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Transition Cow: Non-specific Immune Response

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Abbreviations: E. coli, *Escherichia coli*; IgA, immunoglobulin A; IL, interleukin; TNF, tumour necrosis factor

INTRODUCTION

The immune system of all vertebrates, including bovines, consists of cells and their products, whose prime function is the protection of the host against pathogens, such as bacteria, viruses and parasites; it can also act as an accommodation device to facilitate the development of relatively peaceful associations with foreign organisms which, in some instances and especially in ruminants, could be or could become symbiotic (Morrison, 1986; Halliwell and Gorman, 1989; Poli and Cocilovo, 1996).

The defence of the body against pathogens can be divided into innate and adaptive; both play specific roles in host defence and are essential for health. Pathogens encountered daily during the life of a normal healthy individual are detected and destroyed within hours by defence mechanisms that are not antigen-specific: these are the mechanisms of innate immunity. Only if a pathogen can breach these early lines of defence will an adaptive immune response ensue, with the generation of humoral and cell-mediated antigen-specific effector cells that specifically target the pathogen, and memory cells that prevent subsequent infection with the same pathogen (Morrison, 1986; Halliwell and Gorman, 1989; Poli and Cocilovo, 1996).

The innate immune system, or non-specific immune response consists of physical barriers, humoral factors, and cellular response. The non-antigen-specific defence mechanisms tend to be highly conserved in vertebrates, and these mechanisms in cattle are basically similar to those observed in other species, although they are characterized by some unique aspects (Goodeeris, 1996; Poli and Cocilovo, 1996). The physical barriers consist of skin and mucosae, which provide obstructions to the penetration of pathogens. Humoral factors are an integral and important part of the innate immune system. Plasma proteins of the complement system, some enzymes like lysozyme, interferons, and the acute-phase proteins, such as haptoglobin, are some of the essential elements (Halliwell and Gorman, 1989; Poli and Cocilovo, 1996). In addition to their role in contributing to the direct removal of pathogens, some act as chemotactic factors that cause the influx of inflammatory cells to the site of tissue injury.

Additionally, cattle serum contains conglutinin, a large molecule found in plasma, that is a unique component of the non-specific immune system in ruminants. Conglutinin has multiple binding sites that bind the C3b component of complement on cell surfaces, and thereby clumps particles coated with C3b. This is believed to facilitate phagocytosis and the removal of C3b-coated particles.

The phagocytic cells, the principal cellular element of the innate immune system, are attracted to the site of injury or invasion and are activated, thus stemming the progress of invading pathogens.

The native defence mechanisms are very important for keeping animals healthy. If their function is disrupted for any reason, animals become more susceptible to infections; indeed, in the presence of humoral and/or cell-mediated immune response some of the native mechanisms act more rapidly or more aggressively to help in the elimination of the infection (Morrison, 1986; Halliwell and Gorman, 1989; Goodeeris, 1996; Poli and Cocilovo, 1996).

THE IMMUNE SYSTEM DURING PREGNANCY

The immune system during pregnancy and the exchange of immunity from mother to newborn are unique events in immunological physiology. The uterine implantation of the immunologically foreign fetus is tolerated by the mother's immune response. Successful reproduction requires that the mother and fetus do not reject one another. The ineffectiveness of the immune response is also due to immunosopressive products of the fetus, such as a fetoprotein, or placental-derived steroids, such as progesterone. Periparturient hormonal changes also contribute to impaired immune function. Among other hormones, serum levels of progesterone, oestrogen and cortisol change dramatically at calving (Wettemann, 1980; Detilleux *et al*., 1995; VanKampen and Mallard, 1997;). The physical and metabolic stresses of pregnancy, calving and lactation contribute to the decrease of host resistance and the subsequent increase in disease incidence (Mallard *et al*., 1998).

Pregnancy and the peripartum period result in non-specific immunosuppression in the dairy cow. The magnitude and timing of this reduction depend on many factors such as inadequate hygienic and sanitary management, inappropriate feed and housing, and genetic differences. The transition period, from 3 weeks before to 3 weeks after parturition, is critically important for the health, production and profitability of dairy cows. Most health disorders occur during this time. High reproductive and productive efficiency in dairy cows requires a disease-free transition period. Susceptibility to infectious diseases is generally a multifaceted phenomenon in which distinct features can be recognized; among these is the immune reactivity of the host (Wettemann, 1980; Detilleux *et al*., 1995; Chase, 1996; Shafer-Weaver *et al*., 1996;

VanKampen and Mallard, 1997; Mallard *et al.*, 1998; Drackley, 1999; Roche *et al.*, 2000).

PARAMETERS OF NON-SPECIFIC IMMUNITY IN DAIRY CATTLE

We deal here with the evaluation of some parameters of non-specific immunity in dairy cattle in order to depict important features of the immune reactivity during the transition period and to suggest preventive treatment.

Serum lysozyme

Lysozyme catalyses the hydrolysis of cell wall mucopeptides and induces lysis of some Gram-positive bacteria, but not of Gram-negative bacteria unless antibody and complement are also present. It occurs not only within the granules of neutrophils (except for bovine neutrophils) and macrophages but also in body fluids such as conjunctival secretions, saliva, nasal and intestinal mucus, and milk.

Although lysozyme is of vital importance as a protein capable of exerting antimicrobial activity within phagosomes, it has a role within body fluids in preventing the unregulated multiplication of saprophytic bacteria to which the host is constantly exposed; this is of particular importance in ruminants. Another reported function of lysozyme is its ability to cooperate with IgAs against bacteria. Serum lysozyme in cow can be assessed in a lyso plate assay, according to the protocol of Osserman and Lawlor (Osserman and Lawlor, 1966), using a strain of *Micrococcus lysodeikticus* incubated at 37°C in a humidified incubator for 18 h. Normal range in Holstein–Friesian cow is 1–3 μl/ml (Archetti *et al.*, 1996; Amadori *et al.*, 1997).

Total haemolytic complement

The complex family of complement proteins, many of which are enzymes, is vital to disease resistance. Complement proteins act either directly by cytolysis or opsonization or indirectly during an immune response by cell activation; their two major functions are represented by the principal effector mechanism of antibody-mediated reactions and by the induction of the major inflammatory pathways. Total haemolytic complement can be assessed in cattle according to Seyfarth (1976), using a sheep haemolysin/rabbit erythrocyte haemolytic system. The test can be performed in microtitre plates, modifying the volume of the reagent to a final quantity of 175 μl/well (150 μl serum dilutions + 25 μl of 3% haemolytic system).

Reference standard sera stored at −70°C are used to correct the values of haemolysis for the different batches of rabbit erythrocytes. The normal range in Holstein–Friesian cows is >30 UE CH_{50}/150 μl (Archetti *et al.*, 1996; Amadori *et al.*, 1997)

Serum bactericidal activity

Serum bactericidal activity is due to the presence of natural antibodies and several antibacterial peptides in bovine plasma; most of these are found in the tertiary granules of bovine neutrophils and are different from those described in other species. All of these cationic antibacterial peptides have been shown to be bactericidal for selected bacteria, particularly Enterobacteriaceae, and are believed to be important components of native defence of cattle. Serum bactericidal activity can be detected in cattle according to the method developed by Dorn and colleagues (Dorn *et al.*, 1980) using *E. coli* O119 Weybridge in S phase as test microrganism. Normal range in Holstein–Friesian cattle is >90% (Archetti *et al.*, 1996; Amadori *et al.*, 1997).

The acute-phase response is a physiological response to tissue injury and infection; it occurs within few hours following insult and it induces a vast number of systemic and metabolic changes such as fever and immunosuppression. The acute-phase response in cattle is triggered by pro-inflammatory cytokines such as IL-1, IL-6 and TNF and involves many plasma proteins, the most important of which is haptoglobin, a protein that binds free haemoglobin, ferritin and ceruloplasmin. The C-reactive protein is not considered an acute-phase protein in cattle. Haptoglobin can be measured with a colorimetric test and normal range in Holstein–Friesian cows is <10 mg/dl HbBC (Makimura and Suzuki, 1982; Skinner *et al.*, 1991; Murata and Miyamoto, 1993).

Electrophoretic analysis of given proteins gives a general indication of the function of organs involved in immune reactivity. Specific serum protein profiles in swines and bovines related to age and management indicate individual efficient or non-immune reactivity (Archetti *et al.*, 1996; Amadori *et al.*, 1997).

The principal cellular component of non-specific immunity in cattle is phagocytic cell type whose activity can be evaluated by luminol-enhanced chemiluminescence using zymosan as stimulus, without opsonization, in a luminometer, using the technique described by Zecconi (Osserman and Lawlor, 1966; Zecconi, 1987). To reveal non-specific immunological defects it is important to monitor the proportion of peripheral blood lymphocyte subsets using flow cytometry, particularly in the peripartum period.

Our previous research and that of other authors showed that lysozyme concentration, serum bactericidal activity, serum protein electrophoretic profiles, haptoglobin and complement, with additional analysis of non-specific cellular immunological parameters such as immunoglobulin levels, phagocytic activity and lymphocyte population analysis can give a valuable broader indication of bovine non-specific immune reactivity in different breeding conditions.

In particular, using these parameters it is possible to determine the effects of pregnancy, parturition and lactation on non-specific immune response, especially during the peripartum period, and this may indicate the presence of inadequate hygienic and sanitary conditions of the herd and/or inappropriate feed and management approaches (Zecconi, 1988; Bonizzi *et al.*, 1989; Ponti *et al.*, 1989; Detilleux *et al*., 1995; Chase, 1996; Shafer-Weaver *et al*., 1996; Mallard *et al*., 1998; Roche *et al*., 2000).

We have observed that some of these parameters, such as lysozyme, serum bactericidal activity and complement, decrease in cows and sheep during the transition period but that they can be partially restored, reducing the negative effect of the transition period, with correct diet, feed additives and adequate treatments (Agazzi *et al*., 2001; Capelli *et al*., 2001).

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