Immunity, Stress and Nutritional Support of Immune Function in Stressed Livestock

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Abstract. Progress in understanding the role of nutrition in maintaining the ruminant immune system implies our ability to design nutritional strategies which support this critical function in ruminant livestock. This review focuses on the roles that neutrophils play in the innate immune system, the effects of stress and cortisol on immune function and uses of new nutritional approaches which support and augment innate immunity.

Introduction. The ruminant immune system can be divided into two distinct (yet interacting) systems: the **innate** immune system and the **adaptive** (antibody-mediated) immune system. The innate system functions as the first line of defense against pathogens and consists of several components: 1) physical and chemical barriers to pathogens provided by epithelial lining, gastric acid and digestive enzymes, 2) cells which engulf and digest invading pathogens (e.g., neutrophils), 3) receptors on the surface of these cells which recognize and bind to pathogens, and 4) signaling molecules (cytokines) which communicate sites of infection and regulate expression of immune genes. This review will emphasize the role of neutrophils because of the critical roles they play as a first line-of-defense against many diseases.

Neutrophils. Neutrophils are the first cell to arrive at a site of infection. Dairy cows possess approximately 200 billion neutrophils of which one-half are freely circulating in the blood (Burton and Erskine, 2003). The remainder is held in reserve in bone marrow, their site of formation. As neutrophils circulate, they express an extracellular binding protein (**L-selectin**: Figure 1). L-selectin interacts weakly with the blood vessel wall thereby allowing the neutrophil to "roll" and to search for signals which indicate a local infection (Figure 1). When neutrophils detect a pathogen, they migrate through the endothelial cell wall toward the invading pathogen. During migration, chemical signals originating from the site of infection activate the neutrophil to become a mature "killer cell". The mature neutrophil thereby identifies, sequesters and kills pathogens. Three pathogen-killing mechanisms have been identified in neutrophils. Pathogens may be ingested (phagocytosed) by the neutrophil and then killed by an "oxidative burst" reaction or via fusion with a lysosome. Neutrophils also cast "NETs" (neutrophil extracellular traps) which sequester and inactivate gram positive and gram negative bacteria (Brinkmann *et al.*, 2004).

Dairy producers can observe the actions of the innate immune system in their cows daily by monitoring somatic cell counts (SSC) in milk. Somatic cells are primarily neutrophils which have invaded the mammary alveolus in response to pathogen. High levels of SSC, therefore, reflect an active infection and active killing of pathogens by invading neutrophils.

Stress and immune function. Stress, when it occurs, is an undesirable aspect of livestock production as it results in immunosuppression and increased likelihood of an infection. Common stressors which increase susceptibility to infection include parturition, transport, heat stress and cold stress. Stressors in lactation may also include a high energy diet, ketosis, milk fever, lameness, regular handling, post-partum stress, potential poor feeding practices, (Dobson and Smith, 2000), social isolation when sick animals are placed in a "hospital pen" (Boissy and LeNeindre, 1997) and artificial insemination (Nakao *et al*., 1994).

Burton and co-workers at Michigan State University have identified a key mechanism by which stress suppresses immune function in ruminants. Specifically, they have documented that glucocorticoids (i.e., cortisol) "spike" near parturition and reduce neutrophil L-selectin levels (Weber *et al*., 2001). Loss of L-selectin compromises an essential aspect of an animal's first line-of-defense against pathogens. Specifically, a stressed, immunosuppressed animal has reduced ability to monitor endothelial cell lining for sites of infection and to subsequently attack and sequester pathogens. This creates potential for an infection (Figure 2). Reduced neutrophil function also has potential to bring about other diseases in dairy cattle. For example, Kimura *et al* (2002) have reported that the parturition-associated reduction in neutrophil function may account for increased incidence of retained placenta.

Nutrition and immune function. The immune system, like any other aspect of an animal's physiology, is dependent upon proper nutrition. Inadequate nutrition will compromise the immune system. In the past twenty year's, progress has been made in understanding how nutrients specifically support the immune system. In a recent review Calder and Kew (2002) reported that, in non-ruminants, deficiencies in linoleic acid, vitamin A, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin E, zinc, copper, iron and selenium all have potential to depress immune function.

In dairy animals, less is known about nutritional support of the immune system; however, it is well-known that antioxidants (selenium and vitamin E) play strong roles. For example, vitamin E and selenium supplementation of cows increased ability of isolated neutrophils to kill *S. aureus* and *E. coli* (Hogan *et al*., 1990). High levels of vitamin E supplementation (up to 4000 IU/day) increased neutrophil vitamin E concentrations and reduced incidence of clinical mastitis from 25% to 2.6% in dairy cattle (Weiss *et al*., 1997). Cows with less than 3.0 μg of α-tocopherol in plasma were 9.4-times more likely to develop clinical mastitis during the first seven days of lactation than were cows with over 3.0 μg of α-tocopherol. Other nutrients implicated in maintenance of the immune system in dairy cattle include chromium, cobalt, copper and vitamin A. Spears (2000) has recently published a review on the nutritional support of the immune system in dairy cattle and reported that low levels of cobalt, copper, selenium and vitamin E in the diet reduced ability of neutrophils to kill yeast and bacteria. Copper deficiency reduced humoral immunity (i.e., antibody production) but did not adversely affect cell-mediated immunity. Cobalt deficiency reduced resistance to parasites and vitamin A deficiency reduces immunity partly though its role as an antioxidant. These observations, coupled with data emerging from non-ruminant literature, imply that dairy producers,

veterinarians and nutritionists need to be fully aware of the potential for diet to support the immune system.

Development of nutritional products for immune system enhancement. Wang *et al*., 2004 examined effects of an experimental compound on immune function in sheep. They allotted sixty growing (male and female) lambs to the following five treatments:

1. Control
2. Immunosuppressed with dexamethasone
3. Immunosuppressed plus daily intake of an experimental compound
4. Immunosuppressed plus mold challenge
5. Immunosuppressed plus mold challenge and daily intake of the experimental compound

Immunosuppression followed the model developed at Michigan State University (Weber *et al*., 2001). Specifically, for treatments 2-5, dexamethasone was administered via subcutaneous injection. The experimental compound was added to a dairy-type diet (i.e., high energy, alfalfa-based, rich in corn) at a level of 0.5% (w/w). The mold challenge for Treatments 3 and 5 consisted of addition of heavily-molded wheat mill run (WMR) which had been recovered from a Washington dairy which was experiencing high rates of hemorrhagic bowel syndrome. Wang *et al* determined that the mold infecting the WMR was *Aspergillus fumigatus*, a common mold with potential to cause ruminant mycoses (Sarfati *et al*., 1996; Puntenney *et al*., 2003). Blood samples were taken on a weekly basis and neutrophils were purified. L-selectin and interleukin-1β (IL-1β) concentrations in neutrophils were assessed as markers of innate immune function.

The authors reported that all animals receiving daily injections of dexamethasone (i.e., immunosuppressed animals) grew more slowly ($P<0.05$; Figure 3). These data demonstrate the well-known negative effect that stress exerts on growth. Wang *et al* also found that dexamethasone caused a marked reduction in L-selectin (Figure 4) and completely eliminated IL-1β (Figure 5). These observations document ability of glucocorticoids to dramatically impair immune function. Addition of the experimental compound, whether in the presence or absence of mold, increased L-selectin markedly ($P<0.05$).

A second index of neutrophil function (interleukin-1β) was also examined. IL-1β was increased slightly ($P>0.05$) when the experimental compound was added to the regular diet; however, the addition of the experimental compound to the diet caused a marked increase in IL-1β ($P<0.05$) when *A. fumigatus* was also present.

In follow-up experiments, the authors assessed efficacy of the experimental compound (in meal and pelleted forms) and efficacies of three other commercially-available feed additives (designated as A, B and C) on innate immune function in immunosuppressed sheep. All sheep were immunosuppressed with daily injection of dexamethasone. Treatments consisted of the following:

Treatment	Dexamethasone	Feed Product	Product A	Product B	Product C
1	X				
2	X	X-meal			
3	X	X-pellet 165^{o}F			
4	X	X-pellet 185 ^{o}F			
5	X		X		
6	X			X	
7	X				X

All animals were fed forage free choice and 1.75 lbs of concentrate/day. Final protein was estimated at 15% crude protein. Forage consisted of 50% spoiled grass silage and 50% chopped alfalfa. The experimental compound used in the previous study was added to the concentrate portion of sheep diets at 0.6% w/w (i.e., in Treatments 2, 3 and 4). Products A, B and C were added to the same concentrate mixture at manufacturers' recommended levels. Treatments 3 and 4 included the feed product in a pellet processed at two temperatures (165 and 185 ^{o}F). On the 28th day of the study, blood samples were taken and neutrophils were purified. Neutrophil L-selectin concentrations were assessed and are shown in Figure 6. L-selectin was barely detectable in control (immunosuppressed) sheep. However, the experimental compound, irrespective of whether it was fed as a meal or pelleted, increased L-selectin. Products A, B and C were ineffective in restoring innate immune function.

The experimental compound has now been introduced nationally within the dairy market. Completion of these studies with sheep suggests that its mode of action includes restoration of innate immune function (as indicated by restoration of neutrophil L-selectin and IL-1β).

Summary. Understanding of the ruminant immune system has allowed scientists to develop strategies which support and augment immune function in ruminant livestock. The non-ruminant and human literature is rich with data on how nutrients support the immune system. Additional research into the relevance of these observations to ruminant species should yield additional feed products which support immune function and improve animal productivity.

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Figure 1. Movement of neutrophils through a blood vessel. L-selectin is shown as circles on the surface of the neutrophil. Note shedding of L-selectin and migration of neutrophil into peripheral tissue toward a site of infection (F). Source: Burton and Erskine, 2003.

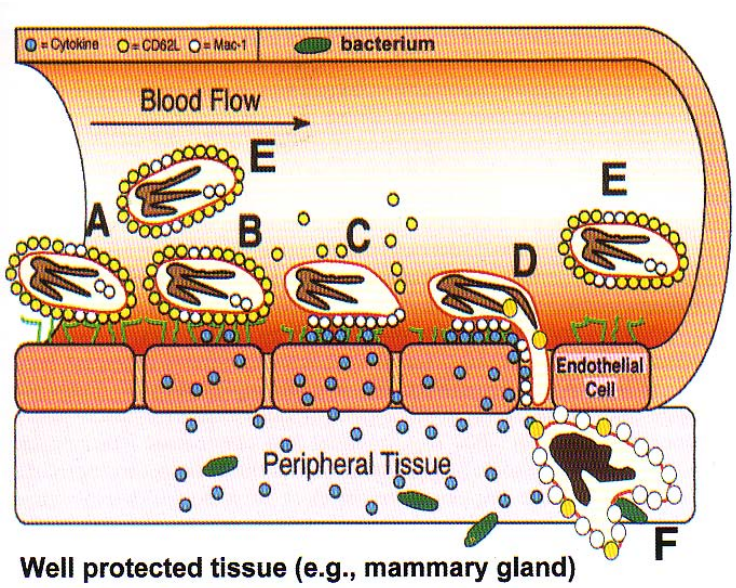

Figure 2. Neutrophils lacking expression of L-selectin in a stressed (immunosuppressed) dairy cow (Source: Burton and Erskine, 2003). Note the inability of neutrophils to sequester invading pathogen.

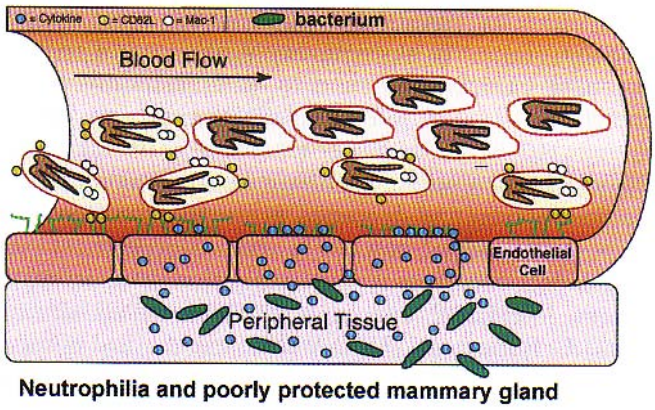

Figure 3. Body weights of sheep on five treatments. Note that all animals receiving DEX had reduced body weights relative to control sheep. The plateau in growth in Weeks 3-4 coincided with a change to cold, rainy weather.

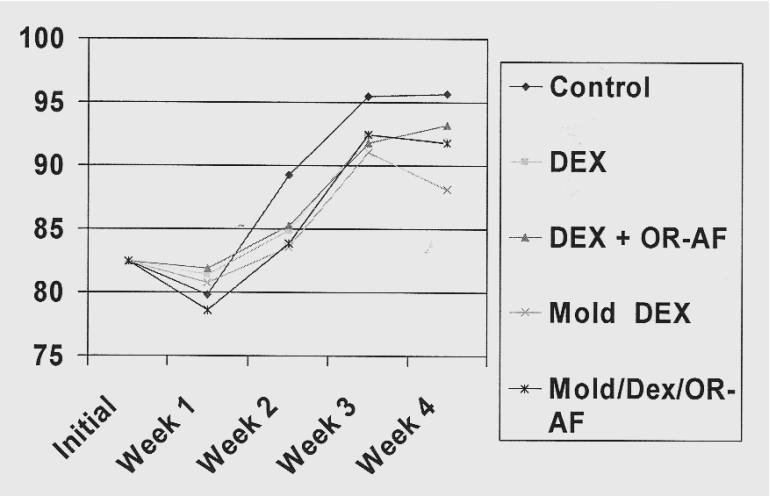

Figure 4. Effects off dexamethasone, moldy feed and experimental compound on innate immune function using. Dexamethasone significantly reduced L-selectin ($P<0.05$) and the experimental compound significantly restored L-selectin levels ($P<0.05$). Data are from Wang *et al*., 2004.

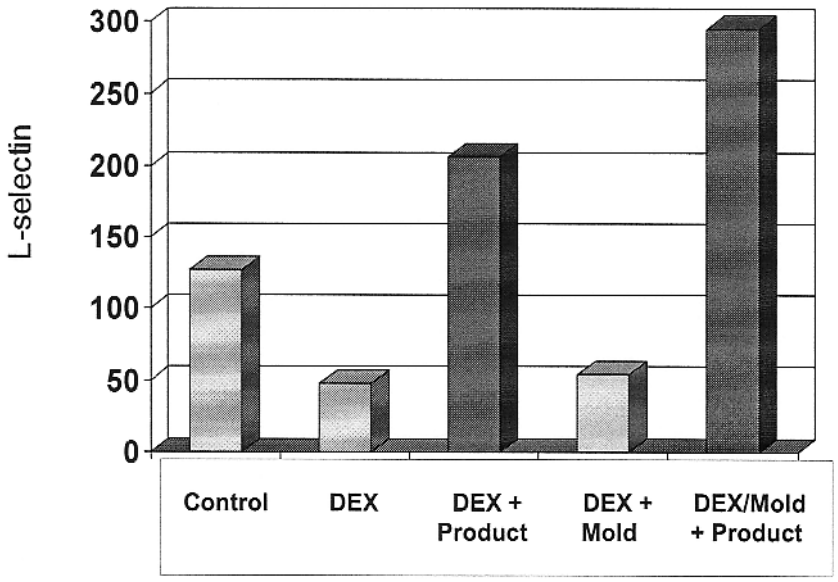

Figure 5. Effects of dexamethasone, moldy feed and experimental compound on innate immune function (using interleukin-1β as an index). Dexamethasone significantly reduced IL-1β ($P<0.05$) and the experimental compound significantly restored IL-1β ($P<0.05$) in the presence of mold. Data are from Wang *et al*., 2004.

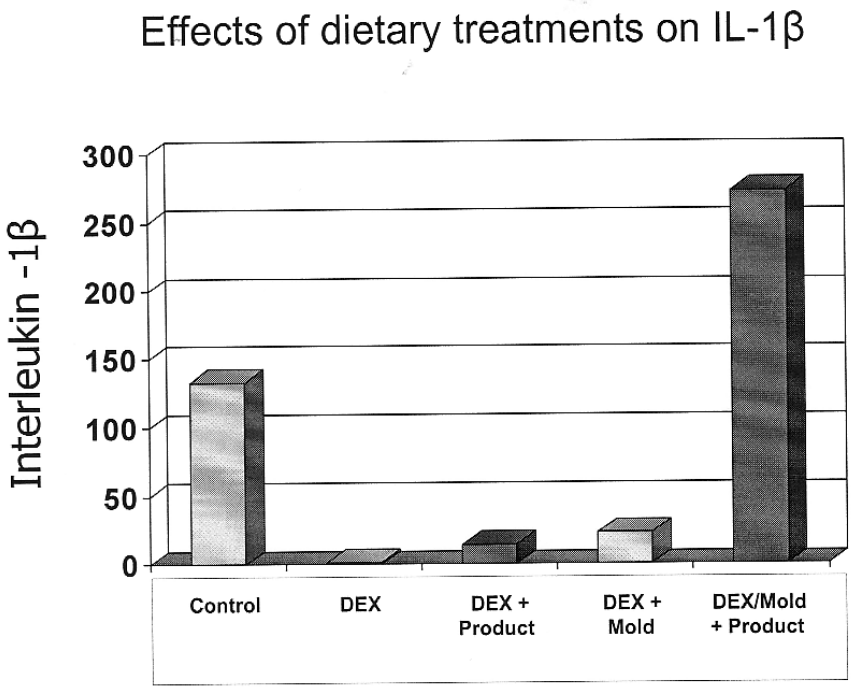

Figure 6. Effects of the experimental compound and of three other commercially-available products on innate immune function (as assessed by L-selectin). The experimental compound was fed as a meal or pelleted at 165 or 185 ^{o}F. Products A, B and C were fed at manufacturers' recommendations in a meal. The product, irrespective of form, increased ($P<0.05$) innate immune function.