandry system (Gregory *et al*., 1990), because such birds may experience more violent accidents, but might improve processing quality. Genetic and housing effects on bone strength are additive (Whitehead, 2006) so selecting birds for better bone strength will benefit birds irrespective of housing system.

Table 1. Performance and tibia characteristics in broilers at 2 weeks of age fed wheat/soybean diets containing different ratios of n-3/n-6 fatty acids resulting from substitution of maize oil (MO) with salmon oil (SO)

Diet content			Livewt	Feed/Gain	Tibia characteristics				
MO (g/kg)	SO	n-3/n-6	at 14d (g)		Breaking strength (N)		Ash (%)	True cortical bone area (mm^2)	Bending stress (N/mm^2)
					1	2			
Experiment 1									
50	0	0.049	327	1.744	87.0a	90.2a	37.3		
25	25	0.271	341	1.628	95.4b	92.8ab	37.9		
0	50	0.778	336	1.616	96.6b	96.0b	38.2		
SED			9	0.099	4.2	1.1	0.5		
Significance			NS	NS	*	**	NS		
Experiment 2									
50	0	0.039	319a	1.826	89.3a	105.8	37.4a	5.69	122.0
40	10	0.148	354b	1.806	106.4b	102.1	38.5b	6.42	120.0
I.	20	0.282	355b	1.750	109.0b	104.0	38.6bc	6.50	120.3
0	50	1.260	359b	1.634	113.5b	106.4	39.5c	6.64	124.1
SED			10	0.085	5.2	4.3	0.5	0.44	5.1
Significance			*	NS	***	NS	***	NS	NS
Experiment 3									
50	0		316a	1.834	63.1a	65.8a	36.3a		
25	25		336b	1.782	72.6b	69.9b	37.4b		
SED			6	0.055	1.9	1.1	0.3		
Significance			**	NS	***	**	**		

1: Values for 3-point breaking strength; 2: 3-point breaking strengths after covariate analysis for liveweight.
Within an experiment and column, values followed by a different letter are significantly different.
* P<0.05; ** P<0.01; *** P<0.001

III. BONE DEFORMITY

Leg weakness is a widespread and longstanding problem in broiler production. The term covers a wide range of pathologies and causes can include genetic predisposition, nutritional and management inadequacies and disease. Almost all forms of leg abnormality, with the exception of femoral head necrosis, involve angular or rotational deformities of leg bones and/or joints and give rise to the type of problems that are likely to lead to bone fracture or splintering during processing or meat recovery.

d) Genetics

Genetic predisposition, as a result of concentration on selection for growth rate, is a common underlying cause of leg problems and can be exacerbated by nutritional practices, either those that maximise growth or are deficient in some nutrients, and management. Some adnormalities, e.g. TD, can directly involve both genetic and nutritional factors. There is now an increasing awareness of the importance of including leg health characteristics among broiler selection criteria. Procedures can include assessing bird gait characteristics and using procedures to identify specific lesions.

TD is a specific abnormality that can be directly selected against using a 'Lixiscope' to identify lesions in real time in live birds. With experience, it is possible to identify large lesions, but small lesions are more problematical. Nevertheless experimental and commercial

use of this approach has led to lines and strains of broilers whose susceptibility to TD is decreased, though not eliminated.

e) Nutrition

Nutrition has an important role in combating leg deformities. Where the primary cause of the problem is genetic predisposition linked to fast growth, nutritional procedures aimed at slowing growth can be effective. This can be achieved by feeding diets of lower nutrient density. This is best done at an early stage of growth, because deformities can be initiated quite early in the chick's life and is often most effective when applied during the second week of life. The optimum procedure can be to give the chicks a fast start during the first week, as this has been shown to stimulate development of the digestive system and give them greater long-term growth potential, then slow growth during the 2nd and 3rd weeks before returning birds to a high density nutritional regime that will encourage compensatory growth.

TD is a common cause of leg bone deformity, with moderate to severe lesions causing a marked bowing of the tibia. A range of nutritional factors have been shown to affect the occurrence of TD, including Ca/P ratio and electrolyte balance, but the most effective means of preventing TD is via vitamin D metabolism. Dietary supplementation with 1,25-dihydroxyvitamin D_3 (1,25D) has been found to reliably prevent TD (Rennie *et al*., 1993). 1,25D in its crystalline form has a low margin of safety between effective (~3.5μg/kg) and toxic threshold (~5μg/kg), depending upon dietary Ca concentrations (Rennie *et al*., 1995). However, herbal extracts containing 1,25D derivatives are becoming available and may have lower toxicity. Another metabolite that can be effective against TD is 25-hydroxyvitamin D (25D). This is available commercially (Hy-D) and effective doses are from 70μg/kg upwards. Earlier studies did not suggest that vitamin D itself was effective against TD, but a more recent study (Whitehead *et al*., 2004) has shown that very high dietary concentrations (up to 10,000IU/kg) can have a marked effect in countering TD. This concentration is above the legal limit of 5000IU/kg in the EU so alternative strategies are needed in Europe. These involve use of 25D and/or water-soluble vitamin D supplements over the first few days of the chicks' lives. It seems to be particularly important to maximise the vitamin D status of the chick during the early stages of growth when TD lesions can first be initiated. The need to provide vitamin D in such high doses raises the question of whether it is perhaps operating by a pharmacological rather than nutritional route. Regulation of gene expression is an important metabolic function of vitamin D. If the genetic component in TD involves dysfunction of a vitamin D-dependent gene, perhaps a gene regulating chondrocyte differentiation, it is possible that a greater signal from vitamin D (or its main metabolite, 1,25D) can overcome the gene defect and prevent TD. An alternative means of providing this signal is explained in the next section.

f) Management

Exercise is well known for its effects in strengthening bones and has also been found to diminish the incidence of leg bone deformities. The latter effect may be related to better muscular development rather than solely to increases in bone strength. Broilers can be encouraged to exercise by such management procedures as increasing to distance between feeders and waterers. Stocking density can also affect a bird's opportunity for exercise. There has been a heated debate in Europe over optimal stocking densities. Using bodyweight as a criterion, quite high stocking densities can be achieved, provided adequate environmental conditions are maintained. However, it is noticeable that the incidence of leg problems

increases at the higher stocking densities, presumably as a result of difficulty in movement (Sorensen *et al*., 2000; Dawkins *et al*., 2004).

Lighting has also been used as a means of improving leg quality, and this effect may also be related to changed patterns of behaviour and movement. Thus intermittent lighting and 'normal' dark periods of 7 to 8 hours have been found to be beneficial.

More specific effects of UV lighting have been reported. Edwards *et al*. (2003) found that exposing birds soon after hatching to UV light from below directly on to the skin of the legs decreased the incidence of TD. It was suggested that this effect was related to endogenous photosynthetic production of vitamin D. Exposure of chicks to UV light in a hatchery is impractical so we have been studying effects of different durations and intensities of UV light in situations that could be applied in a broiler house. In our most recent experiment, day-old chicks were exposed to UV light for 12 h from bulbs suspended 1.5m above them and fed a diet imbalanced in Ca/P to magnify the occurrence of TD.

Table 2. Body weight at 1 and 14 days and tibia weight, breaking strength, stiffness, radiographic density (RD) and ash content at 14 days of age of birds exposed to UV light for 12 h from above

	Control	Treatment	SED	*Significance*
Weight (g) 14d	252.2	267.5	9.92	NS
Tibia Weight (g)	2.186	2.216	0.086	NS
Tibia Breaking Strength (N)	32.4	43.1	2.36	***
Tibia Stiffness (N/m)	24239	32618	1982	***
Whole tibia RD (mm Al. eq.)	0.751	0.875	0.0210	***
Tibia Ash (%)	32.29	37.43	0.668	***
Normal growth plates (%)	29.0	100.0		

*** P<0.001

The results (Table 2) show significant improvements in bone strength and mineralisation and a marked decrease in growth plate abnormalities, mainly TD and rickets. Measurements of 25D showed that plasma concentrations remained elevated in the treated group for several days post irradiation. These results show that it is not essential to irradiate from below; irradiation from above is also effective and did not result in ocular damage. We are now progressing to trials in a broiler house where the normal light bulbs are replaced by UV bulbs for 12h immediately after the chicks are placed. These findings confirm the central role of vitamin D in preventing TD. Whether the effect of UV irradiation is associated solely with the formation of vitamin D (cholecalciferol) or also promotes the formation of other highly-effective hydroxylated metabolites is uncertain at the moment.

IV. CONCLUSIONS

Genetic selection for bone strength and freedom from leg bone deformities should have a high priority in breeding programmes. Nutritionally, careful attention should be paid to Ca, P and vitamin D supply. Fatty acids of the n-3 series are also important. Vitamin D has a central role in prevention of TD and use of vitamin D metabolites, high concentrations of vitamin D itself or induction by UV irradiation can all be effective. Environmental factors such as exercise, lighting pattern and stocking density also affect bone deformities. If, despite attention to these factors, bone splinters still occur in meat products, X-ray detection is a last ditch strategy.

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THE INVOLVEMENT OF MATRIX PROTEINS IN EGGSHELL FORMATION

M. PINES[1]

Summary

The quality of the eggshell is of primary concern to the poultry industry. A simple increase in shell thickness is not a satisfactory goal, since the thickness accounts for only a small fraction of the shell's resistance to breakage. In order to improve eggshell quality, it is necessary to identify the molecular constituents involved in the mineralization of the eggshell that guide eggshell assembly and mineralization. Various strategies were used to identify eggshell proteins such as eggshell extraction and protein purification, evaluating the role of proteins involved in other mineralization processes such as bone and the proteomic approach. At present a few hundred proteins are known to be part of the eggshell. Being an extracellular process, eggshell formation is governed by proteins responsible for calcium transport and establishment of the pH gradient needed for crystal formation and proteins that are secreted out, become integrated into the eggshell, regulate the calcification process and become part of the organic shell matrix. At least three interrelated mechanisms regulate the expression of these genes: the mechanical strain imposed locally by the resident egg; circadian rhythm, probably through systemic hormone secretion; and the calcium flux itself. This occurs in a physiological setting on a daily basis.

I. INTRODUCTION

The quality of the eggshell is of primary concern to the poultry industry. On the one hand, the successful development of a chicken embryo is dependent upon a robust eggshell for mechanical protection, for protection from infection, for prevention of water loss, and as a primary source of calcium for the embryonic skeleton. On the other hand, the commercial production and marketing of eggs exposes them to insults that cause a high rate of broken or cracked eggshells, which impose major economic losses on the egg producer. A simple increase in shell thickness is not a satisfactory goal, since shell thickness affects gas and water exchange, and a thicker shell presents a greater obstacle to the emerging embryo. In addition, the thickness accounts for only a small fraction of the shell's resistance to breakage; therefore other characteristics of the shell should be assessed. In order to improve eggshell quality, it is necessary to identify the molecular constituents involved in the mineralization of the eggshell. Biological molecules guide mineralization processes through a series of specific and definable calcium-biomolecule interactions that lead to the deposition of specific and uniquely oriented crystalline structures (Weiner and Addadi, 1991). Eggshell assembly and mineralization are guided by an array of biomolecules that follow a set of biological principles for the mineralization process. The process of mineralization in the avian eggshell follows a spatio-temporally defined series of events that correlate to specific regions along the oviduct (Arias *et al.*, 1993). Thus, identification of eggshell matrix proteins and their genes, and elucidation of the mechanism and regulation of their synthesis and assembly along the successive segments of the oviduct form a major goal in the process of improving eggshell quality.

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II. EGGSHELL FORMATION

The eggshell is formed during passage of the egg through the oviduct, with the various layers of the eggshell assembled sequentially as the egg passes through the successive sectors of the oviduct. After fertilization of the ovum in the infundibulum, and secretion of albumen in the magnum, the egg enters the isthmus 2-3 h after ovulation. In the isthmus, the granular cells secrete the various components of the shell membranes, such as collagen type X (Arias *et al*., 1991; 1997). Most of the calcium deposition in the eggshell occurs in the shell gland (ESG) (Creger *et al.,* 1976; Stemberger *et al*., 1977). Approximately 5-6 g of calcium carbonate is deposited into the chicken eggshell during its formation; most of it during approximately 17 of the 20-h residence of the egg in the ESG. The rapidity with which this large amount of calcium is deposited makes eggshell mineralization one of the fastest biomineralization processes known.

III. INVOLVEMENT OF MATRIX PROTEINS IN MINERALIZATION

It is widely accepted that the organic matrix components of biologically driven mineralization play a role in the control of crystallization. Extracellular matrix proteins of biomineralized structures influence the strength and shape of the final structure of calcium phosphate (apatite) or calcium carbonate (calcite) by modulating crystal nucleation and growth (Weiner and Addadi, 1991). Various strategies were used to identify eggshell proteins:-

(i) Eggshell extraction and protein purification enabled the eggshell proteins of various avian species to be identified and localized to different regions of the shell. For example, ovocleidin 17 was localized to the palisade and mammillary layers (Hincke *et al*., 1995), ovalbumin to the mammilary knobs (Hincke, 1995), lysozome and ovotransferrin (Gautron *et al*., 1997), dermatan sulfate proteoglycan is found in the palisade region (Carrino *et al*., 1997), keratan sulfate was extracted from the mammilae (Fernandez *et al*., 1997), ovocalyxin-32 (OCX-32), which is present at high levels in the uterine fluid during the terminal phase of eggshell formation, was localized predominantly to the outer eggshell (Hincke *et al.,* 2003), as were ovocleidin-116 (Mann *et al*., 2002) and ansocalcin (Lakshminarayanan *et al.,* 2002). The ability of some of these proteins to aggregate in solution or induce nucleation of calcite aggregates has been studied (Lakshminarayanan *et al*. 2005).

(ii) Phosphorylated matrix proteins such as osteopontin (OPN) are believed to play an important role in the process of bone mineralization. The involvement of OPN in bone calcification was deduced from its tissue distribution, its ability to bind calcium, its localization to electron-dense regions of mineralization, and the regulation of its gene expression by calcitrophic hormones such as $1,25(OH)_2D_3$ and parathyroid hormone. In the hen oviduct, OPN gene expression was detected only in the ESG, where massive calcification occurs, and not in any other part of the oviduct (Pines *et al.,* 1996). The OPN gene was expressed in a circadian fashion during the daily egg cycle, only during the period of eggshell calcification. No OPN gene expression was detected in the ESG of a pre-laying hen before the onset of reproduction, or after forced removal of the egg close to its entrance into the ESG. The epithelial cells of the ESG, which line the lumen, are the source of OPN and, upon synthesis, OPN is immediately secreted out of these cells and localized in the core of the non-mineralized shell membrane fibers in the base of the mammillae and in the outermost part of the palisade). It was suggested that OPN could be part of the mechanism controlling the eggshell calcification arrest (Fernandez *et al*., 2003).

(iii) The recent elucidation of the chicken genome provided an opportunity to explore the matrix proteome of the eggshell biomineral. More than 500 proteins were identified and were divided among a few functional groups (Mann *et al*., 2006). Some of the proteins seem to be unique to the eggshell, some are present in other egg compartments and some are to be found in other tissues as well. Interesting questions emerge, such as the role of lipid-binding proteins in a milieu that is almost devoid of lipids, or the presence of proteins with functions such as apoptosis and angiogenesis in surroundings that lack cells or blood vessels.

IV. REGULATION OF THE SYNTHESIS OF EGGSHELL MATRIX COMPONENTS

The enormous number of proteins found in the eggshell suggests a very complex mechanism of regulation that would be expected to occur in different compartments of the oviduct and at precise time intervals. The unique circadian fashion of eggshell calcification allowed us to compare ESG gene expression at the time when no egg resides in the ESG and no calcification occurs, with that at the time when the egg resides in the ESG and calcification is at its peak. RNA fingerprinting revealed a set of genes that were differentially induced at the time of calcification (Lavelin *et al*., 2001, 2002). Some of them, such as Na-K-ATPase, are probably responsible for ion transport and establishing the pH required for calcification; the role of others, such as the proteoglycan glypican-4 is still unknown.

During the past few years, the effect of mechanical force on the regulation of cell functions has been extensively studied. Various stresses or strains, such as hydrostatic or hydrodynamic pressure, tensile or biaxial stretching and fluid shear stress have been studied. The applied forces caused a variety of physiological responses such as increased bone resorption, changes in matrix protein synthesis, cell differentiation, changes in smooth muscle contractility and increased cell migration, all of which involved multiple signal transduction pathways. It was of great interest to discover that the mechanical strain imposed by the resident egg is coupled to a physiological response and is a major regulator of the expression of genes involved in eggshell calcification. This interpretation was supported by the following observations: the genes are expressed in the ESG only when an egg resides there and imposes a mechanical strain; removal of the mechanical strain caused reduction in the gene expression, and artificial application of a mechanical strain caused their induction to an extent related to the level of the strain (Lavelin *et al*., 1998, 2001, 2002).

V. CONCLUSION

The avian eggshell gland is a tissue specialized in the massive calcium transport needed for eggshell formation. Being an extracellular process, eggshell formation is governed by: (i) proteins responsible for biological processes within the tissue, such as calcium transport and establishment of the pH gradient needed for crystal formation; and (ii) proteins that are secreted out, become integrated into the eggshell, regulate the calcification process and become part of the organic shell matrix. At least three interrelated mechanisms regulate the expression of these genes: the mechanical strain imposed locally by the resident egg; circadian rhythm, probably through systemic hormone secretion; and the calcium flux itself. This occurs in a physiological setting on a daily basis.

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STRATEGIES TO MANAGE WET LITTER

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Summary

The term "wet litter" is generally used to describe, non-specific disease of the gastrointestinal or urinary tract, resulting in compromised water balance, feed conversion inefficiencies and predisposition to secondary disease. Poultry litter becomes wet when the rate of water addition (urine/faeces/spillage) exceeds the rate of removal (evaporation). Anti-nutritional factors, toxins, pathogens and nutrient imbalances may cause wet litter directly by altering the normal physiology of digestion and water balance or indirectly by disturbing normal gut ecology. An integrated and holistic approach is required to ensure that the development and maintenance of gut structure (anatomy) and function (physiology) is integrated with gut microbial community evolution. Antibiotics, enzymes, drinking water acidification, probiotics, prebiotics, immune modulators and mycotoxin binders have all shown promise in this regard.

I. INTRODUCTION

Commercial poultry housing and management practices have been designed to keep birds within their *comfort-zone* at all times. Apart from satisfying the primary concern for bird welfare this also minimises homeostatic activity and ensures the most efficient partitioning of energy for production. Under these carefully controlled conditions water balance is kept positive (growth) or neutral.

Water balance is compromised in healthy animals when a dietary stress exceeds homeostatic mechanism capacity and in disease when the integrity and or function of the cells responsible for water/solute transport are adversely affected. If under such circumstances urine and faecal water loss increases to the point where the rate of water addition to the litter exceeds the rate of removal, the litter moisture content rises until it exceeds desirable levels (25%) and at this point it is deemed "wet". Apart from being an indicator of gastrointestinal upset and feed conversion inefficiencies, wet litter also creates unfavourable house environment conditions.

The term "wet litter" is generally used to describe, non-specific disease of the gastrointestinal or urinary tract, resulting in compromised water balance, feed conversion inefficiencies and predisposition to secondary disease.

II. WATER BALANCE

Water balance is a crucial part of homeostasis and it involves equilibrating *intake* and *synthesis* (metabolic water) with *excretion* via the kidney (urine) and gastrointestinal tract (faeces) and *insensible loss* via the skin and respiratory tract (evaporation). Assuming house environment control is efficient and birds remain healthy, insensible water loss is minimised and excretory water loss is diet dependent. Dietary mineral content, anion-cation balance and several feed ingredient characteristics will affect water intake and feed passage time thus

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altering urine and faecal moisture (Classen 1996; Leeson and Summers 2005; Refstie *et al.* 1999; Sell *et al.* 1983; Sibbald 1979; Weurding *et al.* 2001).

Since feed is generally low in moisture (10%) and metabolic water production is limited by diet formulation, moisture intake is primarily controlled by drinking (~ 80%) (Leeson and Summers 2005). Water consumption is requirement-driven and the thirst centre is stimulated by cellular dehydration (osmoreceptors), extracellular dehydration (mechanoreceptors) and angiotensin II secretion (renin-angiotensin axis) (Goldstein and Skadhauge 2000; Kanosue *et al.* 1990; Kaufman *et al.* 1980).

Insensible moisture loss accounts for 50-80% of total loss but seldom contributes directly to litter moisture since at thermoneutrality, evaporative loss is minimised and water in the vapour form is removed from the house relatively easily (Goldstein and Skadhauge 2000). Water loss as vapour does however increase relative humidity (RH) thus reducing the air's litter drying capacity and could cause saturation and condensation. Once condensed, water requires additional energy (heat of evaporation) and effort (air temperature/humidity control) to remove, so homeostatic stress that increases liquid water loss (faecal and urinary) poses a greater risk to litter moisture control.

Urinary excretion is somewhat unique in the avian species since firstly the ureters open into the coprodeum and secondly the urine passes retrograde up the colon to the caecae before being evacuated via the cloaca with the faeces (Goldstein and Skadhauge 2000). The content of the urine is significantly altered during its passage through the coprodeum, colon and caecae (Rice and Skadhauge 1982).

At standard temperatures broilers will consume approximately 1.75 to 2 times more water than feed (by weight) so it is crucial that the poultry house ventilation system design and operation is efficient enough to prevent the litter moisture content from exceeding an optimal 25%.(Kellerup *et al.* 1965; Leeson and Summers 2005). At stocking densities of 34kg/m^2 huge amounts of water are added to the litter on a daily basis. At six weeks of age for example, 20,000 birds will excrete approximately 2.5t of water into the litter in one day. Relatively minor changes in water excretion rates can very rapidly compromise litter moisture control.

III. WATER IMBALANCE

Urine output increases above normal (polyuria) when intake exceeds requirement (overcorrection of dehydration), the need for solute excretion exceeds normal (mineral and protein loading) or when urine concentrating mechanisms are compromised (nephrotoxins such as ochratoxin, citrinin and oosporin) (Leeson and Summers 2005).

Several feed ingredient characteristics can alter faecal water content directly by increasing ingesta osmolarity, or reducing transit time and absorptive surface area/function thus compromising water absorption and stimulating intake. The resultant increase in faecal water is termed diarrhoea. This is different to enteritis, where inflammation of the gastrointestinal lining negatively affects digestion and the consequent reduction in nutrient and water absorption cause faecal water to increase above normal. The latter is usually associated with pathological conditions involving gut microbiota or feed toxins while the former is usually physiological in nature.

IV. STRATEGIES TO PREVENT WET LITTER

a) Preventing Polyuria

The relatively high levels of potassium in soybean (and molasses) can be sufficient to induce a polydipsia, polyuria and wet litter. In contrast most diets will have added salt and since sodium is the primary extracellular cation, maintenance of sodium balance by the kidney is crucial (Goldstein and Skadhauge 2000; Leeson and Summers 2005). Minor sodium excess is controlled by reducing intestinal uptake but as the concentration in the diet increases renal naturesis follows (Goldstein and Skadhauge 2000). The polyuria induced by sodium excretion is exacerbated by chloride induced osmotic diuresis and can to a degree be countered by partial replacement of salt derived sodium with sodium bicarbonate to reduce chlorine intake (Goldstein and Skadhauge 2000; Leeson and Summers 2005).

Dietary calcium and phosphorus levels are regulated by stringent maximum and minimum specification constraints because both the amount and ratio of these minerals is important to productivity (Leeson and Summers 2005). Elevated blood Ca proportionally increases the calcium concentration of the glomerular filtrate which easily exceeds reabsoption capacity and consequently increases Ca excretion (Clark and Mok 1986; Wideman 1987). Excess calcium excretion can cause renal pathology (calcinosis/urolithiasis) resulting in compromised water retention and diuresis/wet litter (Shane *et al.* 1969; Wideman *et al.* 1985). In addition, dolomitic limestone contains relatively high levels of magnesium (8-10%) and apart from competing with calcium for absorption the Mg excretion can cause diuresis and wet litter (Leeson and Summers 2005).

Nephrotoxins such as ochratoxin (especially type A), citrinin and oosporin, can compromise renal function causing polyuria/polydypsia and wet litter (Leeson and Summers 2005). The avoidance of contaminated ingredients, their dilution with non-contaminated ingredients or the addition of mycotoxin binders are all potential ways of limiting or preventing toxicity.

b) Gut Health Management to Prevent Diarrhoea and Enteritis

The integrity of the gastrointestinal absorptive membrane determines the efficiency of the assimilation process. Development and maintenance of gut structure (anatomy) and function (physiology) has to be integrated with gut microbial community evolution since they are collectively the primary determinants of gut health.

Colonization of the gut with pioneer species that are able to modulate gene expression in the host gut epithelia to assist in creating favourable conditions for the evolution of a stable climax (steady state) community provides a natural form of defence against pathogen challenge (Guarner and Malagelada 2003). The speed with which this "climax flora" develops appears to be important with respect to future/sustained gut health and resilience (Hume *et al.* 1998; Methner *et al.* 1997). With this objective in mind it is possible to identify several management opportunities to enhance gut health and bird productivity including; seeding the hatchling gut with favorable flora; early modification of the gut environment to promote climax flora development; pathogen exclusion (competitive and selective); immune modulation; and ingredient/nutrient management.

Seeding of the gut

It was demonstrated many years ago that a "mature" gut microbial community can reduce the prevalence of wet litter by making it more difficult for pathogens to infiltrate (Pivnick and Nurmi 1982). Steps to control gut health in broilers should ideally start at the

parent flock level because manipulation of parent gut flora can have a beneficial effect on offspring resistance to pathogen colonization (Fernandez *et al.* 2002). These organisms also create conditions that shape development of the climax flora (Dawson 2001).

Similarly, dosing day-old chicks with competitive exclusion products can reduce pathogen infection rate following low grade challenge provided gut colonization is allowed to proceed for at least 4 hours before challenge (Hume *et al.* 1998; Methner *et al.* 1997).

It would appear that by selecting specific pioneer species as probiotic candidates it is possible to create a gut environment that accelerates the establishment of favourable and stable climax flora communities (Dawson 2001).

Gut environment management

1. Acidification

Meta analysis (Partanen and Mroz 1999) and literature review (Ravindran and Kornegay 1993) indicate that water and feed acidification have an important role to play in the avoidance of wet litter through gut flora management. The beneficial effects of commercial acid preparations are thought to arise from the antibacterial properties of ionization. Organic acids are able to diffuse across the bacterial cell membrane rapidly when in the un-dissociated form (Cherrington *et al.* 1990; Cherrington *et al.* 1991). Once internalized the neutral pH of the cytoplasm causes dissociation, thus raising the intracellular concentration of both protons and anions (Cherrington *et al.* 1990; Cherrington *et al.* 1991; Davidson 2001). Bacterial proton-motive forces are exhausted in pursuance of homeostasis and the resultant rise in cytoplasm pH interferes with bacterial cell physiology. At low concentrations organic acids have a bacteriostatic effect but at high concentrations they become bactericidal (when acid concentration causes internal pH to rise to the point where denaturation of bacterial protein and DNA occurs (Davidson 2001; Ricke 2003)

Acid ionization varies considerably according to type, concentration and mix of acids used and is further modified by the pH, buffering capacity and water activity of the feed, water and gut content ((Ricke 2003);

2. Nutrient balance – Intake, absorption and excretion

The low pH of the upper gastrointestinal tract provides a competitive advantage to the acidophilic organisms and is by contrast, relatively hostile to many of the potential pathogens such as *Clostridium perfringens* and *Salmonella spp..* The lower part of the digestive tract is alkaline (pH 7-8) and more hospitable to these potential pathogens but their dominance is limited by intense competition for a limited source of nutrients (Zinser and Kolter 2004). Under such conditions microbial evolution occurs very rapidly and continuously through mutation, selection and takeover thus increasing the propensity for pathogen dominance with nutrient through-flow (Finkel and Kolter 1999; Zambrano *et al.* 1993) High protein diets increase the chance of protein through-flow and downstream gut health challenges since many of the gut pathogens are proteolytic.

Feed retention time and the efficiency of digestion and absorption is reduced by several feed ingredient characteristics including viscosity, particle size, digestibility (starch), and lipid or protein content (Classen 1996; Refstie *et al.* 1999; Sell *et al.* 1983; Sibbald 1979; Weurding *et al.* 2001). To prevent nutrient through-flow from causing wet litter the nutritionist should consider ingredient blend in addition to nutrient specification (Bedford 1996; Collier *et al.* 2003; Iji 1999). Grains such as wheat, rye and barley are rich in water soluble NSPs and there is ample research to demonstrate that this improves digestibility (Choct and Annison 1992; Rosen 2000b; Rosen 2001).

Apart from the direct feed efficiency implication of reduced digestion and absorption, the through flow of undigested nutrients impacts downstream gut ecology (Leeson and

Summers). Potentially toxic compounds such as ammonia, amines, phenols and indoles are generated by the proteolytic and ureolytic activity of the caecal flora on non-digested nutrients that make their way through to the caecal pouches.(Gidenne 1997) These toxic compounds affect flora ecology in the rabbit and the same is likely true for the broiler.

All fats and oils have the potential to become oxidised and the resulting rancid fats have reduced digestibility which can cause gastrointestinal disturbance and wet litter directly (steatorrhoea) or indirectly by affecting gut flora (oxidative). Unprotected fatty acids released by oil seed processing (grinding or chemical extraction) are very susceptible to oxidative rancidity(Leeson and Summers 2005).

3. Antimicrobials

Antibiotics have been an integral part of poultry feed for the past 50 years (Rosen 1995). Decades of research and field use have established the efficiency of antibiotics as growth promoters and in-feed antibiotics have been shown to subtly change the composition of the normal flora. Many antibiotics are excreted via the urine in an active form, are concentrated in the urine and hence caecae, as illustrated by the high concentration of antibiotic in the caecal wall (Akester *et al.* 1967; Knoll *et al.* 1999).

The extensive reviews on in-feed antibiotic use and those covering the various alternatives, have reported on research investigating the response to *first-time-one-off* use of growth promoter strategies in controlled trials under carefully monitored experimental conditions (Collett and Dawson 2001; Hooge 2003; Rosen 1996; Rosen 2000a; Rosen 1995; Rosen 2001). Broiler production is, in contrast, a continuous system. Broiler gut flora determines the composition of the litter/house flora which in turn acts as the seed stock for the gut flora of the next placement (Lilijebjelke *et al.* 2003). While the small-intestine ecology influences the efficiency of digestion and absorption it is the caecal/colon/rectal flora that gives rise to the house flora. While the use of a growth promoter can alter the gut flora within a couple of weeks it takes several grow-out cycles to change the house flora (Avellaneda *et al.* 2003); (Idris *et al.* 2003); (Lilijebjelke *et al.* 2003); (Schildknect *et al.* 2003a; 2003b).

Just like penicillin many of the mycotoxins that commonly contaminate poultry feed likely have antimicrobial properties. Mycotoxin research has focused on host toxicity (Swamy *et al.* 2002a; Swamy *et al.* 2002b) but it is possible that gut flora destabilization and feed efficiency is affected long before symptoms of toxicity appear (Kubena *et al.* 2001).

4. Selective exclusion

Pathogen attachment to the intestinal epithelium is a pivotal first step in the colonisation of the gut and depends on, amongst other things, flagella, type 1 fimbriae and pillus receptors for specific host cell docking sites (Sharon and Lis 1993; Stavric *et al.* 1987) . Adherence has also been associated with mannose resistant haemagglutinins. Scanning electron microscope studies of the caecal epithelium have shown that the organisms of the gut flora form a tightly adherent mat over the gut surface (Giron *et al.* 2002). These organisms are attached to each other and the epithelia by a series of fibrils, which effectively prevents pathogenic organisms from gaining access to epithelial receptors (Giron *et al.* 2002; Sharon and Lis 1993). The adhesive flagella of enteropathogenic E. coli (EPEC) have been shown to be induced by animal cells (Giron *et al.* 2002). While competitive exclusion relies on the ability of live organisms to compete for attachment sites it is also possible to block attachment sites with decoy molecules and change gut flora communities

5. Immune modulation

Any immune response bears a production cost. An appropriate immune response, adequate to contain infectious disease and minimize its impact on productivity, is the cost of health. An inappropriate, excessive or inadequate immune response will depress performance unnecessarily, so in a performance driven broiler industry the prevention of wet litter should include an immune modulation strategy (Kelly 2004; Klasing 1998; Klasing and Barnes 1988; Klasing *et al.* 1987; Klipper *et al.* 2000; 2001).

The gastrointestinal environment is loaded with a plethora of antigens of feed and micro-organism origin, the majority of which pose no threat of infectious disease. An inappropriate adaptive immune response to non-pathogen derived antigens is prevented by the innate immune system (Medzhitov and Janeway 1997). Low level antigen recognition at the gut/ingesta interface probably seldom stimulates systemic/fever response but antigen stimulation of this nature can damage host tissue, thereby causing localized *inflammatory disease* and reduced feed efficiency (Klasing 1998; Klasing and Barnes 1988; Klasing *et al.* 1987; Klipper *et al.* 2000; 2001).

Antigen induced inflammation of the gut cytoskeleton stimulates an increase in mucus secretion, paracellular permeability, and feed passage (peristalsis) (Collier *et al.* 2003; Cooper 1984). The cascade of events that follows is self perpetuating and provides additional advantage to organisms such as *Clostridium perfringens* that are capable of rapid multiplication thus increasing the propensity for wet litter (Collier *et al.* 2003). Both endogenous and exogenous anti-inflammatory agents help to preserve the integrity of the gut and reduce the systemic (fever) response (Choct *et al.* 2004; Ferket *et al.* 2002; Grimble 2001; Kelly *et al.* 2004; Klasing 1998; Korver *et al.* 1998; Korver *et al.* 1997; Parks *et al.* 2001; Surai 2002; Sword *et al.* 1991).

Apart from the obvious inefficiencies of nutrient wastage arising from poor digestibility or rapid feed passage, undigested proteins reaching the caeca are strongly inflammatory and thus further reduce feed efficiency (Klipper *et al.* 2001; 2004; Lillehoj and Trout 1996). This is especially prominent with soluble protein because liquids pass through the digestive tract 15% faster than solids (Klipper *et al.* 2004; Sklan *et al.* 1975; Sklan and Hurwitz 1980).

An inadequate immune response has a negative economic impact long before flock mortality rises. Specific infectious diseases, nutritional deficiencies, toxicity, and stress are all factors that can induce sufficient immune suppression to cause an inadequate response(Ferket *et al.* 1999; Ferket and Qureshi 1992; Qureshi *et al.* 1998; Siegel 1994; Surai 2002; Swamy *et al.* 2002a; Swamy *et al.* 2002b; Sword *et al.* 1991).

Immune modulation can be used to carefully manage the balance between disease resistance and tolerance in order to maintain productivity (Klasing 1998; Klasing *et al.* 1987).

V. CONCLUSION

Urine output increases above normal (polyuria) when intake exceeds requirement (overcorrection of dehydration), the need for solute excretion exceeds normal (mineral and protein loading) or when urine concentrating mechanisms are compromised. Polyuria can be avoided through careful diet formulation and ingredient management.

Gut microbial imbalance is a fundamental cause of wet litter and there are several opportunities for intervention to enhance gut health and productivity by managing this ecosystem:

1. Seeding of the hatchling gut begins with vertical transmission of parent gut flora but is effectively modified with early administration of effective probiotics or competitive exclusion products. To be successful they must initiate the development of a primary

flora which will rapidly evolve into a stable and favorable climax flora by creating suitable gut conditions and excluding unfavorable organisms.

2. Preparing the gut environment (pH, redox potential) for early transition from primary to climax flora through water/feed acidification. Candidates need to be weak acids that are buffered to withstand the neutralizing effect of minerals dissolved in the drinking water and have dissociation characteristics that make them active in the small intestine.
3. Excluding pathogens from colonizing the gut by competitive and selective exclusion. It is important that the selective exclusion product is compatible with (does not exclude) the organisms used for competitive exclusion or as a probiotic.
4. Enhancing resilience by stimulating protective immune response while suppressing the acute phase or fever response.
5. Decreasing nutrient through flow by enhancing nutrient digestion and absorption (exogenous enzyme addition and nutrient modification, feeding and lighting programs, careful use of antibiotics) to avoid caecal flora upset.

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POPULAR ALTERNATIVES TO ANTIBIOTIC FEED ADDITIVES IN MONOGASTRIC PRODUCTION SYSTEMS

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Summary

Throughout Asia regulatory bodies are increasingly considering restricting the use of prophylactic antibiotic use in animal production. Even without regulatory restrictions the use of antibiotic alternatives in Asia is increasing because of growing opportunities for export to European markets and mounting consumer pressure as awareness of environmental and "green" issues increases. The following paper provides a review of products currently available, and being used in Europe, as alternatives to antibiotic feed additives.

I. INTRODUCTION

Pathogenic bacteria are always present in the gut, but the balance between pathogenic and beneficial bacteria determines whether or not disease will occur (Ivanov, 2003). Maintaining a healthy balance between all microflora within the gut is known as eubiosis (Jensen, 1980) and can be influenced by bacteria endemic to the microflora.

In the intestine, bacteria considered beneficial to the gut, including lactic acid forming bacteria like *Lactobacillus* spp, prevent proliferation of pathogens, such as *Salmonella* spp., through competitive exclusion for nutrients and for receptor sites on the gut wall (Thomke and Elwinger, 1998). Beneficial bacteria can also produce an adverse environment for pathogenic bacteria to colonise and grow, for example, by the production of short-chain fatty acids which lower the pH and prevent growth of pH sensitive pathogenic bacteria (Thomke and Elwinger, 1998). The intestine is the biggest immune organ in the body, but to achieve appropriate protection from pathogens a complex gut microflora is essential.

The microflora also have functions in the development of the digestive and immune tissue in the host animal, can produce nutrients that can be used by the host as a nutrient source and also can neutralize some feed toxins and promote an environment in the gut where anti-nutritional factors and toxins are minimised (Dawson, 2001).

In the past, manipulation of the microflora to create eubiosis has been achieved by the use of antibiotic feed additives. However, with the severe restriction of antibiotic feed additive use in the EU and increasing consumer concern in markets such as Japan, alternatives to antibiotic feed additives have been investigated and found to significantly influence this balance also.

II. EUBIOTICS DEFINED

a) Probiotics

The generally accepted definition of probiotics comes from Fuller and states that probiotics are microorganisms that have a positive effect on the host by improving the balance of pathogenic to beneficial bacteria in the gut (Simon, Jadamus and Vahjen, 2001). The benefits of probiotics are based on two main functions, stimulating the growth of beneficial microflora and suppressing the growth of pathogenic bacteria (Wenk, 2003). The

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potential health benefits associated with using a probiotic include improved digestion, stimulation of gastrointestinal immunity and increased natural resistance to enteric disease (Turner, Dritz and Minton, 2001). Although it is known that probiotics do produce these benefits to health a general mechanism of action for probiotics remains unclear, possibly due to the fact that different probiotics (i.e. different types of bacteria) have different modes of action (Turner *et al.*, 2001).

Modes of action that have been suggested include increasing the number of beneficial bacteria in the intestine and therefore improving the ratio of beneficial bacteria to pathogens (Simon *et al*., 2001). When a higher number of beneficial bacteria are present they are more likely to out-compete the pathogens for both nutrients and adhesion sites on the gut wall, a process known as competitive exclusion. Beneficial bacteria, such as *Lactobacillus*, are also known to release short chain fatty acids, bacteriocins and hydrogen peroxide, which have antagonistic effects on pathogenic bacteria (Thomke and Elwinger, 1998; Klein-Hessling, 2001).

An example of the effect of a probiotic on broiler performance is shown on Table 1. Trial A shows a positive effect of a live probiotic product containing *Enterococcus faecium* against a negative control, containing no antibiotic treatment. Both weight gain and feed conversion ratio were significantly improved by administration of the probiotic. Trial B indicates that when the same probiotic was tested against a diet containing antibiotics there was no significant differences in performance. These results demonstrate that probiotics can act as a viable alternative to antibiotic feed additives.

Table 1. The effect of a probiotic (Cylactin) on broiler performance to 42 d in two trials with negative (Trial A) and positive (Trial B) controls.

	Trial A		Trial B	
	Negative Control	Probiotic (Cylactin, 40 mg/kg)	Positive Control (Virginiamycin, 20 mg/kg)	Probiotic (Cylactin, 40 mg/kg)
Number of Animals	800	800	81,000	81,000
42 d Body weight	2392^{b} (100%)	2497^{a} (104.4%)		
Weight Gain (g/d)			44.6 (100%)	44.6 (100%)
FCR	2.076^{b} (100%)	1.994^{a} (96.1%)	1.72 (100%)	1.70 (98.8%)
Livability (%)	96.5% (100%)	97.5 (101.0%)	92.98 (100%)	96.17 (103.4%)

a,b – values within rows with different subscripts are significantly different ($P<0.05$)

b) Direct Acting Gut Flora Modulators

Direct acting gut flora modulators are defined as compounds directly modulating the microflora via growth inhibition and include products such as organic acids and plant extracts.

Organic acids

Organic acids are now routinely used in weaning piglets during the transition from suckling milk to consuming solid feed to aid in acidifying the feed to prevent bacterial growth and improve digestion of feed ingredients (Miller, 2006). The results of a trail investigating the effect of one type of organic acid on performance in weaning piglets are summarized in Table 2. These results showed that addition of Benzoic acid increased growth rate, feed intake and feed utilization efficiency in weaning piglets.

Table 2. The effect of addition of 0.5% benzoic acid on growth performance in piglets from 0 to 32 days post-weaning compared to a negative control (containing no antibiotic feed additives).

	Negative Control	Benzoic Acid (VevoVitall 0.5%)	Improvement
Average Daily Gain (g)	335[a]	379[b]	13.1%
Average Daily Feed Intake (g)	551[a]	581[b]	5.4%
Feed Conversion Ratio (kg/kg)	1.65[a]	1.55[b]	6.1%

a,b – values with different subscripts are significantly different ($P<0.005$)

Use of organic acids is not generally practiced in poultry as the results tend not to be as reproducible or significant as those found in swine (Alcicek, Bozkurt and Cabuk, 2004; Miller, 2006).

Plant extracts

There are many different types of plant extract products available commercially and the definitions vary accordingly. Essential oils (EO) tend to be the oils extracted from plants, but these vary depending on plant species, soil type, climatic conditions, harvesting conditions and storage (Baidoo and Ariza-Nieto, 2005). Essential oil compounds (EOC) refer to the specific active compounds from essential oils (Baidoo and Ariza-Nieto, 2005). Whereas a mixture of essential oils may vary in the active ingredients due to natural variation in the plants from which they were derived, a mixture of essential oil compounds will be consistent, reproducible and measurable.

The modes of action for EO and EOC can vary widely depending on the concentration of the active compound. The principal uses of EOC mixtures in poultry feeding are in regulating gut microflora, specifically limiting the growth of *Clostridia perfringens*, and stimulating digestive enzymes (Baidoo and Ariza-Nieto, 2005). By achieving this EOC mixtures can be used as an alternative to antibiotic growth promoters and result in enhanced performance.

The results of a broiler growth study comparing the effect of an EOC feed additive (CRINA Poultry) with an antibiotic feed additive (Colistin), are summarized in Table 3. There were no significant differences in performance measures between the positive control and the EOC treatment groups, suggesting that CRINA Poultry was as effective in promoting growth as the antibiotic feed additive.

c) Prebiotics

Prebiotics are defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of certain bacteria in the colon resulting in an improvement in host health (Wenk, 2003). Prebiotics are oligosaccharides that are not digestible by the animal, but can be fermented in the intestine and act as a nutrient source for lactic acid-producing bacteria such as specific strains of bifidobacteria (Wenk, 2003).

Table 3. Growth performance of 35 day-old broiler chickens given diets containing either an antibiotic feed additive (colistin, 10ppm) or two levels of an EOC feed additive (CRINA Poultry, a commercial mixture of EOC containing thymol)

	Positive Control	CRINA Poultry (25 ppm)	CRINA Poultry (50 ppm)
Number of Animals	28	28	28
Total Gain (g)	1,451	1,467	1,512
Total Feed Intake (g)	2,648	2,680	2,646
Feed Conversion Ratio	1.83	1.83	1.75

There were no statistically significant treatment differences for any of the performance traits.

IV. CONCLUSIONS

Digestion and immunity are critically dependent on gut microflora. Whilst pathogenic bacteria are always present in the gut, the balance of non-pathogenic and pathogenic bacteria will strongly influence the disease status of the bird. Modulation of these two types of bacteria within the gut to produce a healthy balance of microflora, or eubiosis, can be achieved by a variety of products, including probiotics, direct acting gut flora modulators and prebiotics.

The search for effective alternatives to antibiotic feed additives has intensified as a result of the ban on anitibiotic growth promoters in Europe. Earlier research with these products showed some to have limited efficacy. Now, however, with advancing research and development in this field, some newer products have been shown to give results that are as effective as antibiotic growth promoters in maintaining health status and enhancing monogastric growth performance.

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ENVIRONMENT AND AGE: IMPACT ON POULTRY GUT MICROFLORA

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Summary

The gastro-intestinal tract contains a complex population of bacteria, which can have both negative and positive effects on their host. However, the complexity of these interactions is not yet fully understood. Changes in gut microflora immediately post hatch and modification to achieve lifelong benefit have not been investigated in great detail. This report describes the application of T-RFLP, a microbial profiling technique for examining the chicken intestinal microflora. This DNA based technique is capable of providing a "snap-shot" of the complex bacterial population at any particular time and combined with multivariate statistical analysis has enabled relationships between gut microflora and bird performance to be investigated. These tools are being used to examine changes in the microbial community of the chicken gut associated with environment and age, hence contributing to an increased understanding of the chicken gut microbiota and its role in health.

I. INTRODUCTION

The microorganisms that colonise the gastrointestinal tract during the early post-hatch period form a synergistic relationship with their poultry host. Gastrointestinal microorganisms have a highly significant impact on uptake and utilisation of energy (Choct *et al*., 1996) and other nutrients (Smits *et al*., 1997; Steenfeldt *et al*., 1995), and on the response of poultry to anti-nutritional factors (such as non-starch polysaccharides), pre- and pro-biotic feed additives and feed enzymes (Bedford and Apajalahti, 2001). Microorganisms can also directly interact with the lining of the gastrointestinal tract (Van Leeuwen *et al*., 2004), which may alter the physiology of the tract and immunological status of the bird (Klasing *et al*., 1999). During the first week post-hatch, the chicken small intestine grows rapidly. *In ovo* and early post hatch feeding, as well as altering gut microflora development within the first two weeks post hatch, have been shown to modify gut development in chickens (Smirnov *et al*., 2006; Smirnov *et al.,* 2005). Litter material has been shown to influence bird performance (Grimes *et al*., 2006) but information on the effect of litter material on gut microflora development is lacking.

Therefore, knowledge about the composition of the gut flora, microbial ecology of the gastrointestinal tract and factors affecting its development are still limited. Previous investigations have used culture-dependant approaches, which are limited by knowledge of particular bacterial growth requirements, and generation of sequence information, which is not appropriate for high throughput comparative studies. Alternatively, DNA-based molecular techniques have the advantages of being rapid, relatively inexpensive and capable of monitoring specific gene regions of complex populations. To this end, we have developed terminal restriction fragment length polymorphism (T-RFLP) to examine changes in gut microbial communities in response to a range of parameters. We have previously used this technique to show a correlation between particular gut microflora profiles and bird

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performance associated with diet change (Torok *et al*., 2006). We are presently using this technique to examine the effect of a variety of factors, including age, litter materials and different bacterial load environments, on the development of gut microflora

II. MATERIAL AND METHODS

Total nucleic acid was extracted from chicken gut samples by a modification of a SARDI proprietary extraction method. Tissue, including digesta content, was taken from the indicated gut section from each chicken and the microbial community analysed by T-RFLP. Bacterial ribosomal DNA was amplified with universal 16S bacterial primers, one of which was 5′-labelled with 6-carboxyfluorescein. Amplicons were cut with a four base pair recognition sequence restriction enzyme and separated on a capillary DNA sequencer (ABI 3730, Applied Biosystems). Data were analysed using GeneMapper (Applied Biosystems) to determine positions of terminal restriction fragments (TRF). Prior to statistical analysis the TRF profiles were analysed by a modified method of Dunbar *et al*. (2001) and resulting TRF treated as operational taxonomic units (OTU). OTU were analysed using multivariate statistical models (Primer 5, Primer-E Ltd., Plymouth UK).

III. RESULTS AND DISCUSSION

a) Influence of environment on gut microflora immediately post-hatch

Gut microbial communities were analysed from the caeca of three broiler chicks each aged 2-7 days which were raised either in a low pathogen load isolator or under conventional poultry shed conditions post-hatch (Figure 1). Birds were hatched under identical conditions and fed identical diets. Significant differences in the overall microbial communities between environmental treatments were observed ($P<0.05$). No significant differences where detected in gut microbial community composition between birds of different ages in the same environment, although the small sample size (n=3) used may have prevented detection of a significant effect of age.

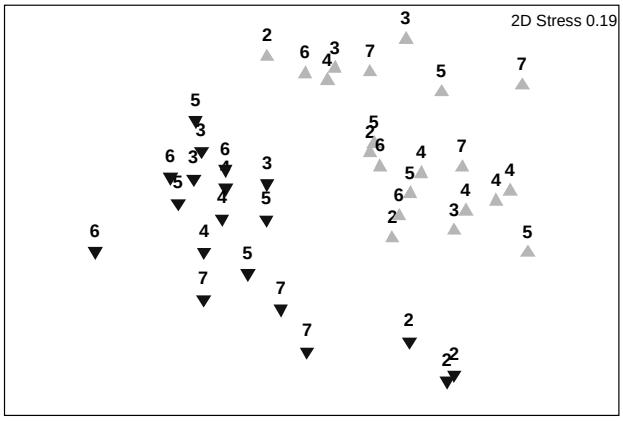

Figure 1. Non-metric multidimensional scaling of OTU's from T-RFLP analysis of caecal microbial communities from chickens raised in a ▲ low pathogen load isolator or under▼ conventional poultry shed conditions. Numbers indicate ages of chickens in days. Each point in the ordination represents the overall microbial profile from individual samples and their relationship to the microbial profiles of all other samples. The closer two points are in the ordination the more similar are the overall gut microbial communities of those two samples.

b) Influence of litter on gut microflora

Gut microbial communities were analysed from the caeca of 12 broiler chickens each raised on seven different litter materials including: softwood sawdust; softwood shavings; hardwood sawdust; shredded paper; chopped straw; rice hulls; and single-batch reused litter based on softwood shavings. Birds were placed on litter materials at day one of age and fed identical diets. Gut samples were collected for microbial profiling at 14 and 28 days of age. Significant differences ($P<0.001$) in caecal microbial community composition were detected between birds aged 14 and 28 days, regardless of litter material used.

Significant differences were observed in caecal microbial community composition of day 14 old birds raised on re-used litter when compared with the six other litter materials used ($P<0.001$). There were also differences in caecal microbial community composition of day 14 old birds raised on rice hulls when compared with softwood sawdust, hardwood sawdust and shredded paper ($P<0.05$), and between hardwood sawdust when compared with softwood sawdust and shredded paper ($P<0.05$).

Significant differences were also observed in caecal microbial community composition of day 28 old birds raised on re-used litter when compared with softwood sawdust, hardwood sawdust, shredded paper, rice hulls and chopped straw ($P<0.05$), but not with softwood shavings. There were also differences in caecal microbial community composition of day 28 old birds raised on rice hulls when compared with shredded paper and chopped straw ($P<0.05$). No significant differences were detected in gut microbial community composition between birds on any of the other litter treatments.

Differences observed in gut microbial community composition between birds raised on re-used litter as opposed to non re-used litter materials may be partly due to re-used litter acting as a bacterial inoculum for gut microflora establishment, particularly in the younger birds.

c) Influence of age on gut microflora

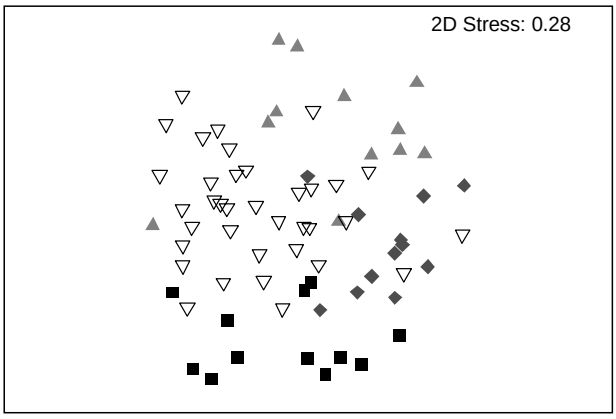

Figure 2. Non-metric multidimensional scaling of OTU's from T-RFLP analysis of caecal microbial communities from chickens from a single commercial flock (n=72) at ▲1, ◆ 2, ▽ 3-5 and ■ 6 weeks of age.

Gut microbial communities were analysed from the caeca of birds from two flocks raised under commercial poultry conditions in South Australia. Over a single growing period samples from 12 birds each aged 1 to 6 weeks were analysed per flock (Figure 2). Significant differences in the overall gut microbial communities of birds aged 1, 2, 3-5 and 6 weeks were observed ($P<0.05$). No significant differences were detected in gut microbial community composition between ages 3-5 weeks. The same trend was observed on two commercial farms, even though overall chicken caecal microbial communities were significantly different between the two flocks ($P<0.05$).

IV. CONCLUSION

T-RFLP has been used to monitor shifts in the chicken gut microbial population associated with environmental and age changes. We have shown that gut microbial community composition changes greatly within the first 2-3 weeks of age before stabilising until age 5-6 weeks, when a final change in community composition was observed. Although previous studies have shown the composition of the gut microflora can vary with age (Knarreborg *et al*., 2002; Lu *et al*., 2003), this is the first report which follows the succession of gut microflora over a six week period within a commercial production setting, and is not dependant on generation of bacterial sequence information for identifying differences in microbial community composition.

Litter material has also been shown to influence development of gut microbial community composition and has confirmed age related changes when comparing birds aged 2 and 4 weeks old. Furthermore, environmental conditions have been shown to affect caecal microbial community development within the first week. This has previously been shown to be a crucial period for gut development and mucin production, which is linked to intestinal bacterial populations. The results indicate that the T-RFLP tool has the potential to contribute significantly to an increased knowledge of the chicken gut microbiota, and hence, a better understanding in its role in chicken nutrition.

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ANTAGONISTIC ACTIVITY OF NOVEL PROBIOTICS AND THEIR EFFECT ON GROWTH PERFORMANCE OF BROILER CHICKENS

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Summary

A total of 294 one-day old Cobb broiler chickens were used to investigate the effects of four lactobacillus strains on production performance. The chicks were assigned randomly to six groups with 7 replicates of 7 chicks per treatment. The six dietary treatments were: (i) basal diet (negative control, T1); (ii) basal diet with added Zinc-bacitracin (ZnB, 50 ppm, T2), iii) one of four strains of *Lactobacillus* (tentatively identified as *L. johnsonii, L. crispatus*, *L. salivarius* and unidentified *Lactobacillus* sp., T3, 4, 5 and 6). The probiotic strains were selected from 235 lactobacilli isolates based on their *in vitro* antagonistic effect against *Clostridium perfringens* and *Escherichia coli*. Results showed that the addition of probiotic *Lactobacillus* spp. to the feed did not significantly improve body weight gain, feed intake and feed conversion ratio of broiler chickens raised in cages during the 6-wk experimental period.

I. INTRODUCTION

Probiotics is a field of science, medicine and business that is growing rapidly. Probiotics, prebiotics, feed enzymes and organic acids have been seen as potential alternatives to antibiotics (Choct, 2002).

The addition of either pure lactobacillus cultures or mixtures of lactobacilli and other bacteria to broiler diets has produced variable results. Han *et al.* (1984) and Kim *et al.* (1988) found an improvement in weight gain and feed conversion ratio from 2 to 6 weeks of age. A consistent improvement in body weight gain of chickens fed a culture of *L. sporegenes* has also been reported (Mohan-Kumar and Christopher, 1988; Kalbande *et al.* 1992). Jin *et al.* (1998) reported that addition to the feed of a single strain of *L. acidophilus* or a mixture of lactobacilli from 0 to 6 weeks of age significantly improved body weight gain and FCR of broilers. There have also been several studies in which no positive results were found. Watkins and Kratzer (1984) and Maiolino *et al.* (1992) did not find any significant difference in the body weight gain of chickens given feed containing host-specific probiotics (KTM, 74/1 and 59) and *L. acidophilus* and *Streptococcus faecium*, compared with those given a non-supplemented diet. Variation in the effects of probiotics on growth performance of broiler chickens may be attributed to differences in the strains of bacteria used as the dietary supplements. In the present study, four strains of *Lactobacillus* spp. previously isolated from the chicken gut were selected based on their antagonistic activity against *C. perfringens* and *E. coli,* and their effect on growth performance of broiler chickens was investigated.

II. MATERIALS AND METHODS

a. Probiotics strains

Lactobacillus spp. were isolated from the ileum and caeca of broiler chickens as previously described (Vidanarachchi *et al.* 2006). The isolates were differentiated using Amplified Ribosomal DNA Restriction Analysis (ARDRA) and representative strains were

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identified by 16 sRNA gene sequencing (Vidanarachchi, 2006). A total of 235 lactobacillus isolates were tested using an antagonistic activity assay as described by Teo and Tan (2005) with some modifications. The lactobacillus isolates were grown in de Man, Rogosa, and Sharp broth (MRS) (Oxoid, CM0359) under anaerobic conditions at 39˚C for 24 h. Similarly, the indicator strains, pathogenic strains of *C. perfringens* and *E. coli*, were grown in Thioglycllate broth (Oxoid, CM0391). The overnight culture of each lactobacillus isolate was streaked onto the surface of Wilkins-Chalgren anaerobic agar (Oxoid, CM0619) using a sterile cotton swap. After anaerobic incubation at 39˚C for 24 h, an overnight culture of *C. perfringens* or *E. coli* was streaked across the same agar plates bisecting the streak line of the lactobacillus isolate (perpendicularly). The inoculated plate was then incubated under anaerobic conditions at 39˚C for another 24 h. The antagonistic activity of test organisms on the indicator bacteria was determined by the appearance of clear zones surrounding the junctions of the streak lines. The width of the clear zone was measured and recorded.

b) In vivo experiment

A total of 294 one-day old male Cobb broiler chickens were obtained from Baiada hatchery, Kootingal (Tamworth, NSW) and allocated to 6 treatments of 7 replicates (7 birds each replicate). The basal diets (starter and finisher) were based on corn, wheat and soybean meal and fed as a one-phase mash feed to avoid inactivation of the probiotics. From results of the antagonistic activity test, four strains of lactobacillus (No 1286 tentatively identified as *L. johnsonii,* No 709 tentatively identified as *L. crispatus*, No 697 tentatively identified as *L. salivarius* and No 461 unidentified *Lactobacillus* sp.) showing the highest degree of inhibition of the test organisms were selected as probiotic candidates and added to the feed to make up four different treatments. Two control treatments were also included, a negative control, with no additives and a positive control treatment, with the antibiotic, Zinc-bacitracin (ZnB, 50 ppm) added. The experimental diets with the probiotic candidates were mixed weekly. The individual strains were grown overnight and harvested by centrifugation (4420 x *g* for 15 min), resuspended in PBS (pH 7.4) and mixed into a premix with the basal diet for 10 minutes using a miniature mixer. This pre-mixture of product with feed (1 kg) was then transferred into a larger mixer (total capacity 300 kg) where the final volume of the weekly feed batch was prepared. The mixer equipment was thoroughly cleaned between the mixing of different treatments by using a vacuum cleaner and a wash diet (basal feed).

c) Analyses and performance measurements

Representative feed samples of each feed batch were tested for bacterial concentrations every week of the experiment. Ten (10) g feed was dissolved in 90ml of peptone water (Oxoid, CM0009) and 10-fold dilutions were performed in Hungate tubes with 9 ml of peptone water. The numbers of lactic acid bacteria in the feed samples were determined on MRS agar inoculated with 0.1ml of diluted sample and after anaerobic incubation at 39˚C for 48 h. Bird performance was measured on a weekly basis by recording the group weight and feed intake for each cage. Mortalities were checked daily and body weight of dead birds was recorded.

Statistical analysis was performed using one-way analysis of variance, and difference between means (StatGraphics Plus version 5.1, Manugistics Inc., Rockville, Maryland, USA).

III. RESULTS AND DISCUSSION

Of the 235 isolates tested, 91 isolates showed no antagonistic activity against both *C. perfringens* and *E. coli* while another 122 isolates showed antagonistic activity against *E. coli* only. The remaining 22 isolates showed antagonistic activity against both *C. perfringens* and *E. coli* (results not shown). Four lactobacillus strains representing four different species (No 461, 697, 709 and 1286) and showing the strongest antagonistic activity against the indicator

organisms, *C. perfringens* and *E. coli*, were selected. The antagonistic acitivity was observed as a truncated clear zone surrounding the intersections of the streak lines of the test and indicator strains (Figure 1). The inhibition zones displayed by the four strains selected were between 3mm and 15mm wide (Table 1).

Table 1. Inhibition of indicator organisms by the probiotic candidates and average concentrations of the probiotic strains in the feed for the broiler experiment

Probiotic candidates[1]	Inhibition of *C. perfringnes* (mm)	Inhibition of *E. coli* (mm)	Treat-ment	Log CFU/g[2] feed (n=6)
LB 461	4	3	Diet 3	6.15 ± 0.25
LB 697	7	12	Diet 4	5.58 ± 1.09
LB 709	7	9	Diet 5	5.44 ± 0.59
LB 1286	15	10	Diet 6	5.50 ± 0.72

[1] LB 461: unidentified *Lactobacillus* sp., LB 697: tentatively identified as *L. salivarius*, LB 709: tentatively identified as *L. crispatus*, and LB 1286: tentatively identified as *L. johnson.i.*
[2] CFU/g: Colony Forming Unit in per gram of feed samples.

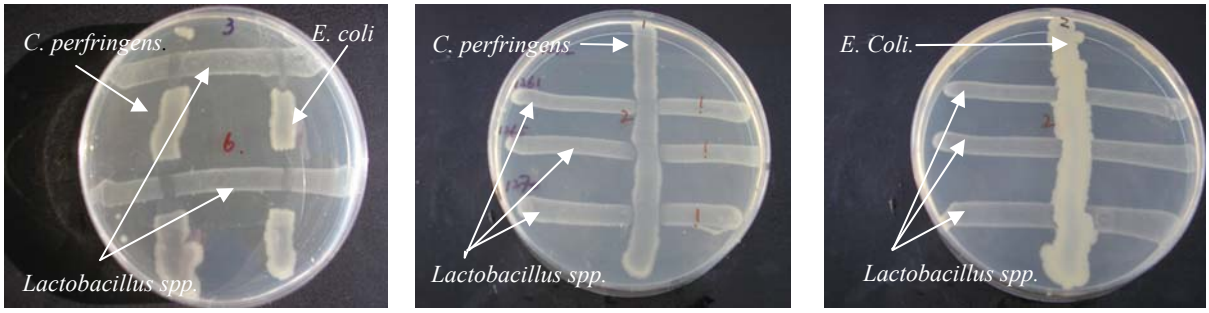

Figure 1: Lactobacilli inhibiting growth of *C. perfringens* and *E. coli* (left) and with no growth inhibition with *C. perfringens* and *E. coli* (middle and right).

Table 2 shows the biological response of birds in the present study. There was no significant effect on body weight gain, feed intake or feed conversion ratio (FCR) of broiler chickens when the probiotic candidates were added to the feed. Similar results were observed by Huang *at al.* (2004) who supplemented either *L. casei* or *L. acidophilus* with or without cobalt in the diets for broiler chickens. Although no significant improvement of growth performance was observed, all four probiotics used in the current study tended to improve body weight gain of birds compared with the negative control diet.

The concentration of the probiotic candidates in the experimental feed (approximately 10^6 CFU/g feed) was lower than levels usually recommended as the inclusion rate of commercial probiotic feed additives (around 10^8 CFU/g). This was due to a limited fermentation capacity for amplification of the probiotic candidates in the current study. It is possible that higher concentrations of the probiotic candidates in the feed may exert a more profound positive response on growth performance, especially if the infection pressure from pathogenic bacteria, such as *C. perfringens*, is high. This needs to be investigated in future studies.

In conclusion, four lactobacillus probiotic candidates with in-vitro antagonistic activity against pathogenic *C. perfringens* and *E. coli* were identified. When supplemented in the feed at 10^6 CFU/g feed, the probiotics did not significantly improve the growth performance of broiler chickens in a cage-scale experiment. However, there may be other beneficial effects associated with the use of microbial probiotics in animal feeds, such as the competitive exclusion of pathogenic bacteria which may improve gut health (Gusils *et al.* 1999). This effect of the probiotic candidates will be evaluated in future studies.

Table 2. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broiler chickens fed the experimental diets[1]

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	SE[2]	P-values
BWG (g/bird):								
W1-3	811	827	791	809	821	826	10.4	0.173
W4-6	1823	1885	1866	1866	1869	1832	52.9	0.954
W1-6	2634	2712	2657	2675	2689	2658	53.8	0.903
FI (g/bird):								
W1-3	1211	1220	1190	1208	1233	1228	14.9	0.309
W4-6	3654	3756	3657	3713	3750	3683	77.4	0.870
W1-6	4865	4976	4846	4921	4982	4910	82.7	0.752
FCR (g feed/g weight gain):								
W1-3	1.44	1.42	1.44	1.43	1.45	1.43	0.02	0.895
W4-6	2.03	2.00	1.95	1.99	2.00	2.03	0.04	0.896
W1-6	1.73	1.71	1.70	1.71	1.72	1.73	0.02	0.914

1. Diet 1: Negative control, with no additives added to the feed; Diet 2: Positive control, with the antibiotic, Zinc-bacitracin (ZnB, 50 ppm) added; Diet 3-6, with one of probiotics No 461 unidentified lactobacilli sp., No 697 *L. salivarius*, No 709 *L. crispatus*, and No 1286 *L. johnsonii* added to the feed, respectively.
2. Standard error.

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EVALUATION OF POTASSIUM DIFORMATE IN NECROTIC ENTERITIS CHALLENGE MODEL

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Summary

The effects of graded inclusion levels of potassium diformate (KDF) in broiler diets were evaluated in the Necrotic Enteritis (NE) challenge model. At an inclusion rate of 4.5 g /kg KDF significantly reduced NE mortalities by 58% (12.7 versus 30.0%) in challenged birds. The reductions in NE mortalities induced by KDF, however, were not associated with decreased jejunal counts of *Clostridium perfringens*, the causative organism. KDF did not significantly influence weight gain or feed efficiency in unchallenged or challenged birds. It is concluded that KDF appears to have promise for the control of NE in broiler chickens, particularly in the absence of antibiotics, and that further investigations are justified.

I. INTRODUCTION

Necrotic enteritis (NE) is a global poultry disease caused by the bacterium *Clostridium perfringens*. Proliferation of *C. perfringens* in the chicken intestine results in the production of toxins that cause necrotic mucosal lesions in the gut, which can result in acute or subclinical disease entities (van Immerseel *et al*., 2004; McDevitt *et al*., 2006). In its clinical form, NE causes high mortalities in broiler flocks and, in its subclinical form, NE is financially damaging because of impaired growth performance of chickens. In-feed antibiotics are routinely used to control the disease, but this practice is coming under increasingly critical scrutiny and has been curtailed in Western Europe due to concerns about the development of antibiotic-resistance in bacteria that are potential human pathogens. A prohibition of antibiotic inclusion in animal feeds is thus likely to lead to an increased incidence of NE in poultry. Consequently, there is a pressing need to identify alternative feed additives to sustain efficient chicken-meat production, without reliance on antibiotics. The inclusion of organic acids in broiler diets is one of a number of strategies to control NE in the post-antibiotic era that are under investigation (Dahiya *et al.,* 2006). Organic acids and their salts are possible alternatives to antibiotics because of their antimicrobial and growth-promoting effects. Also, lactic and propionic acids have been shown to reduce litter contamination with *C. perfringens* (Gornowicz and Dziadek, 2002). Potassium diformate (KDF, Formi®) is a chemical complex, which dissociates into formic acid and potassium formate in the gut, that has shown promise in enhancing growth performance and nutrient utilisation in broilers (Selle *et al.,* 2004). The aim of the present study was to evaluate the potential of KDF to control losses due to NE in broiler chickens caused by *C. perfringens*.

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II. MATERIALS AND METHODS

A total of 1050 day-old male broiler chickens (Cobb) were purchased from the local hatchery, divided into groups of 25 and placed in floor pens with sawdust bedding.
There were 7 treatment groups of 6 replicate pens: (i) unchallenged negative control, (ii) unchallenged plus 4.5 g/kg KDF, (iii) challenged negative control, (iv) challenged positive control [100 ppm monensin, 45 ppm Zn-Bacitracin], and challenged with inclusion levels of (v) 2.25, (vi) 4.50 and (vii) 6.75 g/kg KDF. The birds received a starter diet from day 1 to 7 and again from day 15 to 21 and the finisher diet from day 22 to 35 of the experiment. A high protein diet (50% starter diet and 50% fishmeal) was fed to the birds from day 8 to 14 to facilitate the NE challenge. All diets were pelleted and fed *ad libitum*. The NE challenge followed the model described by Kocher *et al.* (2004) with some modifications. In the present experiment, birds were challenged with NE through infectious litter. Birds were raised on sawdust bedding re-used from a previous NE challenge experiment (Vidanarachchi, 2006) so that unchallenged pens had re-used litter from previously unchallenged birds and challenged pens had re-used litter from previously challenged birds. There was no significant difference in infection pressure between the challenged treatment groups of the present experiment, in aspects of previous NE-related deaths in pens. On day 9 of the experiment, challenged birds were also inoculated by oral gavage of sporulated oocysts of *Eimeria acervulina*, *E. tenella* and *E. maxima*. The birds were inspected daily and dead birds were removed following registration of pen, date and bodyweight. Bird live weight and feed consumption on a pen basis were recorded on days 21 and 35. Feed conversion efficiency (kg feed/ kg weight gain) was calculated after adjustment for dead birds.. Necropsies of all mortalities were conducted to determine the cause of death. On day 15 (peak of NE outbreak), two birds per pen were killed by cervical dislocation and pooled contents from the duodenum, jejunum and ileum were collected and pH was measured immediately. Jejunal contents were transferred into sterile McCartney bottles containing 10 ml of a pre-reduced salt medium. The suspension was poured into a CO_2-flushed plastic bag, homogenised in a Stomacher laboratory blender and serial ten-fold dilutions were performed. C*lostridium perfringens* were enumerated on Tryptose Sulphite Cycloserine (TSC) agar (Oxoid, Agar base CM0587, TSC supplement SR0088 and egg yolk emulsion SR0047) using pour-plating technique and after anaerobic incubation at 39^0C for 24 hours. All data were analysed according to the GLM procedure for ANOVA (SAS Institute Inc., 2001) with treatment as the main factor.

III. RESULTS AND DISCUSSION

The effects of treatment on weight gain and feed efficiency are shown in Table 1. From 1-21 days of age, unchallenged broilers, without and with KDF, and the challenged positive controls performed similarly and significantly out-performed broilers offered the challenged negative control and KDF-supplemented diets. These results indicate that the challenge caused growth depression, which was probably due to the impact of subclinical NE. Overall, while there are numerical differences, KDF was not associated with any significant alterations to growth and feed efficiency in either unchallenged or challenged birds. In contrast, monensin and Zn bacitracin significantly improved performance relative to the negative controls.

Table 1. Weight gain and feed efficiency of broilers from 1-21, 21-35 and 1-35 days of age

Treatment	Weight gain (g/bird)			Feed efficiency (g/g)		
	1-21	21-35	1-35	1-21	21-35	1-35
Unchallenged negative control	730^{a}	1179^{ab}	1910^{ab}	1.43^{b}	1.93^{cd}	1.65^{b}
Unchallenged, 4.50 g/kg KDF	728^{a}	1086^{b}	1814^{bc}	1.44^{b}	2.04^{bcd}	1.68^{ab}
Challenged negative control	599^{b}	1095^{b}	1694^{c}	1.63^{a}	2.48^{a}	1.72^{ab}
Challenged positive control	726^{a}	1266^{a}	1992^{a}	1.36^{b}	1.73^{d}	1.49^{c}
Challenged + 2.25 g/kg KDF	571^{b}	1116^{b}	1687^{c}	1.61^{a}	2.34^{ab}	1.72^{ab}
Challenged + 4.50 g/kg KDF	631^{b}	1120^{b}	1751^{c}	1.67^{a}	2.17^{abc}	1.77^{a}
Challenged + 6.75 g/kg KDF	595^{b}	1159^{ab}	1754^{c}	1.61^{a}	2.27^{abc}	1.65^{b}
SEM	21.1	38.4	48.1	0.05	0.14	0.04
Significance (P =)	< 0.001	0.031	< 0.001	< 0.001	0.012	< 0.001

^{abc}Values within a column without a common superscript are significantly different

Mortality rates attributed to NE are shown in Table 2 where the challenge increased losses from 1.3 to 30.0%, which was counteracted by monensin and Zn bacitracin (2%). Medium and low inclusion levels of KDF reduced NE mortalities in challenged birds. Inclusion of 4.5 g/kg KDF, significantly reduced mortalities by 58% (12.7 versus 30.0%) and 2.25 g/kg KDF tended to reduce mortalities by 31% (20.7 versus 30.0%). While the high inclusion level (6.75 g/kg) did not influence NE mortalities, there was a quadratic response to KDF, which approached significance (r = 0.494; P = 0.053) and the regression equation indicates that the optimum KDF inclusion rate to reduce NE mortalities is in the order of 3.5 g/kg. Counts of *C. perfringens* at the peak of the NE outbreak did not correspond to mortality rates as all treatments, with the exception of monensin and Zn bacitracin, had similar jejunal levels of *C. perfringens.* This is contrary to reports that lower gut levels of *C. perfringens* are found in healthy flocks (Craven, 2000) and implies that the factors that trigger acute NE are not well understood.

Table 2. Necrotic Enteritis mortality rates, *Clostridium perfringens* counts in jejunum (log CFU per g digesta) and pH in small intestinal segments

Treatment	NE mortality (%)	*C. perfringens* (Log CFU/g)	pH		
			Duodenum	Jejunum	Ileum
Unchallenged control	1.3^{d}	6.86^{b}	5.53^{c}	6.36^{cd}	6.91^{ab}
Unchallenged, 4.5 g/kg KDF	3.3^{cd}	6.98^{b}	4.84^{abc}	6.27^{bcd}	6.81^{a}
Challenged negative control	30.0^{a}	6.96^{b}	4.23^{a}	5.53^{a}	6.77^{a}
Challenged positive control	2.0^{d}	3.57^{a}	5.64^{c}	6.57^{d}	7.50^{b}
Challenged + 2.25 g/kg KDF	20.7^{ab}	7.09^{b}	4.73^{ab}	5.84^{ab}	6.62^{a}
Challenged + 4.50 g/kg KDF	12.7^{bc}	6.34^{b}	4.97^{b}	5.98^{abc}	6.56^{a}
Challenged + 6.75 g/kg KDF	28.0^{a}	7.56^{b}	4.65^{ab}	5.74^{a}	6.33^{a}
SEM	3.6	0.45	0.23	0.15	0.22
Significance (P =)	< 0.001	< 0.001	< 0.001	< 0.001	0.005

^{abcd}Values within a column without a common superscript are significantly different

While there was a tendency for KDF to reduce duodenal pH in unchallenged birds it was associated with increased duodenal pH in challenged broilers and, arguably there were no meaningful pH alterations in the jejunum and ileum (Table 2). Gut pH could be indicative

of the amount of the formic acid component of KDF that escapes absorption from the fore-stomach and enters the small intestine.

It is curious that KDF reduced NE mortalities but this was not associated with reduced counts of *C. perfringens* in the jejunum. It is possible, however, that the gut histopathology results (to follow) may prove instructive in this respect. Nevertheless, the significant (58%) reduction in NE mortalities generated by 4.5 g/kg KDF is a promising result.

KDF displayed promise as a 'growth promotant' in an initial evaluation (Selle *et al.,* 2004), but this was not the case in subsequent feeding studies (unpublished data). A review of these experiments suggests that acid binding capacities (ABC) and/or dietary electrolyte balances (DEB) may contribute to the inconsistent responses, possibly by modifying the rate at which KDF dissociates into formic acid and potassium formate in the gut. Also, anecdotally, organic acids will perform more consistently in diets with low ABC. Typical, wheat-based Australian broiler diets routinely contain an anti-coccidial drug and a xylanase feed enzyme and both agents are considered to reduce the predisposition of broilers to NE outbreaks. Therefore, it may prove instructive to determine the efficacy of KDF in the challenge model when used in combination with one or both of these agents. Under practical conditions it may be that KDF, in combination with a coccidiostat and a xylanase, could provide control against NE, which would be beneficial should the use of antibiotics be discontinued. It may also be possible to identify the most appropriate ABC and DEB to enhance the efficacy of KDF as both a growth promotant and as an agent to control NE.

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OCCURRENCE OF REVERSE PERISTALSIS IN BROILER CHICKENS

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Summary

Two experiments were conducted to evaluate the occurrence and possible effects of certain dietary ingredients on reverse peristalsis or reflux in broiler chickens. The first experiment was a preliminary study into the occurrence of reflux while the second experiment investigated the passage of a bacterial marker and Cr-EDTA in birds fed diets of varying viscosities. The results suggest that reflux occurs throughout the digestive tract of both fasted and fed chickens. Reflux appears to be part of normal gut motility and a possible adaptive response to an absence of food, indicating that it serves to extend the digestive process. Dietary ingredients are likely to affect reflux, especially ingredients that increase digesta viscosity in the lumen. In addition, microbial populations may be relocated by reverse peristaltic contractions.

I. INTRODUCTION

The phenomenon of reverse peristalsis, or reflux, has been recorded in a limited number of avian species. As with the overall study of motility, there is insufficient evidence to describe the patterns of these movements or even conclusively associate it with chickens. Digestive reflux, if it does occur in chickens, will have implications for nutrient utilisation, choice of feed ingredients and health. The primary aim of the current study was to investigate the occurrence of digestive reflux, its variation with nature of diet and possible link to bird health.

II. ASSESSING THE OCCURRENCE OF REFLUX USING CR-EDTA AS AN EXTRINSIC MARKER

The soluble marker, Cr-EDTA was injected into the cloaca of 48 broiler chickens at 3 or 5 weeks of age. Due to the preliminary nature of this trial replicates were not employed in the study. Four birds (2 fed and 2 fasted) were then slaughtered at 1, 2, 3, 4, 5 and 24 hours post-marker administration. Digesta samples were taken from the crop, gizzard, duodenum, jejunum, ileum and caeca to analyse for the Cr content.

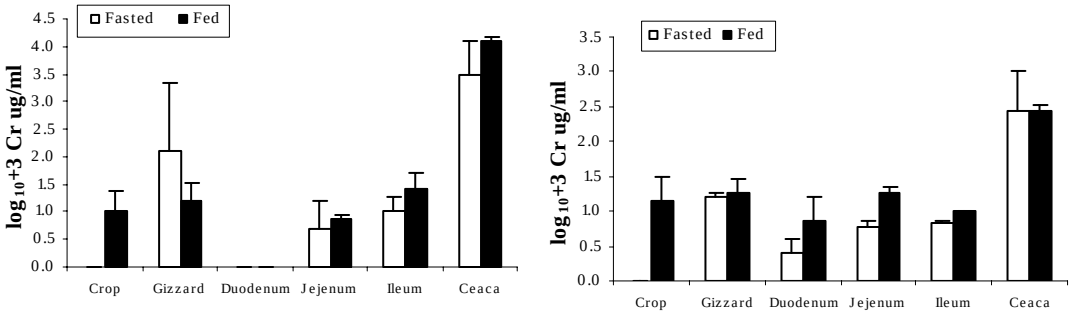

Figure 1. Chromium levels in fasted and fed, 3- (left) and 5-week (right) old birds, one hour post-marker administration. Values are means ± std. error.

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After one hour Cr was detected in all sections of the gastrointestinal tract (GIT) except for duodenum in the 3-week old group (Figure 1.). In both fasted and fed groups at both ages, the majority of the marker remained in the caeca. In the 3-week old, fasted group high levels of Cr were observed in the gizzard, equivalent to the levels found in the corresponding caecal samples.

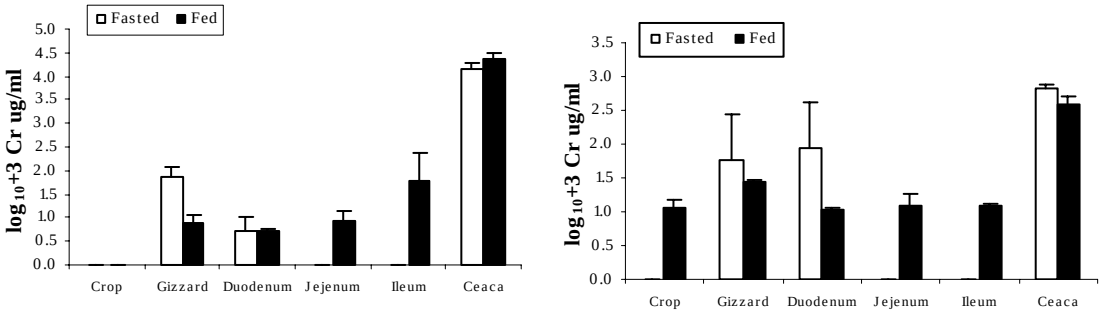

Figure 2. Chromium levels in fasted and fed, 3- (left) and 5-week (right) old birds, four hour post-marker administration. Values are means ± std. error.

In the fasted birds, Cr was absent in the jejunum and ileum, 4 hours after administration in both the 3- and 5-week old birds, as shown in Figure 2. Chromium was present in other regions, except in the crop of fasted birds (both 3- and 5-week old). Cr levels were higher in the gizzard and duodenum for fasted birds at both ages. Levels in the caeca remained the highest for all birds.

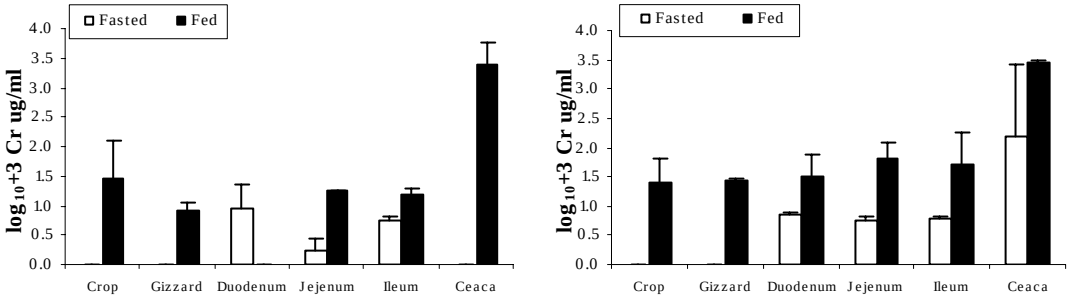

Figure 3. Chromium levels in fasted and fed, 3- (left) and 5- (right) week old birds, twenty-four hours post-marker administration. Values are means ± std. error.

Twenty-four hours after the marker was administered Cr levels remained highest in the caeca for all but the 3-week fasted birds (Figure 3), with levels only slightly less than those observed 1 hour post-administration (Figure 1). In fed birds, Cr was present at all regions with the exception of 3-week old fed birds where Cr was absent in the duodenum. For fasted birds, in both age groups, some regions of the GIT were devoid of Cr.

The steady rise of Cr levels in the gizzard, and the subsequent peak at 4 hours, is more noticeable in fasted birds, and is likely a reaction to prolonged fasting. The high levels may be an indication of a rhythmic oscillating complex (ROC), which has been observed to occur after 4-6 hours of fasting and characterised by re-stimulating fed state motility (Clench and Mathias, 1992; Jimenez *et al.*, 1994). Peristalsis rather than reverse-peristalsis would be expected for the fed state, which might explain why after the peak at 4 hours post-marker administration, Cr levels declined after 24 hours.

The high levels of Cr in the caeca are not surprising, since the marker was injected at a point just beyond the colonic sphincter. Colonic-caecal reflux is well established and may serve to recover nitrogen from urea. The levels observed especially in the 5-week old birds,

suggest initial increasing levels (probably due to Cr entering via the colonic-caecal reflux), then levels decreased (due to reflux towards more proximal areas in the GIT) and finally increased slightly again (material flowing distally under normal peristalsis).

III. EFFECT OF DIET ON THE REFLUX AND THE RETROGRADE MOVEMENT OF A NOVEL ANTIBIOTIC RESITANT STRAIN OF *E.COLI*

The 72 birds used in this study were divided into 6 groups of 12 birds. Groups 1, 2, 3 and 4 were fed diets based on maize, wheat, wheat plus an exogenous microbial enzyme or a commercial diet, respectively. Two markers, Cr-EDTA and a bacterial marker, were administered via the cloaca after 4 hours of feed deprivation at 28 days of age. Group 5 were fed a commercial diet and both markers were administered at 28 days of age via the crop while Group 6 were fed a commercial diet and only Cr-EDTA was administered via the cloaca at 28 days old. The bacterial marker used was a strain of *E. coli*, chosen due to its resistance to two antibiotics, Nalidixic acid and Rifamycin, making it almost impossible to find in nature. Digesta samples were taken from the gizzard, duodenum, jejunum, ileum and caeca, and analysed for Cr content and plated on selective media to analyse for the bacterial marker.

Table 1. Mean Cr-EDTA levels (Cr µg/ml) and *E. coli* (logCFU/g digesta) detected in the gizzard, duodenum and caecum of birds on the four dietary treatments.

	Commercial	Maize	Wheat	Wheat+enzyme	SED
Cr-EDTA					
Gizzard	0.048	0.028	0.043	0.040	0.009
Duodenum	0.038	0.019	0.037	0.041	0.018
Caecum	1.133	2.170	0.844	1.658	0.564
E. coli					
Gizzard	3.238	2.907	3.211	3.051	0.256
Duodenum	3.221^{b}	4.158^{a}	3.671^{ab}	3.036^{b}	0.370
Caecum	5.862	6.408	6.567	5.839	0.531

a,b – Mean values on the same row with unlike superscripts are significantly different ($P<0.05$). SED is standard error of difference between mean values.

Chromium was detected in the gizzard of birds from all four diet groups, with the lowest mean concentration, although not statistically significant, in birds given the maize diet (Table). The antibiotic resistant *E. coli* was present in the gizzard of birds on all four diets but there was no significant difference between the dietary groups.

Analysis of digesta from the duodenum showed that Cr was present in this section of the GIT for birds from all four diet groups but there was no significant difference between the groups. The microbial marker was also detected in the duodenum of birds from all four diet groups. Analysis of variance revealed significant differences ($p=0.0296$) between the maize diet group and both Wheat+enzyme and commercial diet groups, in terms of number of colonies.

The digesta retrieved from the caeca of birds from all four diet groups contained Cr and the antibiotic resistant *E. coli*. Analysis of variance did not reveal significant differences between the Cr levels or *E.coli* counts in birds on the maize and wheat dietary groups.

The results of this experiment confirm the findings of the previous study, with regard to the reverse passage of the soluble marker, Cr-EDTA. As such, it may be concluded that reflux occurs throughout the GIT.

This experiment also substantiates the hypothesis that bacterial communities may be relocated to more proximal sites in the GIT by reverse peristalsis. The antibiotic resistant strain of *E. coli* used in the experiment, was recovered from the gizzard of birds from all four diets, indicating that the bacterium was able to travel the length of the GIT when introduced through the cloaca. The implications of this finding could be considerable, especially when related to the relocation of pathogenic microbes such as *C. perfringens* to areas in the upper tract.

It may be assumed that intestinal viscosities of the maize and enzyme-supplemented wheat-based diets were similar while the wheat control diet would generate a more viscous digesta, particularly in the jejunum and ileum. Consequently, one would expect to see significant differences in the levels of the two markers in the small intestine for the maize and wheat diets. However, this was not the case; significant differences were not observed between the marker levels in the maize and wheat diet. A significant difference was observed in the number of colonies counted from the digesta obtained from the duodenum and this coupled with the strong tendency for different counts in the jejunum (data not shown) between the maize and commercial diet are worth noting. The fact that this difference was observed in adjoining sections where the counts were higher both in the duodenum and jejunum in birds from the maize diet group suggests that this may be a dietary effect. Because viscosity was not measured, it is difficult to explain why significant differences were not observed between maize and wheat-based diets. However, it has been noted that the intestinal viscosity for maize can be higher than for wheat (Maisonnier *et al.*, 2001). The variability in pentosan content of different wheats may be a factor in the results obtained in this study; this, together with digesta viscosity, should be measured in subsequent studies.

IV. CONCLUSION

These findings suggest that reflux is an adaptive response to a lack of feed, as well as being characteristic of normal gut motility occurring throughout the digestive tract. In addition, microbial populations may be relocated by reverse peristalsis.

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ENDO-XYLANASE, A POSSIBLE WAY OF SUPPLYING PREBIOTIC OLIGOSACCHARIDES ?

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Summary

Earlier research on prebiotic compounds mainly focused on inulin and fructo-oligosaccharides. Recent investigations involving *in vivo* studies in monogastric animals and *in vitro* culture fermentations, as well as the availability of more powerful fractionation techniques, have shown the existence of other potential polysaccharide based sources with prebiotic properties. Their effectiveness seems to be related to their molecular structure and the purity of the preparation. This implies that production techniques will require to concentrate on optimal yield and efficient recovery of specific molecular weight fractions.
Arabinoxylans are abundantly present in cereals. Both the water-soluble as the water-insoluble arabinoxylans exert well-described anti-nutritive effects in monogastrics. Endo-xylanases with a pronounced capability to hydrolyze the insoluble fraction into soluble oligosaccharides will convert an omnipresent polysaccharide into beneficial substances. The effect of different EC-registered enzyme preparations, based on endo-xylanase activity, on different xylan substrates was investigated.

I. PREBIOTICS – PLENTY OF SUBSTANCES

The crucial role of the intestinal microbiota in the general health status of man and animal is becoming more and more acknowledged. In animal production, good health is a prerequisite for high performance. Recent years, following the footsteps of human nutrition, considerable effort has been made to reveal the possible effects and working mechanisms of prebiotics, non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria (Gibson and Roberfroid, 1995).

Prebiotics are fermented by micro-organisms in the distal part of the gastro-intestinal tract. Hereby the composition and/or the activity of the microbiota is altered, leading to secondary effects such as: increased gas production (short chain fatty acids (SCFA), lactic acid, hydrogen, carbon dioxide, hydrogen sulphide); a drop in pH; and decreased metabolic activity, (e.g. the activity of 7-α-deshydroxylase, which converts primary bile salts into carcinogenic secondary bile salts, Marteau *et al*., 2004). This shift in microbiota may be translated into macro-effects like an increased resistance against pathogens or an improved growth performance.

So far, most research has been performed on inulin and fructo-oligosaccharides. However, emphasis has widened towards a complete range of oligosaccharides including galacto-oligosaccharides, beta-glucans, soybean oligosaccharides and xylo-oligosaccharides. Up to now, the major part of research on these prebiotics has been performed *in vitro*, despite of the known limitations. Recent molecular techniques indicate that only 20 to 50 % of the bacterial species in the intestines can be cultured (Patterson and Burkholder, 2003). In order to fully assess the potential of a prebiotic substance, trials in the intestinal environment are necessary, where substrate availability, nutrient competition, cross-feeding, and bacterial population levels and niches will influence bacterial metabolism, population dynamics and functional effects in the host (Crittenden *et al*., 2002).

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The structure of the prebiotic substance largely determines its effect. Inulin is known to be fermented faster (resulting mainly in the production of butyric acid) than xylo-oligosaccharides, which are slowly fermented, generating acetic, propionic and lactic acids. There is a large diversity in the responsiveness of the different bacteria. Differences have been noticed between bacterial genera, species and strains (Crittenden *et al*., 2002). Xylo-oligosaccharides tend to stimulate bifidobacteria, but effects are influenced by the degree of polymerisation (DP), the degree of substitution, and the character of possible side-chains.

II. THE MANUFACTURE OF XYLO-OLIGOSACCHARIDES

Recently considerable effort has been put into the refinement of the manufacturing process of xylo-oligosaccharides. One possibility of obtaining these is to use an enzymatic de novo synthesis process, starting from mono- and oligosaccharides (Rastall *et al*., 2002). Up until now costs of production are high, resulting in an expensive product that cannot be used at a large scale in human and animal nutrition.

On the other hand, plant cell wall polysaccharides, rich in arabinoxylans, are abundantly present in nature. This makes them an excellent substrate for producing xylo-oligosaccharides, in a chemical and/or enzymatic way. The xylo-oligosaccharides obtained this way consist of a diversity of components, which need refining. Multiple purification steps (solvent extraction, ultrafiltration techniques) may be necessary in order to obtain a high-purity end-product. (Moure *et al*., 2006).

Looking for an alternative, we considered the fact that feeds for poultry and pigs are mostly based on corn, wheat and/or barley. All of them are raw materials rich in arabinoxylans, a known anti-nutritional factor, countered by the standard addition of endo-xylanase. In the gastro-intestinal tract, the endo-xylanase breaks down the arabinoxylan chains into smaller fragments, with potential prebiotic properties.

III. CHARACTERISATION OF THE HYDROLYSATES FORMED BY ENDO-XYLANASE

a) Materials and methods

Purified xylo-oligosaccharides with an average degree of polymerization of respectively three, four and five, were purchased from MegaZyme, Ireland. Three enzyme preparations containing mainly endo-xylanase activity were compared in the trial: one originating from Bacillus subtilis (xylanase-B), one from Trichoderma Longibrachiatum (xylanase-T) and one from Aspergillus oryzae (xylanase-A). All three preparations are registered for use in the EC..

The synthetic xylo-oligosaccharides with three, four and five xylose units were incubated in the presence of the different endo-xylanase preparations. A negative control group was subjected to the same incubation conditions, without endo-xylanase addition. The average DP of the hydrolysates was determined.

b) Results and discussion

Figure one illustrates the average DP values of the xylo-oligosaccharides of the 12 treatments: all possible combinations of three different xylo-oligosaccharide substrates (DP of three, four and five) and four enzyme treatments (three commercial endo-xylanase preparations and a negative control group). In every substrate, the DP of the negative control

group showed a slight deviation compared to the theoretical value. Therefore, the DP values of the three groups treated with enzymes are compared to the measured values of the corresponding negative control. The endo-xylanase of Bacillus subtilis did not affect the DP when the substrate contained four xylose units or less, so the bacterial endo-xylanase requires a minimal chain length of five xylose units in its substrate prior hydrolytic action to carry on.

The fungal xylanases (-T and -A) started hydrolyzing xylo-oligosaccharides of smaller size, theoretical DP values of respectively three and four were needed minimally before hydrolytic action was initiated. One can conclude that after complete hydrolysis of an arabinoxylan substrate, xylanase-B will result in fragments with an average DP of not less than three and without the concomitant generation of xylose monomers that exert a metabolic (osmotic) stress in monogastric animals. These results were in accordance with prior findings that the *in vitro* degradation of rye water-soluble arabinoxylans by xylanase-B, after 24 hours of incubation, generated hydrolysates of 550 to 1.730 dalton, corresponding to an average DP of respectively three to 12 (unpublished data, Puratos, Belgium).

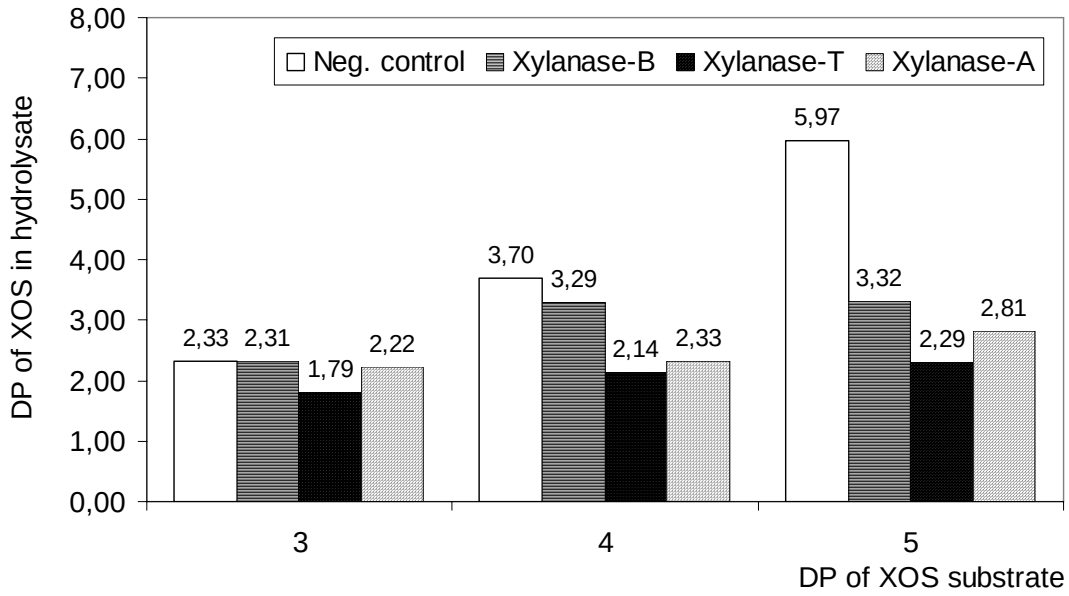

Figure 1. Measured average degree of polymerization of the fragments formed after enzymatic breakdown of xylotriose, xylotetraose and xylopentaose

Courtin and Delcour (2001) carried out similar research on xylanase-B and an endo-xylanase preparation originating from Aspergillus aculeatus. They incubated a standardised water-insoluble arabinoxylan substrate with each of the endo-xylanase preparations and screened the generated solubilised fragments (Table one). Courtin and Delcour determined the amount of reducing sugars, as a measure of the number of fragments solubilised, but did not observe large differences between the two preparations. On the other hand, the total amount of xylose units present in the solubilised fraction was much higher in the xylanase-B hydrolysate (35 times). This can only be explained by the fact that the average size (and thus the DP value) of the xylanase-B hydrolysate was much higher than in the other xylanase preparation. This suggests the following *in vivo* benefits from the use of xylanase-B: a fast decrease of intestinal viscosity, absence of generated xylose monomers and the conversion of water-insoluble arabinoxylans, naturally present in the feed, into substances with prebiotic potential.

Table 1. Enzyme activities that can be defined when incubating endo-xylanase with standardized water-insoluble arabinoxylans (based on Courtin and Delcour, 2001)

Enzyme activity	Endo-xylanase of Bacillus subtilis	Endo-xylanase of Aspergillus aculeatus
Reducing xylose (units/ml)	4195	3831
Total solubilised xylose (units/ml)	867781	24817

c) Conclusion

Most animal feeds contain relatively high amounts of arabinoxylans, present in the fibre fraction of cereals. Diets with corn or wheat inclusions of 50% or more, contain a water-insoluble arabinoxylan fraction of at least 30 g per kg feed. Through the use of a selective endo-xylanase, the *in situ* generation of xylo-oligosaccharides with a specific DP range is possible. This hydrolysis and its resulting hydrolysate product(s) will be characterised by a sequence of parameters such as reaction time, pH, availability and structure of the substrate. An *in vitro* experiment closely matching intestinal conditions, could provide more insight into the structure of the hydrolysates and their potential prebiotic effect.

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THE EFFECT OF DIETARY SHORT AND MEDIUM CHAIN FATTY ACIDS ON THE PERFORMANCE OF BROILER CHICKENS

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Summary

An experiment with 528 non infected and 624 malabsorption syndrome-infected Hybro G broiler chickens was carried out. Birds were housed at day-old in two separated rooms, each with 48 pens. Birds were infected from day 0 by two seeder birds in each pen, which were removed at 21 days of age. The experiment included 3 treatments: T1 was the control group in which feed without antibiotic growth promoter was provided. In T2, 0.75 g/kg of a mixture of sodium butyrate and medium chain fatty acids (MCFA) was added to the starter and grower diets; In T3 1.0 g/kg and 0.5 g/kg of this mixture were added to the starter and grower diets respectively. The results of the experiment showed that the mixture of sodium butyrate and MCFA improved performance in both non infected and infected birds and reduced mortality in infected chickens.

I. INTRODUCTION

The use of antibiotic growth promoters (AGP) has been banned in the European Union from January 2006 on. Because AGP still showed an improvement in the performance of broiler chickens, alternatives were sought for these growth promoters. The positive effect of AGP on bird performance is believed to be caused by a reduction in the incidence of sub clinical infections, a reduction of growth inhibiting metabolites that are produced by the intestinal micro flora, a reduced use of nutrients by the micro flora and an increased absorption and utilisation of nutrients due to a thinner intestinal wall (Gaskins *et al.*, 2002). Recently, it was suggested that the main effect of AGP is due to a reduction of inflammatory responses in the intestinal tract (Niewold, 2006).

Many potential alternatives for AGP have been described (Verstegen and Williams, 2002). Among these, short and medium chain fatty acids (MCFA) affect parameters that are also affected by the banned AGP (Canibe *et al*., 2001; Dierick *et al.*, 2002). Short and medium chain fatty acids have been reported to have antibacterial properties (Santomá *et al.*, 2006), with their antibacterial effect more pronounced in acid-intolerant bacteria such as *Salmonella* (Thompson and Hinton, 1997; Van Immerseel *et al.*, 2004) and *Campylobacter* (Chaveerach *et al.*, 2002). MCFA are digested and absorbed faster than long chain fatty acids and may be very useful when the digestion, absorption or transport of dietary fat is defective (Bach and Babayan, 1982).

An experiment was carried out to determine the effect of short and medium chain fatty acids (C6 to C12, mainly C8 and C10) on broiler chicken performance. Since Ter Huurne and Smits (1999) demonstrated that malabsorption syndrome (MAS) can be used as a model for intestinal disorders in broiler chickens, the experiment was carried out with both healthy and MAS infected birds.

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II. MATERIAL AND METHODS

The experiment involved in total 1152 Hybro G broiler chickens, of which 528 were placed in 48 floor pens in the control room (11 birds per pen) and 624 in 48 floor pens in an identical room that were infected with MAS (13 broilers per pen). Each pen had a floor area of 0.8 m^2 and wood shavings were used as bedding material. Feed was provided *ad libitum* by one feeder per pen and water by 2 nipple drinkers per pen.

Birds were infected with MAS by giving 2 "seeder" birds per pen 0.5 ml of a MAS homogenate at 0 days of age. This homogenate was obtained from the intestines of an earlier flock that was infected with MAS. Before infection, the homogenate was thawed and mixed 50/50 with a phosphate buffer solution. In order to facilitate transmission of infection, plastic foil was put on top of the wood shavings during the first week in order to promote pecking of the excreta. The 2 seeder birds were removed at 21 days of age after weighing. Weight was also determined at 0 and 42 days of age. Feed intake was recorded from 0 to 21 d and from 21 to 42 d and mortality was recorded daily along with the body weight of dead birds to correct FCR for mortality.

In each room, three treatments were applied with 16 replicates per treatment. The treatments included one control treatment without the addition of any antibiotic growth promoter (treatment 1). In treatment 2, 0.75 g/kg of a mixture of sodium butyrate and medium chain fatty acids (Trouw Nutrition, Putten, The Netherlands) was added to the starter and grower diets. In treatment 3, 1.0 g/kg of this mixture was added to the starter diet and 0.5 g/kg in the grower diet. The starter diet contained diclazuril as the coccidiostat and monensin was added to the grower diet.. The starter and grower diets were provided as 2 mm and 3 mm pellets respectively. The composition of the basal diets is given in table 1.

Birds were vaccinated at day-old against Marek's Disease and Infectious Bronchitis and at 20 days of age against Infectious Bursal Disease. A standard temperature schedule was applied, commencing at 30 °C at day-old and gradually decreasing to 20 °C at 28 days of age. Light was continuously provided during the first 3 days of age and thereafter, a light schedule of 20L:4D was applied.

Data was subjected to analysis of variance (general linear models procedure of SAS, 1997). The statistical model included treatment, room (infection) and treatment x room (infection) as factors. Significant differences between treatments were detected by a Least Significant Difference procedure (Snedecor and Cochran, 1967). Differences between treatments were considered significant at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

The results of the experiment are summarised in tables 2 and 3. The MAS infected chickens had a significantly lower live weight and feed intake compared to the non-infected ones. The changes in live weight and feed intake were in line with earlier experiments with MAS infected chickens (Den Hartog *et al.*, 2005). FCR and mortality were not significantly influenced by the MAS infection.

The inclusion of the mixture of sodium butyrate and medium chain fatty acids in the feed resulted in a non significant increase in live weight at 42 days of age, while feed intake was not affected. FCR improved with inclusion of sodium butyrate and medium chain fatty acids. As compared with the control group, this difference was significant when the starter and grower feed contained 1.0 and 0.5 g/kg respectively of the mixture. These results confirm that short and medium chain fatty acids improve performance of broiler chickens and can be considered as important alternatives to AGP (Canibe *et al.*, 2001).

Table 1. Ingredient and calculated nutrient composition (g/kg) of the basal experimental broiler starter and grower diets

Feedstuff	Starter diet	Grower diet
Corn	256.64	
Barley		80.00
Wheat	300.00	502.67
Soybean meal, 48 % cp	348.49	303.59
Sunflower oil	52.00	73.00
Sodium chloride	2.83	2.71
Sodium bicarbonate	1.34	1.52
Calcium carbonate	7.35	11.98
Dicalcium phosphate	21.09	13.84
L-lysine HCl	1.25	1.58
DL-methionine	2.64	2.50
L-threonine	0.37	0.61
Premix, vitamins and trace elements	5.00	5.00
Avizyme 1300	1.00	1.00
Nutrient		
$AME_{broilers}$, MJ/kg	11.72	12.14
Moisture	121.1	120.3
Ash	63.0	59.7
Crude protein	220.0	210.0
Crude fat	73.3	88.1
Crude fibre	23.3	23.6
Digestible lysine	11.0	10.5
Digestible methionine	5.5	5.1
Digestible methionine+cystine	8.5	8.1
Digestible threonine	7.1	6.8
digestible tryptophan	2.3	2.3
Calcium	9.0	9.0
Available phosphorus	4.5	3.5
Sodium	1.6	1.6
Potassium	9.9	9.3
Choride	2.3	2.3

No significant interactions were observed between infection and treatment for live weight, feed intake and FCR. Thus, a mixture of sodium butyrate and medium chain fatty acids improved performance of both healthy chickens and of chickens suffering from an intestinal disorder. Sodium butyrate is the principle energy source of the enterocytes (Isolauri *et al.*, 2003) and may reduce inflammatory responses as with AGP. This might explain why healthy birds also responded to the addition of the mixture. There was an indication ($P=0.074$) of an interaction between infection and treatment for mortality. This was caused by a considerable decrease in mortality in the MAS infected chickens when sodium butyrate and medium chain fatty acids were included in the feed but no effect of SB and MCFA on mortality in the non-infected chickens (Table 3). This reduction of mortality in the infected birds is most likely a response to the antibacterial properties of MCFA (Chaveerach *et al.*, 2002; Van Immerseel *et al.*, 2004) which probably alleviated the poor health status of the infected animals. The results show that sodium butyrate and medium chain fatty acids have an additional beneficial effect when intestinal health status is impaired.

Table 2. Effect of a mixture of sodium butyrate and medium chain fatty acids on performance and mortality of non-infected and MAS infected broiler chickens from 0 to 42 days of age

Main effect	Live weight, day 42	Feed intake, g, day 0-42	FCR day 0-42	Mortality, %, day 0-42
Non-infected birds	2038[x]	3468[x]	1.740	3.97
MAS infected birds	1937[y]	3291[y]	1.738	2.46
Control	1971	3379	1.754[a]	3.69
Fatty Acid 0.75/0.75 g/kg[1]	1987	3372	1.735[ab]	2.41
Fatty Acid 1.0/0.5 g/kg[2]	2005	3388	1.728[b]	3.54
P infection	<0.001	<0.001	0.805	0.259
P treatment	0.115	0.881	0.045	0.668
P interaction	0.862	0.791	0.823	0.074

[1] mixture of sodium butyrate and medium chain fatty acids, 0.75 g/kg in starter and 0.75 g/kg in grower feed
[2] mixture of sodium butyrate and medium chain fatty acids, 1.0 g/kg in starter and 0.5 g/kg in grower feed
[a-b, x-y] means within a column and main effect lacking a common superscript differ significantly (P<0.05).

Table 3. The interaction between infection and treatment on measurements of mortality (%) of broiler chickens from 0 to 42 days of age

Main effect	Non-infected	MAS infected
Control	3.41	3.97
Product, 0.75/0.75 g/kg[1]	4.24	0.57
Product, 1.0/0.5 g/kg[2]	4.24	2.84
Pooled SEM	1.31	

[1, 2] See table 2

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EFFECT OF MANNAN-OLIGOSACCHARIDES ON BROILER BREEDER PERFORMANCE

A. KOCHER[1]

Summary

There has been a number studies of the effects of mannan oligosaccharides (MOS) supplementation of poultry diets, particularly the use of MOS as a growth promoter in broiler production. More recently the use of MOS in broiler breeder diets has received considerable attention. The present paper examines the effects of MOS on egg production, hatchability and fertility as well as chick quality, offspring performance, immune status of progeny and immune response to vaccination.

The combined results from the studies suggest that improved gastrointestinal health of parent birds supplemented with MOS has a significant impact on their reproductive performance as well as a clear follow-on effect on offspring health and performance.

I. INTRODUCTION

Efficient and profitable broiler breeder production depends on laying performance of the breeder flock as well as the vitality of day-old broiler chicks. A number of factors such as the age of the breeder flock, feed and nutrient intake, nutrient absorption and overall health status can influence parent stock performance (Siegel *et al.*, 2006). Furthermore environmental stress, bacterial and fungal contamination and the transfer of maternal immunoglobulins (Ig) can affect embryonic development and vitality of the day-old chick (Stanley *et al.*, 2004). Hence a stable and healthy intestinal microflora to reduce the risk of bacterial imbalance and poor nutrient utilisation and the enhancement of the immune response are critical factors for optimal broiler breeder performance.

A number of reports have focussed on the use of vitamins and trace minerals in breeder diets (Lin and Chang, 2006; Rebel *et al.*, 2004; Siegel *et al.*, 2006). These additives have been shown to be beneficial as a means of enhancing immune function and the performance of breeder hens. However there is uncertainty about the optimal dose of these additives and the combinations that will be most beneficial to breeder performance and economic returns (Rebel *et al.*, 2004).

Continued research into the structure and function of carbohydrates has opened new opportunities for their use as functional feed ingredients in modern poultry diets. The surface of the gastrointestinal tract of chickens harbours a wide range of carbohydrate-based receptors, which interact with intestinal cells, the immune system and the microflora in the intestine. Carbohydrates, such as β-glucans from yeast cell walls have been shown to stimulate both specific (vaccine adjuvants) and non-specific immune responses (Williams *et al.*, 1989) and specific mannan oligosaccharides (MOS) derived from yeast cell wall can assist immunity by several mechanisms. Cotter *et al.*, (2002), speculated that MOS stimulate the production of mannose binding lectin (MBL), an acute phase protein that aids in phagocytosis. It is suggested that surface mannose present on the mannan- oligosaccharides have a positive effect on the production of MBL by stimulating Toll-like receptors (TLR) on the intestinal surface. Furthermore the inclusion of MOS in poultry diets has been shown to inhibit colonisation of pathogens in the gastrointestinal tract by blocking mannan sensitive

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attachment sides (type-1 fimbriae) (Spring *et al.*, 2000). Such activity is linked to better health, nutrient availability and digestibility. Several comprehensive reviews on the use of a specific commercially available MOS (Bio-Mos, Alltech Inc) on broiler or turkey diets have reported significant improvements in weight gain and feed conversion (Rosen, 2006a; 2006b). More recently there has been an increasing number of studies of the effects of the addition of MOS to broiler breeder diets on breeder hen performance, hatchability and subsequent chick quality, broiler performance, immune status of progeny and immune responses to vaccinations.

II. PATHOGEN CONTROL PROGRAMME

Salmonella infections in poultry flocks are an ongoing concern in human medicine because of the possibility of transmission between species. Improvement in hygiene management, the introduction of effective salmonella vaccination and heat treatment of feed have reduced the threat of salmonella infections. However an effective salmonella control programme in poultry must start with the breeder flock. Hooge, (2003), reported that MOS can be an effective tool in controlling Salmonella in broiler breeder operations. It is known that mannose or mannose derivatives will reduce the risk of salmonella infection in chicks under controlled experimental conditions (Spring *et al.*, 2000). Adding MOS to the diet blocks type-1 fimbriae, which are specific to mannose and are used by a large number of Salmonella *spp.* to colonise the intestine.

III. HEN PERFORMANCE, HATCHABILITY AND ECONOMIC BENEFITS

Data from large scale evaluations conducted in Ireland indicate that the addition of MOS to broiler breeder diets between 25 and 39 weeks of age resulted in increased production and hatchability compared to the breed standards (Considine, 2000). Furthermore it was noted that the variability in flocks with MOS was almost halved. The larger number of chicks produced over the trial period resulted in a significant increase in profitability.

Feeding trials from India demonstrated that the addition of MOS to broiler breeders aged 60-67 weeks improved semen traits, hatchability and antibody responses in both the parents and progeny (Shashidhara and Devegowda, 2003). These researchers confirmed that adding MOS (1kg/t) to breeder diets significantly increased hatchability (+1.6%, $P<0.05$) and resulted in a corresponding reduction ($P<0.05$) in dead-in-shell and infertile eggs. Fertility and hatchability are also influenced by sperm quality and the same study revealed a highly significant increase in spermatozoa density (+384 million/ml, $P<0.005$). Furthermore it is known that increased levels of *E. coli* can lead to head-to-head sperm agglutination and subsequently reduced fertility (Monga and Roberts, 1994). This reaction can be partly inhibited by blocking the specific receptor for type-1 fimbriae. Reducing the number of *E.coli* which expresses mannose sensitive fimbriae will lead to reduced interference with sperm fertilisation. MOS has been shown to reduce the prevalence and concentration of those type of *E.coli* (Spring *et al.*, 2000) It is therefore postulated that the improvement in fertility resulting from inclusion of MOS in the diet, is related to improvements in nutrient utilisation and spermatozoa density and a reduction in the levels of *E. coli* and other pathogenic bacteria. A recent study in Holland looked at use of MOS under commercial conditions. Despite the limited number of replicates ,substantial numerical improvements were observed The inclusion of 1kg/t of MOS to Ross 308 broiler parent stock from 81-90 weeks of lay following moulting resulted in a 2% increase in laying performance, fewer infertile eggs and lower levels of embryonic mortality and non-viable chicks leading to an overall increase in the number of viable chicks of 3.7% (de Lange *et al.*, 2007).

The results of a number of studies of the effects of MOS on broiler breeder performance are summarised in Table 1. The data generally showed improved egg production and hatchability. The economic benefits shown were manifested through the production of more saleable chicks per hen housed. de Lange *et al*. (2007), reported increased profit of AU$ 4.50 per hen housed. These data indicate that the inclusion of MOS in broiler breeder diets has potentially beneficial effects on productivity and economic returns.

Table 1. Effect of MOS on laying performance and hatchability

Performance Parameter	Control	Bio-Mos	Difference
(Cragoe and Olsen, 1994)			
Egg production	83.75	85.59	+1.84%
(Considine, 2000)			
Egg production %	72.4	78.6	+6.2%
Hatchability %	85.3	86.2	+0.9%
(Shashidhara and Devegowda, 2003)			
Egg production %	53.15	53.45	+0.3%
Hatchability %	83.51	85.17	+1.66%
(de Lange *et al.*, 2007)			
Egg production %	60.2	62.3	+2.1%
Viable chicks %	87.0	90.7	+3.7%

IV. EGG QUALITY, IMMUNITY AND OFFSPRING PERFORMANCE

In poultry maternal antibodies are transferred from the hen to the chick via the egg. These maternal antibodies are deposited in the egg yolk (IgY) or are present in the egg white (IgA and IgM) (Larsson *et al.*, 1993). Developing chicks absorb the IgY from the yolk as embryos in the shell until two days after hatch (Yoshimura, 2004). Hamal *et al.*, (2006) found that the levels of IgY and antibodies in yolk and subsequently in the circulation of the chick are directly related to the levels in the hen.

Specific functional carbohydrates are important modulators of the immune response (Kelly, 2004). Research with dairy cows, pigs or breeders have shown that MOS have a marked influence on the transfer of Ig or antibody titres from the mother to their offspring, resulting in improved liveability of the offspring (Franklin *et al.*, 2005; Shashidhara and Devegowda, 2003). When feeding MOS to breeders significant improvement in antibody titres to IBD vaccine were found in dams (+15%, $P<0.05$) as well as progeny chicks hatched 5 weeks post-vaccination (Kőrösi-Molnár, 2002; Shashidhara *et al.*, 2003). Titre levels measured by ELISA testing were 27% and 47% higher in chicks from breeders fed MOS.

De Lange *et al.* (2007) speculated that the level of IgY and antibodies deposited in the yolk could be measured indirectly by determining the ratio of egg yolk to egg white. A higher ratio – proportionally higher level of egg yolk compared to egg white – could therefore be used as a useful indicator of transfer of immune status of the newly hatched chick. In his work he found that the egg yolk from hens fed MOS add a ratio of 32% egg yolk to 57.5% egg white compared to eggs from the control group which had a ratio of 30.9% egg yolk to 59.2% egg white. Improved transmission of maternal immunity will protect chicks before their own immune system is developed. Preliminary data on the effects of MOS supplementation of breeder diets on broiler performance and health, indicate an improvement in growth performance and a improvement in overall feed efficiency (de Lange *et al.*, 2007; Peric, 2006). Studies from Serbia and Holland showed improvements in weight gain of +20g

and +60g respectively and an improvement in feed efficiency of +2 points (1.54 control vs. 1.52 MOS).

Further research on the levels of Ig in the plasma of hens and subsequently in the egg yolk (IgY) and egg white (IgA and IgM) will be necessary to establish a clear relationship between the use of MOS in breeder diets and the effects on Ig levels in hens and improvements in immune response and growth performance in chicks.

V. CONCLUSIONS

A review of the available reports has shown that supplementation of broiler breeder diets with MOS could play an important role in maximising broiler breeder health and performance. These findings may be related to lower levels of pathogens, such as Salmonella *ssp.* and *E.coli*, as well as a better developed immune system, and better immune status in both parents and progeny. The benefits are manifested through the production of a greater number of chicks per hen housed as well as improved growth performance of the progeny.

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MANNANOLIGOSACCHARIDES MODULATE THE POPULATIONS OF MUCOSA-ASSOCIATED BACTERIA IN BROILER CHICKENS

Y. YANG[1], P. A. IJI[1], A. KOCHER[2] and M. CHOCT[3]

Previous research showed that mannanoligosaccharide (MOS) supplementation of broiler diets tended to reduce the number of coliform bacteria in the intestine of broiler chickens (Yang *et al*., 2006). The effects of MOS on the populations of mucosa-associated bacteria were further examined using an *E. coli* challenge model.

One hundred and eight (108) day-old chickens were randomly divided into three treatments each consisting of 6 replicate cages of 6 chickens. The treatments were: a negative control; a diet with 2 g MOS/kg (Bio-MOS, Alltech Inc.); and a positive control (50ppm Zn-bacitracin). The birds were offered an experimental sorghum-wheat-based diet. A mixture of four specific strains (E3, E30, E956 and E133) of pathogenic *E. coli* (kindly provided by Prof. G. F. Browning, University of Melbourne, Parkville Victoria) was inoculated into brain heart infusion broth and grown overnight. Each bird was orally gavaged with 1 ml of the culture (10^7 CFU/ml) on d1, 3, 7, and 14 and 2 ml on d21. The challenge was also boosted via water on d2, 5, 12, and 20 at a concentration of around 10^5 CFU/ml. At 1 and 3 weeks of age, one bird per cage was killed, and the mucosal populations of bacteria were counted. Besides coliform, *lactobacillus* spp. were examined as they are considered as beneficial bacteria and can compete with pathogenic bacteria, such as *E. coli*, for the adhesion receptors in the gut wall. *Lactobacillus* spp. were counted on Rogosa agar incubated in an anaerobic condition at 37 °C for 48 h and coliform were counted on MacKonkey agar aerobically incubated at 37 °C for 24 h. The results are shown in Table 1.

There was a significant reduction in the number of tissue-associated coliform bacteria on MOS treatment compared to the negative control in week 1 but there was no difference at week 3. The populations of tissue-associated lactobacilli were unaffected by diet at either age. When pathogens attach to the mucosa, the gut integrity and function will be severely affected (Droleskey *et al*. 1994). Therefore, the inhibitory effect of MOS on the growth of tissue-associated coliform bacteria, indicates that MOS may maintain gut integrity and function when the microflora of young birds is in transition, but not subsequently.

Table 1. The counts (log CFU/g wet tissue) of tissue associated bacteria in the jejunum of birds on the different diets under an *E. coli* challenge [1].

	Control	2g MOS/kg	Zinc-bacitracin	SEM	P value
Week 1					
Lactobacillus	6.55	6.38	6.55	0.22	0.39
Coliform	4.35[a]	3.93[b]	4.06[ab]	0.23	0.05
Week 3					
Lactobacillus	6.64	6.59	6.75	0.32	0.96
Coliform	4.43	4.37	4.85	0.26	0.53

[1]A mixture of *E. coli* (E3, E30, E956 and E133) was used.
[a,b] Means within a row sharing no common superscripts differ significantly (P<0.05).

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EFFECT OF PHYTATE AND PHYTASE ON THE FLOW OF ENDOGENOUS AMINO ACIDS AT THE TERMINAL ILEUM OF GROWING BROILER CHICKENS

A.J. COWIESON[1] and V. RAVINDRAN[2]

Summary

The effect of phytate and phytase on the recovery and composition of endogenous protein at the terminal ileum of broiler chickens was investigated using the peptide alimentation method. Phytate (fed as the sodium salt) was included in a semi-synthetic diet at 8.5, 11.5 and 14.5 g/kg phytic acid (or 2.4, 3.2 and 4.0g/kg phytate-P) and each diet was fed with or without an *Eschericia coli*-derived phytase at 500 U/kg. A control containing no phytate was fed as a comparison to estimate basal endogenous flows. Ingestion of phytate increased ($P<0.05$) the flow of endogenous amino acids and nitrogen by an average of around 47% at the lowest phytate concentration and 87% at the highest. The addition of phytase reduced ($P<0.05$) the inimical effects of phytate on endogenous amino acid flow at all dietary phytate levels. The effects of phytate and phytase, however, varied depending on the amino acid. It can be concluded that the effects of phytase on amino acid digestibility may be mediated both through a route of reduced endogenous loss and improved retention of dietary amino acids.

I. INTRODUCTION

The effect of microbial phytase on the retention of P and to a lesser extent, Ca, is well accepted by the poultry industry (Lei and Porres, 2003; Cowieson *et al*., 2006). However, the effect of phytase on the utilisation of dietary energy and amino acids is less well documented and has not received as wide an acceptance. One reason for this less rapid adoption of energy and amino acid matrix values for phytase is that the data are equivocal (Adeola and Sands, 2003; Selle and Ravindran, 2006). There are a number of reasons for the ambiguity in the data, including variation in the concentration and location of dietary phytate, variation in concentration and 'stringency' of endogenous phytases, nutrient balance of the control diets, differences in animal husbandry, environment and diet manufacture, and the age and species of the animals to which the diet is offered. Although many of these factors serve well to 'muddy the water' as to the true end-user value of phytase, it is fair to say that an increased understanding of the mode of action of phytase in relation to amino acids and energy would be useful in order to increase both the scale and consistency of the response. It has been demonstrated previously that phytate and phytase alter the secretion of endogenous compounds by broilers (Cowieson *et al*., 2004), partially explaining the beneficial effects of phytase noted *in vivo*. It was the purpose of the experiment reported herein to further explore the effects of phytate and phytase on endogenous amino acid flow in the ileum of broilers in order to elucidate the mode of action of phytase on amino acid utilisation by broilers.

II. MATERIALS AND METHODS

Phytate (as the sodium salt) was included in a semi-synthetic diet at 8.5, 11.5 and 14.5 g/kg phytic acid (or supplying 2.4, 3.2 and 4.0g/kg phytate-P) and each diet was fed with or without an *Eschericia coli*-derived phytase (Phyzyme XP; Danisco Animal Nutrition, UK) at

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500 U/kg. A control containing no phytate was also fed as a comparison to estimate basal endogenous flows. A total of 180 3-week old male broiler chicks (Ross) were used in this 7 treatment and 6 replicate study based on the protein alimentation method as described by Moughan *et al*. (1990; 1992), and Ravindran *et al.* (2004). Briefly, this technique uses a semi-synthetic diet based on dextrose. The protein in the diet comes only from enzymatically-hydrolysed casein (EHC) which consists of peptides with a molecular weight less than 5000 Da. The diet contained a total of 200g/kg EHC. This allows endogenous and exogenous proteins to be separated at the terminal ileum by molecular mass resolution. The flow of nitrogen and individual amino acids at the terminal ileum is calculated as milligrams lost per kilogram of ingested dry matter. Two-way ANOVA was employed to determine the main effects (phytic acid and phytase) and their interaction by using the GLM procedure of SAS (1997) using pen as the experimental unit. The data from 200 g/kg EHC diet without added phytic acid was compared with that with 2.4 g/kg phytate-P using a completely randomised design ANOVA. Differences were considered significant at $P<0.05$, although P values up to $P\leq0.10$ are shown in the text if the data suggest a trend.

III. RESULTS

The influence of phytic acid and phytase in the ileal endogenous flows of nitrogen and amino acids is shown in Table 1. The endogenous flow of nitrogen and most of the amino acids were greater ($P<0.05$ to 0.001) in birds fed the EHC diet with phytic acid compared to those fed that without added phytic acid. The flow of histidine, cystine and methionine tended ($P< 0.10$) to be greater in birds fed the EHC diet with phytic acid. No differences were observed for the flow of alanine and phenylalanine. The ileal flow of nitrogen and total amino acids in birds fed the 200 g/kg EHC diet with phytic acid were 1.4-fold greater than those determined for that with no added phytic acid. The increments in the flow of individual amino acids in birds fed the 200 g/kg diet with phytic acid ranged from 11% (methionine) to 97% (threonine).

The effects of dietary phytate concentration on ileal endogenous flow were significant ($P\leq0.05$ to 0.001) for nitrogen and some amino acids. The flow of proline, alanine, valine, tyrosine, phenylalanine, histidine, lysine and arginine were not influenced ($P>0.05$) by phytate concentration. Phytase supplementation lowered ($P<0.08$ to 0.001) the ileal endogenous flow of most amino acids. The exceptions were the flow of alanine, tyrosine and phenylalanine, which were unaffected by added phytase. Phytase responses in the flow of aspartic acid, phenylalanine, histidine,arginine, cystine and methionine, however, were influenced by dietary phytate concentration, as indicated by phytate x phytase interactions ($P<0.06$ to 0.02).

The amino acid profile of endogenous protein (data not shown), expressed as g/100g crude protein, indicates that the concentrations of aspartic acid, threonine, serine and tyrosine were increased ($P<0.05$ to 0.001) and those of glutamic acid, alanine and phenylalanine were lowered ($P<0.05$ to 0.09) when 8.5 g/kg phytic acid was added to the EHC diet. The concentrations of other amino acids in endogenous protein were unaffected ($P>0.05$).

Table 1. Ileal endogenous flows (mg/kg DM intake) of nitrogen and amino acids in 4-week-old broiler chickens as influenced by dietary levels of phytate and microbial phytase[1]

Phytic acid, g/kg	Phytase	N	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg	Cys	Met	Sum of AA
0.0[2]	-	2051	1081	887	944	2798	791	925	425	613	535	631	216	287	230	501	255	246	153	11520
8.5	-	2898	1746	1744	1583	3570	1242	1328	411	885	864	915	370	311	296	747	412	351	183	16983
	+	2284	951	1045	1024	3273	1147	832	449	836	775	770	327	342	323	424	488	292	215	13511
11.5	-	3255	1957	2024	1742	3888	1376	1995	406	852	1056	1165	348	385	317	706	552	396	218	19381
	+	2688	1469	1313	1382	3508	1080	1497	461	748	861	875	472	407	224	584	396	260	169	15695
14.5	-	3643	2223	2085	2386	4176	1603	2089	548	1040	1087	1092	341	416	376	772	584	521	274	21616
	+	2962	1878	1421	1758	4015	1109	1571	459	833	852	870	306	299	231	667	380	310	211	17170
Pooled SEM		124.5	91.3	103.8	100.8	178.2	90.1	101.7	37.9	64.8	64.1	55.9	62.4	30.5	27.7	61.8	49.9	30.4	17.7	6716
Main effects[3]																				
Phytic acid																				
8.5 g/kg		2591	1348	1395	1304	3422	1195	1080	430	860	819	842	362	326	309	586	450	321	199	15247
11.5 g/kg		2971	1708	1668	1562	3698	1228	1746	433	800	959	1020	410	396	270	345	473	328	194	17538
14.5 g/kg		3302	2050	1753	2072	4096	1356	1830	504	937	970	981	323	358	303	720	483	415	242	19393
Phytase																				
0		3265	1976	1951	1904	3878	1407	1804	455	926	1002	1057	362	371	330	742	516	422	225	19327
500 U/kg		2645	1429	1260	1388	3599	1112	1300	456	806	829	838	368	350	259	559	422	287	198	15459
Probability, P ≤[3]																				
Phytic acid (PA)		0.001	0.001	0.01	0.001	0.01	NS	0.001	NS	NS	0.05	0.01	NS	0.09	NS	NS	NS	0.01	0.02	0.001
Phytase		0.001	0.001	0.001	0.001	0.07	0.001	0.001	NS	0.03	0.01	0.001	NS	NS	0.01	0.001	0.03	0.001	0.08	0.001
PA x phytase		NS	0.06	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.04	0.02	NS	0.02	0.06	0.03	NS

[1] Mean values for six replicates of five birds each with their pooled standard errors.
[2] Differences in the flow of nitrogen and most amino acids between birds fed diets with 0 and 8.5 g/kg phytic acid were significant (P<0.10 to 0.001), except for the flow of alanine and phenylalanine.
[3] Data involving 8.5, 11.5 and 14.5 g/kg phytic acid, without or with microbial phytase, were analysed as a 3 x 2 factorial arrangement of treatments.

Phytic acid influenced (P<0.05 to 0.001) concentrations of aspartic acid, serine, proline, glycine, valine, leucine and histidine in endogenous protein (data not shown). The concentrations of all amino acids, except proline, isoleucine, tyrosine, histidine, lysine, arginine and methionine, in the endogenous protein were affected (P<0.06 to 0.001) by phytase supplementation. Significant phytic acid x phytase interactions (P<0.07 to 0.01) were noted for aspartic acid, proline, alanine, phenylalanine, histidine, lysine, arginine, cystine and methionine, suggesting that the changes in the concentrations of these amino acids in endogenous protein to added phytase varied at different phytic acid levels.

IV. DISCUSSION

The data presented herein confirm previous reports that phytate is an antinutrient capable of stimulating an increase in the flow of endogenous material in the small intestine of broiler chickens and that the capacity of phytate as an anti-nutrient is reduced in the presence of phytase (Cowieson *et al*., 2004). It is interesting that the effect of phytate on endogenous amino acid flow was dependent on the amino acid, with arginine, tyrosine, histidine, alanine and valine being less obviously affected by the ingestion of phytic acid than threonine, serine, glycine or nitrogen. Indeed, in contrast to all other amino acids, the ingestion of phytate did not promote (P>0.05) an increase in the flow of alanine and phenylalanine. Furthermore, the effects of phytase were also amino acid-dependent with relatively poor effects on phenylalanine, tyrosine and alanine. The effects of phytate on endogenous amino acid flows is in agreement with earlier work and also is logical based on the amino acid composition of endogenous secretions such as mucins and enzymes (Selle and Ravindran, 2006). Although the mechanisms remain to be elucidated, these data suggest that the effects of phytases on the retention of alanine, tyrosine and phenylalanine in more traditional metabolism studies may be mediated more by improving retention of dietary sources rather than amelioration of endogenous 'cost'.

It can be concluded that the beneficial effects of phytase on amino acid retention in broilers can be partially attributed to a reduction in the effect of phytate on endogenous amino acid losses. Further, the effects will not end at an amino acid 'cost' but will have substantial effects on the net energy value of a diet. These results may partially explain why phytases have been found to improve energetic efficiency in growing broilers.

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EFFECTS OF PRE-PELLETED WHEAT AND PHYTASE SUPPLEMENTATION ON BROILER GROWTH PERFORMANCE AND NUTRIENT UTILISATION

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Summary

As empirical evidence suggested that prior steam-pelleting of wheat may adversely affect phytase efficacy in wheat-based broiler diets, this hypothesis was investigated. Exogenous phytase increased nitrogen (N) retention of broiler diets based on raw wheat (55.0 to 57.4%) but depressed N retention in diets containing pre-pelleted wheat (58.7 to 57.9%) and the treatment interaction was significant (P<0.01). Trends towards similar treatment interactions were observed for AME and weight gain. Pre-pelleting wheat eliminates intrinsic phytase activity but may also reduce the solubility of phytate and protein, which renders phytate less susceptible to phytase hydrolysis and may reduce the extent of *de novo* protein-phytate complex formation. It is also possible that pre-pelleting solubilises non-starch polysaccharides in wheat and increases gut viscosity, which could impede substrate access and absorption of nutrients liberated by phytase. While the hypothesis was not conclusively established, there is the indication that prior steam-pelleting of wheat for inclusion in broiler diets negatively influences phytase efficacy.

I. INTRODUCTION

Because plant phytase activity could compromise responses to phytase feed enzymes, wheat may be separately steam-pelleted (pre-pelleted) to eliminate intrinsic phytase activity in experiments involving exogenous phytase. However, there is empirical evidence that suggests pre-pelleted wheat may negatively influence responses to phytase supplementation. In apparent metabolisable energy (AME) assays in broilers completed in this laboratory there is a significant, negative correlation between dietary proportion of pre-pelleted wheat and AME following phytase supplementation. Thus, the objective of the present study was to determine the validity of this empirical evidence.

II. MATERIALS AND METHODS

Phosphorus adequate diets were formulated from characterised ingredients using wheat (112.0 g/kg protein, 2.20 g/kg phytate-P) that was either 'raw' or steam-pelleted at approximately 90°C (Table 1). Both wheats were ripple-milled and the diets were fed in mash form. A 2 x 2 factorial array of dietary treatments, based on either raw or pre-pelleted wheat, without and with 750 FTU/kg (Natuphos®) phytase was offered to 168 male Cobb chicks from 1-28 days post-hatch. Each treatment consisted of seven replicates of six chicks with unlimited access to feed and water. Growth performance from 1-28 days post-hatch was monitored and total excreta output was collected from 18-22 days post-hatch for AME and nitrogen (N) retention determinations by standard procedures described by Selle *et al.* (2003). Experimental data were subjected to analyses of variance using a general linear models procedure (SPSS® Inc. Chicago, IL).

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Table 1. Composition and nutrient specifications of experimental diets

Composition	g/kg	Specification	g/kg
Wheat (raw or steam-pelleted)	670.02	Protein	209.16
Soyabean meal	115.31	Fat	44.29
Canola meal	90.00	Fibre	35.01
Rice bran	19.60	Calcium	9.45
Meat-and-bone meal	71.18	Phosphorus	6.78
Vegetable oil	13.06	Nonphytate phosphorus	4.03
Limestone	8.90	Phytate-phosphorus	2.75
Salt	1.40	Metabolisable energy (MJ/kg)	12.05
Sodium bicarbonate	2.65	Lysine	11.50
Lysine monohydrochloride	2.95	Methionine	5.20
D,L-Methionine	2.06	Methionine + cystine	9.10
Threonine	0.87	Threonine	7.90
Vitamin, mineral premix	2.00	Tryptophan	2.34
Natuphos® Granulate (plus/minus)	0.15	Arginine	11.99

III. RESULTS

The average weight of the day-old chicks was less than 40g, which may have contributed to the high mean mortality rate of 8.3% from 1-28 days post-hatch. Whilst mortality rates were independent of treatment ($P>0.95$), they did introduce a source of variation.

Growth performance and nutrient utilisation results are shown in Table 2. Pre-pelleted wheat enhanced N retention (58.3 versus 56.2%; $P<0.01$) but feed efficiency was inferior (1.64 versus 1.57; $P<0.01$) and pre-pelleted wheat tended ($P<0.10$) to increase feed intake but reduce AME. As a main effect, phytase supplementation did not significantly influence growth performance or nutrient utilisation. There was, however, a significant treatment interaction ($P<0.01$) for N retention, as phytase increased N retention by 4.4% in raw wheat diets but depressed N retention by 1.4% in diets based on pre-pelleted wheat. Similar trends towards treatment interactions were evident for weight gain ($P<0.20$) and AME ($P<0.15$). The data indicate phytase increased AME of the raw wheat diets by 0.19 MJ, but decreased AME of the pre-pelleted wheat diets by 0.11 MJ. Numerically, phytase addition to raw wheat diets increased weight gain of broilers by 12.5%; in contrast, the response to phytase was only 1.0% in diets based on pre-pelleted wheat. Pre-pelleting essentially eliminated (<90%) the intrinsic phytase activity of wheat.

IV. DISCUSSION

The results of the present study support the hypothesis that pre-pelleting the wheat component of broiler diets negatively influences responses to exogenous phytase. The significant treatment interaction for N retention and trends towards interactions observed for AME and weight gain from 1-28 days post-hatch are consistent with the contention that heat-treatment of wheat reduces the efficacy of exogenous phytase.

Table 2. Effect of wheat processing and phytase inclusion on growth performance from 1-28 days post-hatch and nutrient utilisation of broiler chicks.

Treatment		Growth performance			Nutrient utilisation	
Wheat processing	Phytase (FTU/kg)	Gain (g/bird)	Intake (g/bird)	FCR (g/g)	AME (MJ/kg DM)	N retention (%)
Raw	0	1034	1631	1.58	13.70	55.0
Raw	750	1163	1803	1.55	13.89	57.4
Pelleted	0	1118	1833	1.64	13.69	58.7
Pelleted	750	1129	1853	1.64	13.58	57.9
SEM		44	66	0.02	0.095	0.6
Main effects						
Wheat raw		1099	1717	1.57	13.80	56.2
Wheat pelleted		1123	1843	1.64	13.63	58.3
Phytase 0		1076	1732	1.61	13.70	56.8
Phytase 750 FTU/kg		1146	1828	1.60	13.74	57.7
Significance (P =)						
Wheat processing		0.582	0.066	0.001	0.099	0.001
Phytase		0.126	0.156	0.521	0.672	0.133
Treatment interaction		0.198	0.256	0.486	0.125	0.009

The majority of phytate (96%) in raw wheat is present as *myo*-inositol hexaphosphate (IP_6) and pelleting or extrusion has little effect on the composition of phytate (Pontoppidan *et al.,* 2006). However, heat-treatment of Durum wheat has been shown to increase insoluble protein fractions from 27.7% following extrusion to 74.0% at 50°C, and to 83.2% at 96°C (Ummadi *et al.,* 1995a,b). As demonstrated by electrophoresis, extrusion aggregated albumins, globulins and glutenins via the formation of disulphide linkages. Moreover, the proportion of phytate in the insoluble protein fraction was increased with phytate being bound to the albumin and globulin fractions. These findings indicate that heat-treatment has the potential to reduce the solubility of the phytate and protein components of wheat. It is likely that any reduction in phytate solubility would reduce its susceptibility to hydrolysis by phytase. However, it also follows that decreased phytate and protein solubility may reduce the extent of *de novo* binary protein-phytate complex formation. This complex formation in the fore-gut of broilers is probably an important mechanism whereby phytate negatively influences protein/amino acid digestibility (Selle *et al*., 2000). Thus it is possible that prior steam-pelleting of wheat may reduce phytate hydrolysis by phytase and ameliorate the anti-nutritive properties of phytate in respect of protein digestibility and that this may have contributed to the significant interaction observed for N retention in this study.

It is generally considered that pelleting complete broiler diets is beneficial; however, in this study pre-pelleting the wheat component of the diet significantly reduced feed efficiency and tended to reduce AME. However, increasing pelleting temperatures of wheat-based broiler diets from 70 to 95°C have been shown to increase intestinal viscosity (11.14 versus 4.12 cps), which was attributed to increased non-starch polysaccharide (NSP) solubility (Silversides and Bedford, 1999). In this study, broilers offered xylanase-supplemented diets performed best at pelleting temperatures of 80-85°C. Cowieson *et al.* (2005) concluded that pelleting wheat-based diets in excess of 80°C can compromise bird performance and, in this context, recommended the inclusion of NSP-degrading enzymes.

Therefore, in the present study, it seems possible that increased gut viscosity generated by increased solubility of NSP in pre-pelleted wheat diets may have reduced phytase efficacy by impeding substrate access and/or absorption of nutrients liberated by phytase. Indirectly, it is relevant that the simultaneous inclusion of phytase and xylanase in wheat-based broiler diets has been shown to be advantageous (Ravindran *et al.,* 1999; Zyla *et al*., 1999; Selle *et al.,* 2003), and this approach is commonly adopted in practice.

The hypothesis may not have been conclusively established but the results do suggest that, from an experimental standpoint, the practice of pre-pelleting wheat may be a confounding factor in phytase feeding studies. This study, together with the results of Silversides and Bedford (1999) and Cowieson *et al.* (2005), suggests that the conditioning/pelleting temperatures to which wheat-based diets are exposed may impact on both phytate and NSP and the efficacy of relevant feed enzymes, in addition to influencing the overall nutritive properties of the diet. Conventional steam-pelleting of complete, wheat-based diets may have less impact than pre-pelleting wheat *per se*. This should be investigated as this study suggests that pelleting wheat-based broiler diets at high temperatures may negatively influence phytase efficacy, which could be addressed in practice. A second issue is whether or not phytase efficacy in diets based on 'non-viscous' grains, such as maize and sorghum, would be similarly effected by high pelleting temperatures.

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PHOSPHORUS SUPPLY FROM LAYER DIETS

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Supply of phosphorus (P) in poultry diets is an important aspect of diet formulation. It is well documented that most P in plant ingredients is unavailable to the birds as it is bound in phytic acid and supplementing phytase in diets can release phytate P (Selle *et al*., 2000). The objective of the study was to examine the influence of supplementing phytase to diets with graded levels of available P on production performance of laying hens from 23-47 weeks of age.

The experimental diets were plant based and formulated to contain available P of 4.0, 2.9 and 1.8 g/kg diet with or without supplementary phytase (450 FTU/kg diet). Phytate-P content was similar across all diets. All other nutrients met the nutrient requirements recommended by NRC (1994). The experimental diets were fed to 9 replicates (3 layers each) of ISA Brown laying hens. In addition to production and egg quality parameters, toe ash, the ileal digestibility coefficients of energy (DEC) and amino acids (AADC) were determined. The results are shown in the Table.

Main effect	Egg production (%)	FCR (g feed/g egg)	Shell thickness (mm)	Toe ash (%DM)	AADC	DEC
Avail. P (g/kg)						
4.0	95.8	1.84	0.31	14.07	0.75	0.73
2.9	95.8	1.81	0.31	14.27	0.74	0.72
1.8	94.5	1.83	0.32	14.38	0.76	0.73
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Phytase						
+Phytase	96.2	1.83	0.32	13.98	0.76	0.73
--Phytase	94.8	1.83	0.31	14.50	0.73	0.71
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

There were no significant differences in egg production, FCR, egg shell thickness, toe ash or apparent ileal digestibility of energy and amino acids throughout the experimental period irrespective of dietary treatments (P>0.05). Interestingly, the dietary available P concentrations even at the lowest level (1.8 g/kg diet) evidently met the hens' requirement for P, as indicated by the high level of production recorded and the lack of effect on toe ash. The daily intake of available P (174 mg) on this diet indicates that the available P requirement of laying hens for egg production is lower than the NRC (1994) recommendation of 250 mg/day or the dietary levels of 4.0-4.5 g/kg typically used by industry.

It can be concluded that current dietary usage of P is in excess of layer-type hens' requirement. Refinement of the available P requirement of laying hens is likely to reduce the cost of laying hen diets and environmental pollution through excess P excretion.

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THE RESPONSE OF BROILERS TO DIETARY DIGESTIBLE LYSINE LEVELS IN THE GROWER PHASE

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Optimum broiler performance and profitability depend largely on adequate and consistent dietary amino acids supply. An accurate estimation of dietary digestible lysine requirement is critical in any attempt to apply the ideal amino acid concept in diet formulation. Our early studies demonstrated that the optimal performance of broilers during the starter phase is achievable with dietary digestible lysine content of 12g/kg (Zhang *et a*l., 2006). The aim of the current study was to determine the optimal dietary digestible lysine content during the grower period.

Male broiler chickens (Ross, 22 days old), which had been fed a commercial diet from day-old, were used in this study. Experimental diets contained sorghum, soybean meal, corn starch, feather meal and synthetic amino acids. All dietary digestible amino acids concentrations were based on the ideal amino acid concept (Baker, 1997) except lysine, which was formulated at 6.0, 7.5, 9.0, 10.5 and 12.0 g/kg diet, respectively. All chickens were individually weighed, wing tagged and allocated to five dietary treatments with five replicates of seven birds for each diet. The birds were kept in floor pens and given free access to feed and water. Individual body weight and pen feed intake were recorded weekly and feed efficiency was calculated. On day 42, two birds with body weight close to the pen average were selected for breast muscle and abdominal fat measurements.

Bird body weights were significantly increased from 1784g to 2246g ($P<0.01$) at day 42 when dietary digestible lysine content was increased from 6.0g to 7.5g/kg. Birds fed 6.0 g/kg digestible lysine had significantly lower ($P<0.01$) average daily weight gain than birds on the other four dietary treatments. Feed intake from day 22 to 42 was also significantly increased ($P<0.01$) as the digestible lysine content increased from 6.0g to 7.5g/kg. FCR was substantially reduced from 2.84 to 2.30 ($P<0.01$) when digestible lysine content increased from 6.0 to 7.5g/kg. Although beneficial effects were not significant on production performance with further increase in digestible lysine content to 12g/kg, quadratic responses of average daily weight gain ($R^2 = 0.923$) and FCR ($R^2 = 0.871$) were observed when dietary digestible lysine level increased from 6.0 to 10.5 g/kg. An optimum digestible lysine level of 8.5 g/kg was observed for weigh gain and feed efficiency for broilers from 22-42 days of age. No dietary treatment effects were observed for percentage breast muscle or abdominal fat. The results from this study suggest that a dietary level of 8.5 g digestible lysine /kg is required for optimal performance of broiler chickens during the grower phase.

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APPARENT METABOLIZABLE ENERGY AND ILEAL AMINO ACID DIGESTIBILITY OF FABABEANS, LUPINS AND PEAS FOR BROILER CHICKENS

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Summary

The apparent metabolizable energy (AME) and apparent ileal digestibility (AID) of amino acids in four cultivars of fababeans, three cultivars of Australian sweet lupins, three cultivars of white lupins, four cultivars of peas and a sample of soybean meal for 4-week old broiler chickens were determined using the difference method. Significant (P<0.05) cultivar effects on AME were observed only for fababeans. The average AME of fababean, sweet lupins, white lupins, peas and soybean meal were determined to be 10.2, 6.8, 9.1, 10.3 and 12.5 MJ/kg DM, respectively. The AME of the two lupins were lower (P<0.05) than those of other ingredients. No cultivar effects (P>0.05) were observed for the AID of amino acids in any of the legumes. In general, the AID of amino acids in the two lupins were similar to those in soybean meal. The AID of threonine, cystine, proline and tyrosine were lower (P<0.05) in peas and fababeans compared to those in soybean meal. The mean AID coefficients of 17 amino acids in fababeans, sweet lupins, white lupins, peas and soybean meal were 0.80, 0.84, 0.87, 0.83 and 0.85, respectively.

I. INTRODUCTON

Grain legumes are potential protein sources in poultry diets. The nutrient composition and the feeding value of grain legumes for poultry have been extensively investigated, but published data on the ileal digestibility of amino acids for poultry are limited. The present study was conducted to determine the apparent metabolisable energy (AME) and apparent ileal digestibility (AID) of amino acids in four cultivars of fababean (*Vicia faba*; cv. PGG Tic, Spec Tic, South Tic and Broad), three cultivars of Australian sweet lupin (*Lupinus angustifolius*; cv. Walan, Tanjil and Borre), three cultivars of white lupin (*L. albus*; Promore, Kiev mutant and Ultra) and four cultivars of pea (*Pisum sativum*; cv. Santana, Miami, Courier and Rex) for broiler chickens. A sample of soybean meal was also assayed for comparison purposes.

II. MATERIALS AND METHODS

The assay diets were formulated by substituting the soybean meal and legumes for 50 and 25% (w/w), respectively, of a maize-soy basal diet (Table 1). Legume seeds, with hulls, were crushed to pass through a 1-mm sieve in a hammer mill prior to inclusion into diets. Titanium oxide was added to all diets as an indigestible marker. The basal diet and the assay diets, in mash form, were each fed to four pens (5 birds/ pen) of male broilers from day 28 to 35. From day 32 to 35, feed intake and excreta output were measured quantitatively per pen for the determination of AME.

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Table 1. Percentage composition of the basal diet[1]

Ingredient	g/kg
Maize	590.0
Soybean meal	351.8
Vegetable oil	17.8
Dicalcium phosphate	21.7
Limestone	7.8
Salt	2.0
Sodium bicarbonate	2.3
Trace mineral premix	2.5
Vitamin premix	0.5

[1] Formulated to supply 200 g crude protein/ kg diet. Titanium oxide (3 g/kg) was added to the basal diet and all test diets.

On day 35, all birds were euthanized by intravenous injection of sodium pentabarbitone and digesta were collected from the lower half of ileum. Digesta were pooled within a pen, lyophilised and ground to pass through a 0.5 mm sieve. Samples of diets and digesta were analysed for titanium, nitrogen and amino acids. The AME_n values and the apparent ileal digestibility (AID) coefficients of the grain legumes were calculated based on the assumption of additivity between the basal diet and test ingredient. The data were analysed using the GLM procedure of SAS (1997).

III. RESULTS AND DISCUSSION

The use of 'direct method', where the test ingredient represents the only amino acid source in the assay diet, is the preferred methodology to determine the amino acid digestibility of ingredients (Lemme *et al*., 2004; Ravindran *et al*., 2005). However, the use of this method to determine the AID of feedstuffs with low protein content such as grain legumes is recognised to result in the underestimation of AID coefficients relative to ingredients with high protein content because of the relatively greater proportion of endogenous amino acids in the digesta (Ravindran and Bryden, 1999). For this reason, an alternative method known as the 'difference method', which involves the formulation of both a basal and a test diet, was employed in the present evaluation. The digestibility in the test ingredient was then calculated using the difference in digestibility between the two assay diets and the contribution level of the nutrient in the diets. While this methodology overcomes the limitation of the diect method in underestimating the AID values of grain legumes, it must be noted that the calculations are based on the assumption that there is no interaction between the basal diet and the test ingredient.

Significant (P=0.051) cultivar effects on AME were observed for fababeans (Table 2). The AME values of the four fababean cultivars were determined to be 10.8, 9.2, 12.0 and 8.8 MJ/kg DM. The reasons for these differences are unclear, but possibly related to differences in starch digestibility between the cultivars.

Table 2. Comparison of AME (MJ/kg dry matter) and apparent ileal digestibility coefficient of amino acids in the four legumes and soybean meal (SBM)[1]

	Faba beans	Sweet lupins	White lupins	Peas	SBM
AME	10.2^{b}	6.8^{c}	9.1^{b}	10.3^{b}	11.1^{a}
Ileal digestibility coefficients					
Indispensable amino acids					
Arginine	0.902^{cd}	0.936^{ab}	0.946^{a}	0.914^{bc}	0.886^{d}
Histidine	0.715^{c}	0.789^{b}	0.810^{b}	0.815^{b}	0.882^{a}
Isoleucine	0.829	0.848	0.877	0.841	0.859
Leucine	0.835	0.871	0.894	0.843	0.842
Lysine	0.894	0.867	0.901	0.888	0.886
Methionine	0.811	0.720	0.830	0.816	0.870
Phenylalanine	0.884	0.884	0.923	0.873	0.849
Threonine	0.769^{b}	0.821^{ab}	0.836^{a}	0.781^{ab}	0.817^{ab}
Valine	0.815	0.828	0.847	0.835	0.848
Mean of indispensable amino acids	0.828	0.847	0.874	0.845	0.860
Dispensable amino acids					
Alanine	0.857	0.829	0.846	0.837	0.836
Aspartic acid	0.868	0.839	0.866	0.844	0.815
Cystine	0.564^{c}	0.761^{a}	0.806^{ab}	0.648^{c}	0.748^{b}
Glycine	0.755^{b}	0.823^{a}	0.860^{a}	0.804^{ab}	0.811^{ab}
Glutamic acid	0.881^{bc}	0.910^{ab}	0.925^{a}	0.901^{abc}	0.869^{c}
Proline	0.538^{b}	0.820^{a}	0.852^{a}	0.759^{a}	0.866^{a}
Serine	0.792	0.819	0.846	0.807	0.839
Tyrosine	0.806^{b}	0.839^{ab}	0.878^{a}	0.811^{b}	0.862^{a}
Mean of dispensable amino acids	0.758^{c}	0.837^{ab}	0.860^{a}	0.801^{bc}	0.831^{ab}
Overall mean[2]	0.795^{b}	0.843^{a}	0.867^{a}	0.825^{ab}	0.846^{a}

^{a,b,c} Means in a row not sharing a common superscript differ ($P < 0.05$).
[1] Each value represents mean of four replicates (five 4-week old broilers per replicate).
[2] Average digestibility of 17 amino acids.

In the other three legumes, no cultivar differences ($P>0.05$) were observed in the AME values. The average AME of fababean, sweet lupins, white lupins and peas were determined to be 10.2, 6.8, 9.1 and 10.3 MJ/kg DM, respectively (Table 2) and these AME values were lower ($P<0.05$) than the value of 12.5 MJ/kg DM determined for soybean meal. The AME of sweet lupins was lower ($P<0.05$) than those of other legumes, which may be

attributed to the high contents of non-starch polysaccharides in sweet lupins. Among the two lupin types, white lupins had a higher (P<0.05) AME than sweet lupins, which may related to the higher fat levels in white lupins. The average crude fat contents in white lupins and sweet lupins used in the present study were 113 and 51 g/kg, respectively. In general, the AME values determined in the current study for peas, fababean and the two lupin species are lower than the ranges reported in the literature (Hughes and Choct, 1999). This may be attributed, in part, to the presence of hulls in the legume meals evaluated.

In general, no cultivar effects (P>0.05) were observed for the AID of amino acids in any of the legumes (data not shown). The AID coefficients of amino acids in two lupins were similar (P>0.05) to those in soybean meal (Table 2). The digestibilities of threonine, cystine, proline and tyrosine were lower (P < 0.05) in peas and fababeans than in soybean meal. The mean digestibility coefficients of 17 amino acids in fababean, sweet lupins, white lupins, peas and soybean meal were 0.80, 0.84, 0.87, 0.83 and 0.85, respectively. As anticipated, the AID coefficients for grain legumes determined using the difference method in the current study were higher than those determined using the direct method by Ravindran *et al.* (2005).

Sulphur-containing amino acids are generally the first limiting in grain legumes. The AID coefficients of methionine and cystine were also found to be lower, whereas the AID of lysine and arginine were uniformly high in all grain legumes. Similar trends in ileal amino acid digestibility in grain legumes have been reported in previous studies with broiler chickens (Perez *et al*., 1993; Ravindran *et al*., 2005).

In summary, the ileal amino acid digestibility data highlight the potential usefulness of fababeans, lupins and peas as replacements for soybean meal in poultry diets. The low AME values of lupins will limit their inclusion levels, but this limitation could be overcome, at least in part, by dehulling the seeds and by the use of appropriate exogenous enzymes (Brenes *et al*., 1993; Bryden *et al*., 1994; Hughes and Choct, 1999)

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A COMPARISON OF THE GROWTH RESPONSE OF DIFFERENT SOYBEAN MEALS IN BROILER CHICKS UNDER ENERGY OR AMINO ACID DEFICIENT CONDITIONS

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Summary

Soybean meals of similar proximate analysis have been shown to perform differently in broiler feeding trials. The question is whether the differences can be attributed to higher available amino acids (AA) or higher metabolizable energy (ME) or both. Three dehulled soybean meals from different origins were used to conduct a broiler chick feeding trial under either low AA or low ME conditions. The results showed that broiler chicks fed the AA deficient diet had significantly better feed conversion ratio than broiler chicks fed the energy deficient diet. On soybean meal origins, chicks fed the diet using a commercial Malaysian dehulled soybean meal[1] had significant higher body weight gain than chicks given diets containing commercial US dehulled soybean meal or commercial Argentinean dehulled soybean meal. No diet by soybean meal origin interactions were detected. This suggests that both available AA and ME content of soybean meals tested were important and contributed to better bird performance.

I. INTRODUCTION

There are several reports in the literature to suggest that soybean meal with similar proximate analyses can perform differently in broiler feeding trials (Neoh, 2003; Parsons *et al*., 1991; Vohra and Kratzer, 1991). A chick bioassay using the protein efficiency ratio (PER) was proposed by Mateo and Swick (2003) as a tool for predicting the quality of soybean meals. Subsequently Neoh and Ng (2006) showed a correlation between apparent metabolizable energy (AME), PER and the growth performance of broiler chicks given diets containing different soybean meals. The purpose of this study was to compare the performance of broiler chicks offered diets containing dehulled soybean meal from three different origins with either energy-deficient or amino acid-deficient diets, to determine if differences among soybean meal origins were more related to differences in AME, available amino acids or both.

II. MATERIALS AND METHODS

A 2 x 3 factorial experimental design was used in this trial. Two diets of different nutrient levels and three soybean meals of different origins were used. The energy and amino acid ratio as well as essential amino acids specifications of the diets were formulated according to recommendations from Degussa Feed Additives (2001). One of the diets was formulated to be deficient in metabolizable energy (ME, 12.13 MJ/kg) but adequate in amino acids (digestible lysine 11.13 g/kg). The other diet was deficient in amino acids (AA, digestible lysine 10.59 g/kg) with an "adequate" ME of 12.76MJ/kg. The three commercial dehulled soybean meals were US dehulled soybean meal (US SBM) as control, dehulled soybean meal processed from US soybeans by Soon Soon Oilmills, Malaysia (SS SBM) and Argentinean dehulled soybean meal (ARG SBM). The proximate analysis of the soybean meals are listed in table 1.

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[1] Soon Soon Oilmills, Malaysia

[2] American Soybean Association, Singapore

Table 1. The proximate analysis (as is basis), potassium hydroxide Protein Solubility (KOHPS), Trypsin Inhibitor Activity (TIA) and Total Lysine content of US SBM, SS SBM and ARG SBM

Parameters	US SBM	SS SBM	ARG SBM
Moisture, g/kg	122	119	117
Protein, g/kg	465	466	462
Oil, g/kg	15	17	19
Crude Fiber, g/kg	34	31	32
Ash, g/kg	64.2	60.2	65.8
KOHPS, g/kg	823	854	835
TIA, mg/g	14	19	19
Total Lysine, g/kg	29.2	29.6	28.4

The trial was conducted at the Bangkok Animal Research Center, Thailand. A total of 1500 Arbor Acres High Yield Breed, male chicks were randomly assigned to 6 treatments each with 10 replicates. The chicks were allocated equally over 60 pens and each pen contained 25 birds. All birds were fed *ad libitum* with the above experimental diets from 1 to 21 days of age. Body weight and total feed consumption were measured at the end of 21 days. Body weight gain and feed conversion ratio (FCR) were then calculated.

III. RESULTS AND DISCUSSION

The body weight gain, FCR and livability are shown in table 2. The results show that chicks offered diets with high ME and low AA had numerically greater body weight gain (9 grams or 1.2%, P=0.08) and a significantly better feed conversion ratio (4 points or 3%, P<0.0017) than those offered the low ME-high AA diets. For the soybean meals from different origins, the results showed that chicks fed the SS SBM diets had significantly greater body weight gain than chicks fed the US SBM control diets (18 grams or 2.3%, P< 0.0001) across diet types. Chicks fed the ARG SBM diets had significantly lower body weight gain than those given the US SBM control diets (-13g or -1.6%, P< 0.0001) and SS SBM diets (-30g or -4.0%, P<0.0001). There were no significant differences in livability. Overall, there appears to be no interaction between nutrient levels and soybean meal origins (P=0.86 for body weight gain and P=0.46 for FCR). These results suggest that both available AAs and ME content of soybean meals are critical determinants of the growth performance of broiler chicks. In terms of overall available nutrient content, the soybean meals can be ranked as SS SBM >US SBM >ARG SBM.

Table 2. Body weight gain, feed conversion ratio and livability of broiler chicks when offered US SBM, SS SBM or ARG SBM in diets formulated with low energy or low amino acid requirements.

SBM	ME MJ/kg	Dig. Lys g/kg	Body Weight Gain (g)	Feed conversion ratio	Livability (%)
Main effects					
Nutrient					
	12.13	11.13	763.1	1.391[a]	98.9
	12.76	10.59	772.1	1.351[b]	99.3
Significance of nutrients effects			NS	**	NS
Soybean meal					
US			765.9[a]	1.374	99.6
SS			783.7[b]	1.354	99.2
ARG			753.3[c]	1.386	98.6
Significance of SBM effects			****	NS	NS
Nutrient x SBM			NS	NS	NS
Treatment effects					
US	12.13	11.13	761.3[abc]	1.384[ab]	99.6
SS	12.13	11.13	777.5[cd]	1.383[ab]	99.6
AG	12.13	11.13	750.6[a]	1.406[a]	97.6
US	12.76	10.59	770.6[b]	1.363[bc]	99.6
SS	12.76	10.59	789.8[d]	1.325[c]	98.8
AG	12.76	10.59	756.1[ab]	1.366[abc]	99.6
Significance of treatment effects			***	**	NS
Pooled SE			6.2	0.015	0.54
CV, %			2.57	3.37	1.7

[a, b, c, d] Means with unlike superscripts within either main effects or treatment effects within a column differ *(P<0.05), **(P<0.01), *** (P<0.001), ****(P<0.0001). NS – not significant.

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MEAT AND BONE MEAL, FUTURE NUTRACEUTICALS FOR POULTRY? A REVIEW

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Summary

Food proteins have a wide range of nutritional, functional and bioactive properties. In the last decade, the importance of biologically active peptides in the diet has been recognised, particularly their effect on health (nutraceuticals) for humans and animals. In Australia, meat and bone meal (MBM) is an important feed ingredient for poultry and a possible potential source of bioactive peptides. This review will highlight the current understanding of the potential for bioactives to improve the production and efficiency, health and wellbeing of poultry as well as enhancing food security. Research aimed at improving MBM feeding value (amino acid and mineral retention) and exploring the potential of its bioactive peptides suitable for poultry usage, is thus indicated. An improvement in the feeding value and nutraceutical activity of MBM will give the poultry industry greater confidence in using this by-product, reducing their reliance on imported plant protein ingredients.

I. INTRODUCTION

Biologically active peptides have been identified in many food resources, of both vegetable and animal origin. Peptides are short amino acid segments that have biological activity, providing that they are present at the absorptive site of the gut both "intact" and "active". In particular, there have been a number of bioactive peptides identified in milk and/or milk products, many of which have a number of nutraceutical (health promoting) activities (Rutherfurd-Markwick and Moughan, 2005). Of interest to us, is the limited information available on the presence of bioactive peptides in MBM. MBM is a common feed for livestock; however the risk of transferring bovine spongiform encephalophaty (mad cow disease) has limited its use to non-ruminant livestock only (i.e. poultry). Australia produces around 520,000 tonnes of animal by-products annually and relies heavily on its ability to recycle these products back into animal feed; thereby providing both economical and sustainability benefits. Animal protein meals in poultry diets have been proven to produce better growth and feed utilisation efficiency than diets containing only soybean meal as the sole source of protein (Irish and Balnave, 1993). Therefore, studies on the potential usage of bioactive peptides present in MBM as nutraceuticals for poultry are indicated at this time as they are likely to benefit both the industry and the consumer.

II. BIOACTIVE PEPTIDES

Biologically active peptides are specific protein fragments that have influence on metabolic processes and ultimately have a positive effect on health. Bioactive peptides have specific biofunction after they are released from the parent protein source, either by digestion or prior to consumption by food processing. Once liberated, they are capable of affecting a range of physiological and metabolic processes, such as immune response, behaviour, hormonal and neurological response, and gastrointestinal function. Bioactive peptides are normally comprised of 3 – 20 amino acids residues (Clare *et al.*, 2003). Some peptides that have been isolated and identified have specific potential, such as: antimicrobial and

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immunomodulatory function, Angiotensin Converting Enzyme (ACE) inhibition (anti hypertension), antioxidant activity and opioid peptides.

Concerns about the use of chemicals and/or antibiotics in the poultry industry have forced the industry to consider alternatives in disease prevention and/or treatment that are effective and will not contribute to drug resistance or result in residues in poultry products. One of the alternatives might be antimicrobial peptides. The activity of antimicrobial peptides depends on the interaction of the peptides and cell membrane. Some of the peptides that have been identified are glycomacropeptides, immunoglobulins, lactoferrins and lysozyme.

Glycomacropeptides, which are produced from κ-casein in cheese, play an important role as antimicrobial peptides by binding pathogenic bacteria and thereby preventing them from disrupting the mucosal membrane of the gut (Rutherfurd-Markwick and Moughan, 2005). It also has physiological functions such as the inhibition of bacterial and viral adhesion, promotion of bifidobacterial (i.e. probiotics) growth, and modulation of immune responses through proliferation of splenic lymphocytes (Brody, 2000).

Immunoglobulins (Ig) provide an important defence mechanism against infectious pathogens. Three principal classes of Ig in avian species are IgA, IgG (also called IgY) and IgM. IgA and IgM are transferred into the egg albumen in limited amounts, whereas IgY is transferred across the ovary into the egg yolk during oogenesis, in a similar way to the placental transfer of IgG in mammals, thereby supplying passive immunity to the chick.

Lactoferrin, a glycoprotein that belongs to the transferrin family, has important functions including antibacterial, antiviral, and antifungal activity, while also being identified as an anti-inflammatory, antioxidant and immunomodulatory agent (Lonnerdal and Iyer, 1995). Lactoferrin has been discovered in external secretions, such as saliva, tears, semen, and glandular epithelial cells (Giansanti *et al.*, 2002). It is also present in food products such as milk and milk products, as well as some fish, barley and pumpkin. Ovotransferrin, an analog of lactoferrin in birds, is present in egg white, and it has similar properties to lactoferrin. The antibacterial activity of lactoferrin is associated with its ability to bind free iron, depriving microorganisms of this essential nutrient and inhibiting the attachment of bacteria to the intestinal wall. Lactoferrin also has a direct killing effect by binding to the surface of susceptible microorganisms such as *S. mutans, V. cholerae, E. coli,* and *L. pneumophila* (Tomita *et al.*, 2002).

Lysozyme, an antimicrobial peptide found in harvestable amounts in egg albumen, is a potent antimicrobial against certain gram positive microorganisms. It can lyse bacterial membranes and destroys the cell wall. More recently, lysozyme has also been shown to have a bactericidal effect on gram negative bacteria (Mine *et al.,* 2004). Lysozyme has also been reported to have anti-inflammatory, antiviral, antitumor, antihistaminic and agglutinating properties (Mine *et al.,* 2004).

Immunomodulatory peptides, such as β-casokinins from casein hydrolysate, act by inhibiting ACE, which is responsible for inactivating bradykinin (a hormone with immune enhancing effects). β-casokinins has a role in the stimulation of lymphocyte proliferation including antigen-dependent T-cell proliferation; promotion of antibody development, phagocytic activity of macrophages and neutrophil movement/cellular proliferation; and the inhibition of lipopolysaccharides and phytohemagglutinin-induced propagation of murine spleen cell cultures (Rutherfurd-Markwick and Moughan, 2005).

ACE plays an important part in regulating blood pressure, fluid, and salt balance by converting the inactive angiotensin I into a powerful vasoconstrictor angiotensin II (Zaloga and Siddiqui, 2004). ACE inhibitory peptides play a significant role in controlling body fluid homeostasis of chicken, such as the circumventricular organs and arginine-vasotocin (AVT, avian ADH) producing systems. It also has an indirect effect on the immune system by preventing inactivation of bradykinin (Yamamoto, *et al*, 2003) as mentioned previously.

Antioxidant peptides have an important role in preventing damage to the epithelial cells by oxidants (free-radicals) produced by activated immune cells (macrophages). Carnosine, a peptide found in meat and fish products, has a variety of biologic properties in addition to its antioxidant properties (Zaloga and Siddiqui, 2004). It is a precursor of histidine (histidine containing peptides also have antioxidant properties), stimulates maturation of immunocompetent cells, influences modulation of enzymatic activity, and vasodilation of arteries. The latter may have a significant influence in cardiovascular disease (e.g. sudden death syndrome) and in ascites by preventing pulmonary constriction.

Other important peptides that have been discovered are the opioids, such as casomorphins and met-enkephalin, which are known to modulate social behaviour and to have an analgesic effect in humans (Clare *et al.*, 2003). Opioid peptides found in food sources are absorbed intact into the blood circulation and produce their effect when they reach endogenous receptors located in the spinal cord, digestive tract, pituitary and adrenal glands, and hypothalamus. Opioid endogenous receptors of chickens have been identified in the neurohypophysis, and they are important in regulating feeding behaviour, produced either by stimulating feeding which can result in hyperphagia; or by suppressing food consumption by slowing gastric movement (Bungo *et al*, 2004; Bungo *et al.*, 2005).

III. MEAT AND BONE MEAL

MBM, by definition are rendered products from mammalian tissues, including bone, but typically exclusive of any added blood, hair, hoof, horn, hide trimmings, manure, and stomach or rumen contents. MBM has a crude protein level of approximately 50%, with typical calcium and phosphorus contents of 10 and 5%, respectively. MBM contains a highly balanced source of amino acids; however its quality, in terms of the available or digestible amino acids varies greatly depending on the products rendered and processing conditions (temperature, moisture, pressure and time). There are more than 100 rendering plants in Australia that produce MBM; these plants vary in size, processing capabilities and types of raw materials used. Rendering conditions recognised to minimise protein degradation include: temperatures less than 125°C (Batterham *et al*, 1986); zero psi pressure (Shirley and Parsons, 2000); and for minimal periods of time (Batterham *et al*., 1986).

Heat is an essential component in the production of MBM; the critical factors affecting protein quality are the degree of heating and the length of time this heat is applied. When proteins are exposed to higher temperatures, digestibility is reduced as a result of reactions between amino acids and other compounds and intramolecular reactions between amino acids within the protein molecule that cannot be split by digestive enzymes. Cross linking between amino acids, such as cysteine can have a detrimental effect because cysteine or total sulphur amino acid is the first limiting amino acid in MBM for chicks. In addition, inappropriate processing conditions used to produce MBM can significantly (>20%) reduce the digestibility of lysine.

IV. POTENTIAL NUTRACEUTICAL

The interests in "functional food products" (nutraceuticals) have risen considerably in the last two decades (Rutherfurd-Markwick and Moughan, 2005). Our research will examine the potential of MBM to act as a source of bioactive peptides for poultry. In particular we will try to address the following questions: Are there any bioactive peptides in MBM? If so, how much would be available? What will their effect be on the metabolism of poultry? What can be done to improve the activity and quality of the peptides? These questions arise as an increasing number of bioactive peptides are identified and isolated, especially from milk and

milk products. The discovery of carnosine, as well as ACE inhibitory peptides in meat and fish products has led to our hypothesis that MBM may have the potential to be a significant source of bioactive peptides.

There is considerable potential for developing MBM as a source of nutraceuticals for poultry thereby increasing its feeding value. Improved nutrient retention from MBM will reduce the levels of undigested proteins reaching the lower intestine and causing pathogen proliferation (i.e. reducing the incidence of necrotic enteritis). Consequently, this will be beneficial to the poultry industry to reduce the industry reliance on in-feed antimicrobials and to improve the environmental sustainability of the poultry industry by minimising nitrogen excretion and/or recycling it. Better quality MBM will give the industry more confidence to use it which in turn will reduce the usage of imported plant protein ingredients.

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OPPORTUNITIES AND CHALLENGES FOR EXTENSION WORKERS SERVICING THE POULTRY INDUSTRY

C. BENNETT[1]

Summary

A short overview of the history and principles of agriculture extension programs is given. Some key characteristics of extension programs are described and methods of delivering extension programs are discussed. The advantages and challenges of industry funded extension are outlined. Illustrations from experience from the long-running, industry funded poultry extension program at the University of Saskatchewan are used, including methods of delivering a successful program.

I. HISTORY OF EXTENSION

According Jones and Garforth (1997), modern extension in agriculture has its roots in the agricultural societies that became common in Europe in the early 1800s. These societies or clubs were formed by educated landowners who sought to take advantage of the emerging science of agriculture to increase the profitability of their tenants and the rent that they could tax from them. By the 1840s, these societies were caught up in the general movement of "education for all" and the desire emerged for all farmers and not just the wealthy to benefit from improved production practices. As Jones and Garforth point out, the critical element in educating the "generality of farmers" was the role of the extension specialist or agent in directly advising and encouraging farmers. The birth of agricultural extension occurred when these societies started to hire "itinerant agriculturalists who could meet farmers in their home localities, give instructional talks and demonstrations, advocate superior or new practices, and have discussions with the farmers" (Jones and Garforth, 1997). The dedicated extension specialist who devoted most of his or her time to working with farmers was a key part of the extension movement in agriculture. By the late 1800s, many agriculture extension programs were based around agricultural research stations or universities but working directly on farms and in the farming community was still a major role of the extension specialist.

Today, many extension programs have wandered away from these roots. Some specialists now spend most of their time in the office such as on a university campus instead of working on farms, in hatcheries, or with processing plants. Extension activities may be treated as temporary off-campus events instead of on-going day-to-day activities in industry.

II. SOME EXTENSION PRINCIPLES

a) Definition of extension

The founding principles of extension programs are based around education and motivation of individuals. Boone (1989) states that:

> "Extension is a system of nonformal education. As such, it is a field of professional practice aimed at 1. teaching people in their own context and life situations, how to identify and assess their own needs and problems; 2. helping them to acquire the

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> knowledge and skills required to cope effectively with those needs and problems; and 3. inspiring them to action. Extension education occurs in settings whereby the problems and concerns of those to be helped provide the base for the instruction that occurs."

This definition recognizes three essential characters of extension: informal education, meeting the needs of immediate interest to individuals, and local delivery. Of these three, education is traditionally given the most prominence in the literature. According to Mosher (1978), "the primary task of every extension agent is *teaching*, and for the most part it is teaching *adults.*" Chang (1963) writes that, "Education is a process of bringing about desirable changes in human behaviour…. Whatever we do in extension work is to be viewed as an educational means to bring about these desirable changes." Education is seen as the method by which extension programs can be drivers of accelerated change in the farming community.

b) A key characteristic of the extension specialist

Working with farmers, hatcheries, processors, and feed mills on a daily basis is a key characteristic of a successful poultry extension specialist. Extension workers who routinely deal with the owners, managers, and employees of poultry farms and companies are the people that industry will work with instead of being the people to whom they politely listen and then ignore. At its best, extension specialists, farmers, service people, and industry leaders will work together as "reciprocal colleagues" (Mosher, 1978). People are reciprocal colleagues when they take turns teaching and learning from each other. Reciprocal colleagues respect the talent and experience that each can bring to bear on a problem. In my case, much of what I know has been learnt from working with some very smart farmers, hatchery managers, and service people over the last twenty years. Their experience has helped me to understand that chickens do not read textbooks and simplistic application of research is not enough to successfully apply new knowledge.

The idea of reciprocal colleagues may seem old-fashioned but it is more relevant today than ever. Today's integrated and commercialized industry is run by managers who have been hired to keep the industry on track and solve problems as they arrive. These professional managers may actually find it harder to ask for help in solving problems than traditional owner-operators. Also, because more and more of the expertise in the industry is now learnt on-the-job instead of at school, these people can correctly view themselves as experts and question the value of the outsider – the extension specialist. By working together on a regular basis, people can develop the trust and respect needed to collaborate and solve industry problems.

c) Innovative Diffusion Theory

"Innovative Diffusion Theory" is one of the most widely held theories in agricultural extension (Mosher, 1978; Stephenson, 2003). This theory first gained popularity after research was published on the adoption of new hybrid corn varieties in the state of Iowa in the 1920s. The research demonstrated that when innovative farmers started to plant the new corn, their neighbours saw their success and started to plant the new crop as well. A wave of adoption spread across Iowa, with the "innovators and early adapters" benefiting first and the "laggards" benefiting last. Based on this experience, extension people realized that they could focus their efforts on the innovative farmers and that the remaining farms would learn from them.

As pointed out by Stephenson (2003), the Innovative Diffusion Theory has led to problems as well as successes. Stephenson (2003) writes that extension agents tend to be technically minded people who are naturally attracted to the innovators. The theory can be used as an excuse to ignore the farmers who are viewed as the "laggards" but who may be the most in need of encouragement and assistance.

III. WHAT DOES AN EXTENSION PROGRAM LOOK LIKE?

a) How do you know if you have an extension program?

An extension program is a focused collection of activities designed to turn ideas into actions. Boone (1989) emphasizes the need for extension programs to be "planned and sequenced to produce or effect desired changes(s) in the behavioral patterns of the learners." Mosher (1978) reminds us that people learn best when they receive the information by a variety of teaching methods. Giving a presentation at a meeting or writing a newsletter article will not be sufficient on their own to spark and sustain a wave of change in industry.

As an example of what might constitute an extension program, consider my own program to teach farmers how to ventilate their barns. While a lively lecture or workshop on ventilation is enjoyable for me and the participants, it still cannot achieve the goal of improving the air quality in poultry barns. The workshops and presentations must be followed up with visits to poultry barns to measure and assess the ventilation for individual farmers. Research is also needed to see if new equipment is helpful and field trials are required to demonstrate its effectiveness. Getting inspectors for the poultry industry's HACCP program to monitor ammonia levels in barn air may further reinforce the need to change practices on-farm. To fix some problems, it may be necessary to convince local suppliers to stock different types of fans and control units. A series of ventilation factsheets must be mailed out to farmers to remind them about the ways they can improve their barn ventilation. For extension activities to form an effective program, a whole range of activities and support must be visible. If they are not, you do not have an extension program.

The time frame and nature of the problems addressed are further distinguishing characteristics of extension programs. I like to work on problems with a 3 month to 3 year time frame – a longer time frame than industry can easily address. Industry is very good at solving short-term problems but tends to get knocked off course by everyday events when tackling longer term problems. Problems which are shared by different industry sectors, farms or companies are also good targets for extension programs. It may be more efficient for a poultry specialist to concentrate effort on a common problem compared to each individual farm or company trying to tackle the problem separately. Finally, problems which have limited immediate economic payoff but with long term consequences may be targets of extension programs, often as a precursor to impending pressures such as tighter legislation. My own work on developing large-scale, outdoor composting of end-of-lay hens is an example of an activity that may not be important today but which could suddenly be very important if the rendering industry (the only market for old hens on the Prairies) decides one day to no longer handle these low value carcasses.

b) Extension versus Technology Transfer

Technology transfer is the process of transferring and disseminating the results of research. It is one type of extension activity that is sometimes used synonymously for extension. It may require non-educational activities such as sourcing financing or removing

legislative barriers to business development. Technology transfer emphasizes the diffusion of new knowledge and may not involve helping people learn to solve their own problems (Baker, 1989). As an extension person, I find this approach to be passive and ignore the motivational potential of extension.

Opio-Odongo (2000) writes about causes of ineffective extension in Africa but his comments could very well be about the pitfalls of the technology transfer that is practiced in North America. Opio-Odongo lambastes extension workers who have "tended to treat farmers as if they were empty vessels to be filled with knowledge and expertise." He also complains about extension workers who under-estimate the experience of farmers and their role in adapting new technologies. Opio-Odongo challenges the belief that outside technology and research can be implemented with minimal change or testing under local conditions. My own experience with the failure of dry manure storages on Manitoba egg farms is a good example of how technology that has worked well in Ontario or Ohio may not work well on the Prairies. A lot of time and money can be wasted if you assume that technology or practices from the U.S.A., Europe or elsewhere can be easily inserted into your local industry.

c) How is extension delivered?

Many approaches can be used in extension. The delivery methods that I have seen to be effective are 1) classic researcher to industry flow 2) industry practice to research flow 3) extension specialist – industry collaboration 4) teaching moments 5) transfer of learning strategies and 6) producer-to-producer interaction.

1) *Classic researcher to industry flow*

The adoption of the broiler lighting program by the Saskatchewan poultry industry is an example of the classic model for the extension process. Dr. Hank Classen at the University of Saskatchewan tried out a few novel lighting programs as part of class teaching project in 1986. Encouraged by the apparent improvement in bird health, Hank conducted a series of university trials the following year and demonstrated a significant decline in leg problems with little or no loss in growth rate. I and the extension veterinarian at the university promoted the research results by first convincing a few innovative broiler producers to try the program. An initial factsheet describing the program and a second one addressing the common questions were mailed to all producers in the province. Producers who were worried about frightened birds or "unnatural" dark periods were visited and reassured about the low risk involved in turning off the lights. Every broiler producer in the province was telephoned and surveyed about his or her success or lack of implementation/success with the lighting program. Regional industry meetings detailing experience with the lighting program were held in town halls, schools, and producer homes throughout the province. Within a year, 50% of the producers were regularly using the lighting program and within another six months, 70% of the producers had adopted the program.

2*) Industry practice to research flow*

News of industry experimentation with whole wheat feeding in Europe and neighbouring provinces reached Saskatchewan in the early 1990s. The practice of diluting diets with whole wheat was considered questionable but industry interest in the practice did not fade away. In response, I organized a field trial involving eight broiler farms. With the help of three commercial feed mills, broiler diets were diluted with whole wheat up to a maximum of 30% of the diet. On each farm in the field trial, I weighed birds and submitted mortality to the lab on a weekly basis. Despite concerns about dilution, none of the flocks

"crashed and burned" and average return over feed and chick cost was increased by 1.5 cents/kg. These results were enough to gain support to do a pilot study at the University of Saskatchewan (Bennett, 1995). The university contributed the research barn and flock supervision while I ran the trial and contributed labour. Again, the improvement in return was confirmed. This study was the "proof of concept" that allowed a further $70,000 (a fortune to me) to be raised from government and industry to do a full investigation of whole grain feeding. The net result was a research program which determined how to feed whole wheat and barley to broiler chickens, laying hens, and turkeys. The formal research demonstrated that whole grain feeding did not offer any advantage in nutrient utilization but did consistently improve leg health in chicken and turkeys. The university trials helped several producers decide that milling a coarsely ground mash feed on-farm was more economical than adding whole wheat or barley to the rations. In this case the role of extension was to use industry experience to encourage university action for the benefit of the industry.

3) *Extension specialist – industry collaboration*

The work of Dr. Stan Savage at the University of Georgia is wonderful example of collaborative field research with industry. Stan spent ten years working with processors in Georgia and the southeastern United States on methods of reducing carcass contamination and yield losses. Stan used observation, knowledge of bird physiology, and practical field experiments to develop the basic concepts of using eating patterns, drinking patterns, and "windows of opportunity" to design feed withdrawal programs (Savage, 1998a,b). These concepts were entirely developed and verified as effective in the field. Stan worked directly with the processors, plant managers, and flock supervisors and because of this active participation in the research, the transfer of this knowledge was immediate and effective. The industry decision makers had a vested interested in seeing the results applied quickly. Stan was able to implement programs that helped to improve both carcass weight and feed conversion by approximately 2%. The reduction in shrink for the one billion broilers marketed in Georgia each year made Stan's field research an example of a highly profitable extension program.

4) *Teaching moments approach*

For years I worked with an extension veterinarian at the University of Saskatchewan who displayed an unwavering belief in "teaching moments" – moments when an individual is ready to learn. He did not feel a strong drive to organize workshops, conduct field research, or even visit farms – unless someone called him first. He believed that there was little benefit in working with people until they felt a desire to change. Once someone picked the phone, however, he worked tirelessly to solve the problem. By focusing on the people who were ready for change, he maximized the benefit from his efforts. It was a unique approach

The approach worked even better because the extension veterinarian was part of a team. In that case, I was the team member who enjoyed organizing workshops and field trials and who involved the extension veterinarian in these projects. The roles of the various team members provided a balance - allowed contact and "teaching moments" with a wider range of producers.

5) *Farmers as teachers and transfer of learning*

Recently we held a cracked egg workshop at the University of Manitoba. It was a collaborative effort between the university, the provincial department of agriculture, federal food inspectors, and provincial egg producer board. The workshop was organized because evaluation forms from previous workshops showed a strong interest in learning how to solve cracked egg problems. These same forms indicated that the average producer who attended

the previous workshops had an average of 17 years experience in the poultry industry. Thus the question that arose was, "How do you deliver something relevant to people with so much experience and how do you get them to challenge some of their long held beliefs?"

The answer was to try a different approach to teaching. Instead of lecturing, hands-on laboratories and group problem solving were the major teaching methods. Out of the eight hour workshop, only two hours was devoted to lecturing. The rest of the time was split between group discussion and hands-on training. Approximately 25% of the day was spent with the producers discussing case studies, problem solving, and using their own experience as the major source of information. The hands-on training was in the laboratory, with the producers being divided into groups and being given "mystery eggs" which they had to test and then form an action plan for further investigation of the problem. The result was a workshop that was not just rated as "Excellent" by 85% of the participants but which elicited a much deeper response than normal. One farmer called me a week after the workshop to express his satisfaction with how he could now see a much broader picture of how to approach cracked egg problems. To reinforce this learning, participants are visited after the workshops and assisted with the egg quality measurements on their own farms. The whole experience is effective because it challenges the participants to use their own experience and situation as a basis for learning.

6) *Producer to producer*

Farmers, service people, hatchery managers, and processors have always talked to their peers to share ideas and solve problems. Although it is one of the major methods of transmitting information in industry, these discussions have usually been pretty informal. One of the major purposes of coffee breaks at industry meetings is to facilitate these discussions.

It is possible to give more structure to these discussions to increase their effectiveness. In Saskatchewan, for example, eight chicken farmers organized a producer group that met monthly to discuss their operations and share experience. The farmers set the rules and agenda for the group. They used the extension specialist as a resource but the producers were in charge. The group proved highly effective with some farmers citing a 20% improvement in profitability in one year. Improvement was noticed by both novice and experienced farmers. All of the farmers learnt that people who are in the same business do not necessarily think the same or have the same results. By sharing experience, they gained a broader basis for making decisions on their own farms.

IV. CHALLENGES AND OPPORTUNITIES IN INDUSTRY FUNDED EXTENSION

a) Advantages of industry funded extension

What are the advantages of industry funded extension? It keeps the extension specialist very focused. There is no doubt who you are working for and who you need to keep happy. At the same time, more companies and farms will seek out your services because they are paying the bill and want to see a return on their investment. Companies and farmers are also less likely to view the extension specialist as an outsider and are more likely to openly discuss their problems.

Industry-funded extension can be a very accessible and a lower cost method of delivering technical services. In Saskatchewan, the industry is small and sparsely distributed across a large area. It is very difficult for any one company or consultant to gain enough business to justify specializing in servicing the poultry industry. By funding extension, the Saskatchewan industry gains access to both a poultry specialist and poultry veterinarian on an on-going basis. Both of these specialists can also afford to take a lower wage as employees of

the extension service compared to earning their living as consultants. Because the program costs are paid out of industry levies, farms and companies are not charged each time they access the extension service and they are more likely to make regular use of it.

b) Disadvantages of industry funded extension and overcoming these challenges

What are the disadvantages? The extension specialist must still keep everyone happy. The turkey producers, for example, do not care if you have solved a shell quality problem for the egg producers. It is difficult to keep everyone satisfied especially if they all expect special attention each year.

In Saskatchewan, a team approach has helped to meet industry expectations and to prevent overload. One approach was to have team members lead different areas; for example, in years when I concentrated on feeding programs for broilers or layers, the extension veterinarian might balance this by working with the turkey producers to solve air-sacculitis problems. Positioning the extension team at the university also helped to address an even broader range of problems and keep the service pertinent to the whole industry. The university offered a pool of people with whom to share ideas and information. Many times, extension people were involved in or inspired research projects. Situating the extension program at the university also provided a buffer between the extension service and industry requests for non-extension activities such as counting chickens or running on-farm food safety programs. By working together, both the university and extension programs were more successful and viable over the long run.

How do You Evaluate Success?

For an extension program to succeed over the long term, the industry sponsors and the extension operators must have a clear idea of what they are aiming for and how to evaluate success. If you do not document and promote its success, the support for the program will falter. At some point the industry will demand, “Show me the money!” Unfortunately, a plan for evaluating the program is often not developed and the amount of work needed to document success is under-estimated.

As an example, consider the number of techniques needed to verify the success of my barn ventilation program. An easy but limited approach is to measure producer participation and interest in ventilation workshops. Another tool is recording the number of farmers that ask me to test their ventilation systems. A convincing evaluation, however, requires more than these simple tools. Follow up assessment of the farms that I visit is needed to provide concrete evidence that recommendations have been implemented and poultry house environment has improved. Surveys are needed to determine the success in increasing the number of farms that use misting systems, static pressure gauges, and back-draft dampers. A field demonstration trial must be conducted or an economic model developed to demonstrate cost/benefit of the ventilation recommendations. Considerable effort is needed to document the success of an extension program.

V. CONCLUSION

An extension specialist who seizes the opportunity to work with industry on a daily basis can be a catalyst for accelerated and widespread improvement in industry. An industry that supports this effort will see research, field studies, and training employed in new and effective ways. An extension team based at a university is an effective method for creating an extension program that will earn long-term support from industry.

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THE EFFECTS OF *SACCHAROMYCES CEREVISIAE* ON PERFORMANCE AND BIOCHEMICAL PARAMETERS OF BROILER CHICKS DURING AFLATOXICOSIS

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Summary

The amelioration of aflatoxicosis in broiler chicks was examined by the dietary addition of *Saccharomyces cerevisiae* (SCE). *Saccharomyces cerevisiae* incorporated into the diet at 1 g/kg was evaluated for its ability to reduce the deleterious effects of 1 and 2 ppm aflatoxins (AF) on Ross broiler chicks from 1days to 42 days of age. The AF treatments significantly decreased feed consumption and body-weight gain, and increased feed conversion ratio ($P<0.05$). Serum cholestrol, total protein and albumin decreased significantly ($P<0.05$) in diets contaminated with aflatoxin. Compared to controls, the addition of SCE to an AF-containing diet significantly reduced the deleterious effects of AF on body-weight gain, feed conversion ratio, cholestrol, albumin and total protein. The AF fed groups had higher serum activities of the enzymes LDH and AST and decreased activity of ALP. The addition of SCE to an AF-containing diet reversed the effect of the toxin on the activities of serum enzymes. These results suggest that SCE reduced the adverse effects of AF and should be helpful in a solution to the aflatoxicosis problem in poultry.

I. INTRODUCTION

Aflatoxins (AF) are toxic compounds produced in grains by the fungi *Aspergillus flavus* and *A. parasiticus*. Aflatoxicosis in poultry is characterized by weakness, anorexia with lower growth rate, poor feed utilization, decreased egg production, increased susceptibility to environmental and microbial stressors and increased mortality (Bailey et al., 1998; Kubena et al.,1998). Aflatoxicosis is also associated with biochemical, haematological, and pathological changes. The liver is the target organ of aflatoxins and hepatobiliary damage is associated with alterations in liver enzyme functions. As aflatoxin often contaminates poultry feed, there is a need for a comprehensive tool to counter this problem. Recent biotechnological progress has opened new avenues for tackling this problem. *Saccharomyces cerevisiae* was found to have beneficial effects in poultry during mycotoxicosis (Stanley et al., 1993). A study was conducted to determine the efficacy of inclusion of *S. cerevisiae* in decreasing the effects of aflatoxin on performance and biochemical parameters of broiler chickens.

II. MATERIALS AND METHODS

In this study 480 day-old male chicks (Ross 308) were randomly assigned to pens with 20 chicks in each. The study was a completely randomized design with four replications of each of the following treatments: A- Control without aflatoxin; B- A plus 1g/kg SCE; C- A plus 1 ppm AF; D- A plus 1 ppm AF + SCE (1g/kg); E- A plus 2 ppm AF; F: A plus 2 ppm AF + SCE (1 g/kg).

Aflatoxins were produced on rice following inoculation with fungal spores ($6.5x10^6$ - $7.0x10^6$) of a toxigenic *A. parasiticus* species as described by Shotwell et al. (1966).

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Aflatoxin content of the rice was determined by TLC and HPLC based on the procedure described by Wilson and Romer (1991). Birds were inspected daily and their body weight and feed consumption was recorded weekly and daily, respectively. Feed intake, mean body weight gain, and feed conversion ratio were calculated. Blood samples were collected from the wing vein of birds at 21 (n=240) and 42 (n=240) days of age, and serum was separated for total protein, albumin and cholesterol analysis. Serum enzymes, namely lactate dehydrogenase (LDH), aspartate amino transferase (AST), and alkaline phosphatase (ALP) were measured using commercial kits (Zist Chimi, IR) on an auto-analyzer (Technicon RA-1000). Data were analyzed by the General Linear models procedure of SAS Institute (1994). Means for treatments showing significant differences in the analysis of variance were compared using Duncan's multiple range tests. All statements of significance are based on the probability level of 0.05.

III. RESULTS

The results show (Table 1) that growth rate, food intake and feed utilization efficiency were depressed in birds given aflatoxins (treatments C and E), but that the depression was alleviated in those also receiving SCE (treatments D and F).

The results of serum biochemical parameters presented in Table 2 show that serum cholesterol, total protein and albumin levels were all significantly ($P<0.05$) depressed at 21 and 42 days in birds in groups C and E given aflatoxin without SCE. The depression tended to be greater in group E given 2 ppm AF. In chickens given SCE treated aflatoxin contaminated feed (groups D and F), the serum levels of all three parameters were within the normal range.

Effects of treatments on blood serum enzymes are shown in Table 3. Serum lactate dehydrogenase as well as aspartate amino transferase activities were found to be elevated ($P<0.05$) at both ages in chickens fed aflatoxin contaminated feed (treatments C and E), but addition of SCE to the AF-containing diets resulted in a return to normal enzyme levels (treatments D and F). Serum alkaline phosphatase activity was reduced in birds given aflatoxin treated feed (treatments C and E), whilst again addition of SCE to the AF-containing diets returned activity to near normal levels (treatments D and F).

Table 1. Comparison of performance parameters in chicks fed different rations.

Treatment	Body weight gain	Dietary feed intake	Feed conversion ratio
A	$38.3 \pm .0.6^{a}$	75.4 ± 1.8^{a}	1.97 ± 0.05^{a}
B	38.6 ± 2.2^{a}	74.5 ± 2.2^{a}	1.93 ± 0.02^{a}
C	32.7 ± 1.2^{b}	70.3 ± 2.8^{b}	2.15 ± 0.05^{b}
D	37.9 ± 0.8^{a}	76.5 ± 2.4^{a}	2.02 ± 0.09^{a}
E	28.5 ± 1.1^{b}	65.0 ± 1.7^{c}	2.28 ± 0.07^{b}
F	36.2 ± 1.0^{a}	76.0 ± 1.3^{a}	2.10 ± 0.06^{ab}

Experimental groups are as shown in Table 1. Feeding started from the first day of housing and parameters calculated on 42 days of age. Data presented as mean ± S.E.M. Mean values in same column without common letters (a-c) differ significantly ($P<0.05$).

Table 2. Comparison of serum biochemical parameters in chicks fed different rations.

Treatment	Cholesterol (g/l)		Total Protein (g/dl)		Albumin (g/dl)	
	Day 21	Day 42	Day21	Day 42	Day21	Day42
A	132 ± 5.5^{a}	145.7 ± 8^{a}	2.58 ± 0.26^{a}	3.76 ± 0.23^{a}	0.77 ± 0.82^{a}	1.4 ± 0.20^{a}
B	128 ± 6.4^{a}	146.8 ± 7^{a}	2.7 ± 0.28^{a}	3.82 ± 0.29^{a}	0.82 ± 0.96^{a}	1.38 ± 0.21^{a}
C	89.5 ± 5.2^{b}	133.8 ± 6^{b}	1.78 ± 0.26^{b}	2.45 ± 0.37^{b}	0.54 ± 0.92^{b}	0.92 ± 0.20^{b}
D	127 ± 3.7^{a}	140.5 ± 8^{a}	2.47 ± 0.28^{a}	3.65 ± 0.28^{a}	0.72 ± 0.89^{a}	1.32 ± 0.18^{a}
E	85.5 ± 2.9^{b}	126.5 ± 12.7^{b}	1.65 ± 0.27^{b}	2.3 ± 0.22^{b}	0.43 ± 0.96^{b}	0.77 ± 0.14^{b}
F	124.5 ± 3.8^{a}	136 ± 11.2^{ab}	2.4 ± 0.23^{a}	3.57 ± 0.24^{a}	0.71 ± 0.86^{a}	1.29 ± .16^{a}

Data are mean ± S.E.M. of 10 analyses carried out on samples obtained from 10 individual birds. Chickens were examined at two stages of growth when they were either 21 or 42 days old. Means in the same column without common letters (a-c) differ significantly ($P < 0.05$).

Table 3. Serum enzymes in chickens fed different rations.

Treatments	LDH (U/l)		AST (U/l)		ALP (U/l)	
	day 21	Day 42	day21	Day 42	day21	Day42
A	227.5 ± 6.8^{a}	223 ± 15^{a}	223 ± 5^{a}	255.5 ± 20^{a}	4500 ± 200^{a}	4450 ± 225^{a}
B	226 ± 7.9^{a}	214 ± 6^{a}	219 ± 4^{a}	250 ± 21^{a}	4450 ± 280^{a}	4320 ± 220^{a}
C	272 ± 6.5^{b}	246 ± 11^{b}	260 ± 5^{b}	305.5 ± 36^{b}	3720 ± 450a^{b}	3400 ± 250^{b}
D	224.5 ± 6.5^{a}	213 ± 15^{a}	227.5 ± 7^{a}	265 ± 18^{a}	4200 ± 300^{a}	4160 ± 270^{a}
E	284.5 ± 12.5^{b}	261 ± 9^{b}	278 ± 6^{c}	325 ± 14^{c}	3220 ± 240^{c}	3150 ± 150^{b}
F	235.5 ± 8.5^{a}	217 ± 17^{a}	235.5± 7^{a}	280 ± 38^{ab}	4050 ± 280^{ab}	3860 ± 170^{ab}

Data are mean ± S.E.M. of separate analyses carried out on 10 samples obtained from individual chickens. Enzymes measured at two stages of growth, viz. 21 and 42 days after treatment. U, one unit is equivalent to 1 μmole of lactate, oxaloacetate, pyruvate, or inorganic phosphate released per minute at 25 °C for LDH, AST and ALP, respectively.

IV. DISCUSION

Aflatoxicosis is an important problem in the poultry and livestock industries due to its profound effects on growth and the frequent contamination of feed by these mycotoxins. Mycotoxin producing fungi are responsible for significant financial losses encompassing a broad spectrum of crop and farm animals, and extending through the food chain to the consumer (Shane 1994). Every year a significant percentage of the world's grain and oilseed crops are contaminated with hazardous mycotoxins, such as aflatoxin. Unfortunately, discontinuing the feeding of aflatoxin contaminated grain is not always practical, especially when alternative feedstuffs are not readily available or affordable. Thus, these toxins frequently detected in animal feed, can cause significant production losses in animals.
Dersjant-Li et al. (2003) reported that the reduction in body weight gain in pigs and broilers fed aflatoxin contaminated feed is directly related to the levels of aflatoxin in the diet. They suggested that for each mg/kg increase in aflatoxin in broiler diets, growth rate is depressed by 5%. Our results showed that inclusion of *S. cerevisiae* in rations reduced the effects of aflatoxin in broilers.

This was achieved by comparing different parameters related to aflatoxicosis as well as the production efficiency of broilers. Data presented in this study showed that most of the plasma parameters were found to be affected by aflatoxin but remained within the normal range in chickens given *Saccharomyces cerevisiae*. Unlike the non-enzymatic parameters, marker enzymes particularly lactate dehydrogenase and aspartate amino transferase showed more dependency on the concentration and duration of the exposure to aflatoxins. These data

suggest that serum enzymes particularly AST and LDH can be used as indices for investigating the performance of chickens challenged with aflatoxins. Serum ALP decreased with aflatoxin intoxication whereas, it was unaffected in chickens fed diets containing SCE (Table 3). It has been suggested that the nutritional deficiency induced by aflatoxin can lead to disruption of the activities of digestive enzymes and absorption of essential nutrients (Bolden and Jensen, 1985). The depression in growth and feed utilization efficiency observed on aflatoxin contaminated diets, is likely due to the effects of aflatoxin in impairing nutrient absorption and reducing pancreatic digestive enzyme production (Swamy and Devegowda, 1998). The present results suggest that *SCE* has a potentially important role to play in alleviating the toxic effects of aflatoxin in poultry diets.

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PATHOGENESIS OF TWO STRAINS OF INFECTIOUS BRONCHITIS VIRUS FOR THE OVIDUCT OF UNVACCINATED LAYING HENS

K.K. CHOUSALKAR[1] and J.R. ROBERTS[1]

Summary

The pathogenesis of Infectious Bronchitis Virus (IBV) for different parts of the oviduct was studied in unvaccinated Isa Brown laying hens exposed to T and N1/88 strains of IBV. Two hens from T and one hen from N1/88 infected groups went out of lay. Ultrastructual findings revealed that the infundibulum and magnum were more negatively affected than the isthmus, tubular shell gland and shell gland pouch. However, all parts of the oviduct of hens out of lay showed severe cytopathology. Overall, T strain was more pathogenic for the fully functional oviduct than N1/88. Virus particles were recorded on the 10th day p.i. and severe cytopathology was observed between 10 and 12 days p.i.

I. INTRODUCTION

Infectious bronchitis, which is caused by a coronavirus, can be a devastating disease to the layer industry. Despite the fact that IBV infection can cause serious problems with egg production and quality, very little research has been undertaken to understand and define the pathogenesis of IBV in the functional oviduct. Earlier studies have been conducted on laying hens using the Massachusetts strain of IBV (Sevoian and Levine, 1957) but still the basic causes of watery whites, thin-shelled eggs and cessation of egg production are unresolved. Also, the extent of pathogenesis of Australian strains of IBV for the reproductive tract is still not clear. Our preliminary studies have shown that Australian strains of IBV can cause severe pathology in the oviduct of unvaccinated (Chousalkar *et al*, 2006a) and vaccinated hens (Chousalkar *et al*, 2006b). The present study was undertaken to compare the degree of cellular pathogenesis in the fully functional oviduct of hens challenged with either T or N1/88-strains of IBV.

II. MATERIALS AND METHODS

Isa Brown day-old laying birds (150) were obtained from a commercial hatchery. At day-old, birds received Rispens vaccine against Mareks disease. The birds were divided equally into three groups, one control and two treatment groups. IBV-free status of the birds was maintained by isolation and strict biosecurity. The antibody free status of hens for IBV prior to the challenge experiment was confirmed by antibody ELISA (IDEXX). Birds were fed commercial broiler starter to 3 weeks of age, chick starter to 5 weeks followed by pullet grower up to 16 weeks and layer mash thereafter. All the hens were in full lay at 30 weeks of age when they were challenged with one of two different strains of IBV, T and N1/88 (obtained from Dr. Jagoda Ignatovic, CSIRO, Geelong). Two hens from each challenge group and one hen from the control group were euthanased at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 days post infection (p.i.). At the time that the birds were sacrificed, the position of the egg in the oviduct was noted. Two hens from the T infected group, which stopped laying on the 13th day p.i., were euthanased on the 30th day p.i. Different parts of the oviduct, infundibulum, magnum, isthmus, tubular shell gland (TSG) and shell gland pouch (SGP) were fixed in fixative (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate

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buffer, pH 5.5). The tissues were processed by standard electron microscopy procedures and embedded in epoxy resin. Semi-thin sections from 5 blocks of each tissue of individual birds were collected and stained with toluidine blue. Ultra-thin sections (80 to 90 nm) were then collected on grids and stained with a saturated solution of uranyl acetate followed by lead citrate in a carbon dioxide free environment. Sections were washed in CO_2-free water, allowed to dry and examined under a transmission electron microscope (Joel, JEM- 1200 EX). Some of the above samples, after fixation, were critical point dried, mounted on aluminium stubs, gold coated and observed under a scanning electron microscope (Joel, JSM -5800LV). Samples were also processed for histology as described earlier (Chousalkar *et al*, 2006).

III.RESULTS

a) Autopsy findings of the oviduct

Out of 24 hens killed from the N1/88 infected group, the oviduct and ovary were atrophied in one hen that stopped laying at the 12th day p.i. On the 18th day p.i., a fully formed egg was recorded in the infundibulum of another hen. The remaining 22 hens from this group had visibly normal and fully functional oviducts and ovaries. In the T-strain infected group, out of 24 hens, two hens, which had stopped laying from the 13th day p.i., showed thin and inspissated yolk material in the oviduct. However, their ovaries were functioning normally. No visible abnormality was recorded in the oviduct of the remaining hens except for the occurrence of meat spots in the magnum of 12 hens. None of the hens from the control group showed any abnormality.

b) Infundibulum

Pathology was not observed in the infundibulum of hens from the N1/88 group except for one with an atrophied oviduct. In T-strain infected hens, the histopathological findings in the infundibulum included infiltration of plasma cells and lymphocytes into the lamina propria. There was patchy loss of cilia from mucosal folds in both the anterior and posterior parts of the infundibulum from the 10th day p.i. onwards. The cilia loss was observed until the 14th day p.i. Most of the cilia appeared normal from day 16 until the end of the experiment. The posterior surface of the infundibulum of the hen with an atrophied oviduct was devoid of areas of granular cells. However, cilia loss was not observed in that hen. Patchy loss of cilia in the anterior and posterior infundibulum was observed in the hens which were still not laying even after the 30th day p.i. When tissues were examined under the electron microscope, there were increased deposits of endoplasmic reticulum, irrespective of egg position in the oviduct, in the surface epithelial and gland cell. Endocytosis of virus particles was observed on day 10 p.i. in the granular cells. Virus particles were also noticed in the golgi complex and rough endoplasmic reticulum (RER).

c) Magnum

The histopathological findings were similar to our earlier findings in unvaccinated hens except for the dilatation of tubular glands in N1/88-infected hens observed in the earlier study but not in the present study (Chousalkar *et al*., 2006). Scanning electron micrographs revealed loss of cilia which was extensive in the T group and patchy in the N1/88 group between 10 and 14 days p.i. Cilia in the magnum from both the challenge groups appeared normal for the remainder of the experiment. The loss of cilia was extensive in hens which had stopped laying. Changes observed by electron microscopy were increased deposits of rough endoplasmic reticulum, free ribosomes, and endocytic vesicles. Virus particles were recorded in the Golgi complex and dilated endoplasmic reticulum. Granular cells were devoid of

secretory granules. Both A and B type tubular glands were dilated and type C glands were inconspicuous. The microvilli of dilated glands were disrupted. The secretory granules in the dilated glands were gathered near cell surface and the Golgi complex was non functional. Virus particles were also recorded occasionally in cytoplasmic vesicles. After the 14th day p.i., the only findings were lymphoid nodules in the muscularis area and scanty secretory granules in granular cells in T-infected hens. There was degeneration of mitochondria in surface as well as glandular epithelial cells. Phagocytic vacuoles were frequent during 12 to 14 days p.i. The above changes were also recorded in the magnum of hens killed on the 30th day p.i. The tissues from all parts of the Infundibulum and magnum in control hens appeared normal throughout the experiment.

d) Isthmus

All parts of the isthmus of control hens appeared normal. No prominent microscopic lesions were recorded in the isthmus of the N1/88 group except for mild infiltration of inflammatory cells in the submucosa and muscularis area in one hen on the 16th day p.i.. In T-infected hens, there was patchy loss of cilia in two hens on the 10th and 12th days p.i., respectively. There was no glandular dilatation. Lymphoid nodules were observed in the submucosa in the isthmus of hens at 16, 20 and 24 days p.i. Severe pathology was recorded in the isthmus of hens from the T-infected group which had stopped laying. There was extensive infiltration of lymphocytes into the submucosa and large lymphoid nodules in the muscularis area. In granular cells, RER deposits were increased and some secretory granules were observed at the apex of individual cells. Virus particles were recorded in dilated cisternae of RER, dilated golgi vesicles and cytoplasmic vesicles in surface and glandular epithelium. There was patchy cilia loss and virus particles were occasionally recorded in these cells. Mitochondria appeared enlarged in both ciliated and mitochondrial cells. Type 2 gland cells were evident in all parts of the isthmus. The glands appeared dilated. Necrotic cells, along with an elongated mass of secretion, were observed in the lumen of some dilated gland cells. Phagocytic vacuoles were also observed in glandular cells.

e) Tubular shell gland and shell gland pouch

Most of the changes seen in the TSG and SGP were similar. Pathological changes were not recorded in TSG and SGP of hens from the N1/88 group. In T-strain infected hens, at 20 and 24 days p.i., large lymphoid nodules were recorded in the muscularis area. Severe pathology was observed in the TSG and SGP of hens which were out of production. Cilia loss and increased deposits of RER with virus particles were recorded in ciliated cells. Vacuoloids were not recorded in any granular cells of the SGP. The glands in both TSG and SGP were occasionally dilated. Mitochondria in non-ciliated cells, as well as glandular epithelium, appeared swollen. A few lipid droplets were also recorded in gland cells of the shell gland pouch. The apical border of most of the ciliated cells in the SGP and TSG appeared disrupted and all mitochondria had degenerated. The infiltration of plasma cells and lymphocytes into the submucosa was intense. The microvilli of the surface epithelium and glandular epithelium were not affected. All parts of TSG and SGP in control hens appeared normal.

IV.DISCUSSION

In the present study, the autopsy findings of the oviduct of hens which were out of lay are in accordance with the findings of Jones and Jordan (1972). The present study found that most of the hens recovered from infection and resumed laying but some hens stopped laying and this was associated with presence of virus particles in the oviduct. Such hens, despite appearing healthy, can act as persistent virus shedders. Histopathological findings regarding

the magnum are similar to our preliminary study except that gland dilatation in the magnum of N1/88 infected group and severe pathology was not recorded in the TSG and SGP of T-strain infected hens in the present study, other than in hens which were out of production. This could be attributed to a difference in age or breed of the hens.

Thinning of albumen also observed in this study could be due to dilatation of the tubular glands of the magnum and disturbance in physiology of the Golgi complex and RER. Absence of severe pathology in the lower part of the oviduct indicates that the upper oviduct is more susceptible. However, pathology in the oviduct of hens which were out of production suggests that IBV has the potential to infect all parts of the oviduct in some hens. Pathology in the tubular shell gland could be responsible for formation of abnormal mammillary cores which are the initial templates for the calcified shell, resulting in formation of a poor quality mammillary layer. Moreover, pathology in the shell gland pouch could result in alteration in mineral deposition during egg shell formation. All this could result in formation of wrinkled or thin shelled eggs. However, cytochemical studies are essential to clarify this. Such changes may not be common during infection with T and N1/88 strains of IBV in adult Isa Brown hens, as only two hens which were out of lay showed changes in the isthmus, TSG and SGP.

All the above findings support the view of McMartin (1968) that the response of individual hens during IBV infection varies greatly. Loss of cilia in most parts of oviduct could pose the potential risk of secondary bacterial infection and may affect fertility in breeder hens. It is still difficult to conclude the exact reason for cessation of egg production in some hens, but non-functional Golgi bodies, dilated RER and hence the possible alteration in protein synthesis of affected cells could be a contributory factor. Overall, T-strain was more pathogenic for the fully functional oviduct than N1/88 strain. In the present study, out of 50 infected hens in each challenge group, only three hens, one from N1/88 with a non-patent oviduct and two from the T-infected group with patent oviducts, were out of lay. By way of contrast, Crinion *et al*. (1971), found 26 % non layers with 83 % non-patent and 17% patent oviducts following infection with IBV as young chicks, which supports the view of Bradfoot *et al*., (1956) that the oviduct of younger chickens is more likely to be affected by IBV than older birds.

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EGG AND EGGSHELL QUALITY DURING EXPERIMENTAL IBV INFECTION IN UNVACCINATED LAYING HENS

K.K. CHOUSALKAR[1] and J.R. ROBERTS[1]

Summary

The effect of two strains of infectious bronchitis virus (IBV - T and N1/88 strains) on internal and external quality of eggs was studied in unvaccinated Isa Brown hens in full lay. Overall, there was no decline in egg production in either of the infected groups. Long-lasting effects were observed on egg internal quality of T strain-infected hens. Effects on internal quality in the N1/88 strain-infected group were more short term. The only significant effect of IBV infection on shell quality measurements was paler egg shells from the T-infected birds for the first 5 weeks post-infection.

I. INTRODUCTION

Infectious bronchitis virus (IBV) is a viral disease of poultry and one of the factors responsible for deterioration in egg production and quality. The disease is potentially a major threat to the egg industry as egg quality problems currently cost millions of dollars a year. In Australia, IBV was first reported by Cumming (1962). Australian strains of IBV are thought by some to be a cause of deterioratation of egg and egg shell quality but evidence one way or the other for this is lacking. The effects of Australian strains of IBV on the oviduct of laying hens have received little research attention. The present study was undertaken to study the effects of IBV on egg and egg shell quality in unvaccinated laying hens.

II. MATERIALS AND METHODS

150 day-old female Isa Brown chicks were reared under strict isolation and biosecurity and divided into three groups at 25 weeks of age. At 30 weeks of age, birds were exposed to one of two strains of IBV: T or N1/88 strains and one group was left unchallenged as a control. All eggs were collected at 3 and 2 weeks prior to challenge and then daily during the week immediately before infection to determine any inherent differences among the groups. Eggs were collected and analysed daily up to 5 weeks post infection (p.i.) and again at weekly intervals 6, 7, 8, 9 and 10 weeks p.i. All eggs were analysed for the internal quality parameters albumen height, Haugh Index and yolk colour score. Egg shell quality was measured as reflectivity, egg weight, deformation, breaking strength, shell weight, shell thickness and percent shell. Data were analysed by ANOVA and Fisher's protected LSD was used to distinguish differences between means. Significance was assumed at $P<0.05$.

III. RESULTS

a) Clinical findings

There were no significant effects of challenge or time in relation to challenge on egg production, although one hen from N1/88 and two hens from T-infected groups stopped laying from the second week p.i. Visual loss of shell colour was noticed in both infected groups from 4 to 8 days p.i. Some hens from both T and N1/88 infected groups showed coughing and sneezing from 3 to 9 days p.i. Feed intake did not vary significantly over the weeks after challenge or

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amongst the groups and there were no significant interactions. However, feed intake tended to be lower in the T-infected group from 2 to 4 weeks p.i. There were no visible deformities in egg shells in the N1/88 infected group, but there was an occasional occurrence of black spotted shelled eggs in the T-infected group. Eggs with meat spots and yolks that separated from the albumen during egg breakout were observed mostly between the 10th and 16th day p.i. in the N1/88 and T-infected groups.

b) Egg quality

Data are presented for egg quality measurements only where there was a significant interaction between IBV treatment group and time post-infection.

Egg weight, deformation and percent shell varied significantly over the 3 weeks before challenge. There was a significant main effect of treatment group and week of experiment on breaking strength. However, there was no interaction between treatment group and time post-challenge. The same differences between groups and time in relation to challenge were recorded post-challenge. Overall, shell weight and shell thickness increased significantly over the 10 weeks p.i. Such differences were not recorded before challenge. However, no significant difference was recorded between treatment groups. Over the ten weeks p.i., there were significant main effects and significant interactions between treatment group and weeks p.i. on albumen height and Haugh units (Fig 1).

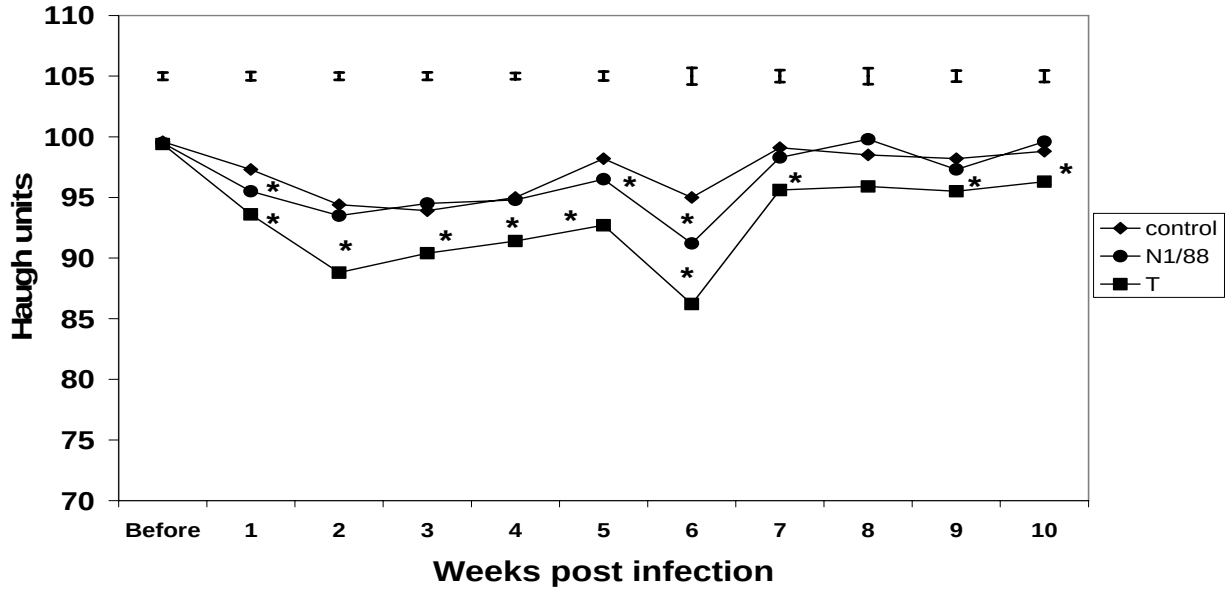

Fig 1. Haugh units before infection and for 10 weeks post infection for the three IBV treatment groups. Bars represent pooled standard errors. T and N1/88 strain means indicated by asterisk are significantly lower than the control group.

Such differences were not recorded within these parameters before challenge. Compared to control hens, the albumen height in N1/88 group was significantly lower at 2, 5 and 6 weeks p.i. and haugh units were significantly low on 1, 5 and 6 weeks p.i. Except for the 8th week p.i., albumen height and haugh units were significantly lower from the first week until the end of the experiment in the T-infected group. Overall there were no significant differences amongst the infected groups for yolk colour score but there was a significant variation over the weeks in relation to challenge and a significant interaction between group and week of experiment. Yolk colour score was significantly lower in T-infected hens from 2 to 4 weeks p.i. (Fig 2).

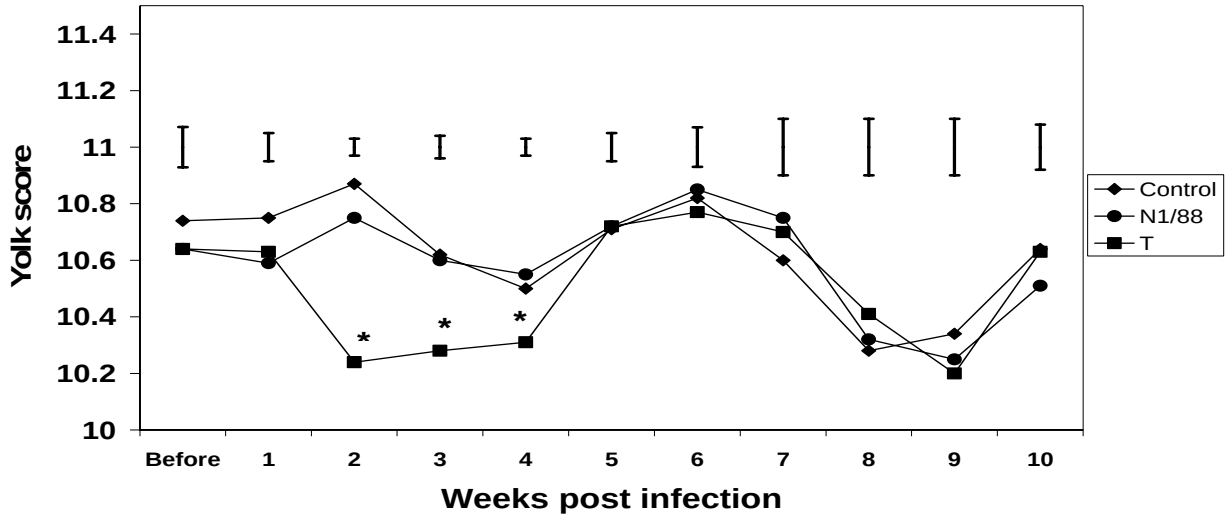

Fig 2. Yolk score before infection and for 10 weeks post infection for the three IBV treatment groups. Bars represent pooled standard errors. T strain means indicated by asterisk are significantly lower than the control group.

Over the 10 weeks after challenge, there were significant main effects of treatment, time in relation to challenge and a significant interaction between treatment group and time on shell reflectivity (Fig 3). In comparison with the control group, shell reflectivity was significantly higher in the N1/88 group in the 2nd week p.i. whereas, in the T-infected hens, reflectivity was significantly higher from weeks 1 to 5 p.i.

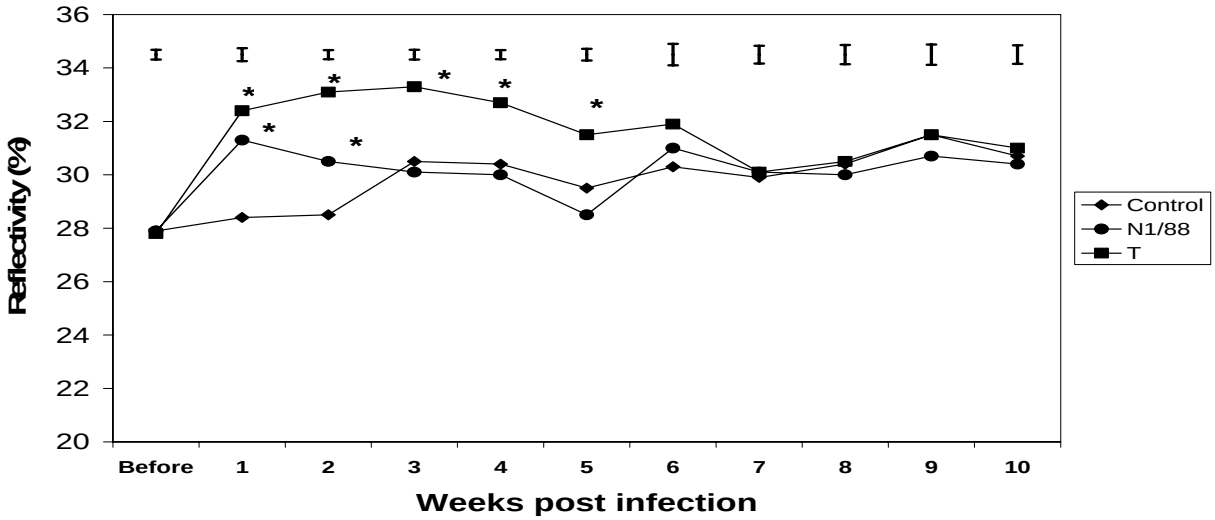

Fig 3. Shell reflectivity before infection and for 10 weeks post infection for the three IBV treatment groups. Bars represent pooled standard errors. T and N1/88 strain means indicated by asterisk are significantly higher than the control group.

IV.DISCUSSION

There were no significant effects on egg production of the infected groups although mean production over the 10 weeks p.i. was highest in the control group (95.0%) and lower in the T (91.6%) and N1/88 (92.0%) groups. The relatively small number of birds in each treatment

group made detection of differences in production due to IBV difficult. IBV clearly has major effects on albumen height and Haugh units and these parameters in the T infected group were significantly lower than controls from the first week p.i. until the end of experiment, except in the 8 weeks p.i. indicating prolonged effects of IBV. The fluctuation in HU the N1/88 group could be due to selective removal of hens showing low albumen height for other studies. This finding is consistent with results of our study regarding ultra structural changes in the magnum during T and N1/88 infection and indicates that both strains of IBV have trophism for the upper reproductive tract. Our findings support the view that IBV is associated with thinning of albumen (Sevoian and Levine, 1957). The significantly lower yolk score in T-infected hens from the 2 to 4 weeks p.i. could be attributed to the transient decrease in feed intake observed in the T group. However, feed intake did not vary significantly within the treatment groups or over the weeks p.i.. Similar findings regarding feed intake were recorded by Roberts (2005).

The significant increase in shell reflectivity in the T-infected group was reported earlier by Jolly *et al*, (2005) in vaccinated HyLine Brown and HyLine Grey hens; there was, however, no effect in vaccinated Isa Brown hens. Temporary loss of shell colour from N1/88 infection has not been reported previously. Reduction in shell colour during IBV infection with other strains has been reported in the past (Cook and Huggins, 1986). In the present experiment reflectivity was measured using a sensitive shell reflectivity meter and pale shells were visibly recorded only between 4 and 8 days p.i. Paler egg shells may not be regarded well by Australian consumers.

Other measures of egg shell quality did not vary significantly. The trend towards the hens in the N1/88-infected group having lower egg weight and higher breaking strength may be due to inherent differences among hens that were present before challenge, although there was no significant variation in egg weight within the groups. Deformation did not alter within the group, a finding that is in accordance with the study of Roberts (2005). Increase in shell weight and shell thickness towards end of the experiment could be due to increase in egg weight and such findings were reported earlier in ISA brown hens by Leary (1999). There was no obvious pattern in shell percentage.

It can be concluded from the study that challenge with either T or N1/88 strains of IBV results in a deterioration in albumen quality and an increase in shell reflectivity which may be relatively short-term for N1/88 strain infected hens but more prolonged in T strain infected hens. This finding explains the uterotropism of Australian strains of IBV for the fully functional oviduct.

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IDENTIFICATION OF AUSTRALIAN POULTRY REARING REGIONS AT HIGH RISK OF AVIAN INFLUENZA DISEASE INCIDENTS

I.J. EAST[1], and S.A. HAMILTON[1]

Summary

Since 2003, highly pathogenic avian influenza due to H5N1 virus (HPAI) has been reported from both domestic poultry and wild birds in 59 countries and has resulted in the direct death or slaughter of over 200,000,000 birds. The risk of entry of this virus into Australia has been assessed as low but real. Resources for surveillance within Australia's estimated 2000 commercial poultry farms are limited and thus identification of high risk areas in order to more effectively target surveillance effort is an urgent priority. This paper describes a GIS-based spatial analysis of risk factors for the occurrence of HPAI and identifies the poultry producing regions of Australia at greatest risk. The risk factors examined included the presence of wetlands suitable for wild bird habitat, wild bird distributions and the historical distribution of known poultry diseases. The model was validated by assessing its ability to predict the locations of the five previous known outbreaks of highly HPAI in the Australian poultry industry.

I. INTRODUCTION

In the past 12 months, H5N1 avian influenza (HPAI) has spread beyond China and south-east Asia to 59 different countries (OIE., 2006) and, the Food and Agriculture Organisation of the United Nations has estimated that the current pandemic of HPAI has resulted in the direct death or slaughter of over 200,000,000 birds (FAO, 2006). The closest occurrences to Australia thus far have been at Manokwari in Irian Jaya Barat province of Indonesia and at Timika in Papua province of Indonesia. Migratory shorebirds have been implicated in the spread of HPAI to Russia, Europe and Africa and, the involvement of migratory birds provides a potential pathway for HPAI to spread from south-east Asia along the Australasian flyway to Australia (Tracey *et al.,* 2004). The most likely pathway of introduction of HPAI into Australian commercial poultry flocks is believed to be the transfer of HPAI from migrating shorebirds to native duck species that subsequently interact with poultry on low security poultry farms. Concern over this route of transfer has lead several European countries including Germany and the Netherlands to mandate that all free range poultry be kept housed during the higher risk migration season (Pro-Med, 2006). Recognised risk factors for HPAI outbreaks on poultry farms include contact with migratory waterfowl and low levels of farm biosecurity (Alexander, 1995; Capua et al., 1999; Tracey et al., 2004).

Early detection of an incursion of HPAI will rely upon an effective surveillance program and in recent times, surveillance for HPAI within Australia has been extended to include wild birds, commercial poultry and the sentinel chicken flocks maintained by various State/Territory Departments of Health. However, with over 2000 commercial poultry farms in Australia, it is not possible to maintain constant surveillance coverage of all farms. This paper describes the conduct of a spatial analysis to determine a relative risk ranking for the introduction of HPAI into the poultry farming regions of Australia.

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II. METHODS

Spatial data sets of the location of significant wetlands within Australia and significant shorebird habitat areas within Australia were provided by the Department of Environment and Heritage. Spatial data sets of geographic features, including locations of lakes, dams and areas of native vegetation, were obtained from GEODATA TOPO 250K Series 3 (GeoSciences Australia, Canberra, Australia) and spatial data sets of wild bird distributions were provided by Birds Australia (Hawthorn East, Victoria). Information on the location of commercial poultry farms was sourced from an unpublished study on movements of poultry and poultry products conducted by Scolexia Animal and Avian Health Consultancy for the Australian Government – Department of Agriculture, Fisheries and Forestry. Where location data of farms was incomplete, the location of farms was determined using the 'White Pages on-line' (http://www.whitepages.com.au/wp/index.jsp), multi-map.com (http://www.multimap.com.au/) and Google maps (http://maps.google.com/). Subsequently, the list of poultry farms generated was cross-checked against lists maintained by the State/Territory Departments of Primary Industries and any discrepancies resolved. The distribution of poultry farms with evidence of infection with either Newcastle disease virus (NDV) or Marek's disease virus were obtained from the national survey of 753 Australian chicken farms for NDV that was conducted in 2000 (East *et al*., 2006).

Mapping studies were completed using MapInfo version 8.5 (MapInfo Corp., Troy, New York). Data sets for each risk factor were converted into raster maps and these maps were subsequently manipulated to produce the final risk map using the Vertical Mapper add-on software package for MapInfo.

III RESULTS

The poultry study conducted by Scolexia identified a total of 2025 poultry farms in Australia of which 142 were either temporarily or permanently closed. These farms were scattered throughout Australia with a distribution similar to that of Australia's human population (Figure 1).

Based on the predicted route of introduction of HPAI into Australia through migratory birds and native ducks (Tracey, 2004), a range of risk factors for the location of HPAI outbreaks were examined. The models examined thus far have included measures of surface water and wetlands, wild bird distributions, location of poultry farms and the historical distributions of NDV and Marek's disease on Australian farms. An example of the risk models produced thus far is shown in Figure 2. This model is based on historical outbreaks of Newcastle disease and Marek's disease and identifies areas of higher relative risk that are coincident with the locations of the five known historical AI outbreaks in Australia (Figure 2). Further models will be presented and discussed during the presentation.

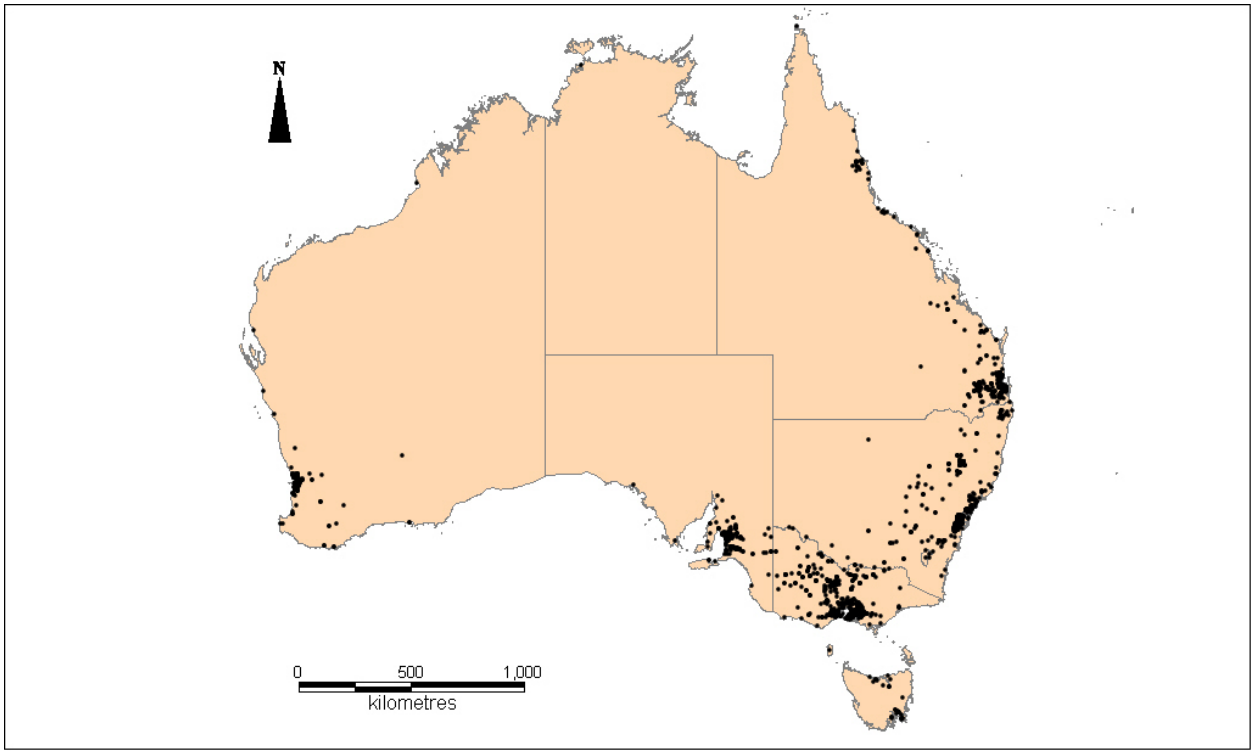

Figure 1. Map of Australia showing the locations of all Australian commercial poultry flocks.

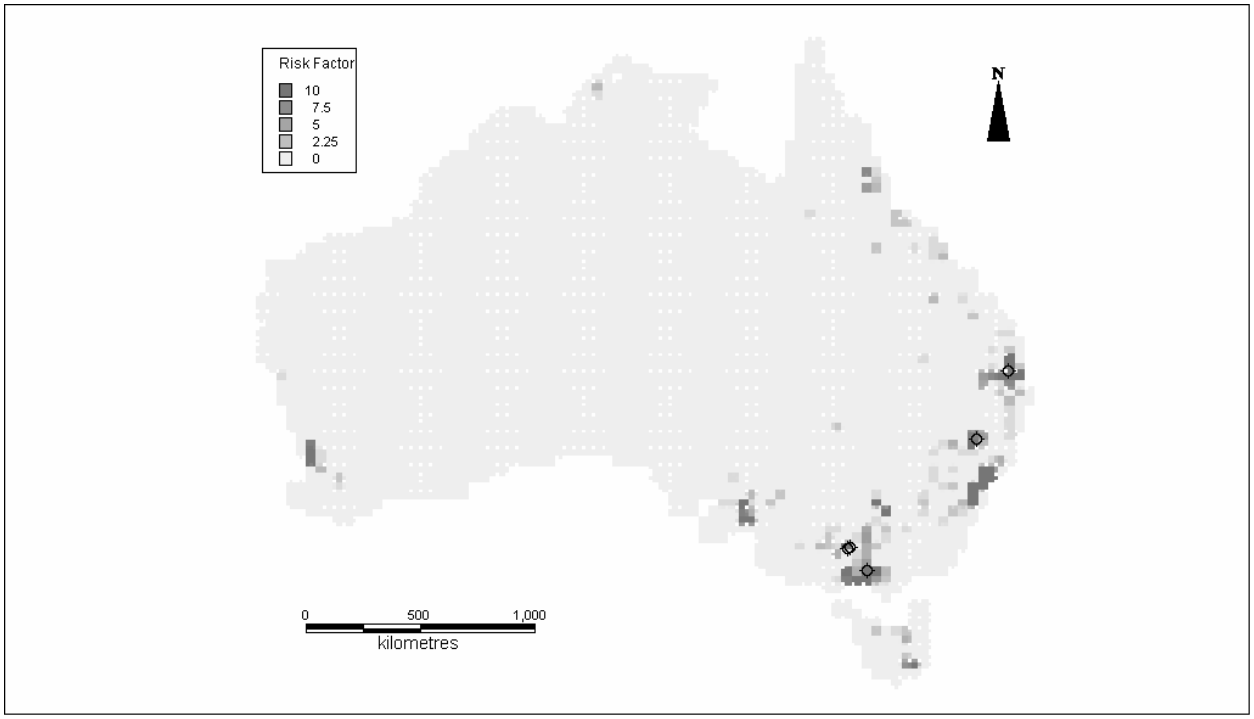

Figure 2. Map of Australia identifying relative risk for avian influenza introduction to commercial poultry and locations of previous known outbreaks of HPAI in Australian poultry flocks (◊).

IV DISCUSSION

Our model predicts areas of Australia that are at a higher relative risk for HPAI infection in commercial poultry and these areas include the location of all five previous known outbreaks of avian influenza in Australia. This suggests that high risk areas for incursions of HPAI in commercial poultry flocks can be successfully predicted. However, these predicted regions include a substantial proportion of Australia's commercial poultry farms and, further work needs to be conducted to identify the farm-level risk factors that make individual farms within these regions vulnerable to infection with AI. Future studies will examine the biosecurity, hygiene and management practices on individual farms that make them more or less susceptible to infection with HPAI.

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SIMULATING THE SPREAD OF HIGHLY PATHOGENIC AVIAN INFLUENZA BETWEEN VACCINATED CHICKENS IN CAGE PRODUCTION SYSTEMS

S.A. HAMILTON[1,2], I.J. EAST[1] and M.G. GARNER[1]

Summary

Emergency vaccination can be useful in eradication programs for highly pathogenic avian influenza (HPAI). However there is a concern that HPAI could circulate inapparently within vaccinated flocks, because vaccination protects against clinical disease but does not induce sterile immunity. Hence animal health organisations advise that vaccinated flocks should be actively monitored for HPAI through serological surveillance or the placement of unvaccinated sentinel chickens within flocks. This paper describes the development of a stochastic state transition model to examine the effect of vaccination efficacy upon the ability of sentinel surveillance schemes to detect HPAI outbreaks in caged chicken flocks. Preliminary results show that even though the time to the detection of an outbreak increased for more effective vaccination strategies, the number of birds that had been infected by the time the disease had been diagnosed decreased because of slower transmission of infection. This would presumably lead to a reduction of the amount of HPAI contamination in the environment, reducing the risk of the infection spreading to other farms.

I. INTRODUCTION

There is controversy regarding the use of vaccination to control the spread of H5N1 highly pathogenic avian influenza (HPAI) in chickens. This is because even though vaccinated chickens have reduced susceptibility to HPAI, are less infectious to other birds, and are protected from developing clinical disease, they can still become infected and shed virus (Swayne, 2003). Hence there are concerns that infection may spread inapparently within vaccinated flocks. Additionally, levels of flock immunity after field vaccination may also vary. In a trial conducted in Hong Kong, 24.2% of 248 flocks had less than 70% of chickens with an adequate haemagglutination inhibition (HI) titre ($\geq$16) after vaccination (Ellis *et al*., 2006). However, there is evidence that chickens with low HI titres (10-40) may have an altered clinical course of disease compared with unvaccinated birds (Kumar *et al.*, in press). Because of this uncertainty, animal health organisations recommend the implementation of active surveillance schemes to monitor vaccinated flocks for evidence of HPAI (FAO and OIE, 2005). One scheme is to place a minimum of 100 unvaccinated sentinel chickens in vaccinated flocks so that they can be monitored for clinical signs (i.e. mortality) and/or laboratory evidence of infection with HPAI (European Commission, 2006).

Experimental studies suggest that HPAI is spread in cage production systems through direct contact between infectious and susceptible birds in adjacent cages (Shortridge *et al*., 1998). This is supported by field studies which have noted that the disease may affect birds in localised areas of a shed before spreading to birds in adjacent cages (Beard, 1998).

In this paper we present preliminary results of a simulation model designed to investigate the potential impacts of varying levels of vaccination efficacy on the dynamics of HPAI transmission in a flock of caged chickens.

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II. METHODS

A stochastic state transition model was developed to simulate the spread of HPAI between individual chickens in daily time steps. Chickens are represented individually in the model and exist in one of five disease states: *Susceptible*; *Latent*; *Infectious*; *Immune* or *Dead.* Three different vaccination states are also included in the model: *Not Vaccinated* (chickens that have not been vaccinated in a flock); *Fully Vaccinated* (chickens that develop adequate HI titres after vaccination); and *Partially Vaccinated* (chickens that develop inadequate HI titres after vaccination). The model is designed so that disease may be spread between birds in the same cage; to birds within adjacent cages on the same tier; to birds in the cage below; and to birds in distant cages through contact with contaminated fomites. Individual chickens transition between disease states after certain trigger events occur, such as direct or indirect contact with an infectious chicken or at the end of the latent or infectious periods (Figure 1). Parameters affecting these transitions are estimated for each chicken from pre-determined probability distributions. Case fatality ratios, levels of susceptibility and distributions of latent and infectious periods vary according to the vaccination status of each chicken. Transmission parameters are weighted according to the vaccination status of the *Infectious* and *Susceptible* birds. Parameters used in this model have been estimated from published challenge or transmission studies using H5N1 HPAI virus or from expert opinion (see Appendix).

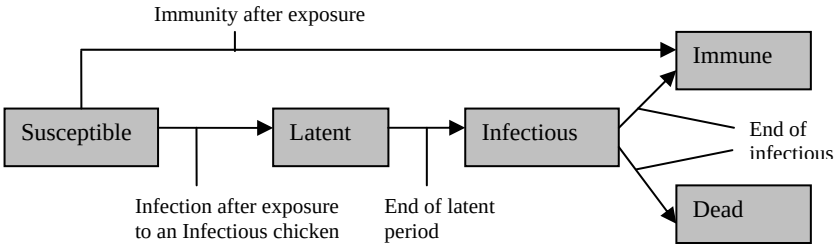

Figure 1. States and transitions in the HPAI model.

Simulations were run using a flock of 9600 chickens kept in 2400 cages that were arranged spatially to emulate the typical structure of a chicken meat farm in Hong Kong. At the start of each iteration, 100 sentinel chickens were placed in randomly selected cages. All other birds in the flock were then probabilistically determined to be *Fully Vaccinated* or *Partially Vaccinated*, using a parameter representing vaccine effectiveness, i.e. the probability that an individual chicken would be *Fully Vaccinated* after vaccination (P_{fv}). Infection was seeded into the model by exposing all birds in a randomly selected cage to disease. Each iteration continued until the disease transmission cycle had ended or a sentinel chicken died. P_{fv} was varied (P_{fv} = 0.5, 0.6, 0.7, 0.8 and 0.9) and the effect upon the proportion of outbreaks that were detected by sentinel surveillance, the time until detection and the number of birds infected during the outbreak were recorded. One hundred iterations were carried out for each value of P_{fv}.

III. RESULTS

The probability of detection was inversely related to the proportion of *Fully Vaccinated* birds (Figure 2). As P_{fv} increased from 0.5 to 0.9, the proportion of outbreaks that were detected through sentinel surveillance decreased from 100% to 78%. The effect of P_{fv} on the distributions of the time until an outbreak was detected through sentinel surveillance is presented (Figure 3a) showing that distributions became more positively skewed as P_{fv}

increased up to 0.9. The median number of birds infected by the time disease was detected decreased from 291 to 13 as P_{fv} increased from 0.5 to 0.9 (Figure 3b). Where the outbreak died out without being detected, relatively few birds were affected: only 5 of 30 outbreaks that died out spread beyond the index cases and the maximum number of birds infected was 12.

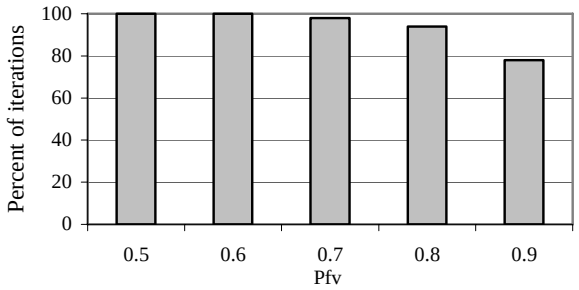

Figure 2. Percent of iterations in which the outbreak was detected

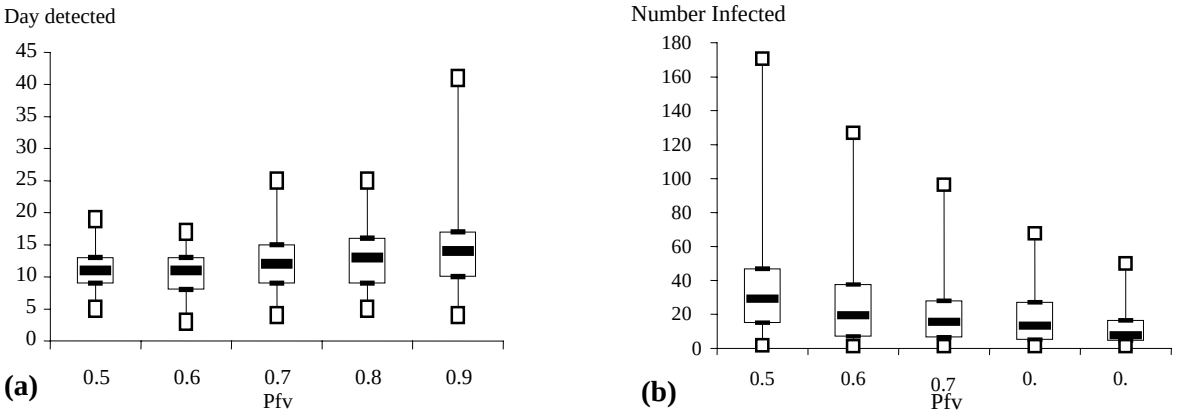

Figure 3.
(a) Quartile plot of the time to detection; (b) Quartile plot of number of birds infected at detection

IV. DISCUSSION AND CONCLUSION

An ideal surveillance system for HPAI in vaccinated flocks would allow the prompt detection of incursion of the infection, before it could spread to other farms. These results show that even for very high levels of flock immunity, surveillance of sentinels appears to be a sensitive mechanism for the detection of HPAI. Although the time until detection tended to increase as vaccination effectiveness increased, this needs to be discussed in the context of spread of infection. As vaccination effectiveness increased, there was a trend towards lower numbers of birds being infected because the disease spread more slowly. This indicates that even though the disease may have been circulating in a flock for some time before detection the actual level of virus production, and hence the level of environmental contamination, will be lower as vaccination effectiveness increases. This would presumably reduce the risk of the disease spreading to other flocks.

Caution must be exercised when extrapolating these preliminary results to the field situation because parameter values have been estimated from a range of experimental studies that used different H5N1 virus isolates and only one transmission study involving vaccinated chickens was found. Furthermore, only one introduction scenario (exposure of all birds within one cage) was used for this study and it is possible that disease may be introduced to

larger numbers of birds, leading to higher incidences of infection. Further work will include modifying the model to incorporate other methods of passive surveillance, to investigate sampling strategies for serological surveillance schemes and to undertake sensitivity analysis on the flock structure and introduction scenarios.

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APPENDIX

Parameter	Value / Distribution	Reference / Comment
Chickens per cage	4	Chow, *pers. comm.*
Number of rows	6	Chow, *pers. comm*
Number of columns	100	Chow, *pers. comm.*
Number of cage banks	4	
Prob of spread within cage	0.5	Est. from Shortridge *et al*. 1998
Prob of spread to adjacent cage	0.5	
Daily P of spread to lower cage	0.3	Est. from Shortridge *et al*. 1998
Birds exposed in distant cages	Poisson (0.5)	Per infectious bird per day
Relative susceptibility (*Fully Vaccinated*)	0.8	Bublot et al. *In Press*
Relative susceptibility (*Partially Vaccinated* and *Not Vaccinated*)	1	
Latent period (*Not Vaccinated*) (days)	1	
Latent period (*Partially Vaccinated*) (days)	Triangular (min=1, mode=3, max=5)	Sims, *unpublished data*
Latent period (*Fully Vaccinated*) (days)	Triangular (min=1, mode=3, max=5)	
Infectious period (*Not Vaccinated*) (days)	Triangular (min=1, mode=1.5, max=2)	Est. from Shortridge *et al*. 1998
Infectious period (*Partially Vaccinated)* (days)	Triangular (min=1, mode=2.5, max=4)	Sims, *unpublished data*
Infectious period (*Fully Vaccinated*) (days)	Triangular (min=1, mode=2.5, max=4)	

Relative risk of spread between infected and susceptible birds

From:	To: *Fully Vaccinated*	*Partially Vaccinated*	*Not Vaccinated*	Reference
Fully Vaccinated	0.1	1	1	Bublot *et al*. In Press
Partially Vaccinated	0.8	1	1	
Not Vaccinated	0.8	1	1	Bublot *et al*. In Press

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