The purposes of this short manuscript will be to review some of the standard nutritional approaches by which producers may maintain immunity in their livestock and to review some results of our recent studies which have shown that a feed additive which we recently developed is also able to modify several important aspects of immunity. First, however, some general background on immunity is provided.

**General Aspects of Immunology**

Higher animals (i.e., vertebrates) are endowed with two aspects of their immune systems. These are the innate system and the acquired (antibody-mediated) system (Janeway et al., 2005). The innate system is an evolutionarily ancient system found in invertebrates as well and consists of a variety of strategies to prevent an infection. Elements of innate immunity include:

- **Epithelial barriers.** The skin and epithelial surfaces of the lungs, gastrointestinal tract and mammary gland provide a first line of defense against pathogens of all types (bacteria, viruses, fungi and parasites).
- **Digestive stomach acid.** The hydrochloric acid of the stomach (or abomasum) reduces the likelihood that a pathogen may pass through this digestive sac into the lower gastrointestinal tract.
- **Complement.** The liver produces a variety of proteins, collectively known as "complement", which are able to bind to pathogens and to thereby mark those pathogens for destruction. Complement proteins may assemble on the cell wall of a pathogen and form a "membrane attack complex" or they may recruit cells of the innate immune system to assist in killing.
- **White blood cells.** Some of the white blood cells (monocytes and their derivative cell type [i.e., the macrophage], neutrophils, eosinophils and basophils are endogenously produced in the bone marrow and are able to identify and kill pathogens which have crossed the epithelial barriers. To detect the presence of pathogens within the body, these cells express on their cell surface (as well as extracellularly and intracellularly) a repertoire of germline-encoded pathogen receptors. These receptors include the Toll-like receptors (of which 11 have been identified in humans), Dectin-1, CD14, NOD-like receptor, peptidoglycan recognition proteins (PGRPs), and mannose-binding lectin (Lippolis, 2008, in press). It is important to underscore the point that this collection of pathogen recognition receptors is able to recognize a limited subset of pathogen molecules because they are "germ-line encoded".

In most cases, the innate system provides adequate protection from an infection. In cases where the innate immune system is "breached", the acquired (antibody-mediated) system becomes activated.

Another type of white blood cell which is part of the innate system is the "dendritic cell". When pathogen invades a tissue, the dendritic cell, via its pathogen receptors, is able to identify it as "foreign" (Janeway et al., 2005). The pathogen is phagocytosed by the dendritic cell after which it is partially digested intracellularly. During this digestion, the dendritic cell migrates the nearby lymphoid tissue (e.g., lymph nodes) and presents portions of the ingested pathogen on its cell surface associated with a group of proteins called the "major histocompatibility complex [MHC]". In lymphoid tissue, naïve B and T lymphocytes come into contact with antigen-presenting dendritic cells and those which display antibodies which bind to the presented antigen become "activated". This process of activation initiates a process of clonal selection whereby those lymphocytes displaying antibodies specific for the membrane-bound antigen begin to rapidly divide. The B lymphocytes activated in this manner become antibody-secreting plasma cells and the selected T-cells exit the lymph node with antibodies tethered to their cell surface. The binding of secreted antibody (i.e., from plasma cells) or of T cells via tethered antibody initiates processes whereby the targeted pathogen is marked for killing.

Additional reading of Janeway et al (2005) is recommended for a thorough overview of immunology.
Nutritional Support of Immunity

Nutrition impacts all aspects of an animal’s physiology; hence, it should not be surprising that deficiencies of most nutrients bring about some form of immune impairment. Calder and Kew published a survey in 2002 which listed all nutrients known to support immunity. In non-ruminants, this list included essential amino acids, linoleic acid, vitamin A, folic acid, vitamin B₆, vitamin B₁₂, vitamin C, vitamin E, zinc, copper, iron and selenium. More recently, studies have shown that calcium and vitamin D also play important roles in supporting immunity (Cantorna, 2006). A challenge in integrating all that is known in how nutrition supports immunity is that there is not a standard method to assess “immunity”. Dozens of methods exist and are acceptable in peer-reviewed journals. Hence, it is often difficult to compare the roles of individual nutrients to other nutrients as no single method of assessing immunity exists.

One of the most intriguing ways in which individual nutrients support immune function is via provision of antioxidants (Chew and Park, 2004). Immune cells such as neutrophils utilize the generation of reactive oxygen species (ROS) in killing of pathogens (Chew and Park, 2004). This high generation of ROS in immune cells places these cells at high risk for oxidative damage. Further, the membranes of immune cells are typically higher in polyunsaturated fatty acids because these are used in formation of signaling molecules used by the immune system (e.g., leukotrienes, thromboxanes, etc). As a result, the high level of ROS generation has potential to also damage membranes through free radical-induced damage. Therefore, any nutrient with anti-oxidant properties is thought to play immune supportive roles. Examples of nutrients which function as anti-oxidants are well known and include vitamin E, selenium, vitamin A and various carotenoids. Minerals also support the immune system as components of enzymes involved in normal immune function. For example, both copper and zinc are used in the generation of ROS. Deficiencies in Cu and Zn thereby bring about immunodeficiency.

In recent years, more specific molecular mechanisms by which the nutrients support immunity have been elucidated. For example, vitamin B6 is required for the formation of lymphocyte receptor which is involved in lymphocyte trafficking between blood, lymphoid tissues and peripheral tissues.

Use of novel feed additives to regulate immune function in livestock

In 2002, we developed a feed additive for livestock (OmniGen-AF) which is now commonly used in the US dairy industry. Since that time, we have conducted approximately two dozen studies in a variety of species (sheep, dairy cattle, beef cattle, swine, poultry and laboratory species [mice and rats]) which have examined the hypothesis that the additive had the ability to augment immune function. We do not have enough room to present all data collected in this short review paper and, so, we with present a synopsis of what we now know about this additive’s ability to regulate immune function.

a. Effects of the additive on molecular markers of neutrophil function

Neutrophils represent the most abundant white blood cell type and are the first cell to arrive at a site of infection. Their arrival to an infection site is mediated by tissue macrophages which secrete chemoattractants (e.g., interleukin-8 [IL8]). To date, we have examined three markers of neutrophil function following the addition of the additive to the diets of various species. These markers include L-selectin (CD62L), interleukin 8 receptor (IL8R) and interleukin 1β (IL1β). Studies have been completed in animals which have been immunosuppressed by daily injection of dexamethasone (Azium) and in non-immunosuppressed animals. In general, the additive increases molecular markers of neutrophil function by 50% to two-fold in normal animals but caused marked increases in these markers in immunosuppressed animals.

L-selectin is an extracellular neutrophil adhesion molecule which enables the neutrophil to adhere to the endothelial lining of blood vessels and to thereby find sources of infection following macrophage signaling. Earlier work by others (Weber et al., 2006) has shown that L-selectin is a “plastic” molecule: i.e., that in stressful situations (e.g., parturition) it is down-regulated. This represents a form of immunosuppression in that neutrophils with less L-selectin expression have reduced ability to seek out and find sources of infection.

In a recent study, we assessed effects of the additive on immune function in immunosuppressed sheep (Wang et al., 2007). The additive increased expression of L-selectin protein concentration and this effect was more evident when sheep were co-stimulated with a moldy feed. IL1β is a cytokine released at a site of inflammation which brings about a variety of actions. It increases vascular permeability thereby increasing movement of fluids (which contain complement) from the blood into the tissue infection site. Further, it provides a feed-forward mechanism from the innate to the adaptive immune system by stimulating...
lymphocyte differentiation (Janeway et al., 2005). Similar to its effects on L-selectin, the additive increased IL-1β protein concentration and this effect was pronounced when a co-stimulatory additive (moldy feed) was also provided.

More recently, we studied the effects of the additive on a broad range of genes which are expressed in bovine neutrophils using microarray analysis (gene profiling). To accomplish this study, we used the bovine total leukocyte array (BoTL-5) which has been developed by the Center for Animal Functional Genomics at Michigan State University. This study (Wang et al., 2008) revealed that, in addition to the above-mentioned markers of neutrophil function, several additional genes were differentially-regulated in neutrophils recovered from periparturient dairy cattle. These included interleukin converting enzyme (ICE) and interleukin 4 receptor (IL4R). These changes explain the increase in expression of IL1β protein reported in Figure 1 (as ICE is a rate-limiting enzyme in IL1β formation) and also explain an observation which we have seen in several studies; that the additive increased concentrations of neutrophils in blood by 20%. IL4R signaling controls apoptosis in neutrophils and the differential expression of this receptor in neutrophils presents one plausible mechanism for this (Wang et al., 2008).

b. Effects of the additive on neutrophil physiology

While it may be exciting to find that a feed additive can bring about changes in molecular markers of neutrophil function, we needed to determine whether these changes brought about meaningful changes in the biology of the white blood cell. To test this, we examined effects of the additive on two markers of neutrophil physiology: phagocytosis and ROS generation. Effects of the additive on phagocytosis of E. coli and Strep uberis were assessed in neutrophils of immunosuppressed sheep and in neutrophils of commercial dairy cattle, respectively. In both cases, consumption of the additive increased the rate of phagocytosis (whether E. coli or S. uberis) by 50-60% (P<0.05).

Effects of the additive on ROS generation in neutrophils of immunosuppressed sheep have also been studied. The additive caused an approximately doubling (P<0.05) in ROS generation indicating that it increases the killing potential of individual neutrophils.

c. Effects of the additive on development of titer

We reasoned that if IL1β secretion by neutrophils is increased by the additive, that this could feed forward and activate the production of antibodies by the adaptive immune system (i.e., as noted earlier, one function of IL1β is to activate adaptive immunity). To test this hypothesis, we conducted a study with Angus beef cattle where were followed the development of J5 titer in IgM, IgG1 and IgG2 fractions following a vaccination program with a J5 bacterin vaccine. Animals were fed three levels of the additive (0, 15 and 30 g/day) for 56 days after which all animals were placed on the 0 g/head/day dose until Day 82 of the study. Animals were vaccinated with J5 vaccine on Days 7, 21 and 35 and blood samples were taken periodically throughout the trial for assessment of titer. We found that the additive had no effect (P>0.05) on development of J5 titer in the IgM fraction; however, it brought about significant improvements (P<0.05) in titer within IgG1 and IgG2 fractions. Specifically, animals fed the additive maintained J5 titer in the IgG1 fraction following removal of the additive from the ration through to Day 82 of the study. Animals which did not receive the additive lost J5 titer during this time. Furthermore, animals which received the higher level of the additive (30g/head/day) had elevated levels of J5 titer within the IgG2 fraction on Day 56 compared to animals which did not receive the additive. Further studies with different vaccines are now on-going.

d. Effects of the additive on animal health

Within the past year, we have embarked on a novel research program aimed at understanding mechanisms by which the additive may bring about improvements in animal health. Our primary focus has been on the incidence of mastitis and, to accomplish this, we have adapted a mouse model of bovine mastitis. Bovine isolates of S. uberis, E. coli and S. aureus have been obtained from field veterinarians in Iowa and Washington and have been deliberately infused into the teat canals of lactating mice (control and additive fed). Results have been promising. It is premature to provide all of the information on these studies in this review; however, results will be presented in detail at the upcoming meeting in Iowa.
Summary

Published (Wang et al., 2007, 2008) and unpublished studies have given us a fairly clear idea of the actions of the product in vivo. The main properties of the product include the following:

• The product increases neutrophil function (increased molecular markers, increased functional properties and increased numbers)
• The product also brings about improvements in titer following vaccination.
• Actions of the product take time to develop (about a month, perhaps longer).
• When the product is removed from a ration, its effects on immunity are lost over a period of about one week.
• Actions are detected in all mammalian species tested to date (ruminants, swine, rodents).
• The mechanism(s) by which the product brings about these changes is not entirely clear. However, our hypothesis is that it is a gastrointestinal-driven event (i.e., the product is detected by receptors lining the GI tract which sets up a cascade of events resulting in immune modulation). Various aspects of this hypothesis are under investigation.

References