An Ecological Approach to Manage Gut Health in Turkeys

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Summary
Gut health has a great influence on the growth performance and welfare of turkeys, as it affects feed digestion, nutrient absorption, protein and energy utilization, immunity and disease resistance, metabolism, and physiology. Genetic selection for faster growth along with public pressure to eliminate antibiotic growth promoters in the production of meat products has made gut health a priority concern for the turkey industry. To cultivate gut health, we must understand the fundamentals ecology and enteric development, particularly during the early growth phase. This presentation discusses three important factors that affect the development of a symbiotic enteric ecosystem in turkeys: 1) Establish the ecological environment by cultivating the physical ecosystem and seeding pioneer colonizers of the microbiome; 2) Establish a symbiotic nutrient-substrate balance (starve the pathogens; feed the symbionts); and 3) Maintain symbiotic microbiota stability (feed-additive microbiome stabilizers). Developing a robust **ecological environment** in the gut is paramount to establishing lasting gut health and nutrient utilization efficiency, beginning by enteric conditioning during the perinatal period. Supplementing the perinatal chick's first meal (the amnion) by *in ovo* feeding greatly advances gut development, skeletal health, immune function, meat yield, and growth performance efficiency. Access to feed soon after hatch also has a critically positive effect on subsequent health and growth performance. Optimizing **nutrient balance** within the appropriate parts of the gut to favor a symbiotic enteric ecosystem is a critical. Poultry depend upon dietary texture and particle size to normalize gizzard function and gut motility, which helps establish distinct microbial ecosystems within the gut that favor the proliferation of symbiotic microflora. Strategic use of supplemental enzymes improves foregut digestion, which starves pathogens of nutrients and leaves fermentable substrates for symbiotic bacteria in the hindgut. Finally, strategic use of feed additives help maintain the **symbiotic microflora stability** throughout the productive life of broilers. Dietary supplementation of herbs, spices, essential oils, and organic acids have been shown to reduce the microbial load of pathogens, while probiotics and functional carbohydrates favor of microflora that symbiotically support gut health. The strategic combination of factors affecting early development, feed manufacturing, and feed additives formulation is necessary to cultivate gut health in poultry.

Introduction
Gut health has a great influence on the growth performance and welfare of turkeys, as it affects feed digestion, nutrient absorption, protein and energy utilization, immunity and disease resistance, metabolism, and physiology. Indeed, gut health may be of greatest concern among turkey producers because of its impact on economic sustainability, and their customers' concern about food safety and traceability. Gut health and nutrition are intricately dependent upon one another. Optimum dietary nutrient utilization cannot be realized unless the gut is in a healthy state.

Traditionally, intestinal health has been largely dependent on prophylactic and therapeutic uses of antibiotics. According to the Center of Disease Dynamics, Economics and Policy (https://cddep.org), "more antibiotics are used in poultry, swine, and cattle to promote growth and prevent disease than are used by the entire human population (CDDEP, 2015). Global use of antibiotics in animals are expected to increase between 2010 and 2030 in countries such as China, Brazil, India, and Russia (White, 2015). However, the voluntary or legislated limits on the use of antibacterial feed additives for poultry are requiring changes in the methods to maintain good intestinal health. Today, the focus needs to be on earlier establishment of immunity and intestinal integrity if birds are to reach their maximum potential for growth and feed efficiency. In modern turkeys, attention to the first weeks of life is critical to achieve the best possible performance later. High quality nutrients provided early in life are needed to ensure the development of immunity, microbial intestinal maturity and proper tissue building in the intestinal tract. Therefore, understanding the role of feed formulation to optimize gut development and health is vital for achieving future sustainability and for improving the efficiency and environmental acceptability of poultry production. The objective of this paper is to review nutritional strategies to optimize gut development and health and control pathogen colonization with emphasis on potential natural alternatives to antibiotics.

The Microbial Ecosystem of the Avian Gut

Finding alternatives to antibiotics to control gut health can be more easily understood if one considers it from an ecological perspective. It has much to do with maintaining a stable ecosystem in the hindgut for symbiotic bacteria to flourish rather than allow conditions that favor the proliferation of pathogenic or competitive microbes. Birds can harbor an extensive and diverse microflora throughout the digestive tract, but they are mainly hind-gut fermenters, accommodating large numbers of different microbes in the ceca. The microorganisms in the intestinal tract become attached to mucosal surfaces or food particles, or they remain free-living in the lumen. Micro-communities in different parts of the tract develop only after the successive establishment of some types of organisms and a decline of others, which characterize symbioses (Hentschel et al., 2000). In poultry, bacterial populations resembling those of adult small intestine are present within two weeks of hatching, but it takes 30 or more days in the ceca to develop a stable and dynamic population (Barnes et al., 1972). The slow rate of development appears to be due to the highly sanitized hatching and rearing conditions, the lack of contact with the mother hen, and the use of antimicrobial feed additives commonly used in commercial poultry production.

Factors that affect ecosystem structure and function

Whether an ecosystem is as robust as the Amazonian jungle or as fragile as the far northern tundra, the three factors that influence ecological structure and function most are: 1) the ecological environment; 2) nutrient balance; and 3) symbiotic flora stability. Likewise, these three general factors are important to any farmer who wishes to yield a bountiful crop; he must cultivate the land and plant good seed (prepare the ecological structure), feed the crop a good balance of nutrients at agronomic rates (nutrient balance), and maintain the crop free of weeds and pests (symbiotic flora stability). Managing the enteric ecosystem is a similar process to yield a good crop of chickens.

Develop a robust ecological environment (Cultivate and Seed the Gut Ecosystem)

Developing a robust **ecological environment** in the gut is paramount to establishing lasting gut health and nutrient utilization efficiency. Conditioning the ecological environment that supports a healthy gut depends on early development and digestive function, colonization of symbiotic microbiota that will resist the colonization of pathogens, and physical structure of the diet to stimulate “normal” gut motility that helps maintain the distinct microenvironments through the length of the intestinal tract.

Establishing a robust enteric ecosystem all begins by appropriate adaptive conditioning during the perinatal period: the first 3 days before and 3 days after hatch. Incubation distress (excessive temperature and low oxygen) due to improper ventilation and air exchange adversely affects enteric development. In contrast, we (Uni and Ferket) have demonstrated that supplementing the perinatal chick’s first meal (the amnion) by *in ovo* feeding greatly advances gut development, skeletal health, immune function, meat yield, and growth performance efficiency (Uni and Ferket, 2003; US Patent No. 6,5692,878).

The benefits of *in ovo* feeding on early growth and development of broilers and turkeys have been demonstrated by several experiments in our laboratory (Uni and Ferket, 2004). *In ovo* feeding (IOF) has increased hatchling weights by 3% to 7% (P<.05) over controls, and this advantage is often sustained at least until 14 days post-hatch. The degree of response to *in ovo* feeding may depend upon genetics, breeder hen age, egg size, and incubation conditions (i.e. the epigenotype). Above all, IOF solution formulation has the most profound effect on the neonate. The positive effects of IOF has been well documented in a review by Peebles (2018). IOF clearly advances the digestive capacity, energy status, and development of critical tissues of the neonate by about 2 days at the time of hatch. Using scanning electron microscopy, Bohórquez *et al*. (2008) observed that *IOF* significantly increased functional maturity and mucus secretion of goblet cells of villi of ileum and ceca of turkey poults. Associated with these goblet cells was the colonization of lactobacilli. Therefore, *IOF* may help improve the colonization resistance of enteric pathogens of neonatal chicks and poults. Based on the rapidly growing number of peer-reviewed publications from around the world, IOF consistently shows promising benefits, especially if applications can be done without compromising hatchability.

Probiotics: A probiotic (direct-fed microbial) is defined as "a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). In a sense, probiotic application "seeds" the microflora with beneficial organisms that promote a symbiotic enteric ecosystem. Lactobacillus and Bifidobacterium species have been used most extensively in humans, whereas species of Bacillus, Enterococcus, and Saccharomyces yeast have been the most common organisms used in livestock (Salminen et al., 1998). More recently, *Clostridium buytricum* has been shown to be a particularly effective probiotic for poultry post-hatch (Yang et al., 2012) and by in ovo application (Abousaad et al., 2019). These symbiotic microorganisms competitively exclude (Nurmi and Rantala, 1973) pathogenic microorganisms by the following possible mechanisms: 1) lowering the pH through production of fermentation acids; 2) competing for mucosal attachment and available nutrients; 3) producing bacteriocins; 4) stimulating the gut associated immune system through cell wall components (Nousiainen and Setala, 1998); and 5) increasing the production of short-chain fatty acids, which have bacteriostatic and bactericidal properties (Fuller, 1977) and stimulate intraepithelial lymphocytes, and natural killer cells (Ishizuka and Tanaka, 2002; Ishizuka et al., 2004). In a study that challenged turkey poults with *Salmonella* spp., Rahimi et al. (2009) observed a probiotic supplement increased the barrier function of the enteric mucosa, such as increased mucin secretion and more organized villi morphology that support the concept of gut health and function.

Probiotics have some disadvantages in comparison to other modulators of enteric microflora (Fooks et al., 1999; Isolauri et al., 2004). Relatively few species of microorganisms can be considered for use in probiotics products due to their limited knowledge of cultivability and required conditions for application and storage, such as extreme anaerobiosis. Probiotics have a short shelf-life and most are labile to excessive heat and pressure during feed processing. Some probiotic microorganisms may be reduced or eliminated by the low pH in the gizzard, and thus have little effect in the lower intestinal tract where pathogens pose problems. If a probiotic is added to the drinking water, the chlorine sanitizer may adversely affect its survivability. Acidification would be a better sanitizer than chlorine when delivering a probiotic via the drinking water.

Particle grind size of feed important to establish health gut motility: Good gut motility is necessary for proper food digestion, nutrient absorption, and maintaining a healthy gut ecosystem. Textural properties of feed (fiber content, particle size, and particle integrity) are important for proper gizzard musculature and motility. The gizzard is the "pace-maker" of normal gut motility in birds (Duke, 1994). Unlike mammals, vigorous gut refluxes (reverse peristalsis) are normal in birds as an adaptation to compensate for a short intestine. The refluxes serve to re-expose intestinal digesta to gastric secretions, vigorously mix digesta with enzymes to enhance digestion, enhance nutrient absorption over a short segment of the gut, and discourage microbial proliferation that may cause disease or compete for nutrients. Enteric disorders, such as diarrhea, swollen proventriculus, and gizzard erosion may be partially a consequence of dysfunctional gut motility associated with processed feed characteristics. The primary objective of modern feed manufacturing (grinding, post-mix grinding, steam conditioning, expansion, and pelletizing) is to reduce the bird's "work" of feed prehension and enhance digestion for the sake of maximizing feed conversion efficiency. Although all this mechanical work invested into processing feed reduces the work load of the gizzard to grind the ingested food, it also leads to atrophy and malfunction of the gizzard and associated gut motility (Cumming, 1994). Poor peptic digestion by pepsin in the gizzard will result in less efficient peptic digestion by trypsin and chymotrypsin in the duodenum. Consequently, more undigested proteins end up in the hindgut where they are subject to microbial fermentation. Poor protein digestibility will cause an undesirable shift in the hindgut microflora towards proteolytic and pathogenic bacteria.

Managing Nutrient Balance of the Enteric Ecosystem (Feed the Symbionts; Starve the Pathogens)

Dietary enzyme supplementation has become a standard practice in the poultry industry, largely driving by the rising feed ingredient costs, particularly sources of dietary phosphorus, energy, and protein. Increasing use of grain and oilseed processing co-products that have lower nutrient digestibility has also created greater incentives for use of supplemental enzymes, especially in feeds that are not supplemented with pharmaceutical antimicrobial feed additives. Supplemental enzymes in the feed are used to achieve one or all the following objectives: (a) increase the animal's own supply (Schaible, 1970); (b) alleviate the adverse effects of antinutritional factors, such as arabinoxylans, β-glucans, etc; (c) render certain nutrients more available for absorption and enhance the energy value of feed ingredients (Classen and Bedford, 1991; Lyons, 1993), and (d) modulate intestinal microflora to a healthier state (Engberg et al., 2004).

The major enzymes used in animal feeds are hydrolytic protease, amylase, lipase, phytase, NSP-degrading enzymes, and cellulase. Commercial enzymes products are typically a blend of several different enzymes that are effective on a wide variety of substrates. The enzymes with proven efficacies for animal husbandry include xylanase, arabinoxylanase, β-glucanase, cellulase, phytase, and mannanase (Ferket, 1992; Choct and Kocher, 2000). Amylase and lipase are enzymes commonly used in corn-SBM based diet to supplement endogenous enzymes of the animal, thus improving nutrient digestibility and growth performance characteristics (Ferket, 1993). Phytate is a universally antinutrient present in all plant material that irreversibly chelates divalent cations and interferes with amino acid absorption in the gastrointestinal tract of birds. Supplementation of poultry diets with enzyme mixtures, including proteases and amylases, has produced significant improvements in growth performance (Greenwood et al., 2002; Burrows et al., 2002). Greenwood et al. (2002) reported that supplementing a corn-SBM broiler starter diet with an enzyme preparation containing a mixture of xylanase, protease, and amylase increased body weight at 14 and 42 days of age. The effect of exogenous xylanases in improving dietary nutrient availability is more complex than phytase. Endoxylanase degrades the xylan backbone of arabinoxylan into smaller units, which has several beneficial consequences. It renders the xylose units more available to monogastrics (Odetallah, 2000). It disrupts the water holding capacity of the NSP (Scott and Boldaji, 1997), and reduces the viscosity of the digesta in the small intestine (Bedford and Schulze, 1998; Choct et al., 1999). Reduced digesta viscosity increases the diffusion rates of nutrients and endogenous enzymes enabling the bird to digest and absorb more nutrients (Pawlik et al., 1990). Endoxylanase releases entrapped nutrients for the digestion by the endogenous enzymes of the bird (Chesson, 2000). Endoxylanase inhibits the proliferation of the fermentative microorganisms in the small intestine by increasing the digesta passage rate and nutrient digestion (Choct et al., 1999). Thus, nutrient utilization is improved by reducing the competition between the host and its enteric microflora.

Many authors have shown the interaction between pentosans, microflora, and enzyme supplementation. Fischer and Classen (2000) reported that bacterial count from the small intestine of broilers fed wheat-based diets was lower in xylanase-supplemented birds than the unsupplemented ones. Because enzymes supplementation reduces the microbial population in the small intestine (Choct et al., 1995; Dunn, 1996), the entire intestinal ecosystem can change. These conditions in the intestine alter the composition and activity of intestinal microflora (Vukic-Vranjes and Wenk, 1996). When the microflora profile changes after enzyme supplementation, there is a decrease in the adverse effects of microbial fermentation. Some of the adverse effects of active microbial fermentation include: deconjugation of bile salts reducing fat digestion (Langhout, 1999); competition between the host and the microflora for nutrients (Bedford, 1995; Langhout et al., 2000); atrophy of the intestinal villi and enlargement of digestive organs (Brenes et al., 1993; Viveiros et al., 1994). Additionally, Santos (2006) showed that dietary supplementation of NSP-degrading enzymes (endoxylanase and complementary enzymes blends) reduces the adverse effects of dietary NSP on nutrient digestibility, and increases the variety of non-starch oligosaccharides that serve as substrate for a more diverse microflora, thus augmenting the positive effect of NSP on ecosystem stability and discouraging Salmonella colonization in turkeys.

Maintaining a Symbiotic Enteric Microbiome

Several strategies have been proposed as a means to manage intestinal microflora and gut health through diet formulation. Growth-promoting antibiotics (AGP) work in part by decreasing the microbial load in the intestinal tract, resulting in a reduction in energy and protein required to maintain and nourish the intestinal tissues; thus, more nutrients are partitioning toward growth and production. In contrast, most "natural" feed additives do not reduce overall microbial loads, but they alter the intestinal microflora profile by limiting the colonization of unfavorable bacteria and promote the activity or growth of more favorable species. AGP alternatives modulate gut health by several possible mechanisms: altering intestinal pH; maintaining protective intestinal mucins; selection for beneficial intestinal organisms or against pathogens; enhancing the fermentation volatile short-chain fatty acids; enhancing nutrient uptake; and increasing the humeral immune response (Ferket, 2003). Although there is growing scientific support for many of these antibiotic replacements (Yang et al., 2009), the claim of efficacy is in many cases inadequately substantiated (Rosen, 2003). The search has been for a single intervention or product to replace antibiotics, and this has shown to be less efficient than a multi-factorial approach (Collett, 2004). A number of options are available for enhancing the performance of poultry in the absence of specific feed-additive antibiotics. However, an alternative strategy or program must yield comparable economic return, and production efficiency must be sustainable if it is to be accepted for commercial use.

Herbs, spices and essential oils have been used to make human foods more appetizing for centuries, and many of them are recognized for their health benefits. Essential oils have long been recognized for their anti-microbial activity (Lee et al., 2004a), and they have gained much attention for their potential as alternatives to antibiotics. Lee and Ahn (1998) found that cinnamaldehyde, derived from the cinnamon essential oil, strongly inhibit *Clostridium perfringens* and *Bacteroides fragilis* in vitro, and moderately inhibit *Bifidobacterium longum* and *Lactobacillus acidophilus* isolated from human. Also, a wide range of in-vitro anti-microbial activities of essential oils derived from cinnamon, thyme and oregano have been published (Deans and Ritchie, 1987; Lee et al., 2004a). Although the exact anti-microbial mechanism of essential oils is poorly understood, it may be associated with their lipophilic property and chemical structure (Lee et al., 2004b).

Helander et al. (1998) investigated how two isomeric phenols, carvacrol and thymol, and the phenylpropanoid, cinnamaldehyde, exert their antibacterial effects on *E. coli* O157 and *S. Typhimurium*. Both carvacrol and thymol disintegrated the membrane of bacteria, leading to the release of membrane-associated materials from the cells to the external medium. Conversely, cinnamaldehyde exhibited its antibacterial activity due to its lipophilicity of terpenoids and phenylpropanoids, which can penetrate the membrane and reach the inner part of the cell and impair bacterial enzyme systems. Therefore, these plant-based phenolic compounds have antimicrobial effects similar to antibiotic compounds produced by fungi. As with antibiotics, continued use of these plant-based antimicrobials may result in the development of resistance in some pathogenic bacteria (Lee et al., 2004a). However, more research is necessary to confirm this risk. To be as effective as growth promoters, these herbal antimicrobial compounds must be supplemented to the feed in a more concentrated form than found in their natural state, which will increase usage costs.

Acidifiers and organic acids have been used for decades in feed preservation, protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feeds (e.g. silages). Because organic acids have strong bacteriostatic effects, they have been used as Salmonella-control agents in feed and water supplies for livestock and poultry (Ricke, 2003). The most common organic acids in animal nutrition are citric acid, propionic acid, fumaric acid, lactic acid, formic acid and benzoic acid. Additionally, some other available acidifiers and organic acids have been shown to have some antimicrobial activity (Russell, 1992). The antibacterial activity of organic acids is related to the reduction of pH, as well as their ability to dissociate and easily enter the microbial cell by both passive and carrier-mediated transport mechanisms. Once in the cell, the organic acid releases the proton H^+ in the more alkaline environment, resulting in a decrease of intracellular pH. This hinders microbial metabolism by inhibiting the action of important microbial enzymes and forces the bacterial cell to use energy to export the excess of protons H^+, ultimately resulting death by starvation. In the same matter, the protons H^+ can denature bacterial acid sensitive proteins and DNA. Generally lactic acid bacteria are able to grow at relatively low pH, which means that they are more resistant to organic acids than more pathogenic species. Lactic acid bacteria, like other Gram-positive bacteria, have a high intracellular potassium concentration, which counteracts acid anions (Russell and Diez-Gonzalez, 1998). The use of organic acids has not gained as much attention in poultry production, partly because limited positive responses in weight gain and FCR (Langhout, 2000). However, Vogt et al. (1982) reported a positive influence on either FCR or growth performance by dietary supplementation of fumaric acid, propionic acid, sorbic acid and tartaric acid in broiler diets. Dietary supplementation of coated sodium butyrate was also found enhance growth performance of broilers, attributed to better mucosal development (Malheiros and Ferket, 2010).

Mannan oligosaccharide (MOS) is derived from mannans on yeast cell surfaces and is not used as a substrate in microbial fermentation; but, it still exerts significant growth-promoting effect by enhancing the animal's resistance to enteric pathogens. Based on the literature, MOS enhances an animal's resistance to enteric disease and promotes growth by the following means: 1) inhibits colonization of enteric pathogens by blocking bacterial adhesion to gut lining; 2) enhances immunity; 3) modifies microflora fermentation to favor nutrient availability for the host; 4) enhances the brush border mucin barrier; and 5) reduces enterocyte turnover rate. MOS act as high affinity ligands, offering a competitive binding site for a certain class of bacteria (Ofek et al., 1977). Gram-negative pathogens with the mannose-specific Type-1 fimbrae attach to the MOS instead of attaching to intestinal epithelial cells and they move through the gut without colonization. Spring et al. (2000) observed five of seven strains of E. coli and 7 of 10 strains of Salmonella typhimurium and S. enteritidis agglutinated MOS and Sac. cerevisiae cells. However, strains of S. choleraecuis, S. pullorum, and Campylobacter did not lead to agglutination. Although MOS does not bind clostridia, it does reduce clostridia numbers in some trials, possibly by enhancing the mucin barrier or stimulating gut-associated immunity.

Conclusion

Although AGP have served the poultry industry well in maintaining efficient production and animal welfare, the availability of this valuable production tool will become limited in the future because there will be no such products that will be developed in the future and use of remaining AGP will be constrained because of government regulations or consumer demands. Indeed, finding alternatives to AGP to maintain gut health and efficient growth performance in poultry is a priority. Strategic use of these alternatives is dependent upon understanding their modes of action and how they influence the dynamic enteric ecosystem. A stable enteric ecosystem, particularly in the hind gut of poultry, is essential as symbiotic microflora competitively excludes the adverse effects of more pathogenic species. Establishment of that stable ecosystem depends on uncompromised early gut development, gut motility conditioning by the structural properties of feed, and strategic use of organic acids, essential oils, prebiotics, probotics, and enzymes.

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