

New issues and science in broiler chicken intestinal health: Emerging technology and alternative interventions

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Primary Audience: Researchers, Veterinarians, Nutritionists, Flock Supervisors

SUMMARY

Intestinal health is important for maximizing the health, welfare, and performance of poultry. Traditionally, due to lack of available technologies, our knowledge of the diversity of microbial communities within the chicken intestine had been oversimplified. Recent progress in technology suitable for microbial community analysis has evolved our understanding. New molecular technologies allow for a detailed molecular and physiological assessment, including quantification of individual microbial species and their metabolites. One area of research has assessed the microbial shifts that occur in response to supplementation of broiler diets with alternative products intended to promote broiler intestinal health. As a result of consumer awareness and the development of antibiotic resistant organisms, there is increasing social pressure for use of nonantibiotic products to promote broiler health, including: prebiotics, probiotics, organic acids, essential oils, enzymes, and volatile fatty acids, among others. Molecular technologies can be utilized to help advance our understanding of the mechanism of action of these alternative products by evaluating the changes in the intestinal microbiome and immune function following supplementation with these novel products. Advancing the selection of these products for practical application in the broiler industry is necessary to promote broiler chicken intestinal health, minimize public health risks, and maximize broiler production in the absence of antibiotics.

Key words: broiler, intestinal, microbiome, technology, quantification, molecular, alternative

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DIAGNOSTIC TOOLS

Until recently, knowledge of the intestinal microbial community (MC) of poultry had been limited to information obtainable from culture-dependant techniques, which are prone to selectivity bias due to the nature of the isolation

media used [1]. In addition, culture-dependant techniques are limited to the detection of readily cultivated bacteria, numbers of which remain low due to lack of knowledge of nutritional requirements and stress imposed on bacteria by these techniques [2–4]. It has been estimated that 99% of microbial species cannot currently be cultured under laboratory conditions [5]. These limitations have been overcome through

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development of sophisticated nonculture-dependent, molecular technology suitable for MC analysis [6]. These new technologies also aid in differential diagnosis of emerging enteric health issues of poultry flocks. Studies using these technologies are based on the direct extraction of bacterial DNA from intestinal samples and subsequent sequencing of the taxonomically relevant genes. Using this extraction method, researchers have differentiated many more bacteria in the chicken gastrointestinal tract than had been previously identified through culture-dependant methods [6]. These new technologies include: PCR assays, denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis, terminal-restriction fragment length polymorphism, microarrays, next-generation sequencing, ultra pressure liquid chromatography, mass spectrometry, metagenomic sequencing, meta-metabolomics, and FLX-titanium amplicon pyrosequencing [7–10].

These techniques are briefly described herein, but specific details can be found elsewhere [7–10]. The basis for the PCR technique, developed in 1983, is the amplification of DNA segments. Since its development, PCR has been widely used for sequencing, identification of functional genes, and the detection of specific organisms. DGGE and temperature gradient gel electrophoresis are both genetic fingerprinting techniques based on the amplification of the 16S rDNA segments of DNA, followed by separation of the DNA molecules based on guanine and cytosine content to create a banding pattern that is indicative of the diversity of microbial populations [3]. The two techniques differ in that temperature gradient gel electrophoresis relies on temperature dependent changes in structure for separation, whereas DGGE relies on a chemical gradient. DGGE and other molecular fingerprinting methods identify microbes based on differences in sequence integrity or length between microbial taxa to create molecular banding patterns which characterize a community. Unfortunately, overlaps and similarities in sequence integrity or lengths exist between members of microbial taxa [5]. Techniques which rely on the differentiation of 16S rDNA and molecular cloning are accurate, as these sequence patterns change slowly and are rarely transferred

between microbial species [5]. Although DGGE technology has proven useful for assessing shifts in MC diversity, they fail to provide information related to microbial function [13]. Terminal-restriction fragment length polymorphism is another molecular technique which can be used to characterize microbial communities. This high-throughput, high-resolution microbial analysis technique involves fingerprinting of DNA fragments (16S ribosomal gene regions); resulting PCR products are digested with restriction enzymes, followed by separation of terminal restriction fragments using a DNA sequencer. Terminal-restriction fragment length polymorphism is particularly useful in poultry intestinal microbiome research because of its capacity to identify operational taxonomic units, particular bacterial species or taxonomically related groups of bacteria, which can be identified and related to bird performance [10]. Ref. [10] has demonstrated the utility of terminal-restriction fragment length polymorphism analysis for investigating the response of intestinal MC profiles of broiler chickens to dietary changes. Microarrays, an additional technology with utility analyzing the poultry intestinal microbiome, involves simultaneously measuring gene expression levels, including assessment of which genes are active and inactive. Microarrays are particularly useful for comparative assessments of gene expression levels in the chicken host when exposed to different experimental conditions [7].

The advancement of genome sequencing technology to overcome previous limitations has led to the development of next-generation sequencing, a high-throughput, high-capacity, and lower-cost DNA sequencing technology that can be divided into several categories based on the sequencing platform used, including 454 pyrosequencing and Illumina sequencing. Next-generation sequencing has been useful for profiling MCs, in addition the identification of functional genes. The use of next-generation sequencing for metagenomic analyses of the chicken intestinal microbiome has improved the understanding of spatial organization, biodiversity, functional traits, and the association of MCs with intestinal disorders [11]. Metagenomic sequencing is a whole community analysis method which uses high-throughput, nonspecific microbial DNA sequencing to inventory genes and

allow researchers to compare functional diversity. Recovered gene sequences may be assigned to one of various functional categories, including: carbohydrate metabolism, membrane transport, and metabolism, among others. For example, Ref. [12] recently used metagenomic sequencing and analysis to study the function of intestinal microbial populations in poultry, specifically, the functional diversity of the chicken cecal microbiome. Ref. [13] also investigated the role of the chicken cecal microbiome using comparative metagenomic pyrosequencing; however, no known studies have used this technology to research nonantimicrobial alternatives. Metagenomics can only determine the potential function, and not actual function being performed by a MC [5].

To achieve knowledge of actual function, other whole community analysis techniques including metaproteomics and metatranscriptomics can be used to monitor what genes are expressed [5]. In addition, metabolomics, a newly developed technique which involves the identification and quantification of all metabolites in a biological system can be used to study intestinal microbiome. One limitation of this technique is the inability to incorporate genomic information. Nuclear magnetic resonance or mass spectrometry are the principle detection techniques that are utilized to identify and quantify the metabolome; these techniques represent useful tools for understanding the relationship between the genome and metabolome [5, 8]. Mass spectrometry can be coupled with chromatography to analyze complex mixtures extracted from various matrices. This combined approach has been used in metabolomics, where it has proven useful for the identification of large molecules [8]. The creation of ultrahigh pressure liquid chromatography, which uses high pressure, has improved the amount of detectable analytes. The advent of a new technology, FLX-titanium amplicon pyrosequencing technique, also allows for a rapid, quantitative assessment of microbial composition, in addition to taxonomic identification of members of the bacterial community at a high level of resolution [9].

The benefit of microbial technologies include being able to: enhance our understanding of composition and function of intestinal microbiota, identify shifts in MC by rapidly identi-

fying and enumerating the presence of a wide range of microorganisms, enhance understanding of relationships which exist between hosts and microbiota [14], and help to advance the selection of alternative products which promote intestinal health [15]. Interpretation of MC analyses using these advanced molecular techniques can be complex as a result of interactions which may exist between resident microbes, between microbes and the host, due to the impact diet composition may have on the MC. Additionally, due to our ability to only culture some bacteria and the potential for microbial groups to escape detection, an underestimation of microbial numbers and diversity may result [4, 5, 16].

CONTROL AND TREATMENT USING ALTERNATIVE PRODUCTS

A growing body of research demonstrates that the continued use of antibiotics in poultry production contributes to the selection of resistant organisms [18]. Drug residues and antibiotic resistance can have critical consequences for the environment, food safety, and animal welfare. Increasing consumer interest in these issues and government regulations concerning prudent antibiotic-use practices has stimulated research which seeks acceptable replacements [6]. The effect of nonantibiotic alternatives on microbial composition, intestinal architecture, and innate immune response have been researched. These products include: probiotics, prebiotics, and synbiotics [15, 19], enzymes [4, 19], essential oils (EO) [20], and volatile fatty acids [21, 22]. These products are particularly useful in alternative production systems, including organic farms [8]. Evaluation of changes in intestinal MCs and immune function following treatment with these novel products is an important aspect of comparing the efficacy of alternative products to antibiotics [23]. The efficacy of alternative products in chickens is often assessed using a number of parameters pertaining to: performance, such as feed intake, FE, and weight gain; intestinal integrity, often assessed as a measure of high villus height and villus height: crypt depth ratio, evaluation of lesion scores, or count of goblet cell numbers; assessment of innate immune response; and counts of pathogenic organisms.

Where possible, disease prevention and control efforts by practitioners and producers should focus on prevention and control of various enteric illnesses in the poultry flock using a combination of approaches, including: assessment of management protocols, use of alternative non-antibiotic products, and where necessary, targeted and prudent antibiotic treatment following veterinary advice on withdrawal times and targeted use. A flock health program should be developed and reviewed annually with a veterinarian to ensure incorporation of new research. In some cases, assessment and implementation of good husbandry, management, and biosecurity protocols can help to prevent or control further disease spread and reduce reliance on antibiotic use. Management factors which limit introduction and spread of disease have been summarized by the Canadian Food Inspection Agency [24]. Feed management adjustments including use of non-antibiotic feed additives can be utilized to support immune function and strength [25]. These types of alternative control measures are discussed in more depth including their mechanism of action and potential efficacy.

Probiotics

Probiotics, also known as direct-fed microbials, have been extensively researched and reviewed [15, 19]. Although the practice of feeding probiotics as prophylactic agents to improve intestinal health is not new, it has received renewed interest [26]. A probiotic is defined as 'live organisms which, when administered in adequate amounts, confers a health benefit on the host' [27]. Commonly used probiotics in livestock and poultry production include: *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Escherichia*, *Lactobacillus*, *Lactococcus*, *Saccharomyces*, and *Streptococcus*, and mixed, undefined cultures [15, 26, 19]. In addition, yeasts such as *Saccharomyces cerevisiae* and fungi such as *Aspergillus oryzae* have been used [26]. Although *Lactobacillus* is a commonly used probiotic, Johnson [28] suggested that excess abundance of certain *Lactobacillus* species in the avian intestine may be responsible for displacement of other bacteria important for intestinal microbiota development, thereby resulting in slower bird development.

The mode of action of probiotics has been reviewed [2, 15, 19] and is related to key concepts, including: competitive exclusion of bacterial pathogens, immune modulation, and possibly by a third concept termed cross feeding, whereby beneficial bacterial that already exist in the intestine utilize metabolites produced by probiotics to multiply. For example, Ref. [25] demonstrated using quantitative real-time PCR that a combination of probiotics and organic acids altered toll-like receptor-2 and cytokine profiles, effector molecules which activate immunity. A review by [15] highlights other beneficial modes of action for probiotics, including: regulation of intestinal microbial homeostasis, stabilization of the gastrointestinal barrier function, expression of bacteriocins, enzymatic activity inducing absorption and nutrition, immunomodulatory effects, and interference with the ability of pathogens to colonize and infect the mucosa. To investigate mechanism of action of a probiotic containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Enterococcus faecium* (*E. faecium*), Ref. [29] used PCR amplification of broiler intestinal contents to demonstrate that probiotic treatment significantly increased the proportion of *Lactobacillus* species in the ileum compared with the control group. In addition, mucin mRNA analysis was carried out using real-time PCR, revealing that probiotic treatment altered the processes of mucin biosynthesis, a modification which may influence intestinal function and health, including nutrient uptake.

Evaluation of the efficacy of alternative compared to antibiotics products has been cited as important. Ref. [30] demonstrated that birds receiving a probiotic containing two *Lactobacillus* strains in addition to a single *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strain had a FCR that did not differ significantly from birds receiving an antibiotic, avilamycin. In addition, birds receiving probiotic in feed, and feed and water, had concentrations of bacteria belonging to *Bifidobacterium spp.*, *Lactobacillus spp.*, and Gram-positive cocci which were significantly higher ($P < 0.05$) compared to control and antibiotic treatments, as determined by a bacterial culture and enumeration method, indicating altered microbial composition. Ref. [31]

assessed the efficacy of 2 probiotics, *E. faecium* and *Clostridium butyricum* (*C. butyricum*), on growth performance, lipid metabolism, and caecal microbiota. Broilers supplemented with *C. butyricum* had improved ADFI and weight gain ($P < 0.05$), and higher breast and thigh intramuscular fat content at 42 d age ($P < 0.05$). The only difference found in predominant bacterial populations using real-time PCR quantification of DNA from caecal content was that birds in the *C. butyricum*-supplemented groups had lower ($P = 0.030$) relative abundance of *Bacteroidetes* at 21 d age. Growth performance and fat deposition were not affected by *E. faecium* supplementation. Ref. [32] reported that birds which were administered direct-fed microbials in starter ration had longer villus length and perimeter in jejunum compared with control birds. Direct-fed microbials also increased intestinal muscle thickness by 33% vs. controls. Ref. [33] demonstrated *in vivo* that probiotic treatment significantly reduced caecal colonization of the pathogenic organism *Campylobacter jejuni* using a culture-dependant technique. Ref. [34], using a nonspecific microbial analysis technique, also reported that the supplementation of broiler diet with the direct-fed microbials *Bacillus subtilis* increased intestinal health in birds challenged with *Campylobacter perfringens* (*C. perfringens*) by significantly decreasing *C. perfringens* counts, and increasing both villi length and villi length:crypt depth ratio ($P < 0.05$). On the contrary, Ref. [35] used terminal-restriction fragment length polymorphism to characterize the intestinal MC of broiler chickens challenged using a necrotic enteritis model and contrast the profiles between birds subjected to one of several treatment groups, including: supplementation with antimicrobials zinc bacitracin or monensin, an organic acid, probiotic *Lactobacillus johnsonii*, probiotic sham (phosphate-buffered saline), and challenged or unchallenged controls. Administration of *Lactobacillus johnsonii* tended to improve intestinal lesion scores and feed conversion; treatment with an organic acid decreased clostridial load and led to an improvement in feed conversion. However, the administration of neither probiotics nor organic acid was found to be associated with improved growth or reduced mortality.

Prebiotics

Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of bacteria in the colon [15]. A prebiotic must meet 3 criteria: not be hydrolyzed or absorbed in the stomach or small intestine, must select for beneficial commensal bacteria in the large intestine, and fermentation of the substrate should induce beneficial effects within the host [15]. Prebiotics have the advantage over probiotics that bacteria stimulated from their use are those which are naturally present in the intestinal environment [19]. Prebiotics have a short history of use in broiler chickens [19]. The predominant prebiotics used in poultry are fructo-oligosaccharides (**FOS**), mannan-oligosaccharide (**MOS**), oligofructose, and inulin [19]. Others investigated include gluco-oligosaccharides, stachyose, and oligochitosan [19].

Prebiotics have been shown to elicit beneficial effects on intestinal health including modulation of microbiota by promoting selective increases in beneficial bacteria in coordination with decreases in undesirable bacteria [8]. Using DGGE analysis of universal 16S rDNA after amplification with PCR and short-chain fatty acid profile, Ref. [3] determined that inulin altered the caecal microbial metabolic activity without having a major impact on the composition of intestinal bacterial communities. Few studies have investigated the immune mechanisms behind prebiotics. Ref. [23] demonstrated that prebiotic supplementation with MOS and FOS had immunomodulatory effects on gut associated lymphoid tissue, similar to those in an antibiotic-treated group, without having a negative effect on production. Supplementation with MOS may improve performance by enhancing the microbial profile by acting as a substrate and energy source for *Lactobacillus* spp., and as a competitive binding site to prohibit the attachment of Gram-negative bacteria to the intestinal wall [22]. In turkey poult, MOS supplementation accelerates early intestinal development, including: increased villi height, surface area, and goblet cell numbers [36].

The effects of prebiotics on broiler host intestinal health and microbial composition are variable, and highly dependent on type [19].

Whether prebiotics are a suitable replacement for antibiotics has also been contradictory. The modulation of intestinal microbial composition by prebiotics to one which is dominated by beneficial bacterial species, such as *Bifidobacteria* and/or *Lactobacilli*, has been demonstrated in literature. Ref. [37] assessed changes in intestinal microbiota following supplementation with FOS and MOS using quantitative real-time PCR. Prebiotic supplementation significantly ($P < 0.05$) changed the small intestine microbial profile of broiler chickens, specifically, diets containing 0.25% FOS and 0.05% MOS led to an increase in the lactobacillus community diversity in the ileum. Ref. [38] reported that MOS conferred intestinal health benefits to chickens by improving morphological development and microbial ecology, microbial analysis was performed using culture-dependant techniques. In contrast to birds fed diets containing the antibiotics virginiamycin and bacitracin, those birds fed diets containing 0.2% or 0.5% MOS consistently had higher ($P < 0.05$) villi height and goblet cell numbers in all intestinal locations, in addition to significantly higher *Bifidobacteria* concentrations. Interestingly, compared with controls, birds fed virginiamycin, bacitracin, and 0.2% MOS also had lower numbers of *Campylobacter* spp. in the caecum. These changes were reported to show no clinical significance as demonstrated by performance characteristics which did not differ between treatment groups. Other positive effects which have been reported as a result of supplementation with a combination of MOS and β -glucans have included: mitigating stress response caused by an *Escherichia coli* challenge and transport in turkey poults [21], quicker recovery of feed consumption and egg mass and numbers following disease challenge in laying hens [22], and higher FCR and Newcastle disease titers in broiler chickens demonstrating a potential immune stimulatory effect of β -glucans [39], as well as higher BW gains and productivity [37].

Synbiotics

Synbiotics are defined as a mixture of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary sup-

plements in the gastrointestinal tract [15, 19]. Fructo-oligosaccharides and bifidobacteria, and lactitol and lactobacilli are examples of synbiotics [19].

The mode of action of synbiotics is favoring the survival and growth of the probiotic organism, by providing a substrate available to the probiotic for fermentation [19]. Limited research on the efficacy of different combinations has been performed. One known study by Li et al. [40] found that FOS and *Bacillus* administered in combination to broiler chickens improved performance measures and decreased mortality.

Enzymes

Enzymes are catalysts which speed up reactions in specific substrates or reactants [4]. Enzymes improve the digestibility of a number of feed components, including: fiber, phytate, protein, and others [19]. Enzymes used in animal production have included: phytases, carbohydrases, proteases, and lipases, among others [4]. Carbohydrase enzyme is composed of 2 dominant enzymes: xylanase and glucanase [4, 41]. A new generation of enzyme supplements, including multicarbohydrase blends, also exists [41].

The mechanism of action of enzymes can be divided into 2 phases. The first phase occurs in the ileum, where enzymes decrease bacterial numbers by increasing rate of digestion and limiting the substrate available to the microbiota. The second phase occurs in the caecum, where enzymes produce soluble, poorly absorbed sugars that feed beneficial bacteria. These bacteria produce volatile fatty acids which may be useful for decreasing *Salmonella* numbers, and possibly *Campylobacter* spp., but also serve as an energy source for the host [19]. A major function of enzymes is to decrease the viscosity of the diet, allowing an increase in nutrient uptake and improved animal performance, as has been shown with xylanase [19]. A number of mechanisms for decreasing diet viscosity have been reviewed by Ref. [4]. These mechanisms include: hydrolysis of specific chemical bonds in feedstuffs which are poorly degraded by the host's own enzymes; elimination of nutrient-encapsulating effect of the cell wall of partially broken down

polysaccharides, therefore increasing the availability of starches, amino acids and minerals; breaking down of anti-nutritional factors present in many feed ingredients, such as phytic acid; solubilization of insoluble feed for more efficient lower intestinal fermentation and thus improved overall energy utilization; and complementation of the enzymes. Ref. [41] reported the enzyme, multcarbohydrase, added to broiler diets improved growth performance and mitigated effects *C. perfringens* challenge. Ref. [42] reported that the enzyme xylanase included in a wheat-based diet tended to alleviate impairment of the intestinal mucosal barrier in birds challenged with *C. perfringens* by decreasing ($P = 0.09$) intestinal lesion scores in challenge birds compared to controls and also by significantly increasing villus height:crypt depth ratio in jejunum ($P < 0.05$) and similarly having a tendency to increase this ratio in the ileum ($P = 0.087$).

Essential Oils

EO are defined as aromatic, oily substances obtained from plant material and often associated with herbs and spices, including: oregano, rosemary, thymol, thyme, garlic, carvacrol, and cinnamaldehyde [20]. A display of antimicrobial, antioxidant, digestive stimulant, antiviral, antitoxigenic, antiparasitic, and insecticidal properties of essential oils suggest these products may be useful feed additives. EO can also stimulate the release of digestive enzymes to aid in effective digestion [18]. A limited number of studies have demonstrated mixed results concerning the performance of EO [20]. Various studies have demonstrated no effect on broiler performance of: oregano, garlic, thymol, cinnamaldehyde, and pepper. Conversely, by examining the diversity of predominant cecal bacteria using DGGE of 16S ribosomal RNA gene PCR amplicons, Ref. [7] demonstrated that in the presence of a coccidiosis challenge, two types of EO blends modulated intestinal microbial communities better than birds treated with the anticoccidial monensin and the antibiotic bacitracin, by avoiding dramatic shifts in MC postchallenge. Ref. [43] found significant but variable effects of 2 EO blends in birds challenged with mixed *Eimeria* infection. Birds not vaccinated for coc-

cidian and fed an EO blend had similar feed conversion ratios to uninfected, unmedicated controls. Ref. [9] used pyrosequencing to evaluate the effects of coccidiosis challenge on intestinal microbiota of broilers, and indicated a positive modulation of microbiota by EO blends. Ref. [44] also used DGGE to assess intestinal MCs in broiler chickens for 8 different treatment groups, including positive (bacitracin) and negative controls, 3 separately administered probiotics, and an EO blend at varying concentrations. Broilers fed different probiotic treatments all had similar MC profiles. However, no treatment group was found to significantly affect weight gain, feed intake, or mortality. Ref. [18] determined that broiler chickens challenged with *C. perfringens* and treated with EO or a combination of sodium butyrate and EO had significantly better weight gains, higher villus length, improved ratio of villus length:crypt depth, and lower lesions scores compared to control group, demonstrating the potential of EO to be used for prevention of Necrotic Enteritis (NE).

Volatile Fatty Acids

Butyric acid, a volatile fatty acid is well-known for both its antimicrobial activity and function in intestinal epithelium development [45]. Butyric acid demonstrates antimicrobial activity by freely diffusing across the bacterial membrane of Gram-negative bacteria, lowering the pH to result in energy deficiency and osmolarity issues [45]. Butyric acid is regarded as one of the most important alternatives to antibiotic growth promoters in poultry. Butyric acid administration has been shown to: lower shedding of Gram-negative bacteria; treat intestinal bacterial infections, including salmonellosis [46, 47]; improve performance; increase resistance to NE [47, 48]; and change villus morphology in chickens [49].

APPLICATION OF ALTERNATIVE PRODUCTS IN THE INDUSTRY

Antibiotics that are currently effective for treatment of intestinal health diseases in poultry should be reserved for confirmed cases as part of a prudent approach. However, control without relying on antibiotics is becoming

increasingly necessary to preserve the efficacy of existing antibiotics. Prophylactic use of alternative products in feed to promote intestinal health is important for sustaining the future of broiler production. Although the efficacy of these alternative products has been contradictory, these treatments generally have demonstrated effects which produce financial benefits to producers. These effects include: improved feed conversion, early development of innate immunity, stimulation of immune response, increased vitality, and decreased mortality [50]. Discrepancies in results between studies demonstrating how well alternative products work may reflect the fact that environmental management becomes increasingly critical when antibiotics and anticoccidials are not used. Alternative products also promote improved animal welfare and the elimination of food safety hazards. The inclusion of alternative products in poultry feed may be economically justifiable because they lead to a potential reduction in production costs, although the extent of the economic benefit is highly dependent on the cost of the antibiotic being replaced. Lastly, the cost of disease is much higher than the cost of preventing disease through the use of alternative products.

CONCLUSIONS AND APPLICATIONS

1. A significant barrier to studying the intestinal microbiome and performing differential diagnosis of enteric disease has been the inability to effectively identify and quantify microbial species, their metabolic end products, and the mechanisms by which they affect host health.
2. Recently developed molecular technologies provide further understanding and differential diagnosis of emerging microbial ecology issues in poultry. These technologies have revealed a far more complex chicken intestinal microbiome than once thought.
3. The development of antibiotic resistance has heightened the need for alternative products, including: enzymes, probiotics, prebiotics, EO, volatile fatty acids, and so on which promote intestinal health in broiler chickens.
4. Molecular technologies have been valuable in advancing the selection of alternative products. Studies have demonstrated some utility of these products at replacing antibiotics.
5. Additional research using modern microbial analysis techniques is needed to assist in the further development of alternative products to better promote broiler chicken intestinal health, minimize public health risks, and maximize broiler production in the absence of antibiotics.

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