Nutrition and Immunity in Dairy Cattle: Implications to Hemorrhagic Bowel Syndrome

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Introduction

Nutrition impacts every physiological process in the body. Hence, it should not be surprising that nutrition also has important implications to immunity and incidence of disease. While scientists have known for many years that nutrition influences immunity, only in recent years have specific mechanisms by which nutrients affect immunity become apparent. The purpose of this review will be to acquaint readers with some general concepts of immunology and to review what is now known about how specific nutrients benefit the immune system of ruminant livestock. This review will then focus on an emerging disease in dairy cattle (hemorrhagic bowel syndrome: **HBS**) and nutritional strategies which may be useful in preventing the disease.

General Concepts of Immunology

The immune system consists of two distinct *arms* which work in tandem to prevent infections. These are termed the *innate* immune system and the *acquired* immune system. The innate immune system, as its name implies, consists of readily-available mechanisms which *fight* the first stages of infection. This system essentially provides the first line-of-defense against pathogens; whether bacterial, viral, protozoal, or fungal. By providing this front line barrier, the innate system provides the time required by the acquired system to develop an antibody response against a specific pathogen. Developing the antibodies against specific pathogens requires several days to several weeks.

Innate Immunity

The innate immune system is an evolutionarily ancient mechanism to fight disease. It consists of several anatomic, physiologic, and cellular components. Anatomic aspects include the epithelial barriers to infection provided by the skin, lung, mammary, and gastrointestinal (**GI**) tract. Secretion of hydrochloric acid and digestive enzymes by the GI tract also aids in preventing entry of pathogens into the body. But, in addition to these, animals possess a cellular component of innate immunity. The cellular component consists of phagocytic cells (*e.g.*, neutrophils and macrophages) which locate sites of infection and then attack and kill pathogens before they have the opportunity to proliferate and cause a significant infection.

The term *innate immunity* implies that it is stable or unwavering. But, this is not the case. Innate immunity, although always present to some degree, is regulated and may be *strengthened* or *weakened* by plane of nutrition and by stress.

Innate immunity is a non-selective immune system. It does not modify itself depending upon the type of pathogen challenge. Instead, it prevents infection by targeting general properties of pathogens. For example, few pathogens can withstand the low pH of the stomach (abomasum) and most should be digested by the digestive enzymes of the GI tract.

The cellular component of innate immunity identifies pathogens by their presentation of distinct pathogen-associated molecular patterns (**PAMPs**). Specifically, pathogens contain molecules not typically found in mammalian cells and, via this strategy, cells of the innate system are able to recognize foreign cells. Examples of molecules associated with pathogens which are recognized by innate cells include lipotechoic acid, double-stranded RNA, CpG DNA sequences, unusual sugar residues (e.g., mannans) among others. Figure 1 provides a list of molecules expressed on the surfaces of pathogens that are detected by specific Toll-like receptors present on the surface of innate immune cells. Binding of PAMPs to Toll-like receptors initiates killing mechanisms by the neutrophils and macrophages.

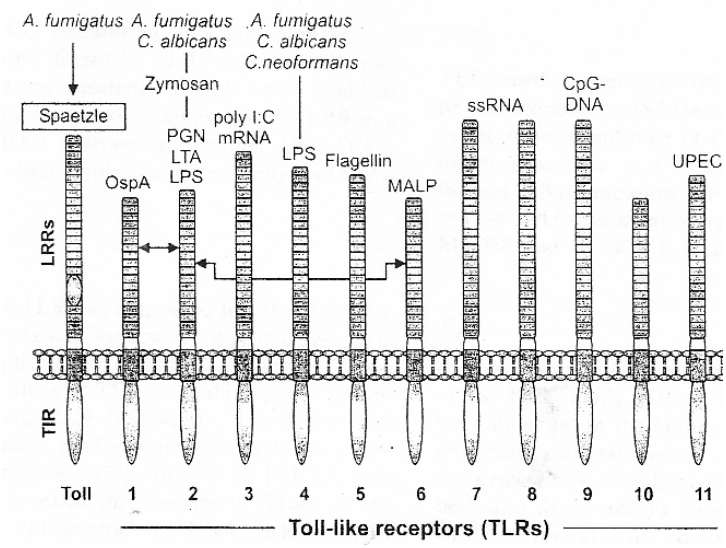


Figure 1. Toll-like receptors in mammalian cells. **Source**: Roeder et al., 2004. Note that 11 Toll-like receptors have been identified and each binds to a specific PAMP. Toll-like receptors 2 and 4 appear responsible for the identification of common molds (e.g., *A. fumigatus* and *C. albicans*) as foreign.

Acquired (Adaptive) Immunity

The second arm of the immune system is termed the *adaptive system* and is characterized by the production of antibodies, which are directed against specific antigens. Pathogens can be phagocytosed and digested by antigen-presenting cells (*e.g.*, macrophages, B lymphocytes and dendritic cells). Digested pieces of pathogens are presented on the surface of the antigen-presenting cell to a T-helper cell (**T_H cell**). The T_H cell may then stimulate clonal expansion of a B-cell lineage, which then secretes antibodies (Figure 2). Alternatively, the T_H cell, in response to a cytokine termed *interleukin-2* (**IL-2**) will develop into a cytotoxic T-lymphocyte (**CTL**). CTLs express antibodies tethered to their cell surface and can mediate destruction of cells infected with pathogen (Figure 2).

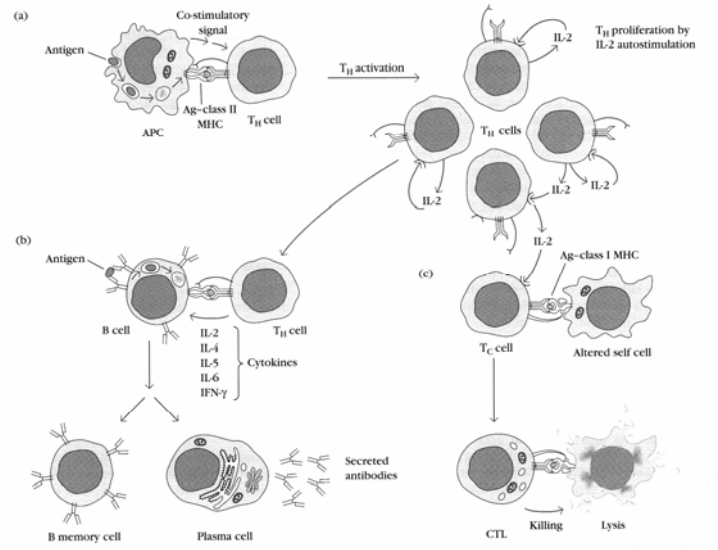


Figure 2. Cellular interactions involved in induction of immune responses. Activation and proliferation of T_H cells (a) is required for generation of a humoral response (b) and a cell-mediated response to altered self-cells (c). APC = antigen presenting cell; Ag = antigen. **Source:** Janeway et al., 2004.

Antibodies may take on a variety of forms and are referred to as immunoglobulins (**Ig**). The most common Ig isotopes include IgM and IgG. IgMs are the first antibodies to be produced by the immune system in response to an infection. Although they *arrive on the scene* quickly following an infection, they possess relatively low affinity against antigen. The more-*powerful* IgG isotypes (IgG_1, IgG_2, IgG_3 and IgG_4) require additional time for their development.

Relationship of the Innate and Acquired Immune System

Discussion of innate and acquired arms of the immune system separately implies that these systems function independently. However, we now know that the two arms communicate with one another and; to some extent, rely upon similar communication molecules. In the past ten years, for example, we have learned that up-regulation of the innate system provides an important feed-forward system for antibody production. For example, activation of neutrophils by invading pathogens causes neutrophils to release IL-1β which, in turn, stimulates the acquired system.

Nutrition and Immunology

Methodology

A challenge facing those of us with interest in the interface between nutrition and immunity is: What are the best predictors of immune status? *Immunity* is an extremely broad concept and there are literally dozens of methods available for assessing immune function. No one laboratory is capable of completing all assays of immunity and, as a result, it is often difficult to make comparisons from one study to the next. The general strategies which have been used to assess impact of a treatment or nutrient on immune function have included the following (Chew and Park, 2004):

A. ***Immunoglobulin production***. One may assess total IgM and IgG concentrations in blood as indexes of immunity. These do not provide very definitive information on immunity; however, because the total IgM or IgG fraction represents the combined titer against all antigens to which an organism has been exposed. Nutritionists should be wary of studies which examine total IgG or IgM responses. More useful information is derivable from titer.

B. ***Titer***. Titer provides specific insight into concentrations of antibodies (whether IgM or IgG) directed toward a specific antigen.

C. ***Immune cell proliferation***. T- and B-cells can be induced to proliferate by the addition of specific chemicals (mitogens) to their environment. The rate of cell division induced by the mitogen provides an index of the ability of the humoral immune system to *ramp-up* following an infection. Typical mitogens added to stimulate T- and B-cell division include poke weed mitogen and lipopolysaccharide.

D. ***Killing activity of lymphocytes*** (Lymphocyte cytotoxic assay). This assay determines the ability of T cells to kill target cells.

E. ***Cytokine production***. Communication among cells which mediate immunity is carried-out by a large number of hormones called *cytokines*. Common signaling cytokines include IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, tumor necrosis factor-alpha (**TNF_α**) and interferon-gamma (**IFN_γ**). Measuring concentrations of cytokines in biological samples can provide useful information on activities of specific aspects of the immune system.

F. ***Delayed-type hypersensitivity (DTH) assay***. A standard means of assessing a cell-mediated immune response is to perform a DTH test. This is done by injecting an antigen of interest intradermally and then determining if there is a delayed induration or swelling reaction at the site. The level of DTH reactivity is determined in humans and guinea pigs by the diameter of induration 48 h after antigen injection or in mice by the amount of ear or footpad swelling 24 h after antigen injection (Nichols et al., 2002).

G. ***Molecular diagnostics***. Availability of genomic sequences for pathogens and new molecular screening techniques has given rise to methods which assess specific components of immunity. Our laboratory at Oregon State University is currently developing and relying upon such indexes of immunity. Examples will be provided later in this manuscript.

Nutrition and immunity

Calder and Kew published a review in 2002 on nutrients with known effects on immunity. In non-ruminants, essential amino acids, linoleic acid, vitamin A, folic acid, vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, zinc, copper, iron, and selenium affect one or more indexes of immunity (Calder and Kew, 2002). Tam et al. (2003) have also published a review on potential roles of magnesium in support of the immune system. Vitamin E and zinc have received the most attention as immunostimulatory nutrients (Calder and Kew, 2002).

Less is known about the nutritional regulation of immunity in ruminant livestock. But, it may safely be assumed that nutrients, at the tissue level, will have similar effects on immunity in ruminants as in non-ruminants. Perhaps dietary sources of the immunostimulatory B-vitamins (B_6, B_{12}, folic acid), vitamin C, and the essential amino acids are less important in ruminants as these are either endogenously synthesized (*i.e*., vitamin C) or provided by a healthy microbial population (*i.e*., essential amino acids, B-vitamins). Spears (2000) reported that selenium, vitamin E, chromium, cobalt, copper, and vitamin A have immune regulatory properties in cattle.

How Do Specific Nutrients Affect Immunity in Dairy Cattle?

Research within the past decade is describing how individual nutrients affect immunity. A general mechanism by which nutrients support the immune system is via provision of antioxidants. Immune cells are characterized by high levels of reactive oxygen species (**ROS**) which are used, in part, to kill ingested pathogens. As a result, immune cells are susceptible to ROS-mediated cellular damage (Chew and Park, 2002). Nutrients with anti-oxidant properties (carotenes, vitamin E, vitamin C, zinc, and selenium), therefore, support immunity. A brief summary of how individual nutrients affect immune function in ruminants is given below.

Carotenoids. Carotenoids are plant pigments and anti-oxidants which include β-carotene, lutein, canthaxanthin, lycopene, and astaxanthin (Chew and Park, 2002). Carotenoids (*i.e*., β-carotene) have traditionally been viewed as a source of vitamin A via the cleavage of β-carotene precursor into active forms of vitamin A. However, studies in the past two decades have shown that carotenoids have immunostimulatory properties independent of their roles as precursors of vitamin A. Bendich and Shapiro (1986) were the first to document immunostimulatory properties of carotenoids. Specifically, they reported that canthaxanthin increased mitogen-stimulated lymphocyte proliferation in rats. Since then, dozens of additional projects have documented mechanisms by which carotenoids, independent of vitamin A, benefit immunity. In ruminants, for example, supplementation with β-carotene at dry-off reduced mammary gland infections (Chew, 1987). β-carotene increased lymphocyte blastogenesis (Daniel et al., 1990) and increased neutrophil killing activity (Michal et al., 1994; Tjoelker et al., 1988).

Vitamin E and selenium. Vitamin E and Se play overlapping and essential roles in support of the immune system in ruminant animals. A large portion of the benefits of these nutrients is related to their functions as anti-oxidants. Feeding elevated levels of Se to ruminant animals reduces incidence of diseases (including intra-mammary infections: *e.g*., Smith et al., 1984) and several studies have identified potential mechanisms. For example, Hogan et al. (1990) reported that Se enhanced neutrophil killing activity. Maddox et al. (1999) have reported that Se deficiency increases neutrophil adherence and Spears (2000) has speculated that altered adherence could affect ability of neutrophils to attack and sequester pathogen. Politas et al. (1995) reported that vitamin E prevented the peripartum reduction in neutrophil superoxide anion production and impaired IL-1 production by monocytes. Two studies have reported that vitamin E supplementation increases lymphocyte proliferation (Reddy et al., 1986; Garber et al., 1996) and Cao et al. (1992) reported that Se and vitamin E increased antibody responses of dairy cattle. In a more recent study, Parnousis et al. (2001) reported that injection of Se either alone or in combination with vitamin E significantly improved the production of specific antibodies against *E. coli*, and that the production of specific antibodies was greater after the administration of Se alone. Perhaps studies with these two nutrients have shown the most consistent effects on the ruminant immune system.

Omega-3 and -6 fatty acids (ω-3 and ω-6). Dietary fatty acids can affect immunity through the production of the cytokines (Lessard et al., 2003). A mechanism by which fatty acids affect immunity is through production of eicosenoids (*e.g*., prostaglandins) and leukotrienes. Diets rich in the ω-6 fatty acids, such as linoleic acid (C18:2), lead to the formation of arachidonic acid; whereas diets rich in the ω-3 fatty acids (such as linolenic acid, C18:3, flaxseed, and fish oils) lead to the formation of, for example, eicosapentaenoic acid (**EPA**). Eicosenoids synthesized from arachidonic acid tend to have strong

inflammatory potential; whereas those synthesized from EPA have lesser potential. Hence, feeding fatty acid mixtures which are enriched in the ω-3 fatty acids reduces inflammatory reactions and reduces production of proinflammatory cytokines including IL-1, IL-6, and TNF_{α}.

Three recent studies have examined the value of feeding flaxseed, a source of ω-3 fatty acids, on dairy immune function. Petit and Trawiramungu (2002) reported that flaxseed modified prostaglandin (**PG**) production and reproduction. A more recent study (Lessard et al., 2003) reported that flaxseed increased progesterone and PGE_4 concentrations, but did not have consistent effects on other indexes of immunity (proliferation responses of lymphocytes, titer to ovalbumin immunization, INF_{γ}, or PGE_2). In the third study Lessard et al. (2004) re-investigated effects of flaxseed on markers of immune function during the periparturient period. They documented that transition was associated with reduced lymphocyte proliferation in response to mitogen stimulation. Furthermore, transition was associated with reduced IFN_{γ} and increased TNF_{α} and nitric oxide (**NO**). Modulation of dietary ω3/ω6 ratio with flaxseed had limited effects on these parameters and the authors concluded that more work was needed to fully explore potential for use of fatty acids to regulate immunity in dairy cattle.

Diets enriched in the ω-3s have been introduced into the pet food market as a strategy to reduce inflammation and a product with by-pass of high ω-3/ω-6 ratios is available for improving reproduction in dairy cattle. However, we do not yet have enough information on the value of modifying fatty acids in ruminants as a strategy to benefit immunity.

Chromium (Cr). Spears (2000) has reviewed studies on the value of adding Cr to livestock diets vis-à-vis immune health. Individual studies have yielded conflicting results, which Spears (2000) attributed to variations in Cr status, supplementation protocol, and physiological states of animals.

Several studies have indicated that supplementation of Cr to dairy cattle, in a biologically-available form (*e.g.*, Cr-amino acid complex or Cr-yeast), benefits immunity. For example, Burton et al. (1993) reported increased Con-A induced blastogenesis in Cr-supplemented peri-parturient cattle. Chang et al. (1994) reported increased blastogenesis in lymphocytes recovered from sick calves. However, this effect was not detected in lymphocytes taken from healthy calves. Burton et al. (1993) reported that Cr increased development of titer to ovalbumin immunization and in 1994 reported increased titer in Cr-supplemented cows following immunization with IBRV antigen (but not parainfluenza virus Type 3). More-recently, Fladyna et al. (2003) reported that Cr, fed as a chelate, increased IgG_2 antibody response to tetanus toxoid.

Many studies have reported that Cr supplementation did not affect immune parameters (as cited in Spears, 2000). A common theme among studies which *have* detected a benefit to Cr supplementation may be the presence of a stressor (shipping, parturition, weaning). It is possible that stress and consequent immunosuppression are required for clear benefits of Cr supplementation to be detected.

Copper (Cu). Natural Cu deficiency increases susceptibility of ruminant animals to disease (Spears, 2000). However, experimental models of Cu deficiency often fail to increase incidence of disease. Several studies have investigated effects of Cu on immunity in ruminant animals. It should not be surprising that Cu supports immunity as it is associated with many proteins. However, specific studies have yielded equivocal results (Spears, 2000). Some studies have shown that supplementation of Cu-deficient diets augments markers of immune function, whereas others do not. Mechanisms by which Cu specifically supports immune function have not been described in ruminants.

Jones and Suttle (1981) published one of the first studies with ruminant animals, which indicated that Cu supports immunity. Specifically, Cu increased neutrophil killing activity of a common mold: *Candida albicans*. Low Cu status reduced mitogen-stimulated blastogenesis following weaning and IBRV challenge (Wright et al., 2000). Ward et al. (1997) reported that Cu enhanced cell mediated immunity (DTH-response) and Salyer et al. (2004) reported that neither supplemental Cu nor Zn affected performance or morbidity of lightweight, newly received heifers; however, source of both Cu or Zn affected the humoral immune response to ovalbumin immunization. Beyond these studies, there are few which directly implicate Cu in immune function and many which have produced equivocal results (Spears, 2000).

Zinc (Zn). In a recent review, Rink and Gabriel (2000) summarized the known effects of Zn on immunity in non-ruminants. Specific roles that Zn plays in support of immunity are plentiful, well-established and too numerous to report in this brief review. However, it should not be surprising that Zn

plays an essential role as an *immunonutrient* as it is associated with over 300 proteins. Clearly, a Zn deficiency has opportunity to impact a large number of cellular events which might compromise immunity.

As one example, Zn plays an important role in transcriptional control through its action as a Zn-finger motif. Cells deficient in Zn have reduced ability to proliferate. The immune response requires rapid proliferation of cells (e.g., T- and B-lymphocytes) in response to specific antigens and; therefore, Zn deficiency prevents this aspect of immunity from developing.

Spears (2000) reported that, in contrast to studies with humans and laboratory animals, marginal Zn deficiency has little effect on immune function in ruminant animals, but that Zn supplementation may be beneficial. For example, Salyer et al. (2004) reported increased antibody response to a Quali-Tech bioavailable Zn supplement when fed to beef heifers and Galyean et al. (1995) reported that morbidity from respiratory diseases was reduced by addition of Zn to weaned calf diets.

Hemorrhagic Bowel Syndrome (HBS)

In 1991, Dr. Bruce Anderson (U. Idaho) reported a new disease in dairy cattle (then termed *point source hemorrhage*; Anderson, 1991). It was characterized by a rapid influx of blood into the small intestine of dairy cattle with sudden death. Since its initial report, incidence of the disease has increased. In a recent review Van Metre (2005) reported that HBS is a "newly emerging, highly fatal intestinal disease of adult dairy cattle. It is characterized by sudden, progressive and occasionally massive hemorrhage into the small intestine with subsequent formation of clots within the intestine that create an obstruction". A report on the disease (Kirkpatrick et al., 2001) referred to HBS as a multi-factorial disease (i.e., one requiring two or more factors to bring it about); one which would presumably occur in animals with a weakened immune system.

Initially, it was believed that the disease was caused by *Clostridium perfringens* Type A; however, more recent studies in our laboratory at Oregon State University have also implicated a common feed-borne mold, *Aspergillus fumigatus*. Van Metre (2005) proposed that *C. perfringens* is not responsible for the *initial* insult to the gastrointestinal tract which causes the disease, because this organism typically results in maceration of the luminal surface of the intestine via release of its toxins. Instead, an alternative pathogen, possibly *A. fumigatus*, may be responsible for this disease because the hemorrhage initiates sub-mucosally.

In 2004, in tandem with Dr. Don Sockett and colleagues at the Wisconsin Veterinary Diagnostic Laboratory (**WVDL**), we conducted a survey of twenty-five cows which had died from a variety of GI diseases. Samples were assayed for pathogens including *Salmonella*, BVDV and *C. perfringens* Type A at the WVDL. The samples were also sent *blind* to our laboratory at Oregon State University, where we conducted quantitative PCR assays of *A. fumigatus* DNA in blood and tissue samples. Results were then forwarded to WVDL for collation (Sockett et al., 2004).

Results of the collaborative study are shown in Table 1. We determined that a high percentage of cows which died, whether from HBS or other GI diseases, were associated with *C. perfringens* type A (Table 1). However, *A. fumigatus* was associated only with cows which had died from HBS, not from other GI diseases. Attempts were made to conduct further research into the syndrome; however, the focus of federal funding of bovine-oriented research has been directed toward Johne's disease.

Van Metre (2005) recommends that prevention is the most likely strategy for dairy producers whose cows are afflicted with HBS. His recommendations include "analysis of transition and fresh cow management to identify problems with cow comfort, nutrition and disease control that might impact disease resistance at peak lactation, review of ration formulation, feed management, effective fiber and soluble carbohydrates, assessment of feed bunk and pen management, and review of commodity handling and silage management to, particularly, eliminate molds". Current research in the Van Metre laboratory is focusing on the potential role that a unique *C. perfringens* toxin (β2 toxin) may play as a contributing factor in HBS.

Nutritional Strategies to Augment Immunity and Reduce Incidence of HBS in Ruminants

In the past three years, we have investigated the ability of a new feed additive (OmniGen-AF® Prince-Agri Products, Quincy, IL) to affect various immune parameters in ruminant animals. Goals of our first

Table 1. Detection of pathogens associated with cows dying from HBS or other GI diseases.

Cause of Death	Number of Samples	Number *C. perfringens* Positive[1]	Number Salmonella Positive	Number BVDV Positive	Number *A. fumigatus* Positive[2]
HBS	16	14	1	0	13
Other Gastrointestinal Tract Diseases	9	6	4	0	0

[1] Chi square = 0.532 ($p \leq 0.466$)
[2] Chi square = 12.153 ($p \leq 0.001$)

study were to investigate the ability of this product to augment indexes of innate immune function in immunosuppressed sheep. In this study, five groups of sheep were utilized and Groups 2-5 were immunosuppressed via daily injection of dexamethasone (**DEX**; a synthetic stress hormone). Group 1 served as a non-immunosuppressed control group. Groups 3 and 5 received the additive daily and Groups 4 and 5 also received feed highly-infected with *A. fumigatus*. After 28 days on these diets, we recovered blood samples from all sheep, purified neutrophils form the blood and then examined markers of neutrophil function. Markers included L-selectin (a neutrophil adhesion molecule) and IL-1β, a cytokine produced by neutrophils and which activates the acquired immune system (i.e., it provides a form of communication between innate and acquired arms of the immune system). Concentrations of neutrophil L-selectin and IL-1β were assessed using Western blotting.

When animals were injected with DEX, we noted the neutrophil L-selectin and IL-1β were markedly reduced ($P<0.05$; Figures 3 and 4). In fact, daily injection of sheep with DEX eliminated all traces of IL-1β. Addition of the additive to the diets of DEX increased L-selectin ($P<0.05$) but had no effect ($P>0.05$) on IL-1β. When animals were fed feeds high in *A. fumigatus* content, little difference in L-selectin and IL-1β were noted; however, addition of the additive to diets containing mold restored normal and consistent levels of both L-selectin and IL-1β (Figures 3 and 4).

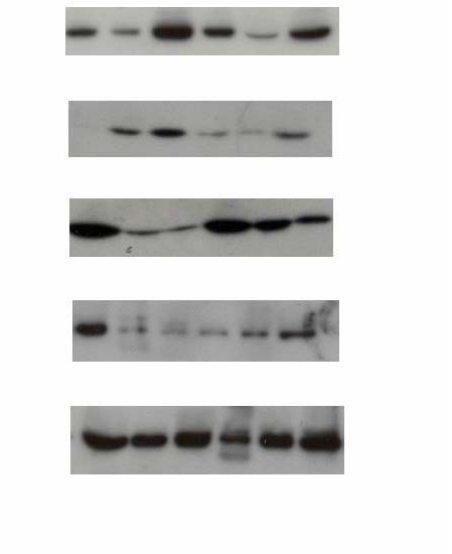
We concluded from this study that the feed product had the ability to restore normal levels of markers of neutrophil function in immunosuppressed sheep. The limitation of this research is that we had imposed a massive and artificial degree of immunosuppression upon the sheep. Hence, in our next study, we used the natural stress of parturition to assess the value of the feed product in augmenting immune function in periparturient dairy cattle. (Wang *et al*., 2004).

Eight Jersey cows were maintained on a control diet or on a diet containing 56 g/day of the additive for approximately 28 d prior to calving. Fifteen hours after calving, 500 ml samples of blood were taken via the jugular and neutrophils were purified. The RNA was purified from the neutrophils and used in a microarray experiment to assess global effects of the additive on neutrophil gene expression in dairy cattle. The work was completed in the Center for Animal Functional Genomics at Michigan State University with Dr. J. Burton.

Microarray analysis identified at least 20 neutrophil genes which were differentially regulated in dairy cattle fed the additive. Examples included numerous receptors and proteins which appear to regulate apoptosis (programmed cell death). We conducted quantitative reverse transcriptase-PCR analysis of some of the candidate regulated genes to confirm the ability of the additive to regulate gene expression in neutrophils and determined that three genes (interleukin-1 converting enzyme [**ICE**], interleukin-8 receptor [**IL-8R**], and interleukin-4 receptor [**IL-4R**]) were significantly ($P<0.05$) altered by the additive. ICE is the rate-limiting enzyme in the conversion of pro-IL-1β to active IL-1β. Hence, the ability of the additive to increase neutrophil IL-1β in the previous study with sheep may be due to its ability to increase expression of the mRNA encoding the processing enzyme (ICE) in neutrophils.

Increased expression of mRNA encoding IL-8R is exciting because neutrophils use IL-8 signaling as a mechanism to identify sites of infection. Specifically, neutrophils migrate toward IL-8 which is produced locally in response to pathogen infection. Increased expression of IL-8R on neutrophils implies that they may be more sensitive to IL-8 signaling

Figure 3. Effects of dexamethasone (DEX), OmniGen-AF®, and mold on expression of L-selectin in sheep neutrophils.



Panel 1: control

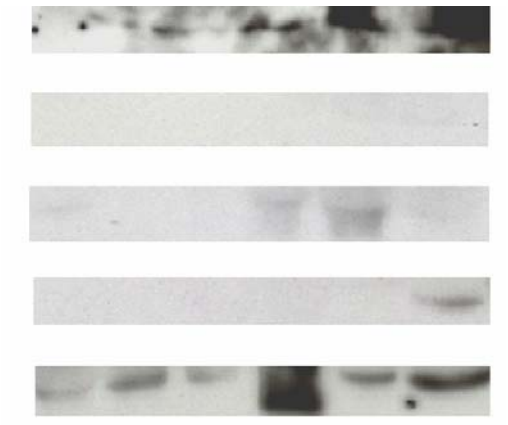
Panel 2: immunosuppressed with DEX

Panel 3: immunosuppressed plus dietary OmniGen-AF

Panel 4: immunosuppressed plus moldy feed

Panel 5: immunosuppressed, OmniGen-AF, and moldy feed

Figure 4. Effects of dexamethasone (DEX), OmniGen-AF®, and mold on expression of interleukin-1β in sheep neutrophils.



Panel 1: control

Panel 2: immunosuppressed with DEX

Panel 3: immunosuppressed plus dietary OmniGen-AF

Panel 4: immunosuppressed plus moldy feed

Panel 5: immunosuppressed, OmniGen-AF, and moldy feed

and; thereby more able to migrate toward and kill pathogen. Finally, neutrophils have short half-lives (<12 h). They undergo a process of programmed cell death (apoptosis). IL-4 is a regulator of apoptosis in neutrophils and altered expression of IL-4R in neutrophils implies that the provision of the additive in the diet may reduce apoptosis of neutrophils and, consequently increase their concentrations. In separate studies with sheep, we have determined that feeding the additive increases the concentrations of various white blood cells (including neutrophils: data not shown). It is possible that enhanced neutrophil concentration, particularly if the neutrophils are sensitized by increased expression of IL-8R, could facilitate capture and killing of pathogens.

Several studies have been completed on dairies with the additive. Two of these were *before-and-after* studies on the incidence of HBS on Wisconsin dairies. Because of the design of these studies, statistical analysis could not be completed. However, on both dairies, incidence of HBS was almost completed eliminated by the addition of the additive to the feeding program. While we cannot be certain of the precise mechanism(s) be which the additive elicited these responses, we may speculate that its above-mentioned effects on innate immunity may enhance ability of stressed cows to *fight* infections by *A. fumigatus*, *C. perfringens*, and, possibly, other pathogens.

Conclusions

Use of feed additives to enhance immune function represent a new strategy in nutrition of livestock. Whereas, scientists have traditionally focused on the known nutrients and examined their effects on various aspects of immunity, research is now revealing novel ways in which immune function may be manipulated and possibly enhanced *in vivo*. This is the interface between nutrition and immunity with added knowledge of immunity regulation. Research in this area is on-going and is likely to yield exciting new discoveries in the next decade.

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