

Variation in the digestive capacity of the broiler chicken

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Summary

A diet for broiler chickens that provides the nutrients essential for maintenance and growth of the flock as a whole may also have chemical and physical properties which are detrimental to the processes of ingestion, digestion, absorption, transport and utilization of nutrients. The digestive capacity of the chicken can be modelled according to interactions between feed-related and bird-related factors known to affect energy metabolism.

The results of recent experimental work in Australia to examine these interactions provide evidence that gut morphology and bacterial colonisation of the gut are at least partially dependent on the sex of the chicken. Clearly, gut microflora have a highly significant impact on between-bird variation in the digestive capacity of the broiler chicken. This has very important commercial implications for the nutrition and management of broilers. Sex-related differences may be important in uptake and utilization of energy and other nutrients, and in responses to anti-nutritional factors, feed enzymes, prebiotics, probiotics, other feed additives, and vaccinations against gut pathogens.

Keywords: broilers, dietary energy, digestion, microflora, intestine, gut morphology

Introduction

The Australian chicken meat industry is highly dependent on dietary energy from wheat and barley which are known to vary widely in nutritive value (Hughes and Choct 1999). For example, surveys by Mollah *et al.* (1983) and Rogel *et al.* (1987) indicated a range in apparent metabolizable energy (AME) values of 10 to 16 MJ/kg DM. Hughes and Choct (1997) reported values ranging from 9 to 15 MJ/kg DM for a single sample of wheat containing a high level of soluble arabinoxylan when given to 40 individually caged broilers hatched and reared under identical conditions. Hughes *et al.* (2001a) observed AME values ranging from 12.3 to 13.5 MJ/kg DM for a single sample of

barley fed to 96 single-sex groups of six broilers. Hence it can be concluded that the 'low ME' phenomenon in wheat and barley is a multi-faceted problem involving highly variable responses by individual chickens to anti-nutritive factors in grains.

This paper reviews the various feed-related and bird-related factors affecting energy metabolism in chickens and develops the hypothesis that these factors interact to influence the digestive capacity of the individual chicken in a variable manner.

Feed-related factors influencing energy metabolism

In a recent review, Hughes and Choct (1999) concluded that grains such as wheat and barley, combined with legumes and oilseed meals, provide not only the bulk of essential nutrients for commercial poultry production and reproduction, but are also the prime source of anti-nutritive components which are likely to have significant bearing on how effectively all dietary components are utilized by poultry. Sources of variation in the physical and chemical characteristics of grains used in poultry diets include variety, seasonal effects and growth sites, crop treatment and grain fumigants, and post-harvest storage conditions and period of storage. Variation in the available energy and protein content of grains can be attributed to a wide range of anti-nutritive factors such as non-starch polysaccharides (NSP), tannins, alkyl resorcinols, protease inhibitors, α -amylase inhibitors, alkaloids, phytohaemagglutinins, saponins, and lathyrogens. The relative importance of such factors will differ between types of grain.

Of the known anti-nutritive components of grains, soluble NSP stand out as a major determinant of the availability of energy and other nutrients for poultry (Choct 1999; Hughes and Choct 1999; Hughes *et al.* 2001b). One of their modes of action is to form a viscous gel in the gut which in turn affects the rates of digestion and absorption of nutrients. Also, rates of gastric emptying and transit time of digesta are thought to be

influenced by increased digesta viscosity, thereby providing hind-gut microflora with an opportunity to colonise the small intestine to the detriment of the host in terms of diminished use of nutrients and reduced ability to ward off ingested pathogens (Choct 1999).

Bird-related factors influencing energy metabolism

Tivey and Butler (1999) described the digestive capacity of an animal as the integration of residence time of digesta, enzyme secretion, absorptive mechanisms, microbial activity, surface area, and barrier function. The importance of each of these determinants of digestion and assimilation of nutrients is discussed in relation to chickens in the following sections.

Gut structure

Net utilization of energy by the chicken will be influenced by the requirements of the gut for growth and maintenance (Choct 1999) and by its total surface area. The latter will be determined by gross morphological features such as length and cross-sectional area of the duodenal, jejunal and ileal segments, and by finer morphological features such as villus height and surface area of the epithelium in each of those segments (Jin *et al.* 1998; Iji 1999).

Rate of passage of digesta

The extent of opportunities for contact between ingested food, digestive enzymes and bile salts, and the time available for contact between digested particles and absorptive surfaces are likely to influence energy uptake by the chicken. Rate of gastric emptying of solids and liquids, and transit time in the small intestine are known to influence the nutritional status of human subjects (Tivey and Butler 1999). Growth performance and nutrient absorption are influenced by rate of passage of digesta in chickens (van der Klis and van Voorst 1993; Uni *et al.* 1995) and rats (Gohl and Gohl 1977).

Clench and Mathias (1992) observed a reversal of the flow of digesta in response to fasting in adult cockerels. They described the phenomenon as a rhythmic oscillating complex which could be an adaptive mechanism for the return of undigested food in the caeca to the small intestine during a period of inadequate food intake. Godwin and Russell (1997) noted that the reverse peristalsis was highly effective in fasted birds but appeared to have little effect in the fed animal. The reversal of flow of digesta could result in the entry into the small intestine of uric acid, other potentially toxic waste products, and harmful micro-organisms. The reflux of uric acid could have a detrimental effect on gut epithelial function. Langar *et al.* (1993) reported a reduction in villus height in the small intestine with an associated decline in nutrient absorption in poultry given diets containing 1 to 1.75%

uric acid. On the other hand, reflux of volatile fatty acids with bacteriostatic action (Corrier *et al.* 1990) may be beneficial in blocking microbial proliferation in the small intestine. Furthermore, Choct and Kocher (2000) reported that the caecal flora of the broiler chicken produce some xylanase and β -glucanase which may be refluxed into the small intestine where these enzymes could cause variable gut viscosity. The nutritional implications of bacterial enzyme activity in the gut warrant investigation.

Digestion and absorption

Capacity to digest and absorb carbohydrates develops during incubation, providing the newly hatched chick with a relatively mature system for utilization of starch (Moran 1985) which is the main carbohydrate in the diet of poultry. On the other hand, the capacity to utilise fat can take 10 days or so to develop in broiler chickens due to a lag in lipase secretion by the pancreas (Jin *et al.* 1998).

Uni *et al.* (1995) reported differences in ability to digest starch in the period 0 to 4 days post-hatch between two strains of broiler chicken. However by day 14, starch digestion was greater than 90% in both strains. These changes are consistent with those noted by Vieira and Moran (1999) who concluded that the full capacity of the small intestine to digest and absorb nutrients took up to two weeks to develop. They attributed this in part to the initial orientation of enterocytes towards absorption of maternal antibody from remnants of the yolk sac. In contrast, Uni *et al.* (1996) had concluded that nutrient supply from yolk was less crucial than a lack of feed in the first 36 h post-hatching, which subsequently delayed normal intestinal development for several days.

Uni *et al.* (1998) studied changes in the structure and function of the duodenum, jejunum and ileum in broilers from hatch to 14 days of age. They noted that development of the small intestine was rapid from day 2 after hatch but that the rates of development differed between the segments of the small intestine. Villus volume in the duodenum reached a plateau after 7 days but continued to increase in the jejunum and ileum. Indices of tissue activity, ribosomal capacity, and cell size decreased with age but at differing rates in these intestinal sections. Sucrase-maltase activity was low in the duodenum at hatch, increased to a maximum at day 2, then decreased. Enzyme activity in the jejunum and ileum was at its highest at hatch. Density of the enterocytes changed little from 0 to 14 days post-hatch.

As with the physical changes in gut structure observed in the two-week post-hatch period, major biochemical changes in the development of the gut also seem to have stabilised within a relatively short time after hatch. Nevertheless, it seems plausible that the subtle differences in gut structure and function between individual chickens may be sufficient to affect the uptake of energy-yielding nutrients in a variable manner.

The effect of the sex of the individual animal on its functional capacity to digest and absorb nutrients has received little attention by researchers. Indeed, much of the knowledge about nutrient utilization by commercial broiler chickens has been gained from study of males only. Yet there are tantalising hints in the scientific literature that males and females differ in unexpected ways. Sex effects in chickens were noted by Guirguis (1975, 1976) in metabolism of energy in oats but not in wheat, and energy in tallow and fish meal. More recent examples include the transient post-weaning differences observed in piglets by Dunshea *et al.* (1998), and the conclusion of Chicurel (2000) that “studies in several organisms have shown that gender and environment affect the behaviour of genes”.

Barrier function

Like other epithelial surfaces of the body, one of the functions of the gut mucosa is to resist penetration by harmful organisms and toxic substances. Lillehoj (1997) described the immune response by gut-associated lymphoid tissues to microbial pathogens as a complex interaction of soluble factors, leucocytes, epithelial cells and other physiological mechanisms.

Klasing (1996) discussed the homeorhetic responses which take place when a chicken is faced with a bacterial challenge. These include decreased appetite, the partitioning of dietary nutrients away from growth, skeletal muscle accretion in favour of metabolic processes which support the immune response and disease resistance, and alteration of nutrient requirements during and after the infectious challenge. During the challenge, the requirements for amino acids and most trace minerals are decreased whereas these are increased later to repair damaged tissue and to accelerate growth. Klasing (1996) recommended an increase in dietary carbohydrate to compensate for the decrease in appetite in order to assist the recovery and lift the performance of immune-stressed animals.

Microflora

It is likely that dietary factors which lead to increased activity of gut microflora will depress energy utilization (Choct *et al.* 1996; Choct 1999), apparent protein digestibility (Smits *et al.* 1997), and availability of amino acids (Steenfeldt *et al.* 1995).

Inclusion of an antibiotic in the diet of pigs can result in a reduction in the weight and the morphology of the small intestine (Parker and Armstrong 1987). These changes were represented by elongated villi and a higher villus:crypt ratio, which was indicative of a lower rate of enterocyte-cell migration from the crypt to the villus. It was suggested that reduced microbial activity in digesta or microbial activity at the level of the brush border would reduce both the damage to enterocytes and the need for cell renewal in the gut.

Williams (1995) has pointed out that gut microflora can significantly influence metabolism of gut tissue

which in turn will affect absorption of amino acids. Protein supplements with poor digestibility will undergo more microbial fermentation than highly digestible material. For example, differences between ileal and faecal digestibilities in intact compared with caeectomised cockerels were minor for cereals and oilseeds, but were large for some animal meals. Nevertheless, relatively small differences between ileal and faecal digestibilities in grains observed by Williams (1995) could become significantly more important when comparing differences between different samples of grain.

Smits (1996) provided unequivocal evidence that the mechanism by which soluble NSP depress fat digestibility in chickens hinges on the reduction of bile salts following bacterial proliferation or overgrowth of the small intestinal contents. He also demonstrated that reduction in fat digestibility was particularly severe in the case of animal fats which contained a high proportion of saturated long chain fatty acids. It seems likely that withdrawal of other essential nutrients by microbial proliferation would immediately compromise the growth performance and feed efficiency of the animal, and ultimately lead to health problems through general inflammation of the gut and invasion of tissue by pathogenic organisms.

The concept of competitive exclusion (CE), as proposed originally by Nurmi and Rantala (1973), involves the establishment and maintenance of a normal population of gut microflora to afford protection from colonisation by organisms pathogenic to chickens (e.g. *Salmonella* spp.) or to humans (e.g. *Campylobacter* spp.) or to both. The topic of CE has been reviewed recently (Cox and Chung 2000). The original concept of CE can be widened to include non-living entities such as fructo- and mannan-oligosaccharides. Iji and Tivey (1998) recently reviewed the role of oligosaccharides in the regulation of gut microflora. They suggested that the regulatory mechanisms involving oligosaccharides could include (a) provision of alternative binding sites for pathogens, thus preventing invasion of the gut tissue, (b) direct stimulation of the blood immune system after crossing the intestinal mucosa barrier, (c) preservation of the systemic immune system by blocking translocation of pathogens, (d) fermentation of carbohydrates to produce short-chain volatile fatty acids with bacteriostatic properties, (e) direct stimulation of the villus-crypt axis, and (f) induction of intestinal microflora to produce glycolytic enzymes.

Concept of digestive capacity of the individual chicken

It is evident from the preceding sections that feed- and bird-related factors interact in a complex manner. The digestive capacity of an individual chicken can be conceptualised as shown in Figure 1.

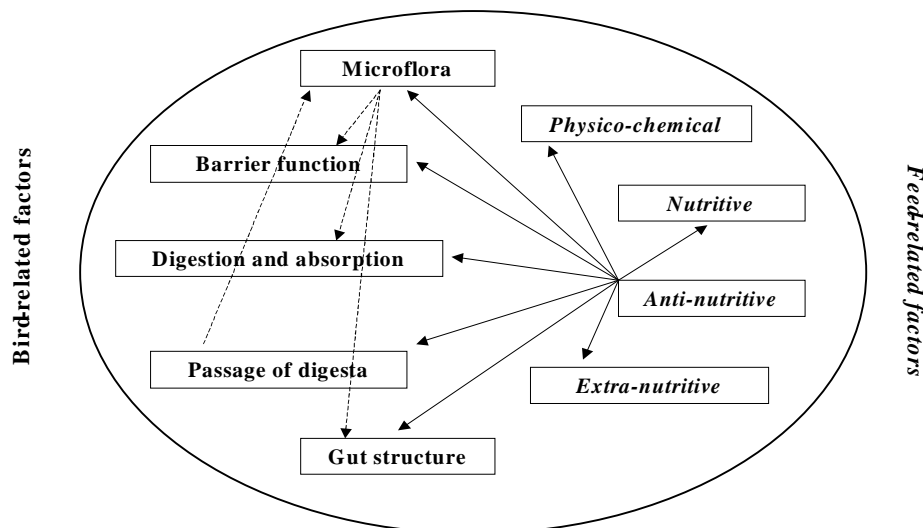


Figure 1 Digestive capacity of an individual chicken.

The particular example shown in the diagram represents the anti-nutritive effects of soluble NSP (feed-related factor) acting on all other factors. It also shows a cascading effect of metabolic activity of microflora on three other bird-related factors, as well as an effect of changed rate of passage (due to increased viscosity associated with soluble NSP) on microbial proliferation in the small intestine.

Some results from recent Australian studies to examine the effects of NSP on gut morphology and gut microflora are discussed in the following sections.

Recent findings on gut morphology

The results of recent studies by Hughes and coworkers at PPPI on probable causes of intestinal dysfunction in commercial broilers strongly imply that there is a highly significant bird component in the problem. These observations are consistent with the hypothesis that wide between-bird variation in gut function persists in commercial breeds of broiler chickens despite heavy selection for economically important traits such as lean tissue growth and feed efficiency, and despite usage of feed enzymes in broiler diets. Furthermore, recent experimental evidence from PPPI supports earlier findings by Guirguis (1975, 1976) that fundamental differences exist between males and females in their respective capacities to digest and absorb energy. These aspects were investigated in a 7-day metabolism study with chickens 21 or 22 days of age at the start; values obtained for AME results are summarised in Figure 2.

The breed effect (14.4 vs 14.2 MJ/kg dry matter) was not significant ($P>0.05$), whereas females were superior to males (14.6 vs 14.9 MJ/kg dry matter,

$P<0.05$). Significant differences ($P<0.05$) due to breed and/or sex were detected in villus height of the mucosa in different sections of the small intestine, but not in crypt depth (Figure 3).

Stepwise regression analysis of pooled data indicated that up to 33% of the variation in AME was associated with morphology of the small intestinal mucosa. AME was negatively correlated with crypt depth which was the key determinant ($P<0.01$; $r = -0.42$). Hence there is good reason to believe that gut morphology may be a limiting feature of energy metabolism, but other aspects are collectively more important given that 67% of the variation in AME remained unaccounted in this experiment. Further work is required to determine whether energy uptake in broilers is limited by gut functionality as well as gut morphology.

Recent findings on glycanase enzymes produced by gut microflora

Choct and Kocher (2000) concluded that between-bird variation in AME was associated with the ability of gut microflora to produce xylanase which degraded NSPs and lowered viscosity of excreta. These results led to the ideas that xylanase and β -glucanase activities are up-regulated in caecal microflora by the presence of high concentrations in caecal digesta of arabinoxylans and β -glucans, respectively, and that excreta viscosity is an indicator of microbial enzyme activity.

To test these hypotheses, practical diets based on wheat or barley were fed to single-sex groups of five chickens 22–29 days of age housed in 48 metabolism cages. Viscosity of ileal and caecal digesta

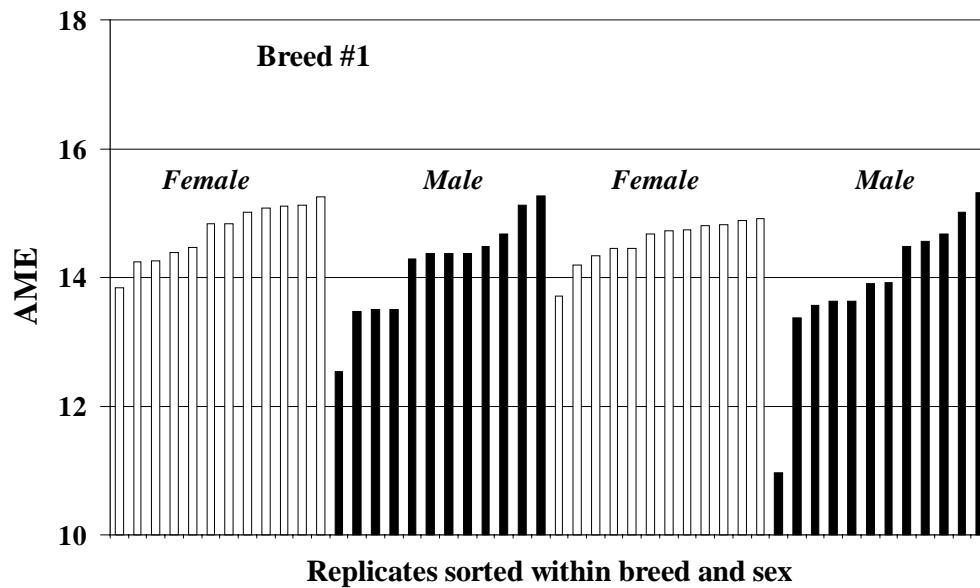


Figure 2 Variability in AME (MJ/kg dry matter) of a wheat diet given to male and female chickens of two commercial breeds. Adapted from Hughes *et al.* (2001a).

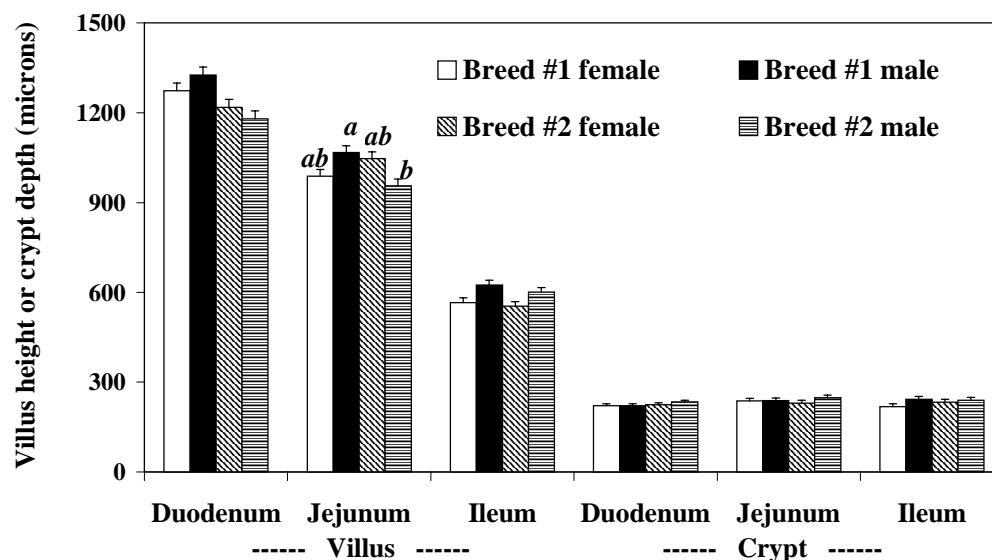


Figure 3 Effects of breed and sex of chicken on villus height and crypt depth in intestinal mucosa (means \pm SD) (Hughes, Tivey and Butler, unpublished data). Jejunal villus means with a common letter are not significantly different ($P > 0.05$).

was measured at the end of the 7-day experiment, as were short-chain fatty acids in freshly collected excreta. An increase in viscosity of excreta relative to viscosity of ileal digesta (Figure 4) is indicative of microbial production of xylanases capable of cleaving insoluble NSP in wheat but not barley.

Clearly, this response was not evident in all chickens given the wheat diet. Also, production of acetic and butyric acids differed widely between male and female chickens and between wheat and barley diets (data not shown). If these observations represent changes in the metabolic activity of enteric bacteria in these chickens then it follows that variation in production of microbial enzymes could contribute to the variability in energy uptake by birds through

mechanisms associated with the effects of digesta viscosity on digestion and absorption of nutrients (Smits *et al.* 1997; Williams 1995), use of nutrients from digesta to support microbial proliferation (Hughes *et al.* 2001b), and effects on gut motility and rate of passage of digesta through the gut (Tivey and Butler 1999).

Recent findings on microbial fermentation of undigested carbohydrates

AME and ileal digestible energy (DE) values for a selection of samples of barley, oats, sorghum, triticale and wheat were measured in a series of five experiments

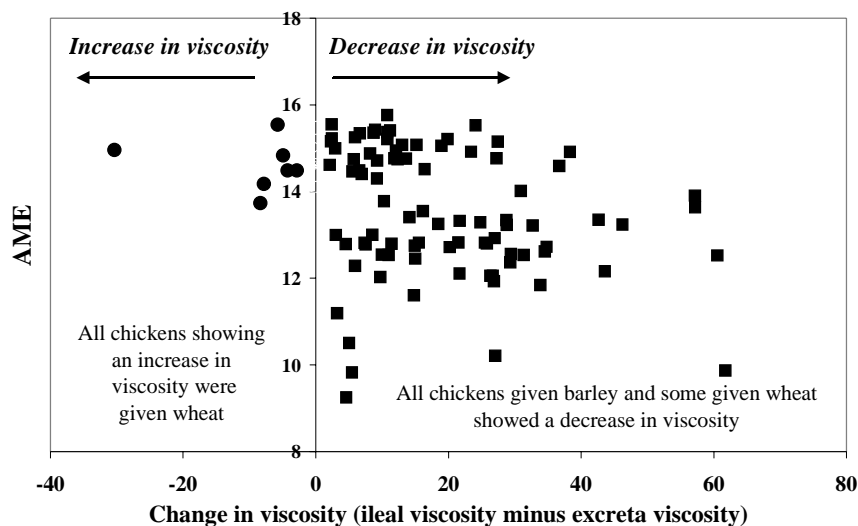


Figure 4 Association between AME (MJ/kg DM) and increase in viscosity as digesta pass through the caeca (Hughes, Choct and Kocher, unpublished data).

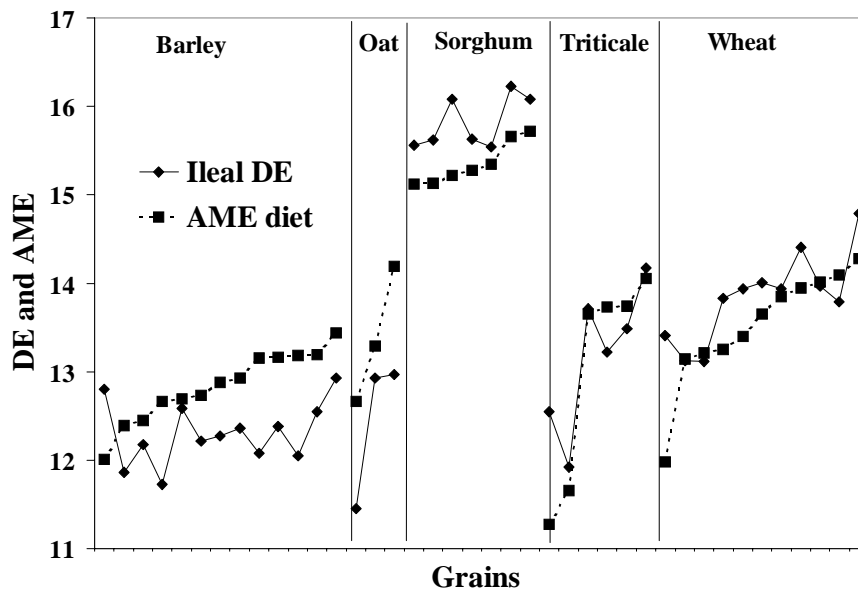


Figure 5 Ileal DE (MJ/kg dry matter) and faecal AME (MJ/kg dry matter) values for cereal grains fed to broiler chickens for seven days commencing at 22 days of age. From Hughes *et al.* (2001a).

(Hughes *et al.* 2001a). The results (Figure 5) indicate a pattern for barley and oat samples in which AME exceeded ileal DE by about 0.4 MJ/kg, whereas for sorghum samples the ileal DE was approximately 0.3 MJ/kg higher than AME. Furthermore, the responses differed between males and females (results not shown). In particular, AME and DE values for barley were generally lower for males than females, whereas for sorghum, only the AME values were lower. These differences resulted in a widening of the gaps between AME and DE for males. There were no obvious patterns for wheat or triticale.

Hughes *et al.* (2001a) concluded that for barley and oats, microbial proliferation in the hindgut utilized

energy from non-digestible carbohydrates which reduced the gross energy content of the excreta when volatile fermentation products were lost, as discussed by Choct (1999). In the case of sorghum, there was little loss of energy through microbial proliferation in the hindgut, and the difference between DE and AME represented endogenous energy losses.

Hughes *et al.* (2001) reasoned that if microbial overgrowth of viscous digesta in the small intestine can be avoided by use of feed enzymes in order to reduce variation in energy metabolism, then therapeutic use of antibiotics in the feed should have a similar effect by reducing the population of gut bacteria. This hypothesis was tested in a 7-day energy balance experiment with

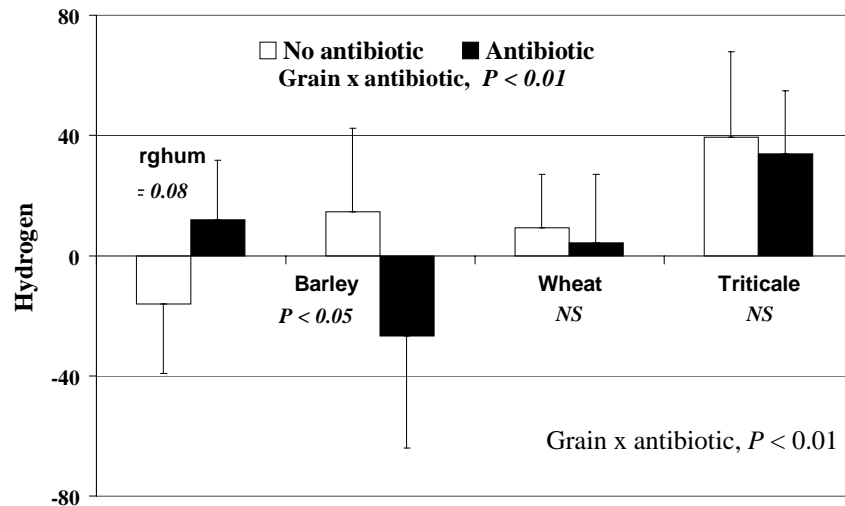


Figure 6 Effects of grain type and dietary antibiotic treatment on increase or decrease in concentration of hydrogen (in ppm) in breath collected on day 6 relative to concentration on day 0 (means \pm SD). From Hughes *et al.* (2001a).

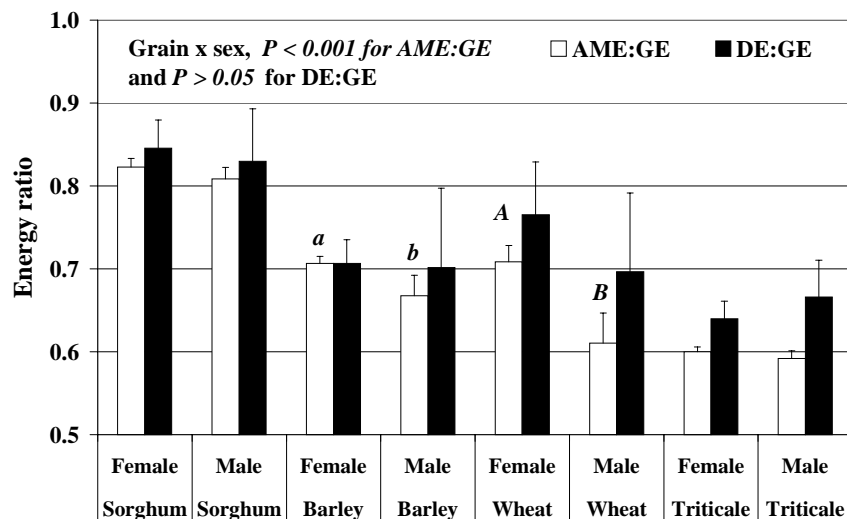


Figure 7 Effects of grain type and dietary antibiotic treatment on the change in breath hydrogen concentration (in ppm) in chickens from day 0 to day 6 (means \pm SD). Upper and lower case letters indicate significant differences ($P < 0.05$) due to sex for wheat and barley, respectively. From Hughes *et al.* (2001a).

single-sex groups of six chickens 22–29 days of age in 48 metabolism cages. Hydrogen content of breath from one chicken per cage was measured on days 0 and 6 to gauge the metabolic activity of the gut microflora. Change in hydrogen concentration was calculated by subtracting the concentration at day 0 from that at day 6 for each chicken. The results are summarised in Figures 6 and 7. The inclusion of antibiotics in the feed did not significantly affect AME or ileal DE values, but did improve the weight gain and feed conversion for each cereal type, except triticale (results not shown).

Antibiotic treatment resulted in an increase in hydrogen concentration in breath of chickens given sorghum but a decrease in chickens given barley

(Figure 6) compared with the respective control diets. Differences in the amounts of hydrogen produced are indicative of changes in the numbers and/or species of bacteria in the gut population, and/or changes in the metabolic activities of those bacteria. Furthermore, these changes in hydrogen concentration in response to antibiotics were also dependent on the type of grain used in the diet. Presumably, the differential flow of undigested nutrients into the hindgut created different growth media for those hydrogen-producing species of bacteria that survived antibiotic treatment. It is also evident from these results that antibiotics did not bridge the gap between AME and DE values and reduce the variation in AME, as had been expected.

Association between sex of chicken and metabolic activity of microflora

The lack of a significant difference ($P>0.05$) in the DE:GE ratio between males and females on barley and wheat diets (Figure 7) implies that digestive and absorptive processes in the small intestine were unaffected by the sex of the chicken, at least in this experiment. On the other hand, male chickens had significantly lower AME values than females when given barley and wheat diets (Figure 7). However, the differing effects of sex on DE and AME values of these grains strongly imply that post-intestinal processes and events associated with gut microflora were affected by the sex of the chicken. This poses questions as to what occurs at a cellular level that enables the gut microflora to change so radically in terms of numbers, species or activities according to the sex of the host animal? What chemical messengers are involved? Presumably, a clear understanding of these processes will enable us to control the colonisation of the gut in newly-hatched chicks, and to maintain an ideal microflora for the life of the bird which would be beneficial for the health, welfare and performance of commercial flocks.

Conclusions

Up to one third (33%) of the variation in AME was associated with physical features of the small intestinal mucosa. Ileal crypt depth, negatively correlated with AME, was the single most important feature of the small intestinal mucosa associated with that variation. Villus heights of the mucosa in the jejunum and ileum were significantly affected by the breed and sex of chicken, respectively. Re-modelling of the villus/crypt axis, presumably in response to dietary NSP in the wheat, differed in male chickens depending on breed, but there were no differences observed in female chickens. These results are indicative of sex-related differences in gut morphology which is a key factor influencing the digestive capacity of the chickens. Whether effects of sex extend into biochemical functioning of the gut remains to be determined.

The influence of gut microflora on the nutritive value of different cereal grains is at least partially dependent on the sex of the chicken. That is, there is circumstantial evidence of sex-dependent 'communication' between the host and gut microflora which has a differential effect on metabolic activity of the bacteria, and possibly also the host tissue.

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