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TRIENNIAL GROWTH SYMPOSIUM: A review of science leading to host-targeted antibody strategies for preventing growth depression due to microbial colonization[1,2]

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ABSTRACT: In this review, the science used to develop host-targeted therapies for improving animal growth and feed efficiency is presented. In contrast to targeting the microbiota of the host, endogenous host proteins are targeted to regulate an overactive inflammatory response in the host. Activation of the immune/inflammatory systems of an animal is costly in terms of growth and feed efficiency. For example, reduced rates of BW gain and poorer feed efficiency in vaccinated animals compared with nonvaccinated animals have been well documented. Also, the growth rate and feed efficiency of animals colonized by microorganisms is only 80 to 90% of their germ-free counterparts. Further evidence of a cost associated with immune activation is that strategies that enhance the immune capability of an animal can reduce animal growth and feed efficiency. Research now indicates that the growth-promoting effects of antibiotics are indirect, and more likely the result of reduced immune activation due to decreased microbial exposure. Studies of mechanisms by which immune/inflammatory activation reduces animal growth and feed efficiency have shown that cytokines of the acute inflammatory response (i.e., IL-1 and tumor necrosis factor α) are key triggers for host muscle wasting. Cytokine-induced muscle wasting is linked to PG signaling pathways, and it has been proposed that regulation of the PG signaling pathways provide host targets for preventing an overreactive or unwarranted inflammatory event. Intestinal secretory phospholipase A_2 ($sPLA_2$) has been found to be a useful and accessible (i.e., found in the intestinal lumen) host target for the regulation of an overreactive inflammatory response to conventional environments. This review presents the science and strategy for the regulation of intestinal $sPLA_2$ using orally administered egg yolk antibody against the enzyme. Clinically healthy animals fed egg antibodies to $sPLA_2$ had improved growth and feed efficiency. Literature presented indicates that use of host-targeted strategies for regulating the overexpression of inflammatory processes in an animal may provide new mechanisms to improve animal growth and feed efficiency.

Key words: antibiotics, conjugated linoleic acid, egg antibody, immunity, inflammation, phospholipase A_2

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[2]J. Pimentel and C. Miller conducted early experiments showing that egg antibodies to host targets can be fed. D. Jerome and M. Yang were key in moving antibody technology into the commercial sector. D. E. Butz, D. L. Trott, and D. Barnes played critical roles in discovery. S. M. Huebner, E. B. Bobeck, E. Hellestad, J. Parrish, M. Schwartz, and J. M. Sand are valued egg antibody-host target explorers (all from University of Wisconsin, Madison). Collaborators C. G. Scanes (University of Wisconsin, Milwaukee), K. W. Koelkobeck (University of Illinois, Urbana), M. Etzel (University of Wisconsin, Madison), K. Roberson (Michigan State University, East Lansing), and J. Gumpez (University of Wisconsin, Madison) moved the knowledge of egg antibody beyond the author's limits. K. Meyer, J. W. Bishop, J. M. Sand, and S. M. Huebner helped with figures. L. A. Colson, E. B. Cook, J. L. Stahl, and J. M. Sand (all from University of Wisconsin, Madison) provided editorial support. Research support was from Wisconsin Alumni Research Foundation, DCV Biologics (New Castle, DE), College of Agricultural and Life Sciences (UW), Robert Draper Technology Innovation Grant, UW's Industrial and Economic Development Research program, Midwest Poultry Consortium (St. Paul, MN), Homeland Security (Washington, DC), aOva Technologies (Madison, WI), and Hatch. M. E. Cook has an ownership interest in aOva Technologies (Madison, WI), which has licensed his antibody patents reported in this publication.

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INTRODUCTION

Immune defense holds a privileged status relative to animal growth. An animal that is challenged by an infectious organism will divert nutrients away from growth and development to defense processes. Immediate redistribution of nutrients during an inflammatory response (i.e., acute phase response; **APR**) is marked by decreased nutrient intake, wasting of skeletal muscle, and the synthesis of select proteins for defense (Klasing, 1988). Nutrient redistribution during immune challenge is so dramatic that it is not uncommon for some animal species to lose 10% of their BW in 24 h after acute exposure to an immune stimulant (e.g., vaccine, endotoxin, sephadex; Miller et al., 1994).

Modern agricultural practices that focus on efficient production of animal products have been designed to minimize activation of immune defenses and acute inflammatory responses, whether intentionally or inadvertently. For example, simple management procedures including sanitation and the use of all-in, all-out practices are well known to improve animal growth and feed efficiency. Good husbandry practices not only limit the outbreak of clinical infectious disease, but also minimize the activation of immune defenses that slow animal growth and reduce feed efficiency. Despite genetic selection of animals for improved growth and feed efficiency, immune defense remains a priority over growth in the domestic animal just as it is in the wild counterpart. Hence, management of unwarranted immune activation continues to be consistent with good animal husbandry procedures. Usefulness of procedures and products for animal production is hindered if said procedures or products induce unwanted immune responses. One management procedure that does have an adverse effect on animal production is vaccination. In the event of a disease outbreak, the cost of not providing protective immunity far exceeds the loss in productivity that accompanies vaccination. In the discussion that follows, mechanisms for immune-induced growth depression will be reviewed, and an example of using dietary antibodies to "host-target" inflammatory events will be described as a possible strategy to improve animal growth and feed efficiency.

COST OF IMMUNE ACTIVATION

For many years it has been known that the germ-free animal grows at a greater rate and converts feed more efficiently than the animal exposed to commensal organisms (i.e., often referred to as a conventional animal). Lev and Forbes (1959) showed that germ-free chicks exposed to select bacteria had a BW of only 89% of that of the germ-free counterpart. The decrease in growth due to microbial colonization in the modern broiler has not changed since 1959. For example, Drew et al. (2003) compared the BW of modern broiler chicks hatched into germ-free or conventional environments. The BW of the modern broiler in the conventional environment was also only 88.5% of the modern broiler in the germ-free environment. In addition to reduced growth rates, animals colonized by normal microbial species were 11% less efficient at converting food into BW gain (Muramatsu et al., 1988). The decline in growth rate and feed efficiency is not limited to potentially harmful bacteria. Germ-free pigs fed a milk-based diet monoassociated with the gram-positive bacteria *Lactobacillus paracasae* had 17% poorer BW gain and 16% poorer feed efficiency than their germ-free counterparts (Loynachan et al., 2005). Thus, when animals transition from their sterile environment to become colonized by commensal microbial species, they are no longer capable of growing at their true genetic potential. Added exposure to microbial loads can further reduce animal BW gain and feed efficiency. For example, Roura et al. (1992) showed that the BW gain and feed efficiency of chicks in dirty battery cages was reduced 4 and 18%, respectively, compared with clean battery cages. Dirty environments were also shown to decrease growth 14% in swine when compared with clean environments (Renaudeau, 2009). Not surprising was the discovery of improved animal growth when animals were placed in newly built poultry facilities (Hill et al., 1952; Lillie et al., 1953). Maintenance of animal facilities at a level of sanitation that prevents growth depression is generally impossible for cost-effective animal production. Hence, there is a critical need to understand the mechanisms by which the microbial environment influences growth.

Because animals are intentionally immune-stimulated through the use of vaccination programs, vaccination induces additional losses in productivity. Chamblee et al. (1992) reported that vaccination of broilers resulted in "decreased final body weights, poorer feed conversion ratios, and greater 8d and 42d mortality [than unvaccinated broilers]...in the absence of overt disease." In a study investigating the effects of vaccination on the final carcass yield of ducklings, vaccination using a gram-negative bacterin reduced final carcass weight and breast meat yield 9 and 13%, respectively, when compared with unvaccinated ducklings (M. E. Cook, unpublished results).

Losses in productivity due to microbial colonization or vaccination can be estimated, assuming that these losses occur throughout the productive life of the animal. For example, the 16% decreased feed efficiency observed when milk fed germ-free pigs were exposed to *L. paracasae* (Loynachan et al., 2005) would indicate that an immune-stimulated pig would need 40 kg less corn during its growing period to achieve a typical market weight, assuming the improved feed efficiency was maintained throughout the growth phase. A total savings just on corn consumption could exceed $500 million in the United States per year. Additional cost associated with losses due to sanitation, vaccination, and increased time to market weight demonstrate an

urgent need to address the problem of immune-induced growth depression.

INTESTINAL CHANGES DURING THE TRANSITION FROM GERM-FREE TO CONVENTIONAL ENVIRONMENTS

As animals transition from a germ-free environment to one colonized by commensal bacteria, the number of lymphocytes and other immune cells begin to increase in the intestine. Pabst and Rothkotter (1999) showed that the number of lymphocytes per enterocyte doubled in a 2-mo period in germ-free pigs, but increased 10-fold in pigs housed in conventional environments. Increased immune cellularity in the intestines of conventional vs. germ-free housed animals partly explains why intestinal weights doubled during bacterial colonization (Waxler and Drees, 1972). Because immune cells in the intestine are for defensive purposes, it was not surprising that cytokine secretion increased when immune cells were exposed to bacteria in the lumen of the intestinal tract. Splichal et al. (2007) orally gavaged germ-free pigs with a virulent and an avirulent mutant (i.e., aroA) of *Salmonella enterica* serovar Typhimurium. Whereas no IL-18 was found in the intestinal lumen of the germ-free animals, 704 and 244 pg/mL of IL-18 was found in the intestinal washings of pigs exposed to the virulent and avirulent forms of the bacteria, respectively. These findings were remarkable for 2 reasons. First, even though the aroA mutant had very low invasive capacity (i.e., 6% of that of the virulent wild-type), IL-18 concentrations in the intestinal lumen were markedly increased when pigs were orally exposed to the mutant. Second, the release of cytokines into the intestinal lumen (none was found in the plasma) may indicate that cytokines have a functional role in intestinal secretions. This latter observation would indicate that the cytokines released into the intestinal secretions could be targeted by dietary strategies that function in the lumen (e.g., enzymes, binding agents, which will be discussed further subsequently).

The majority of the microorganisms in the body of an animal are in the gastrointestinal tract (i.e., 1 to 2% of the BW of an animal is intestinal bacteria). The intestine represents a critical interface between the residing microbes and the animal. This interface is complicated by the need for nutrient absorption on the one hand and defense against potentially pathogenic organisms on the other. Hence, the gastrointestinal tract must maintain a considerable defense, which includes cells and products used for signaling and destroying harmful bacteria. Unlike the intestine of the germ-free animal, nutrients consumed by the conventional animal are used 1) by the colonizing bacteria, 2) to maintain the function of the immune defense, and 3) to repair tissues that may be damaged during an inflammatory response. Additional needs of the conventional animal for nutrients at the intestinal level, when compared with the germ-free animal, may explain poorer feed efficiency in conventional animals.

USE OF ANTIBIOTICS AS GROWTH STIMULANTS

In 1946, Moore and coworkers at the University of Wisconsin–Madison observed that chick growth was stimulated by streptomycin (Moore et al., 1946). These findings were reproduced in many laboratories and the use of antibiotics for stimulating growth in animals grew rapidly. Early attempts to determine the mechanism by which antibiotics stimulated growth led to investigations using germ-free animals. In the study reported by Lev and Forbes (1959), it was apparent that antibiotics did not stimulate growth in the absence of bacteria, however approximately 50% of the BW loss associated with the colonization of the animal with bacteria could be prevented through the feeding of antibiotics. Roura et al. (1992) showed that dietary antibiotics were effective at stimulating growth in the unsantitized environment, but not in the clean environment. Similarly, when new poultry facilities were built in the 1950s, it was noted that antibiotics were less effective at stimulating growth (Coates et al., 1952; Hill et al., 1952). Growth suppression resulting from increasing the chick density in a housing unit could be prevented through the feeding of antibiotics (Dafwang et al., 1987). In addition to changes in growth rate, investigators also found that chicks fed antibiotics had gut thinning. The weight of the intestine of a chick fed antibiotics was reduced 20% (Dafwang et al., 1996). Thus, it would appear that antibiotics prevented the increase in gut weight associated with microbial colonization (Waxler and Drees, 1972). In a study conducted by Yaguchi et al. (2006), the influence of 2 antibiotics on intestinal lymphocyte cell numbers was investigated in mice. Mice injected with the antibiotics cefmetazole or imipenem had decreased (>50%) bacterial numbers in intestinal washings and similar numbers of intestinal Peyer's patches as the noninjected controls; however, the number of lymphocytes per Peyer's patch was reduced 38 and 26%, respectively.

Many theories attempted to describe the mechanism by which gut microbes decrease animal performance and dietary antibiotics stimulate growth (see Visek, 1978, for a complete review of these theories). Most theories speculated that antibiotics stimulated growth by depressing disease causing microbial populations or their toxins or both, or by reducing microbial use of nutrients. Others speculated that gut thinning allowed for more efficient nutrient absorption. It may be concluded from the studies published that antibiotics have both an effect on the microbial population (reducing bacterial exposure) and the animal (reduce intestinal lymphocytes). Without a clear mode of action, the use of antibiotics in animal feed expanded to include approximately 50% of all antibiotics manufactured by

1978 (Van Houweling, 1978; Commission on Life Sciences, 1980). However, this expansion did not occur without strong objection (Van Houweling and Gainer, 1978). Witte (1998) made a plea to the scientific community for the development of alternatives to antibiotics as growth stimulants. The general concern was that feeding antibiotics to animals may adversely affect human health. Although it is not the intention of this review to consider the value of antibiotics as growth stimulants in animal agriculture, it becomes clear that understanding how antibiotics stimulate growth may create the opportunity to develop new strategies to improve animal growth and feed efficiency.

HYPOTHESIS TESTING

The response of the animal to microbial colonization and antibiotics serves as a useful opportunity for developing a hypothesis to test new methods for improving animal growth. If indeed the efficiency of an animal was reduced by as much as 20% due to microbial colonization and environmental management, an effective alternative would have considerable value. The observations described above could lead one to hypothesize that improving the ability of the animal to mount an inflammatory or immune response would make the defense mechanisms more robust and more likely to resolve a potential invasion quickly, thus returning the flow of nutrients to growth. Early attempts were made to improve the immune response of the animal to make the animal healthier and thereby more efficient.

Cook (1991) reviewed the literature involving pharmacological amounts of vitamin E as a potential means of enhancing immune response. Tengerdy et al. (1972) showed that chicks fed dietary vitamin A or E in excess of the nutrient requirement had increased antibody responses. Pharmacological amounts of vitamin E also proved effective in reducing *Escherichia coli*-induced mortality (Tengerdy and Brown, 1977). These studies and others by this Colorado State University research team indicated a possible means for testing the hypothesis that improved immunity (i.e., antibody production and phagocytosis) would improve animal health and efficiency of production. Unfortunately, BW gain and feed efficiency were never reported in the Colorado studies. When field trials were conducted using the guidelines set forth by the Colorado studies, unsatisfactory responses (i.e., poor growth) were observed. An experiment was conducted comparing turkey growth rates when fed vitamins E and A at 10 times the requirement (NRC, 1984), as compared with smaller dietary amounts (M. E. Cook, unpublished data). Increased quantities of both vitamins suppressed growth 8%. Huff et al. (2004) also found that turkeys supplemented with increased vitamin E in water had a decreased growth response. Even though chickens have no dietary requirement for vitamin C, studies have shown that supplemental vitamin C can increase immune responses and decrease *E. coli*-induced mortality (reviewed in Cook, 1991). Gross (1992) observed a linear decrease in feed efficiency with increasing vitamin C in the diet even though ascorbic acid was effective at reducing lesions caused by *E. coli*. Not all nutrients that increase immune function appear to have an effect on growth. The work of Tsiagbe et al. (1987) indicated that as dietary methionine was increased to elevate measures of immune function, there were no adverse effects on animal performance. Nonetheless, it would appear that the hypothesis that more immunity yields improved animal growth may be problematic.

An alternative hypothesis to the one that enhanced immunity may improve performance emerged; the alternative hypothesis is that the immune response to microbial colonization and the microbial environment may be causing decreased animal performance. Although this hypothesis may not lead to a useful method to improve animal performance, it provided a framework to discover immune-related regulation of animal growth. An important body of work that supported the concept of immune regulation of growth began to surface early in the 1980s with the discovery of key inflammatory cytokines, IL-1 and tumor necrosis factor α (**TNFα**). Early studies showed that pure forms of IL-1 (also known as endogenous leukocytic mediator, Dinarello, 1984) and TNFα (also known as cachectin, Cerami et al., 1985) were endogenous host mediators of BW loss and skeletal muscle catabolism during acute inflammation. Using the studies provided above, one can reasonably postulate immune regulation of growth because there was increased infiltration of lymphocytes into the gastrointestinal tract (**GIT**) during microbial colonization and the cytokines these immune cells released were responsible for diverting nutrients away from animal growth and into maintaining host defense. When antibiotics were fed, microbial abundance was decreased (Wise and Siragusa, 2007), and thus, there was no need to maintain the level of defense observed in the conventional animal. Under this condition immune cells would retreat from the GIT, causing gut thinning. An immune regulation of growth hypothesis might also explain why increasing immune activity could also exacerbate the diversion of nutrients from growth to host defense.

In a series of critical studies conducted by K. Klasing at Cornell University and then the University of California–Davis, the hypothesis of immune regulation of growth was supported. First, Klasing and Austic (1984) showed that even the injection of sheep red blood cells into the chicken suppressed growth. Their work eventually illustrated that the immune stimulant did not have to be microbial in nature. Klasing and Austic (1984) then focused on how endotoxin (i.e., lipopolysaccharide from gram-negative bacteria) affected growth. When chicks were injected with endotoxin, macrophages released inflammatory cytokines into circulation and growth rate and feed intake was reduced. Reduced growth was not directly related to decreased feed intake (pair-fed chicks grew faster than endotoxin-injected chicks); however,

the growth reduction and decreased feed intake could be mimicked simply by injecting IL-1. To further support the role of immune function in controlling growth, one would have to show that the immune capacity of the animal was directly related to growth rate. High- and low-producing antibody lines of chickens were developed (Siegel et al., 1982). After many generations of selecting birds for their antibody response, it was found that birds with the greatest antibody response grew 13% slower than birds with a low antibody response. In an elite breeding line of ducks selected for growth rate, Cook (2004) reported that cell-mediated immunity was inversely related to BW gain. Miller et al. (1992) also observed that selection of ducks for certain growth traits adversely affected immune responses. These examples provided considerable evidence to support that the immune response was capable of regulating growth and that selection for growth rate can adversely affect immune function.

Studies to this point indicated that there were 2 primary methods to stimulate growth. The first was to decrease the agents that induce an immune or inflammatory response. Useful procedures included sanitation, the use of antibiotics, or other feed agents that minimized the ability of GIT microbes to stimulate the immune system (pre- and probiotics, enzymes, and microbial binding agents). Although these supplements were shown to be effective agents in stimulating growth, they would not prevent growth suppression caused by certain vaccination procedures. A second method effective in stimulating growth was through the regulation of immune and inflammatory responses. Frank suppression of immune function was potentially growth stimulating, if no infectious disease process developed. A more appropriate means of suppressing immunity or inflammation was through select targeting of the inflammatory cascade. Immune regulation is the foundation of many human and animal pharmaceuticals in the treatment of inflammatory diseases (e.g., cyclooxygenase inhibitors or cortisol). Indeed, young pigs fed aspirin (Xu et al., 1990) or injected with dexamethasone (Gaines et al., 2002) have improved growth rates. A third possible method to improve growth rate of animals involved reducing the effects of the immune cells and their products on the nonimmune tissues. For example, if the mechanism of decreased BW gain caused by injection of IL-1 could be targeted, perhaps an adequate immune response could occur with a minimal decline in growth and feed efficiency. To discover approaches that minimize the collateral effects of immune activation on animal growth and feed intake, a basic understanding of immune-induced wasting had to be explored.

MECHANISM OF IMMUNE-INDUCED GROWTH DEPRESSION

In an attempt to understand the mechanism by which IL-1 induced growth depression, researchers conducted studies on the effect of IL-1 on muscle turnover.

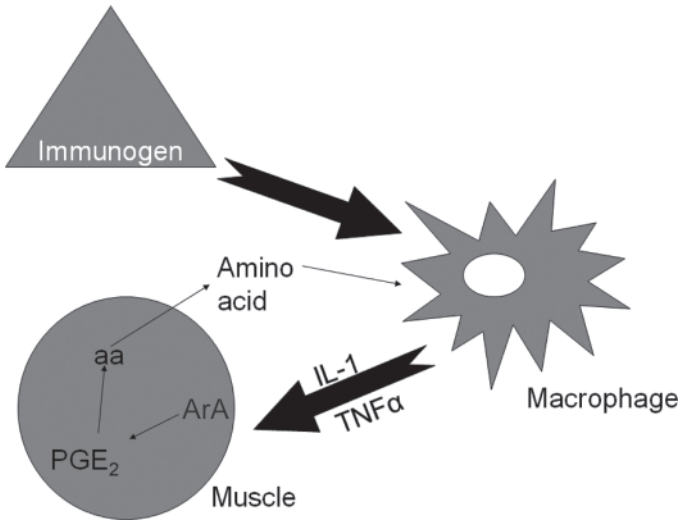

Figure 1. The response of an animal to systemic immune activation. Immune activation results in the release of immune proinflammatory products, such as IL-1 or tumor necrosis factor α (TNFα). Inflammatory cytokines induce muscle catabolism through a PG-mediated pathway. Prostaglandins are derived from essential fatty acids (e.g., linoleic acid) found in the diet, fatty acids which are modified to immediate precursor of PG (e.g., arachidonic acid; ArA). When arachidonic acid is released from the cell membrane by action of cytosolic phospholipase A_2, it is converted to PG that in turn mediate the release of AA from muscle into circulation. Released AA can be used for synthesis of immune and acute phase proteins.

Skeletal muscles from rats were incubated with IL-1, and then protein synthesis and degradation were measured (Goldberg et al., 1984). The addition of IL-1 to muscle in vitro increased protein degradation almost 2-fold with no change in the rate of muscle synthesis. Interleukin-1-induced muscle degradation was also observed in the chick (Klasing et al., 1987). This research supported a role for IL-1 as the mediator of muscle wasting during the APR (reviewed by Klasing 1988; Cook, 2004). Goldberg et al. (1984) also showed that when muscle was treated with IL-1, there was a release of PGE_2 from the muscle. Direct application of PGE_2, an eicosanoid, to cultured muscles also induced muscle wasting (Rodemann and Goldberg, 1982). Thus, during an immune or inflammatory response, systemically released IL-1 (and TNFα) induces muscle wasting through an eicosanoid-dependent pathway (Figure 1, Baracos et al., 1983; Butz et al., 2009). If eicosanoids were the secondary signaling transduction pathway that resulted in IL-1-induced muscle wasting, a dietary mechanism for protecting muscle during an inflammatory event could be envisioned (Cook et al., 1993).

The eicosanoids are derived from common dietary fatty acids: linoleic acid for 2-series PG and α-linolenic acid for 3-series PG (Hwang, 1989). The 2-series PG are synthesized from an arachidonic acid product of linoleic acid by action of cyclooxygenase (**COX**). It is well known that 3-series PG are less inflammatory and could modify some physiological effects of IL-1 (Hellerstein et al., 1989). Using synthesized structural and geometrical derivatives of linoleic acid, known as CLA, experiments were conducted in the early 1990s to deter-

mine if endotoxin-induced BW loss could be prevented through dietary means. Dietary CLA was found to protect against immune-induced BW loss in chicks (Cook et al., 1993) and mice (Miller et al., 1994). In addition, it was found that CLA actually enhanced measures of immune function (Miller et al., 1994) and protected against the collateral damage of immune-related disorders, such as type 1 hypersensitivity (Whigham et al., 2001), lupus (Yang and Cook, 2003b), cancer cachexia (Graves et al., 2005), and arthritis (Huebner et al., 2010). Studies with CLA (not reviewed herein) provided important insight in the development of new dietary approaches to improve the growth rate and feed efficiency of the inflamed animals (Cook and Pariza, 1998; Cook, 1999).

LESSON LEARNED FROM THE REGULATION OF INFLAMMATION BY CLA

Acute inflammatory cytokines are released within the first hour of an inflammatory initiating event (i.e., preformed and independent of transcription) and again approximately 4 h after the initiating event (i.e., dependent on transcription and translation). Dietary CLA (*t*10, *c*12-CLA isomer) was found to regulate the transcriptional/translational dependent production of IL-1, TNFα, inducible nitric oxide synthase and COX-2 4 to 5 h after endotoxin exposure (Li, 2004; Li et al., 2005, 2006). Using COX-2 as a surrogate marker of inflammation, Li et al. (2005) showed that CLA affected the inflammatory signaling cascade upstream of the nuclear factor-κB pathway. Yang and Cook (2003a) supported the upstream regulation of cytokines by CLA when they found that release of preformed TNFα within 1 to 2 h, in response to endotoxin challenge, was also affected by dietary CLA. Hence, the regulation of inflammation by CLA appears to be upstream of transcriptional events. Using an airway hypersensitivity animal model, Whigham et al. (2001) showed that dietary CLA affected the initiation of an inflammatory cascade (i.e., PG) as early as 90 s after immunogen stimulation. These works indicated that upstream regulation of PG synthesis was key to downstream cytokine release.

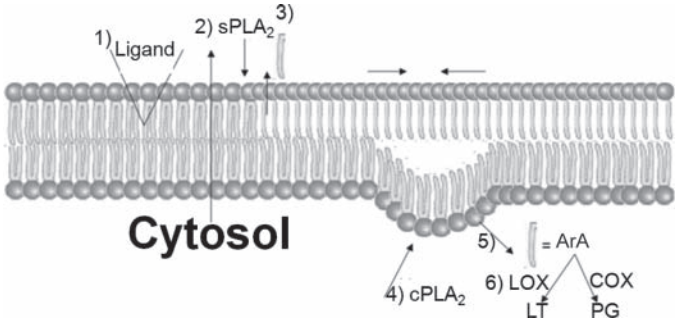

Figure 2. An illustrative model of secretory phospholipase A_2 ($sPLA_2$) and cytosolic PLA_2 ($cPLA_2$). 1) After cellular binding of an inflammatory ligand, 2) $sPLA_2$ is released to an extracellular position, where 3) $sPLA_2$ cleaves the fatty acid located in the *sn*-2 position of cell membrane phospholipids in the outer leaflet yielding a release of FFA and lysophospholipids (e.g., lysylphosphatidylcholine; LPC). 4) Action of $sPLA_2$ permits cytosolic phospholipase A_2 ($cPLA_2$) to 5) cleave arachidonic acid (ArA) from the *sn*-2 position of phospholipids on the inner leaflet of cell membranes. 6) Arachidonic acid is then acted on by either cyclooxygenase (COX) or lipoxygenase (LOX) to produce PG or leukotrienes (LT), respectively.

Research on the anti-inflammatory mechanism of CLA indicated that it may be possible to regulate immune-induced growth depression with no adverse effect on immune response. Demonstration that CLA may be regulating the inflammation at initiation of the inflammatory cascade supported identifying upstream targets for preventing immune-induced growth depression. It seemed reasonable that the most appropriate location to target immune-induced growth depression was where the immune system and the immune stimulants were most likely to interface, which was the GIT.

REGULATION OF SECRETORY PHOSPHOLIPASE A_2 TO IMPROVE THE EFFICIENCY OF ANIMAL PRODUCTION

Cook (2004) proposed a model for growth suppression due to the colonization of the GIT of the animal. In this model, the movement of bacteria to the GIT during the transition from germ-free to conventional environments was marked by increased movement of immune cells into the intestine, which suppressed growth and feed efficiency. Lessons learned from CLA indicated that the inflammatory process, and perhaps the reduced growth and feed efficiency, could be attenuated if key inflammatory initiation steps were regulated. If PG were involved in the initiation of a cascade of inflammatory events, as indicated by the CLA studies, regulation of PG might be useful for growth stimulation. Grossman et al. (2000) had shown that secretory phospholipase A_2 (**$sPLA_2$**) played a significant role in endotoxin-induced PG synthesis in intestinal epithelial cells. Although at the time there was no link between $sPLA_2$ and CLA in the regulation of eicosanoid production, nor was there a clear link between eicosanoids and the early release of preformed inflammatory cytokines (i.e., IL-1 and TNFα), $sPLA_2$ was selected as a host target to regulate the inflammatory process in the GIT.

The family of $sPLA_2$ is complex and beyond the limits of this review. Secretory PLA_2 is released from the cell during appropriate stimuli, where it begins to cleave fatty acids from the sn-2 position of phospholipids in the outer leaflet of cell membranes (Figure 2). A previous report indicated that cytosolic PLA_2 (**$cPLA_2$**) release of arachidonic acid from the inner leaflet of the cell membrane was $sPLA_2$ dependent (Hernández et al., 1998). A reasonable hypothesis indicated that inhibition of $sPLA_2$ may be an effective means for regulating PG production in the mucosal immune cells stimulated with endotoxin (see Figure 3). The ability of dietary CLA to inhibit PG release from allergen-stimulated trachea within 90 s (Whigham et al., 2001) was also

supportive of value in regulating $sPLA_2$, and recent work showed that CLA regulates $sPLA_2$ (Stachowska et al., 2007). Thus, $sPLA_2$ was targeted to improve animal growth.

Several years after work targeting $sPLA_2$ had begun, papers were published that provided credence to the importance of regulating $sPLA_2$ in the GIT. Conventional animals (as compared with germ-free animals) were adversely affected by $sPLA_2$ release in the intestine (Rozenfeld et al., 2001). More importantly, acute, severe systemic endotoxemia in rats induced a release of $sPLA_2$ into the lumen of the GIT, and $sPLA_2$ was responsible for a loss in gut barrier function (Zayat et al., 2008). Inhibiting $sPLA_2$ in the gut lumen maintained barrier function.

An ideal regulator of $sPLA_2$ for animal agriculture should have the following characteristics if it is to be rapidly adopted: 1) the inhibitor should leave no residues in animal tissues; 2) the inhibitor should be generally recognized as safe and naturally found in the environment; 3) the environment ideally is not affected by use of the inhibitor; 4) the inhibitor would need to be mass-produced in a cost-effective manner, and 5) the inhibitor should improve growth and feed efficiency in the absence of clinical infectious disease.

Antibody against $sPLA_2$ (**$aPLA_2$**) was selected as a method to inhibit the enzyme. Antibodies are natural products of many foods and, therefore, have a long history of safe use. Antibodies can be made to be highly specific for a given target. The protein nature of antibodies indicated they would create no environmental concerns. A considerable number of reports showed that antibodies provided orally have biological activity in the GIT (Cook and Trott, 2010). Antibodies are not absorbed to any significant extent through the intestine after postnatal gut closure. Methods to produce antibodies in large quantities were possible. Large-scale antibody production can be achieved through transgenic means involving plants and microbial fermentation. A system of mass antibody production using laying hen was recently reviewed and served as the primary means for producing egg $aPLA_2$ for improving animal growth (Cook and Trott, 2010).

Cook (2004) reported the effectiveness of egg antibodies to host targets, including $sPLA_2$, for stimulating the growth of healthy chicks. In this review, the improved growth response resulting from the feeding $aPLA_2$ in five 3-wk chick battery trials averaged 5.4%. As of this date, 15 battery trials using $aPLA_2$ have been conducted and the average improvements in BW gain and feed efficiency were 5.3 and 3.8%, respectively (M. E. Cook, unpublished results). Currently, new techniques are being used to generate antibodies to $sPLA_2$ peptides that have increased effectiveness in improving performance in broiler. Barry and Yang (2008) tested a commercial $aPLA_2$ product designed for rainbow trout on growth and feed efficiency. Growth was increased in a dose-response manner with increasing quantities of the commercial $aPLA_2$ product (27.8%). Additional studies in pigs have also shown evidence of improved growth (Corrigan et al., 2007).

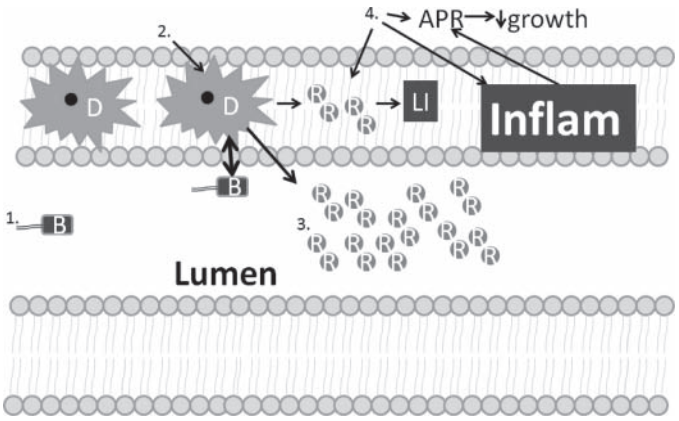

Figure 3. A hypothetical model for growth depression caused by intestinal inflammation. Bacteria (B) enter the intestinal lumen and interface with defensive immune cells (D). Immune cells release host response products (R) into the surrounding tissue space and the intestinal lumen. Although a local inflammatory response (LI) may be limited, in an inflammatory reaction of significant magnitude, R, can move down the intestinal lumen, amplifying the inflammatory response to a systemic inflammatory response (Inflam) and an acute phase response (APR). The Inflam induces muscle wasting through cytokine action that in turn slows animal growth. The inflammatory cascade can be interrupted by 1) the removal of microbial immune stimulates (e.g., antibiotics), 2) suppression of the response of D (immune suppression), 3) targeting and disabling R (e.g., anti-phospholipase A_2), or 4) systemic use of antiinflammatory agents (e.g., cortisol).

Although reports on the effects of $aPLA_2$ appeared to support the hypothesis that protection against the collateral effects of the inflammatory response would improve growth, the mechanism for $aPLA_2$ growth improvement remains under investigation. Secretory phospholipase A_2 can participate in inflammation through several different mechanisms. As reported by Zayat et al. (2008), sPLA2 was involved in disruption of the intestinal barrier function through a mechanism not described. Disruption of barrier function would likely result in the movement of potent inflammatory stimulants (e.g., endotoxin) to resident immune cells in the intestinal mucosa. If the disruption was severe, the release of proinflammatory cytokines into general circulation, such as observed in studies by Splichal et al. (2007), could induce systemic wasting. Secretory PLA_2 can also influence proinflammatory cytokine production through a $sPLA_2$ receptor-mediated process (Hanasaki and Arita, 2002). A third mechanism by which $sPLA_2$ can mediate inflammatory cytokine production is through the production of lysophospholipids, which can be presented via the CD1d receptor on antigen presenting cells to natural killer T cells (Fox et al., 2009). In this latter study, the addition of $aPLA_2$ egg antibody to human peripheral mononuclear cells prevented cytokine production by natural killer T cells during mixed culture.

A potential problem in attempting to regulate host immune/inflammatory processes is its effects on disease resistance. Use of general immunosuppressive drugs after transplantation increases the susceptibility of the

host to bacterial and viral diseases (Rubin, 2001). In contrast, people given anti-tumor necrosis factor therapy for the treatment of inflammatory bowel disease are not at an increased risk of serious microbial infection (Peyrin-Biroulet, 2010). Thus, generalizations of increased risk of infection as a result of targeting host immune products or pathways should be avoided. In the case of phospholipases, certain classes of $sPLA_2$ (e.g., systemic $sPLA_2$ group IIA) have antimicrobial activity (Nevalainen et al., 2008), whereas other forms of PLA_2 have a role in the pathogenesis of microorganisms (Sitkiewicz et al., 2007). Studies involving commercially produced $aPLA_2$ in over 100 trials have not resulted in increased incidence of infectious disease (M. Yang, aOva Technologies Inc., Madison, WI, personal communication). When $aPLA_2$ was fed to chicks infected with coccidia, clinical signs of the disease were not exacerbated (Schwartz et al., 2006). In a model of *Salmonella* Typhimurium, turkeys fed $aPLA_2$ had decreased clinical signs of associated with the infection (Scanes et al., 2008).

ADVANCING HOST TARGETED APPROACHES TO IMPROVE ANIMAL GROWTH

Cook (2004) provided examples of several host targets that, when inhibited by egg antibody, improved animal growth and feed efficiency. Three aspects of antibody regulation of host targets warrant additional consideration by the scientific community. First, the use of dietary antibody could be a powerful tool in the study of key host targets that regulate animal growth. It is apparent that the host releases mediators of inflammation into the lumen of the GIT that may regulate animal growth. The reason the host releases cytokines and select peptides into the GIT lumen may not be directed at the intestinal microbial community, but may be involved in host intercellular signaling. Further studies in these areas might prove illuminating.

SUMMARY AND CONCLUSIONS

Host target approaches to improve animal growth and feed efficiency may provide a new approach in investigating alternatives or adjunct for the use of antibiotics as growth stimulants. Scientists have developed several exciting approaches to alter the microbial load and physiology of intestinal microbial species for the purpose of improving growth and feed efficiency. Additional attention to the response of the host to the microbial flora could provide alternative mechanisms to improve animal growth. Host targeting of inflammatory processes might provide improvement in animal performance that is additive or synergistic with microbial targeting. Systemic application of pharmaceuticals to target unwanted inflammatory processes in humans and animals have been developed and widely administered. Perhaps the initiation of inflammatory processes can be controlled within the lumen of the intestine. Regulation of inflammation within the intestinal lumen could have many benefits in animal agriculture because it would minimize concerns with regards to food safety (e.g., drug residues) and such therapy could be easily applied using dietary means.

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