INFLUENCE OF GUT HEALTH ON NUTRITION

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I. INTRODUCTION

The gut harbors more than 600 different species of bacteria, contains over 20 different hormones, digests and absorbs the vast majority of nutrients, and accounts for 20% of body energy expenditure. It is also the largest immune organ in the body (Kraehenbuhl and Neutra, 1992). Thus, anything that affects the health of the gut will undoubtedly influence the animal as a whole and consequently alter its nutrient uptake and requirements.

Gut health is a complex term that can include the macro- and micro-structural integrity of the gut, the balance of the microflora, and the status of the immune system. Further complexity arises because of their interactions and the resulting changes in gene expression and possibly endocrine regulation. This, in turn, may affect the way nutrients are partitioned and utilized for organ development, tissue growth and immune system maturation (Kelly and Conway, 2001; Kelly and King, 2001).

This paper will attempt to discuss the link between gut health and nutrition, mainly using data generated in poultry studies.

2. FEED CONSTITUENTS AND GUT DEVELOPMENT

Most feed ingredients of plant origin contain considerable amounts of fiber (non-starch polysaccharides – NSP plus lignin), with the majority being insoluble (Bach Knudsen, 1997). Insoluble fiber has traditionally been regarded as an inert nutrient diluent with little or no nutritive value in monogastric animal diets. However, recent findings suggest that this is not true; instead it has various roles in improving gut health, enhancing nutrient digestion and modulating behavior of animals (Hartini *et al.*, 2002; Hetland *et al.*, 2003). Indeed it is postulated that monogastric animals have a "fiber requirement" as their gut development requires physical stimulation by hard, solid particles of feed (Hetland *et al.*, 2004a).

A number of recent reports show that chickens consume a considerable amount of their bedding material (Hetland *et al.*, 2004a) and a laying hen fed a finely ground diet lacking fiber consumes feathers, be it her own or a fellow hen's (Hetland *et al.* 2004b). A sow obtains up to 10% of her intake from the bedding material (van Barneveld *et al.* 2003). Bedding materials, such as straw, sawdust, wood chips and wood shavings, are composed primarily of hard fiber (insoluble NSP plus lignocellulose compounds). Hetland *et al.* (2003) demonstrated in laying hens that consumption of 4% of feed as wood shavings resulted in a 50% heavier gizzard.

The gut is not only the major organ for nutrient digestion and absorption, but it works as the first protective mechanism to exogenous pathogens which can colonize and/or enter the host cells and tissues (Mathew, 2001). The gut is also the largest immunological organ in the body. Thus, it is often implied that a more robust gut will make a healthier animal, which, in turn, digests and utilizes nutrients more efficiently. This link between enzyme activities, gut weight and growth performance has been elucidated by Hetland and his colleagues (Hetland and Svihus, 2001; Hetland *et al.*, 2003) where the inclusion of oat hulls in a wheat-based broiler diet increased the gizzard weight, which coincided with a signification improvement (from 97 % to 99 %) in the digestibility of starch - the most important energy source in broiler diets - in the ileum. It was probably due largely to the massive increase in the amount of starch-degrading enzyme, amylase, secreted. In addition, the gizzard bile acid level increased in proportion to the amount of wood shavings retained in the gizzard (Table 1). Since bile acids enter the intestine through the posterior duodenal loop, their levels in the gizzard contents give a good indication of gastroduodenal reflux, supporting the hypothesis that digesta reflux between the gizzard and the duodenum is increased by inclusion of insoluble fiber. Bile acids are strong emulsifiers and they facilitate nutrient solubilization in the gizzard by effective emulsification of liberated lipids. Lipids are released continuously from the diet by water dilution and protein degradation. An incomplete emulsification of dietary lipids could lead to formation of a protective lipid coating of nutrients in the lumen, resulting in impaired solubility, and hence eventual digestibility, of nutrients. The improvement in starch digestibility may, in part, be due to enhanced emulsification of lipids as a result of more bile acids being available.

Table 1. Performance, jejunal digestive components and starch digestibility in broilers (Hetland *et al.* 2003).

	Wheat diet, no oat hulls	Wheat diet, oat hulls added
Weight gain, g/21 d	1463	1435
Feed intake, g	2293	2357
FCE[1]	0.64	0.66 (0.61)
Gizzard weight, g/kg	20.6	26.0
Ileal starch digestibility	0.97[b]	0.99[a]
Pancreas, g	3.7	4.0
Pancreas, g/kg live weight	2.1	2.2
Amylase, U/g jejunal DM	146[b]	255[a]
Bile acid, jejunum mg/g	11.7[b]	18.0[a]

[1]FCE is presented as corrected means for total dietary fibre contents of oat hulls and uncorrected means in parentheses.

There is another side to the relationship between gut health and nutrition. That is, an infected gut (coccidiosis, necrotic enteritis etc.) is not a healthy gut, and is not efficient in digesting and transporting nutrients. As presented earlier, a heavier and more muscular gizzard appears to relate closely with better utilization of nutrients; there is also much evidence suggesting that a well-developed gut is essential for the ability of poultry to resist disease (Ao and Choct, 2006). This may mistakenly lead to the notion that a "heavy gut" represents a "healthy gut". It is not so. For example, the size of the intestine is reduced and the mucosal layer is substantially thinned when antibiotics are added to animal diets (Hill *et al.*, 1957; Henry *et al.*, 1986). This suggests

that gut health is related not only to the physical development as a result of stimulation by food and solid particles, but it is determined by the organisms harbored in the gut.

3. GUT MICROFLORA ON HEALTH AND NUTRITION

The diversity in bacterial species in the gut is one of the most important factors for the establishment of a stable ecosystem in the intestinal tract. This is suggested by the observation that until the bacterial populations are fully established, young animals have fewer bacterial species in the intestinal tract than adult birds, making their gut microflora more susceptible to disturbances than that of adult animals (Mead, 1989). A stable flora is essential for an animal to resist infections, particularly in the gut. This phenomenon has been described as bacterial antagonism (Freter, 1956), bacterial interference (Dubos, 1963), colonization resistance (van der Waaij *et al.*, 1971), and competitive exclusion (Lloyd *et al.*, 1977).

The evidence for the protective role of the indigenous microflora of animals against infections of pathogenic micro-organisms has been obtained predominantly from studies with either germ-free or antibiotic-treated experimental animals, which are much more susceptible to infections with intestinal pathogens than conventional animals (Hentges, 1980). Collins and Carter (1978) demonstrated that a germ-free mouse can be killed with ten cells of *Salmonella* enteritidis, but it requires 10^6 cells to kill a conventional mouse. The presence of the gut microflora is an important factor in this difference because the LD_{50} (half live dose) for germ-free and conventional mice is the same if the animals are challenged intravenously or intraperitoneally. In these cases, the antimicrobials suppress the protective microflora, allowing the pathogen to survive. Furthermore, colonization resistance against pathogens may partly result from improvement of the immune system. Data in the literature indicate that the microflora affects the immune status of the bird through its influence on the intestinal wall. In the present context, immunity of the animal is the ability of the animal to build up resistance against invasion of pathogenic bacteria. Bienenstock and Befus (1980) suggest that the immunity of the animal is affected after a change in the gut microbial activity. The numbers of lymphocytes, plasma cells and intra-epithelial lymphocytes are lower in germ-free animals than in conventional animals (Crabbe *et al.*, 1970). In addition, Peyer's patches in germ-free animals are smaller and do not show fully developed germinal centers as in their conventional counterparts (Crabbe *et al.*, 1970). Peyer's patches are lymphoid tissues containing all components needed to stimulate an immune response, which are located along the intestinal tract.

Perhaps the most commonly used method to modulate the gut microflora is the use of live bacteria considered to be beneficial to the host (Morland and Midtvedt, 1984; Perdigon *et al.*, 1990; Havenaar and Spanhaak, 1994). Pollman *et al.* (1980) showed that the inclusion of Lactobacilli in the diet of gnotobiotic pigs activated the immune system through an increase in the number of leucocytes. Also the addition of Lactobacilli to a diet of pigs (Fuller, 1989) or mice (Perdigon *et al.*, 1987) stimulates the production of antibodies and the activity of phagocytes against pathogenic bacteria in the intestine. The presence of antibodies, in particular secretory IgA, is considered to confer a primary line of defense against pathogenic invasions (Fubara and Freter, 1973).

The naturally-established protective flora is very stable, but it can be influenced by dietary, disease and environmental factors. For example, hygiene conditions (clean vs dirty environment, pathogen load of the ingredients, humidity of the shed, litter type and usage etc.), feed additives (antibiotics, coccidiostats, buffers or acidifiers that influence gut pH), and stress (change of feed, sudden disturbances, heat or water stress) can also affect gut microflora. However, diet is perhaps the most important factor influencing gut microflora. Dietary factors, such as composition, processing, digestibility, and feeding method, may all disturb the balance in the gut ecosystem, especially in young animals (Choct *et al.*, 1996; Langhout *et al.*, 1999, 2000; Apajalahti *et al.*, 2004). It certainly appears to be the case in poultry that the fermentative characteristics of the gut microflora can be manipulated by diet. Thus, Choct *et al.* (1996) demonstrated that addition of soluble NSP to a broiler chicken diet drastically increased volatile fatty acid production in the ileum, which was easily reversed when the NSP were depolymerized with an enzyme. As shown in Table 3, the VFA levels in the ileum were negatively correlated with apparent metabolizable energy (AME) and starch digestion. Interestingly, the antibiotic (amoxil) had little effect on any of the parameters measured in the study.

Table 3. Volatile fatty acid (VFA) concentrations (uMol/g fresh digesta) in the ilea and ceca of broilers fed NSP-enriched diets with or without enzyme or antibiotic (after Choct *et al.*, 1996)

Diet*	Ileum	Ceca	AME (kcal/kg DM)	Ileal Starch Digestibility (%)
Control	8.3b	312.3b	3293a	90a
NSP	118.2a	369.0b	2596b	56b
NSP + enzyme	5.1b	930.0a	3377a	92a
NSP + antibiotic	178.9a	413.5b	2416b	50b

ab Means followed by different letters within a column differ at $P<0.05$. *Values are means of 8 replicates.

In a more recent study, Torok *et al.* (2006) fed broiler chickens a barley-based diet with or without a β-glucanase, an exogenous enzyme that degrades the anti-nutritive viscous NSP – β-glucans. They used the T-RFLP (terminal restriction length polymorphism) method to profile the gut microbial communities. Their data suggest that the two diets resulted in two distinct gut microbial communities. It is, however, too early to draw a conclusion as to a definitive link between a well-performing flock with a "good microflora" and a poorly-performing flock with a "bad microflora" because it is not known what actually constitutes good or bad flora. Dawson (2001) suggested that an ideal flora should promote the absorption of nutrients during the digestive process, whilst also ensuring that the host is capable of mounting an effective immune response in the event of pathogenic challenge. However, defining what an "ideal microflora" means in terms of their interactions with each other as well as with the host to yield favorable health and nutritional outcomes remains a key challenge. This is because even with the advent of molecular techniques making it possible to identify up to 640 different species of bacteria in poultry gut, 90% of these species are previously unidentified organisms and their role and functions are totally uncharacterized (Apajalahti *et al.*, 2004).

4. GUT MICROSTRUCTURE AND NUTRITION

A newly hatched chick today increases its body weight by 25% overnight and 5000% by five weeks to a 2kg body weight. This astonishing performance of the modern chicken comes from: (a) intensive selection for growth rate; (b) meticulous attention to health and husbandry, and (c) advances in feed formulation, matching the nutrient contents of the feed with the nutrient requirements of the bird. As the growth period is progressively shortened and feed efficiency continuously improved, the health care and nutrition of the bird are becoming more demanding. This makes it more important to pay attention to the minute changes that occur in the gut, which are often overlooked because the damage is subtle and usually characterized by microscopic changes in the mucosal layer of the gut. But these minute changes underpin the efficiency of nutrient assimilation because underneath the mucosa is a vast surface of epithelial cells of the absorptive type essential for the transport of nutrients into the enterocytes.

The surface of the mucous membrane over and between the folds is studded with tiny projections called villi. The surface of each villus is covered by simple columnar epithelium, with cuticular borders, and resting upon a core of connective tissue, the lamina propria. Between the villi are deep pits, the crypt, extending to the muscularis mucosae. Scattered lymph nodules appear in the lamina propria in all part of the intestine. The villi of the duodenum and jejunum are broader and tongue shaped; they become finger shaped in the ileum. In general, length and surface area are maximal at the beginning of the small intestine and they decrease gradually to reach a minimum in the ileum just before the ileo-cecal junction.

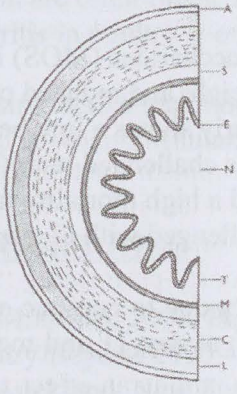

Figure 1. Diagrammatic representation of the basic structure of the digestive tract: N. lumen; E. epithelium of the mucous membrane; T. lamina propria; M. muscularis mucosae; S. submucosa, within which occurs the submucosal nerve plexus; C. and L. circular and longitudinal layers of the muscularis externa. Between these layers is found the myenteric nerve plexus; A. the serosa (Hodges, 1974).

The development of the gastrointestinal tract (GIT) and nutrient utilization are intricately related. Hydrolysis of macromolecules in the small intestine is achieved, to a large extent, by pancreatic enzyme activities, which are correlated with body weight and intestinal weight (Sklan and Noy, 2000). It is reported that early access to nutrients and water stimulate the activity of the GIT and digestive organs (Sklan, 2003). Development of GIT is an important aspect of growth, especially during the early post hatching period (Sell *et al.*, 1991). Segments of the GIT and digestive organs increase in size and weight more rapidly in relation to body weight close to and after hatch than do other organs and tissues (Lilja, 1983; Noy and Sklan, 2001). Morphological measurements of the small intestinal mucosa in chicks indicate that villus height increases twofold in the 48h after hatching and reaches a plateau at six to eight days in the duodenum but

after ten days or more in both the jejunum and ileum. The width of the villus also increases slightly; thus the growth in surface area tends to mirror the change in villus height. From these data the total surface area of the various segments can be estimated and parallel increases were shown to occur in all segments until three days after hatching. After this time the jejunal area continues to increase more rapidly than that of the duodenum and ileum. With the growth of the villus the number of enterocytes per villus also increases (Geyra *et al.*, 2001).

A fast growing broiler devotes about 12% of the newly synthesized protein to the digestive tract. An increase in cell proliferation will reduce the age and maturity of the goblet cells, which might affect the quality of mucin produced by these cells. As a consequence, the absorption of nutrients may be reduced (Hampson, 1986). In addition, a fast turnover of these cells will increase the energy requirement for maintenance of the digestive tract. Changes in intestinal morphology as described above can lead to poor nutrient absorption, increased secretion in the gut, diarrhoea, reduced disease resistance and impaired overall performance (Nabuurs *et al.*, 1993). For instance, stressors present in the digesta can lead relatively quickly to changes in the intestinal mucosa due to the close proximity of the mucosal surface and the intestinal contents (Nabuurs *et al.*, 1993). Changes in intestinal morphology such as shorter villi and deeper crypts have been associated with the presence of toxins. A shortening of the villi decreases the surface area for nutrient absorption. The crypt can be regarded as the villus factory and a large crypt indicates fast tissue turnover and a high demand for new tissue. Demand for energy and protein for gut maintenance is high compared to other organs. Cook and Bird (1973) reported a shorter villus and a deeper crypt when the counts of pathogenic bacteria increase in the GIT, which result in less absorptive and more secretory cells (Schneeman, 1982).

Savage *et al.* (1997) observed that dietary inclusion of manooligosaccharides (MOS) increased the duodenal and jejunal goblet cell numbers, elevated the villus height and reduced crypt depth in poults. A recent experiment (Choct *et al.*, unpublished data) clearly shows a link between gut morphology, disease resistance and performance in broiler chickens challenged with *Clostridium perfringens*. As shown in Table 4, poor growth, depressed FCR and a high mortality rate due to NE coincide with a low villus to crypt ratio in broiler chickens challenged with *C. perfringens*.

Table 4. Effects of MOS, monensin and Zn-bacitracin on mortality due to necrotic enteritis, NE lesion scores, body weight (BW) and feed conversion ratio (FCR) at day 21 and morphological development of the jejunum (villus/crypt ratio at day 16)

Treatment	NE (%)	NE Score	21-d BW (g)	21-d FCR (g/g)	Villus/Crypt ratio
Unchallenged control	0.7	0.00^{b}	716^{bc}	1.403^{b}	11.5^{a}
Challenged control	13.3	0.33^{ab}	607^{d}	1.540^{a}	5.3^{c}
Monensin+Zn-bacitracin	0.0	0.00^{b}	774^{ab}	1.338^{b}	11.7^{a}
MOS	14.7	0.17^{ab}	621^{cd}	1.609^{a}	8.9^{b}
MOS+Monensin	1.6	0.67^{a}	721^{bc}	1.375^{b}	11.4^{a}
MOS+Monensin+Zn-bacitracin	0.0	0.17^{ab}	837^{a}	1.343^{b}	11.9^{a}

[1]Means of 6 replicates for treatments.

[a,b]Means within a column with different superscripts are significantly different ($P<0.05$).

5. CONCLUSION

Health and nutrition are interdependent. The interaction between the two occurs largely in the gut. Thus, the term "gut health" is a very broad topic that requires a multi-disciplinary approach involving gut physiology, endocrinology, microbiology, immunology and nutrition. If gut microflora is taken as an example, the science determining the roles of microorganisms in health and nutrition is still in its infancy despite tremendous development in molecular techniques for characterization of microorganisms. Questions, such as what constitutes an ideal flora and how organisms interact amongst themselves and with the host, will continue to intrigue scientists for many years to come.

6. TAKE HOME MESSAGES

1. To study gut health, a multi-pronged approach is necessary. It should be considered from the point of view of immunology, microbiology and nutrient supply.

2. The impact on gut health often comes from microbial imbalance in the gut, which will be exacerbated if antibiotics are withdrawn from feed.

3. Any gut damage caused by pathogens will lead to poor gut health, which, in turn, affect nutrient utilization efficiency. Subclinical forms of infections with obvious signs of lesion are often financially more devastating than acute, short-term infections. Necrotic enteritis in poultry is one such example.

4. Dietary factors that modulate the immune system and gut microflora should be borne in mind in when formulating diets and managing feeding practices.

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