Host-targeted approaches to managing animal health: old problems and new tools

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ABSTRACT
Our fellow medical and regulatory scientists question the animal producer’s dependence on antibiotics and antimicrobial chemicals in the production of animal products. Retail distributors and consumers are putting even more pressure on the animal industry to find new ways to produce meat without antibiotics and chemicals. In addition, federal funding agencies are increasingly pressuring researchers to conduct science that has application. In the review that follows, we outline our approach to finding novel ways to improve animal performance and health. We use a strict set of guidelines in our applied research as follows: (1) Does the work have value to society? (2) Does our team have the skills to innovate in the field? (3) Is the product we produce commercially cost-effective? (4) Are there any reasons why the general consumer will reject the technology? (5) Is it safe for the animal, consumer, and the environment? Within this framework, we describe 4 areas of research that have produced useful products, areas that we hope other scientists will likewise explore and innovate such as (1) methods to detect infection in herds and flocks, (2) methods to control systemic and mucosal inflammation, (3) improvements to intestinal barrier function, and (4) methods to strategically potentiate immune defense. We recognize that others are working in these areas, using different strategies, but believe our examples will illustrate the vast opportunity for research and innovation in a world without antibiotics. Animal scientists have been given a new challenge that may help shape the future of both animal and human medicine.

1. Changes in antibiotic policy in the United States

The US Food and Drug Administration (FDA) has provided guidance regarding the use of antibiotics deemed as medically important in animal feeds (FDA Guidance 209, 213, and Veterinary Feed Directive) [1]. In these US guidelines (mandatory after voluntary changes have been made), medically important antibiotics should no longer be used for the purpose of improving animal growth and feed efficiency, and when medically important drugs are used, their use should be under the care of a licensed veterinarian. Medically important drugs that have been sold “over the counter” will now be sold under a “Veterinarian Feed Directive Status.” FDA Guidance for Industry 3152 Appendix A lists antimicrobials that are considered important to human medicine (eg, penicillins, cephalosporins, carbapenem, quinolones, fluoroquinolones, aminoglycosides, macrolides, tetracyclines, and glycopeptides) [2]. Ionophores and bacitracin, both considered antibiotics, and the latter used in human medicine, are not included in the guidance.

Consumer expectation for antibiotic-free products exceeds FDA regulations. USDA does not approve claims for “antibiotic free” but will accept claims for “no antibiotics administered” or “raised without antibiotics.” In the United States, poultry-fed ionophores for the prevention of
coccidiosis cannot be used with products labeled as “raised without antibiotics” because ionophores are antibiotics. New US policies and regulations, as well as pressure from consumer groups and food service providers, have placed demands on scientists to find alternatives for antibiotics in the United States. Ideas will likely come from developments in the European Union, where strict policies on antibiotic use have been operational for more than a decade, and some will be the result of continued discovery around the globe. In some cases, the industry may be reluctant to accept new approaches for concern about animal welfare and human health [3].

2. Historical perspectives on antibiotic use in agriculture. A guide for moving forward

The value of antibiotics as growth stimulants in poultry occurred nearly simultaneously with their discovery as therapeutics in the treatment of disease [4]. Early studies showed that chick growth in germ-free environments was not stimulated with antibiotic use; however, germ-free chicks had growth rates and feed efficiency 10% to 15% greater than those grown in a conventional environment [5]. The use of antibiotics partially restored slower weight gains associated with microbial colonization of the gastrointestinal tract [6] by a mechanism proposed by Cook [4]. Sanitation, subclinical disease, and vaccination also prevented animals from performing to their genetic potential. Losses were also minimized through the use of antibiotics [7]. Even though microbial resistance was linked to antibiotic use beginning in the 1940s, resistance was managed within the animal agriculture sectors. Evidence that growth-stimulating effects of antibiotics did not diminish over many years of use [8–10] argued against resistance concerns. Indeed, penicillin continues to markedly improve broiler growth even today (+17%) [11].

For many years, there was a lack of evidence to support a ban of antibiotic use in animal production. However, sound science on the potential of spreading antibiotic resistant microorganisms from animals to humans appeared in 1986 [12]. In this study, researchers followed the movement of a resistance plasmid to a newly introduced antibiotic in swine diets. After 2 yr of using nourseothricin in swine diets, 33%, 18%, 17%, and 16% of the Escherichia coli isolates from pigs, pig farm employees, family members of pig farm employees, and outpatients from the village, respectively, had nourseothricin resistant E coli. Nourseothricin-resistant microbes were not found in regions where the antibiotic was not used. Witte [13] also describes that the resistant plasmid was also found in Shigella (a human pathogen). Witte’s conclusion was that antibiotics as growth promoters should be phased out and scientists needed to develop alternatives to antibiotics for agricultural uses.

During the 2000s, research in Europe was showing clear links between the use of antibiotic in animal agriculture and resistant organisms in pig farming. Rinsky et al [14] conducted a study in the United States to estimate the contribution of antibiotics used in farmed pigs and poultry on the presence of methicillin and multi-drug resistant Staphylococcus aureus (MRSA and MDRSA, respectively). In their study, farmers and family members were placed into 2 groups, those working on farms using antibiotics and those working on farms where antibiotics were not used. The incidence of MRSA in the farm workers was similar in the 2 farm groups, 32% of the farm workers from the antibiotic use farms had MDRSA, whereas 22.9% from the antibiotic free farms had MDRSA. However, household members of farmers working on antibiotic use farms had 28.6% MDRSA, whereas none had resistant strains when household members were associated with farmers that worked on an antibiotic free farm. Tetracycline-resistant S aureus was increased nearly 20-fold in farmers on antibiotic use farms compared with farmers on nonuse farms. Although agricultural use of antibiotics has received increased scrutiny in recent years, there is no question that the over-prescribed use of antibiotics, and perhaps biocides (not alcohols), directly used by humans, is also having major impact on conserving the long-term usefulness of antibiotics [15–17]. However, the human antibiotics and the resultant increase in antibiotic resistance does not diminish the responsibility of animal scientists to pursue new approaches to antimicrobial use in animal agriculture. The last decade has resulted in a number of new products and strategies to reduce antimicrobial use. Animal scientists must continue to explore novel technologies that advance both animal and human health.

3. Host targeting. A strategy to reduce antimicrobial use in animals and humans

Antibiotics and other antimicrobials have been effective in improving the efficiency of animal production and health by directly targeting pathogens. When therapeutic approaches were not available or effective (ie, viruses), vaccination was a means of generating immunity to control infection. The loss of certain antibiotics due to new FDA policies in the United States and the increasing demand for antibiotic free animal protein has increasingly put pressure on animal producers to find new approaches to maintain animal health and efficient production systems without the use of antimicrobials. Vaccination has limitations in that, depending on the vaccine, vaccination can negatively impact animal performance and in some cases have delivery limitations [4,17,18]. Animal industries need scientists to discover new technologies and strategies for assuring the health of animals.

In the last 2 decades, scientists have been effective in bringing forth those new products, and the use of these new products has been widely accepted and expanded. The use of pre- and pro-biotics for the exclusion of infecting pathogens is now commonplace in animal production units. Enzymes have played beneficial roles in improving nutrient digestion and favoring a healthy intestinal microbiota. Feed acids and essential oils, as well as microbial and plant products, have been identified as useful for controlling pathogens and improving feed efficiency.

Increasingly there is interest in moving toward host-targeted approaches as a means of enhancing efficient animal meat production and health [7]. Table 1 lists research areas that our laboratory has been exploring in an attempt to improve animal health and efficient meat production. Our targets include infection detection measuring host
biomarkers, products that regulate host molecules involved in systemic and mucosal inflammation, a host product to increase barrier function, and host-targeting to potentiate immune defense. In our research pursuits to develop new products, we have focused our developments in areas that will likely be acceptable to both regulatory agencies and consumers. Our approach and solutions are within our research capacity but always mindful of industry needs, value, and cost (Table 2). As the food consumer becomes more accustomed and trusting of other approaches (eg, transgenic animals), we believe the approaches suggested in Table 1 and results achieved with some of these approaches may serve as a guide in developing efficient, disease resistant animals that are safe for the consumer.

Section 4 will be restricted to host-targeted research conducted in our laboratory and products that have been commercialized for animal and human use. Approaches listed focus on measuring or controlling host inflammatory/immune responses in contrast to controlling the microbial population directly. Products created have the potential for reducing or replacing the use of antibiotics in animal agriculture. In some cases, the products described are simply designed to improve animal growth and feed efficiency independent of antibiotic use. We believe that by specifically targeting the host, as opposed to the microbe, we can achieve useful products with limited development of resistance among pathogens.

4. Host-targeted approaches to managing animal health

4.1. Infection detection

In humans and farmed animals, clinical signs are used as an indicator of infection. Signs may be as subtle as poor rates of gain, production, or feed efficiency in farmed animals (often referred to as subclinical infections) to pathophysiological changes. The lack of tools available to detect the onset of an infectious process, and the considerable losses that have occurred by the time the infection is observed, often encourage animal veterinarians and medical doctors to use prophylactic antimicrobial agents. For some diseases, the probable occurrence of disease is so high that preventative approaches are essential (eg, coccidiosis in poultry, and bovine respiratory disease in cattle). However, the continued use of antimicrobials as preventive strategies drives resistance and often requires strategic "shutting" or "rotation" of drugs to assure protection [19]. The epidemic growth of antibiotic resistance due to overuse in humans is estimated to represent a societal cost of $55B in the United States alone [20]. Screening and surveillance for infection is often problematic due to continuous need for sampling and the cost associated with testing. Is there value for a cost-effective, simple, and safe procedure to detect the onset of infection in an individual or population?

Beginning in the mid 1990s, we began exploring the use of breath carbon isotopes as a means of detecting the onset of infection [21]. Dependable early detection, particularly if it precedes clinical signs of disease, would allow for strategic antimicrobials use at infection onset or avoidance of use when no infection is present. Breath carbon isotopes of CO₂ were selected as a biomarker because breath CO₂ naturally contains $^{13}$C and $^{12}$C carbon. During the onset of an infection, proinflammatory cytokines (eg, interleukin [IL]-1 and tumor necrosis factor [TNF]) are rapidly released from immune cells (eg, macrophages) inducing muscle wasting. The natural abundance of $^{13}$C in these amino acids is approximately 1% of the carbon. Enzymes that metabolize the amino acids to CO₂ discriminate against $^{13}$C amino acids in accordance with the kinetic isotope effect [22]; hence, breath becomes light (enriched with $^{12}$CO₂) and newly synthesized acute phase protein heavy (enriched with $^{13}$C; Fig. 1) [23].

Experiments involving mice, chicks, and pigs showed that breath isotope ratios were effective at detecting a challenge with lipopolysaccharide (LPS) within hours after injection [21,23,24]. Interest in technology for monitoring flocks increased during avian flu outbreaks, particularly with strains that could infect people. One of the technologies under consideration was thermal imaging. We did a study comparing the surface temperature of the shank and cloaca to that of breath carbon isotopes in adult laying hens undergoing an immune challenge. In this experiment, adult laying hens were injected intraperitoneally with 1 mg/kg

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**Table 1**

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Benefit</th>
<th>Current approach</th>
<th>Company involved</th>
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</thead>
<tbody>
<tr>
<td>Infection detection</td>
<td>Treat when infected</td>
<td>Breath analysis</td>
<td>Isomark LLC</td>
</tr>
<tr>
<td>System anti-inflammatory</td>
<td>Muscle accretion</td>
<td>Conjugated linoleic acid</td>
<td>BASF</td>
</tr>
<tr>
<td>Mucosal anti-inflammatory</td>
<td>Improved feed efficiency</td>
<td>Anti-sPLA₂ oral antibodies</td>
<td>OvaBio LLC</td>
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<tr>
<td>Intestinal barrier function</td>
<td>Improved feed efficiency</td>
<td>sIgA</td>
<td>Wisconsin Alumni Research Foundation</td>
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<tr>
<td>Immune potentiation</td>
<td>Enteric disease prevention</td>
<td>Anti-interleukin-10 oral antibody</td>
<td>Ab E Discovery LLC</td>
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Abbreviations: sIgA, secretory immunoglobulin A; sPLA2, secretory phospholipase A₂

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**Table 2**

Guidelines for product innovation.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
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<tbody>
<tr>
<td>Value</td>
<td>Does the innovation solve a new problem and is there a “market”?</td>
</tr>
<tr>
<td>Compatibility</td>
<td>Does our research team have the skills and models to innovate?</td>
</tr>
<tr>
<td>Cost</td>
<td>Is the innovative solution economically feasible?</td>
</tr>
<tr>
<td>Acceptability</td>
<td>Will the consumers accept the innovation?</td>
</tr>
<tr>
<td>Safety</td>
<td>Will scientists and society find the innovation safe for animals, humans, and the environment?</td>
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Product users, regulatory agencies, and consumers are increasingly scrutinizing products developed and used in animal agriculture. The criteria used in the table have served as a useful guide in development of products produced by our research team.
Fig. 1. Carbon fractionation during the acute phase response. Within the first 2 hr after the onset of an infection, proinflammatory cytokines (TNFα and IL-1) are released. These cytokines induce muscle wasting in an attempt to provide amino acids to fuel the immune response and synthesis of acute phase proteins. Approximately 1% of the carbon in the liberated amino acids is 13C. Enzymatic metabolism of amino acids discriminates against metabolites containing 13C through a process known as the kinetic isotope effect. Metabolites with 13C reside with the body to form acute phase molecules, whereas metabolites composed of 12C are preferentially burned to CO2. The fractionation of mobilized carbon during the acute phase response results in a decreased relative abundance of 13C/12C.

Body weight of E. coli LPS, and at various times after LPS injection, a noncontact infrared thermometer (for thermo imaging temperature) was used to record the temperature of the shank and cloaca surface and breath samples were collected from the hens for the determination of breath carbon delta value (ratio of 13C/12C). The goal was to compare the ability of surface temperature versus breath delta in the early detection of the acute phase response. Hen breath was collected by placing a laying hen in a closed container for 30 s, after which the headspace containing expired breath was collected and analyzed using a Picarro Cavity Ring-Down Spectrometer. Shank temperature and cloacal temperature began showing a rise at 2 and 1.5 hr, respectively, after LPS and saline injection; however, changes in surface temperature tracked with ambient temperature (environmental temperature rose from 24 to 26°C within the first 2 hr of study) and did not differ between LPS- and saline-injected treatments. Breath delta value was stable the first 2 hr after injection, then declined rapidly in the LPS-injected hen (saline-injected control remained stable, Fig. 2). The detection of the acute phase response using breath appeared more accurate that using surface temperature and was not influenced by environmental temperature conditions. Breath detected the onset of the acute phase response within 2 hr of injection, a finding consistent with our published data [23]. Internal cloaca temperature was also imaged, but little difference was shown between those injected with saline or LPS (not shown). We believe that breath biomarkers offer a unique capacity to detect infection onset of herds and flocks in confinement. Current confinement barns commonly contain 2500 ppm CO2, where ambient atmospheric CO2 levels are around 400 ppm. The difference between CO2 levels in the atmosphere and the animal housing structure provides adequate differences for detecting infection-induced carbon fractionation. Use of sensing technology could also prove useful in minimizing use of antibiotics as prophylactics because animals could be treated only at the onset of infection.

Although the breath technology was originally developed for animal agriculture, its use has yet to be adopted by animal producers. Meanwhile, the technology was launched as a medical device for noncommunicative patients (newborn, ventilated intensive care patients). Boriosi et al [25] showed how the breath delta value was a useful early diagnostic of infection onset in intensively cared patients. The technology is currently being developed and marketed by Isomark LLC (isomark.com). Advances in laser technology are likely to drive new innovations in the use of breath biomarkers for the early detection of infection. Preliminary data suggest that in the near future, breath biomarkers could also be useful in biofeedback techniques for energy balance in humans and animals [26].

4.2. Systemic anti-inflammatory strategies

The effects of inflammation on animal growth and muscle accretion are well documented [27]. Although chronic inflammation is generally not a concern in young animals grown for meat, acute inflammatory processes have a profound short-term effect on growth rates; rates of growth that may not be compensated over the short life of the animal. Cook [7,28] described reduced performance due to microbial colonization at birth (hatch), under poor sanitary environments, and after vaccination. He proposed that these costs were mainly the result of immune activation. Although estimates of reduced gain caused by acute inflammation vary widely, data support more than a 10% loss in genetic potential for growth and feed efficiency due to immune activation. Cook and Sand [28] estimated that reduced feed efficiency due to colonization (−12%), sanitation (−10%), and vaccination (−7%) could actually add to 29% if each loss was additive. Can systemic anti-inflammatory agents minimize immune-induced losses in feed efficiency?

Gaines et al [29] found that barrows (not gilts) injected with dexamethasone shortly after birth had increased growth rates (10%). Dexamethasone is a potent inducer of protein catabolism, hence improved growth rates were counterintuitive. The authors suggested that dexamethasone-induced improvement in growth rates may be linked to changes in the somatotropic axis or increased colostrum intake (neither measured). Effects of dexamethasone on inflammatory processes were not considered. Xu et al [30] showed that pigs fed 125 ppm aspirin had as much as a 14% growth response and a 4.4% improvement in feed efficiency. Aspirin acts as an anti-inflammatory agent through cyclooxygenase (COX) inhibition of eicosanoid synthesis. Inhibiting eicosanoid signaling via COX can have a profound effect on the downstream release of proinflammatory cytokines, such as IL-1 and TNFα, as well as signal...
Fig. 2. Comparison of hen shank and cloaca surface temperature versus change in carbon delta value in hen injected with saline (control) or lipopolysaccharide (LPS or experimental treatment). Hens were injected with saline or LPS (1-mg E coli/kg body weight). Surface shank (A) and cloacal (B) temperatures were measured using a noncontact infrared thermometer at times indicated. Hens were also placed in a closed chamber for at least 30 s and chamber air was analyzed for carbon delta value using a cavity ringdown spectrometer. Change in delta value from time zero is shown (C). Data demonstrate the inability to distinguish saline versus LPS injected hens using surface temperature. Changes in surface temperature reflected changes in ambient temperature. Change in breath delta value demonstrated the detection of LPS-injected hens 3 h after injection. LPS, lipopolysaccharide.
transduction through the NFκB pathway [31]. Long-term systemic administration of dexamethasone or COX inhibitors can have adverse effects on animal health (eg, dexamethasone can induce muscle wasting, and blockade of the eicosanoid pathways can induce intestinal ulceration). In the 1990s, conjugated linoleic acid (CLA) was found to have potent anti-inflammatory effects without adverse effects associated with other systemic inhibitors of inflammation.

Early data on CLA showed that it was effective in preventing weight loss associated with acute inflammatory responses [32–35]. Dietary CLA reduced TNFα release [36,37], NFκB signaling [38], and COX-2 protein upregulation [38–40]. Inflammatory and autoimmune diseases such as lupus [41,42], airway disease [43], cancer-induced cachexia [44], and arthritis [45,46] were reduced in CLA-fed rodents. Although CLA was found to have positive effects on growth and efficiency in pigs, there is limited evidence that these beneficial attributes are related to regulation of inflammatory processes. Dietary CLA may have limited benefit in animals raised for meat in the absence of a sustained systemic inflammatory response. The dairy cow may be different. These long-lived animals may benefit from the anti-inflammatory properties of CLA. Recent research has shown that dairy cattle–fed CLA have increased reproductive performance [50,51]. Although increased reproductive performance has been related to CLA’s effects of energy sparing (eg, reduced milk fat), CLA as an anti-inflammatory in dairy cattle should be considered as a potential mechanism of improved reproductive performance in dairy cattle.

Pharmacological inhibition of inflammatory processes often target downstream pathways (eg, COX-2 inhibition) as opposed to upstream pathways of inflammation (eg, CLA). COX-2 inhibitors differ from CLA in that COX-2 inhibition occurs after a transcriptional/translation event (downstream), whereas CLA is an upstream attenuator of secretory phospholipase A2 (sPLA2), the first event in the inflammatory cascade [75,76]. Upstream regulation of inflammation (COX-1, a constitutive enzyme essential for gut homeostasis) using aspirin or nonselective COX inhibitors led to gastric ulceration [53], hence the desire to find downstream regulation of inducible COX-2. Although selective inhibition of COX-2 prevented gastric ulcers, its use has been associated with adverse events including myocardial infarction via a thrombosis [54,55], osteopenia [56], delayed bone repair [57], and increased inflammation after prolonged use [58]. CLA, on the other hand, has been found to protect against gastrointestinal inflammation [59], is antiatherothrombotic [39], increases bone mineral content [60,61], and has prolonged anti-inflammatory effects in a chronic model of inflammation [45,46]. Hence, although both COX-2 inhibitors and CLA reduce inflammatory diseases (eg, arthritis) through the same inflammatory cascade, the adverse events are markedly different. It is our belief that the lack of adverse events associated with CLA, as compared with COX-2 inhibition, is related to difference in upstream versus downstream regulation of inflammation.

Inflammation induces perturbations in secondary metabolism [62]. Downstream inhibition of COX-2 results in a loss of secondary metabolites critical for the feedback regulation of secondary metabolism. Losses of key metabolites result in aberrant metabolism that leads to adverse events. Attenuation of inflammation upstream (eg, CLA) restores perturbations in metabolism to normalcy (Fig. 3). Currently, we have been using a model of collagen-induced arthritis and an inflammatory metabolome to study metabolism differences between the regulation of inflammation using CLA (upstream) and COX-2 inhibitors (downstream). Preliminary analysis has shown that hypoaemia induced by chronic inflammation is prevented through the use of dietary CLA. Although these metabolic changes may have minimal effect on young animals grown for meat production, the adult animals used for the production of milk, eggs, and breeding stock could benefit from dietary CLA.

4.3. Mucosal anti-inflammatory strategies

During acute inflammatory processes, proinflammatory molecules are released into the lumen of the gastrointestinal tract. One of these molecules is sPLA2 [4,7]. Secretory phospholipase A2 has been shown to be involved in sepsis progression [63,64]. Increased tissue expression and circulating levels of sPLA2 in mice during endotoxic shock has been reported [64]. More importantly, recent findings have shown a 3-fold increase in sPLA2 secretion into the lumen of the intestinal tract during endotoxemia [65]. Secretory phospholipase A2 released into the gastrointestinal tract is responsible for a loss of intestinal barrier function, permitting antigen translocation into systemic circulation [65]. In models of rat and mouse endotoxic shock, inhibition of sPLA2 with chemical inhibitors decreased gastric permeability and increased survival. Mice deficient in a receptor for sPLA2 have also been shown to be resistant to endotoxic shock after a lethal dose of endotoxin [66]. Analysis of expression patterns of sPLA2 identified 2 major sPLA2s in the gastrointestinal system, sPLA2–IIA and sPLA2–IB.

All attempts to inhibit the enzyme in models of severe sepsis (sterile endotoxemia) showed marked improvements in health; however, there appeared to be failure when these models were translated into models of infection. We believe the reasons chemical inhibitors of sPLA2 failed in infection models were because chemical inhibition of sPLA2 was systemic and nonspecific (several classes of sPLA2, both at the mucosal and systemic level). Recent data showed that sPLA2 in the intestinal lumen was a feed-forward driver of severe inflammatory response. When sPLA2 is released into the gastrointestinal tract, barrier function loss and gut leakage drives forward the inflammatory process that ultimately kills the animal (human). This leakage can be stopped using chemical inhibitors of sPLA2. However, because the action of these inhibitors is
not confined to the intestinal lumen and because the inhibitors are promiscuous (acting on several classes of sPLA2), the systemic antimicrobial action and normal biochemical function of the sPLA2s is lost. We developed an oral antibody (IgY) to sPLA2 1B with the intent of improving animal performance when fed [67,68]. Fed anti-sPLA2 antibody should only effect the enzyme in the gastrointestinal lumen because oral antibodies are not absorbed into general circulation. Hence, the antimicrobial action and normal physiological functions of sPLA2 in circulation could be maintained when oral anti-PLA2 is used.

During our animal trials with anti-PLA2, it became apparent that host-targeting sPLA2 improved growth of agriculturally important animal species. When anti-sPLA2 was fed to young broiler chicks, improved feed efficiency of 4% was observed [4]. When the antibody was tested in other animal species (ie, trout), fish performance was increased by more than 20% [69]. We discovered in a model of salmonellosis, anti-sPLA2 blocked excessive fluid excretion in the gastrointestinal tract of turkey poults [70]. In invitro experiments using murine macrophages, anti-sPLA2 blocked LPS-induced release of PGE2 and TNFα (unpublished). Additional studies suggested that anti-sPLA2 blocked CD1d lipid presentation by human mononuclear cells to natural killer T cells [71] and prevented inflammatory cytokine production. These experiments clearly demonstrate that there are novel ways to improve animal growth and health by targeting inflammatory molecules of the host’s mucosa.

Fig. 3. An illustration of a biochemical cascade at homeostasis (top left, A), during the inflamed state (top right, B), in the presence of a downstream inhibitor (bottom left, C), or an upstream attenuation of an enzyme (bottom right, D). This image was created to display a concept where circles indicate a metabolite found in the plasma or tissue (size is a function in a change in metabolite concentration), the straight lines between metabolites indicates an enzyme action, and curved lines indicate a regulatory effects (feedback or feed-forward) on an enzyme action. When circles in a cascade are equal in size, the cascade is balanced. When circles in the cascade have unequal sizes, the cascade in unbalanced. These figures visualize a hypothesis that downstream inhibition of an enzyme leads to unbalanced feed-regulatory events, whereas upstream attenuation results in a balance state during the inflammatory cascade.
Our laboratory has also used the oral antibody strategy to target other host targets in the gastrointestinal tract. Gut peptides such as cholecystokinin (CCK) are released into the lumen of the intestinal tract [72], but the biological relevance of luminal CCK has yet to be clearly defined. Work had shown that IL-1 increased the release of CCK (systemically) and CCK in turn reduced food intake [73]. We were able to show that oral antibodies to CCK could improve broiler feed efficiency and growth [4,74]. Receptors and transporters on the apical surface of enterocytes might also be useful host targets. We recently published methods to strategically alter the function of luminal receptors using oral antibodies [75]. Toll-like receptors (TLRs) are expressed on epithelial cells in the intestinal lumen and are known to be important molecules for sensing intestinal bacteria. Toll-like receptor 2 senses gram-positive bacteria and is known to be expressed in low levels in non-inflamed intestines; however, it is upregulated in inflamed intestines. Toll-like receptor 4 senses gram-negative bacteria, is minimally expressed in non-inflamed intestines, and is upregulated in inflamed intestines [76]. These TLRs sample luminal microbial antigens. Because toll receptors are critical initiators of secondary signaling processes during inflammatory events, these receptors may also represent potential targets to regulate inflammatory processes in the intestine that may suppress growth and feed efficiency. Indeed, targeting TLR-4 using oral antibodies was effective at improving growth and feed efficiency [77].

Proliferative molecules in the lumen of the gastrointestinal tract and mucosal microenvironment may explain potential mechanisms by which some growth promoting feed additives act. Unfortunately, host molecules in the luminal milieu and their biological roles are poorly understood and inadequately investigated. Considerable opportunity appears to exist in exploring this area and developing new tools using current and novel models of inflammation.

4.4. Intestinal barrier function

In our attempt to discover useful products that can reduce both mucosal and systemic inflammation, our attention turned to natural mechanisms that animals use to reduce antigen translocation across the intestinal barrier. Secretory IgA stood out as a critical barrier support molecule to prevent local and systemic inflammatory processes [78]. Deficiencies in secretory immunoglobulin A (sIgA) production were often associated with intestinal microbial overgrowth and intestinal inflammation [79]. Although a deficit in sIgA production in agriculture animals has not been described, we asked if oral supplementation with heterologous sIgA may provide protections that have economical value (eg, animal growth and feed efficiency). Literature showed that sIgA binds antigen in a non-inflammatory manner (eg, does not activate complement). Secretory immunoglobulin A plays a critical role in intestinal microbiota structure, encouraging growth of select microbial populations over others [80]. The finding of Perez-Bosque et al [81] that spray-dried animal plasma (a rich source of immunoglobulins) was effective at protecting barrier function led us to hypothesize that sIgA may also be a useful oral supplement for reducing intestinal and systemic inflammatory process. Swine sIgA was selected as a potential source of exogenous immunoglobulin for oral feeding studies. Using data from the study by Bourne et al [82], mucosal sIgA in the pig was estimated to be 30–70 g per pig. Secretory immunoglobulin A was prepared in pure and crude (ie, Cosatein) fractions and tested in animal models for animal growth and feed efficiency. Using a marginal broiler diet (low in energy and essential amino acids), we found that as little as 20 g of Cosatein significantly improved broiler feed/gain ratios (control = 1.81 vs Cosatein = 1.57, \( P = 0.006 \)). No difference in growth response was observed.

Secretory immunoglobulin A deficiency has been associated with atopic dermatitis [83]. Using a murine model of dermatitis [84], we showed that swine sIgA prevented and reversed ulcerative dermatitis in our rodent model. Recent work has shown that direct-fed microbial products (probiotics) enhance sIgA secretion [85,86]. Perhaps the beneficial effects of direct-fed microbials in animal feeding systems could be attributed to the increased secretion of host sIgA and reduced mucosal and systemic inflammation.

4.5. Immune potentiation

Immune potentiation as a means to improve animal growth and feed efficiency may at first appear to contradict strategies previously discussed; however, recent findings in our laboratory and others suggest consideration is needed. Immune potentiation probably has its greatest benefit in overcoming the growth suppression caused by infection. Our introduction to this concept occurred while conducting experiments to enhance gastrointestinal inflammation.

Interleukin-10 is an anti-inflammatory cytokine that regulates other cytokines typically associated with reduced animal performance (eg, TNF and IL-1) [87]. Interference with IL-10 production or function systemically is associated with uncontrolled inflammatory processes (Cytokor and Tuner 2011). For this reason, interfering with IL-10 function would appear to be contraindicated for improving animal growth and feed efficiency.

The discovery of an apical receptor for IL-10 on enterocytes [89] led us to explore the biological effects of oral IL-10 neutralization as a means of enhancing gastrointestinal inflammation. In experiments with coccidia-infected chicks, use of an oral antibody to IL-10 conferred protection of chicks from the growth suppressive effects of infection [90]. To our surprise, oral antibody to IL-10 was not proinflammatory, and, if anything, improved animal performance.

Recent literature showed that select pathogens upregulate IL-10 production as a strategy to suppress innate immune responses and evade the development of adaptive immune defense (Fig. 4A) [88]. Inhibition of IL-10 within the intestinal lumen (or microenvironment of the mucosa) appeared to maintain an active immune defense against pathogens such as Eimeria spp (Fig. 4B). Jang et al [91] found that generating immunity against the putative Eimeria signal that upregulates host IL-10 (Eimeria migratory inhibition factor) was also protective against the
growth depressive effects of coccidia infection. Hence, strategic immunopotentiation may have benefits in controlling infectious processes, thereby preventing pathogen-induced growth suppression. Strategic immunopotentiation cannot be generalized. Cook [7] gave examples where vitamins E and C, when used at pharmacologic levels, had immune potentiating effects, increased disease resistance, but had adverse effects on animal performance [92]. These examples clearly demonstrate that there are fruitful areas of research where the immune system can be manipulated for the benefit of animal health and growth, but the pathway of doing so needs to be strategic.

5. Concluding remarks

In this review, we presented new areas of animal immunology that can be studied in an attempt to improve animal production efficiency and health in an era of restricted antibiotic use. Detecting the onset of inflammation could be useful in treating only infected individuals, herds, or flocks. Inhibition of systemic inflammation may have value in long-lived animals, whereas management of mucosal inflammation may serve as a useful strategy to improve growth and feed efficiency in the young growing animal. In this review, we provide evidence of

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**Fig. 4.** Proposed mechanism by which oral anti-interleukin-10 interferes with pathogen-induced downregulation of immune responses. In (A), a pathogen invades and either directly releases an IL-10 mimic or signals key immune regulatory cells to secrete IL-10. Secreted IL-10 serves as a "stand down" molecule for immune responses relative to the pathogen. The pathogen infects unencumbered by immune defenses. In (B), the mucosa microenvironment is "flooded" with antibody that neutralizes IL-10. When the pathogen secretes an IL-10 mimic or signals IL-10 secretion, IL-10's activity is inhibited by the anti-IL-10 and immune defenses can react against the invading pathogen. IL, interleukin.
host-targeting key inflammatory processes in the mucosal/luminal microenvironment. Current knowledge of how inflammatory molecules in the intestine effect animal growth and feed efficiency is lacking, and this lack of information will hamper the advancement of new technologies to replace antimicrobials as growth promotants. It will be critical for the agricultural animal scientists to be well versed in the basic knowledge involving all animal species including humans and rodents to adequately make progress in animal agriculture. Areas proposed for additional study are as follows: (1) host proinflammatory molecules in the mucosa; (2) molecules critical in maintaining barrier function; and (3) immune products regulated by pathogens that may interfere with normal adaptive immune responses. These areas should be considered in addition to current studies investigating the interface between the intestinal microbiota and the immune system.

Critical needs in developing basic knowledge of mucosal inflammation should be coupled with applied research that develops cost-effective solutions to mitigate the adverse effects of the host’s interface with the microbial environment. In this review, we provided examples of products we have developed to improve animal growth and feed efficiency. We have found the oral egg antibody strategy is a useful platform for developing new tools, particularly when used to target host molecules [93]. It is unclear why this platform has not gained broader use among animal scientists.

The growth of animal agriculture has been the direct result of industry and academic scientists’ animal health innovations. Advancements in antimicrobials, whether natural or synthetically derived, will continue to play important roles in producing food for a growing population that demands animal products. Our research colleagues in the medical sciences and government have identified problems associated with the use of antibiotics in animal agriculture. Society has followed their lead and has increasingly expressed their interest in animal products raised without the use of antibiotics and chemicals. Animal scientists have the opportunity to play lead roles in the discovery of novel approaches in disease management. As we develop new products to manage animal health and improve efficiency, we should forever be mindful of the consumer, who is increasingly concerned about the types of products used in animal feeds.

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References

Cook ME, DeVoney D, Drake B, Pariza MW, Whigham L, Yang M, Xu ZR, Kornegay ET, Sweet LA, Lindemann MD, Veit HP, Watkins BA, Gaines AM, Carroll JA, Allee GL, Yi GF. Pre- and postweaning per-
cachexia, macrophage TNF.

Cook ME, Miller CC, Park Y, Pariza M. Immune modulation by

Cook ME, Sand JM. Dietary egg antibodies to gastrointestinal host targets to overcome inflammatory induced losses in growth and feed efficiency. 9th mid-Atlantic Nutrition conference, 2011 pp 26–35.


Yang M, Cook ME. Dietary conjugated linoleic acid decreased weight loss and extended survival following the onset of kidney failure in NZB/W F1 mice. Lipids 2003;38:21–4.


Scher JU, Pillinger MH. The anti-inflammatory effects of prosta-

Bassaganya-Riera J, Reynolds K, Martino-Catt S, Cuy I, Hemighausen L, Gonzalez F, Rohrer J, Benninghoff AJ, Hontecillas R, Activation of PPAR gamma and delta by conjugated linoleic acid mediates protec-


Wojciech AM, Dow-KoWA R, Miller J, Vogel HJ, Jirik FR. An inflam-


