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Immunological profiles of lactating and mastitis crossbred cattle

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ABSTRACT

In order to assess the immune profiles of lactating and mastitis crossbred cows, blood samples were collected weekly for one month from early, mid and late lactating cows and once from clinical and subclinical mastitis cows. Immune markers nitric oxide, tumor necrosis factor α and immunoglobulin G were estimated in blood plasma. A concurrent significant ($P<0.05$) fall in IgG concentration was noted in clinical mastitis cows and was highest during mid lactation in lactating cows. The clinical and subclinical mastitis cows had significantly ($P<0.05$) high levels of TNF-α and were non-detectable in lactating cows. Plasma NO level exhibited a massive rise ($P<0.05$) in mastitis cows (clinical and subclinical), the levels were significantly ($P<0.05$) higher than in the normal lactating states.

Key Words: Immunoglobulin (IgG), Lactation, Mastitis, Nitric Oxide (NOx), Tumor necrosis Factor-α (TNF-α).

INTRODUCTION

Health disorders can have a major impact on the profitability of a dairy herd resulting in loss of milk production during the time of illness and often for the entire lactation. The beginning of lactation might be a strong stressor for every cow. Immediately after calving, cows' physiology is modified to produce milk. Approximately 75% of disease in dairy cows typically happens in the first month after calving e.g. mastitis and endometritis, occurs in early lactation (Stabel *et al*., 2003). Mastitis is the costliest disease of the dairy industry today that can decrease total milk production by 15 to 20%. (Bray 2011). During the past decade, the research priorities have focused in understanding the physiologic and immunologic factors controlling the susceptibility of cow to disease that may lead to more rational approaches for prevention and control of diseases. The use of certain blood parameters as indicators of the physiological, nutritional, metabolic and clinical status of farm animals is gaining a wider application. The mediators involved in inflammatory reactions can be identified and targeted to reduce adverse effects of diseases. Nitric oxide (NOx) and Tumor necrosis factor (TNF-α), acts as mediators of many inflammatory reactions and play a vital role in the pathophysiology associated with diseases (Kushibiki *et al*., 2003). Keeping in view the important role of these immunological variables in assessing the health of an animal, the present work was proposed.

MATERIALS AND METHODS

Selection of animals: Apparently healthy crossbred cows KF (Karan Fries) with parity ranging from 2-4, were selected from National Dairy Research Institute, Karnal herd. The animals were kept under normal routine management and feeding followed at the Institute's farm. The water was available ad lib to these cows.

Experiment I

Twenty four KF cows were divided into 3 groups each with eight cows, on the basis of their stage of lactation. Early lactation represented 55-85 days; mid lactation represented 150-200 days and late lactation represented 230 days and above, after calving, respectively.

Experiment II

Sixteen mastitis cows were divided into two groups clinical and subclinical with eight cows suffering from subclinical mastitis were identified using mCMT (modified Callifornia Mastitis Test). Eight cows suffering from clinical mastitis were selected on the basis of clinical symptoms.

In experiment I, blood samples were collected from lactating cows in each of the stage of early lactation, mid lactation and late lactation weekly for one month. In experiment II, blood was collected once from cows suffering from mastitis (clinical and sub clinical). After restraining the animal at ease and sterilizing the area to be punctured, approximately 20 ml of blood was drawn in heparinized vacutainer tubes by jugular vein puncture. Immediately after blood collection, the tubes were transported to the laboratory in ice for further processing. The blood was centrifuged at 3000 rpm for separation of plasma. Plasma obtained was liquated in different fractions and stored at -20°C for analysis of nitric oxide, TNF-α, IgG.

Nitrite (NO_2) and nitrate (NO_3) are stable end products of nitric oxide metabolism and thus, serve as indirect markers for the presence of nitric oxide in plasma. The NOx

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levels were quantified using modified Griess Reaction as described by Shoker *et al.* (1997).

Tumor necrosis factor-α was estimated by Bovine tumor necrosis factor-α ELISA kit from Cusabio Biotech Co., Ltd. The kit contained microtitre plate precoated with goat anti-rabbit antibody, HRP- conjugated TNFα and antibody preparation specific for TNFα. The sensitivity of the test was 0.05 ng/ml. All the kit contents as well as sample or standards were brought to room temperature before use. IgG EIA kit was supplied by Mybiosource.Com. The estimations were carried out as per the standard procedure specified along with the kits.

Statistical Analysis: All the data generated was analysed using Systat 7.0 version software package. Analysis of variance of the data was done using CRD.

RESULTS AND DISCUSSION

Nitric oxide levels in plasma were found significantly (P<0.05) higher during early lactation as compared to mid and late lactation (Table 1). Plasma TNF-α levels were not detectable in normal lactating cows at any stage of lactation (Table 1). The IgG levels were found significantly higher during mid lactation as Compared to the early and late lactation. (Table 1)

A significantly (P<0.01) increased level of the plasma NOx concentration was seen in clinical mastitic cows (Table 1). Plasma TNF-α levels were found significantly (P<0.01) higher in clinical cases (Table 1). Average plasma IgG levels were significantly (P<0.01) lower in the plasma of mastitis cows as compared to sub clinical mastitis cows (Table 1).

Stress in form of disease e.g. mastitis or physiological state e.g. lactation may cause NO production. Nitric oxide, produced in increased amounts during infections and endotoxin challenges, has important metabolic and endocrine side-effects and supports bactericidal effects of macrophages. In mastitis cows (clinical and subclinical), the levels were higher than lactating stages. Overproduction of NO was observed in several inflammatory diseases (Dawson and Dawson, 1995 and Hobbs *et al.*, 1999). Massive NO overproduction, however, may be detrimental, i.e., may be associated with severe clinical output. Nitric oxide production during endotoxin-induced mastitis resulted from the activity of the inducible form of nitric oxide synthase. iNOS is the primary isoform that contributes to the majority of changes in NO production during pregnancy and labour as compared to other NOS (Yallampalli and Dong, 2000).

The levels of TNF-α were highest in clinical cases followed by sub clinical cases and were non detectable during all three stages of lactation. The rise of blood plasma TNF-α production may be a severe event because it is well known that this cytokine initiates an array of immunologic and metabolic reactions which can be detrimental locally and to the host if produced in excessive amounts. This is in accordance with other studies (Hirvonen *et al.,* 1999) in which evidence was presented that the most marked acute-phase reactions in responses to *E. coli*-induced mastitis are accompanied by the greatest TNF-á responses in blood plasma. It appears that large quantities of LPS must be produced continuously to induce and maintain elevated TNF-α concentrations in blood plasma because i.v. administration of LPS causes only a transient rise of TNF-α in blood plasma of cattle (Kahl *et al.*, 1997). The enhanced ability of localized cell populations to produce this potent proinflammatory mediator around the time of calving may explain the greater frequency of acute clinical coliform mastitis during the periparturient period. An ability to modify its production during coliform mastitis may lessen the morbidity and mortality associated with the acute form of this disease.

Severe cases of coliform mastitis are accompanied by the highest increase in blood plasma concentrations of both TNF-α and NO (Kahl *et al.*, 1997 and Hirvonen *et al.*, 1999). The increase in NO and TNF-α during *E. coli* mastitis can't be inhibited by antibiotic treatment (Blum *et al.*, 2000) indicating that its release is LPS mediated. Increases in oxidative factors are correlated with the down-regulation of CYP450 and were caused by TNF- α and NO induced the oxidative damage to mammary glands of cows with mastitis (Komine *et al.*, 2004).

A concurrent fall in IgG concentration was noted in infection (clinical) cases. During acute inflammation there is destruction of the blood-to-milk permeability barriers in the mammary gland, resulting in increased passive transport of IgG into milk as postulated by Mackenzie and Lascelles (1968). Acute inflammation inhibits the IgG's selective transport mechanism (Butler *et al.*, 1972).

Thus, after the acute phase of the infection, the selective transport mechanism may recover and operate for a period of time at a rate greater than that of normal lactating tissue. An additional explanation would be that there was an increased local production of IgG (Butler *et al.*, 1972). Hence

TABLE 1: Average values (± S.E.M.) of immune parameters during different stages of lactation in crossbred cows.

Immune parameters	Early lactation	Mid lactation	Late lactation	Clinical mastitis	Sub-clinical mastitis
Plasma NO (µM)	$37.23^{c} \pm 1.3$	$33.08^{d} \pm 0.32$	$33.41^{d} \pm 0.79$	$58.43^{a} \pm 1.13$	$40.50^{b} \pm 1.34$
Plasma TNF-á (pg/ml)	Non Detectable	Non Detectable	Non Detectable	$843.60^{a} \pm 52.69$	$450.03^{b} \pm 19.03$
Plasma IgG (mg/ml)	$46.71^{b} \pm 0.87$	$53.37^{a} \pm 1.14$	$40.04^{c} \pm 1.40$	$25.70^{d} \pm 2.14$	$46.34^{b} \pm 1.64$

Values with different superscripts differ (P<0.05) in same row.

Nitric oxide, produced in increased amounts during infections and endotoxin challenges, has important metabolic and endocrine side-effects and supports bactericidal effects and In mastitis cows the levels were higher than lactating stages. A concurrent fall in IgG concentration was noted in infection (clinical) cases. The levels of TNF-á were highest in clinical cases and were non detectable during normal lactation. These observations are of potential value to the control of bovine mastitis.

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