

Changes in colostrum of Murrah buffaloes after calving

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Accepted: 10 December 2008 / Published online: 24 December 2008
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Abstract Colostrum samples were collected from 8 Murrah buffaloes on days 1, 2, 3, 4 and 5 after calving. Levels of IgG averaged 54.0 mg/ml at calving, then decreased significantly ($P < 0.01$). IgA and IgM on day 1 were 3.22 mg/ml and 5.22 mg/ml, respectively; both decreased during the first five days after calving. Values of IgA and IgM were higher than those reported in cows. SCC values, which were high at calving (500 000 per ml), reduced significantly ($P < 0.01$) on day 2, then decreased slightly until day 5 (180 000 per ml). At calving, macrophages were the most prominent cells in buffalo colostrum, followed by lymphocytes and neutrophils. Phagocytic activity was 23% at calving and reduced significantly ($P < 0.01$) to 14% on day 5. Phagocytic index was highest in the first colostrum, and then decreased non-significantly.

Keywords Buffalo · Colostrum · Immunoglobulin · SCC · DLC · Phagocytic activity

Introduction

There are more than 75 million buffaloes in northern India and Pakistan, and in India they contribute about 55% of milk production. It is essential that buffalo

owners take proper care of calves so that they develop into healthy adult buffaloes and achieve a high lifetime milk production. Producers must consistently provide calves with sufficient high-quality colostrum within the first few hours of life.

Colostrum is considered to be “liquid gold” as it contains maternal antibodies that help protect the neonates from disease. Besides this, colostrum is a rich source of energy, protein, vitamins and minerals. Details of the chemical composition, immune and growth factors present in cow colostrum are available (Georgiev 2008). Three types of immunoglobulins are particularly important; IgG, the smallest but most common antibodies have a critical role in fighting bacterial and viral infections; IgA, which protect body surfaces that are exposed to outside foreign substances; and IgM, the largest antibodies, are the first type of antibody made in response to an infection.

Several studies have estimated the levels of total immunoglobulins and IgG in buffalo colostrum (Singh et al. 1993), but there is no report on IgA and IgM levels. The present study was undertaken to analyze by ELISA all three immunoglobulin fractions in the milk of buffalo cows during the first five days after calving.

A previous study (Dang et al. 2007a) observed increased phagocytic activity in colostrum samples from buffalo cows collected on day 1 after calving; this decreased significantly by day 7. A secondary objective in the present experiment was to evaluate the changes in phagocytic activity during days 1, 2, 3, 4 and 5 after calving.

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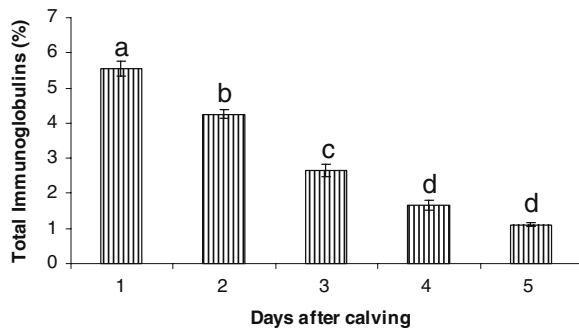


Fig. 1 Total Immunoglobulin in colostrum of Murrah buffaloes after calving

Materials and methods

Eight pregnant Murrah buffaloes, approaching parturition were selected for the study. They were kept in individual pens and subjected to routine management, which included *ad lib* green fodder and calculated amounts of concentrate mixture. Fresh tap water was available *ad lib*.

Colostrum was collected by hand milking on days 1, 2, 3, 4 and 5 after calving. A 20 ml sample was taken in a 100 ml glass beaker, heated in a water bath to 37°C, and then 0.5 ml of 0.5% Rennet solution (250 mg Rennet in 50 ml distilled water) was added. After 10 minutes the clotted sample was mixed by a glass rod and filtered through Whatman No.42 filter paper overnight.

IgG, IgA and IgM levels were estimated by enzyme-linked immunosorbent assay (ELISA), using a kit from Koma Biotech, Korea. The samples were stored at -4°C and estimated within 15 days. Somatic cell counts (SCC) were measured microscopically by the method of Dang et al. (2007a). Differential

leucocyte counting (DLC) was carried out to determine the numbers of lymphocytes, neutrophils and macrophages, and isolation of polymorphonuclear neutrophilic leucocytes from milk was performed as described by (Dang et al. 2008).

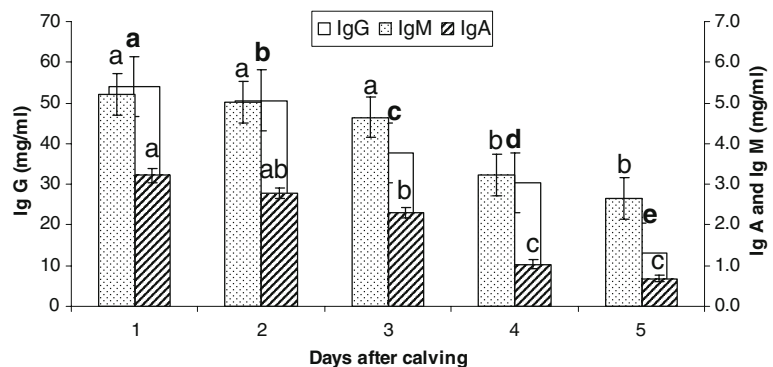
Yeast (*Saccharomyces cerevisiae*) was added to the culture of colostrum neutrophils to study their phagocytosis. The percentage of phagocytosing neutrophils was recorded as percentage phagocytosis or phagocytic activity (PA) and average number of yeast cells per neutrophil was recorded as mean phagocytosis or phagocytic index (PI), as described by Guidry et al. (1976). Fat, protein and lactose levels were estimated using the Lactoscan Milkometer (Mega-Netco, Bulgaria, MMB-965-3100). Total immunoglobulins were estimated by the method of (McEwan et al. 1970).

Results

The results of total milk immunoglobulins are presented in Fig. 1. The elevated level of about 5.6% which was observed at calving decreased significantly ($P < 0.01$) on day 2, and further decreased (non-significantly) during days 3, 4 and 5.

The results of the various immunoglobulin fractions are presented in Fig. 2. The mean level of IgG was 54.0 mg/ml at calving, and there was a significant decrease ($P < 0.01$) on days 2, 3, 4 and 5 after calving. The mean levels of IgA and IgM on day 1 were 3.22 mg/ml and 5.22 mg/ml respectively. IgA decreased non-significantly from day 1 to day 2, then further decreased significantly on days 3 and 4; on day 5 the decrease was non-significant. IgM levels decreased non-significantly during the first three 3 days after calving, then decreased significantly

Fig. 2 Immunoglobulin fractions in colostrum of Murrah buffaloes after calving



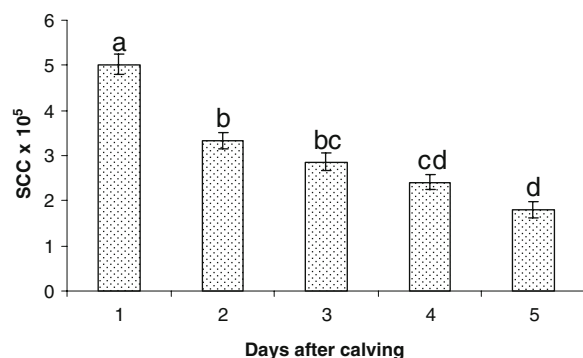


Fig. 3 Somatic cell counts in colostrum of Murrah buffaloes after calving

($P < 0.01$) on day 4; a non-significant reduction was observed on the day 5. Our results indicate that there is a sharper and more significant decrease in IgG compared to IgA and IgM.

Figure 3 shows that SCC averaged 500 000, 333 000, 280 000, 240 000 and 180 000 per ml on days 1, 2, 3, 4 and 5 respectively. SCC values, which were high at calving, reduced significantly ($P < 0.01$) on day 2; these values then decreased non-significantly on days 3, 4 and 5.

The results of DLC are presented in Fig. 4. Colostrum neutrophils increased significantly ($P < 0.01$) on day 2, then reduced significantly on days 3 and 4. Macrophages decreased significantly ($P < 0.01$) on days 2 and 3 and then non-significantly afterwards. Lymphocytes showed no significant change during the first two days and then increased significantly on days 3, 4 and 5.

The phagocytic activity and phagocytic index of buffalo colostrum are presented in Fig. 5. PA

decreased significantly ($P < 0.01$) on all the first five days after calving. A substantial phagocytosis promoting effect was found in colostrum on day 0, followed by day 1. Maximum activity was observed at parturition, and this gradually decreased with time as well as with decreasing IgG level. PI was also highest on day 1, then decreased non-significantly and became almost constant from day 4 onwards indicating that neutrophils were active but were not phagocytosing at the same rate as on the first 3 days.

Postpartum SCC were found to be positively correlated with total immunoglobulins. Milk macrophages were negatively correlated with lymphocytes but positively correlated with total IgG. PA was positively correlated with PI and total IgG.

The results of milk fat, protein, lactose and ash levels are presented in Fig. 6. Milk fat and protein levels were significantly high, and lactose significantly low, in milk samples collected at parturition. The levels of both protein and ash decreased significantly ($P < 0.01$) up to day 5. Milk SCC was positively correlated with protein and total milk immunoglobulins, but negatively correlated with milk lactose. Ash levels were also high, indicating that colostrum is full of nutrients required by the calf.

Discussion

As reported for cows, the major immunoglobulin fraction in buffalo colostrum was found to be IgG. In buffalo colostrum, IgG, IgM and IgA occur in the ratio of 86%, 8% and 5%. Larson et al. (1980) reported a ratio of 85–90%, 7% and 5% in dairy cows.

Fig. 4 Differential leucocyte counts in colostrum after calving N = Neutrophils, M = Macrophages, L = Lymphocytes

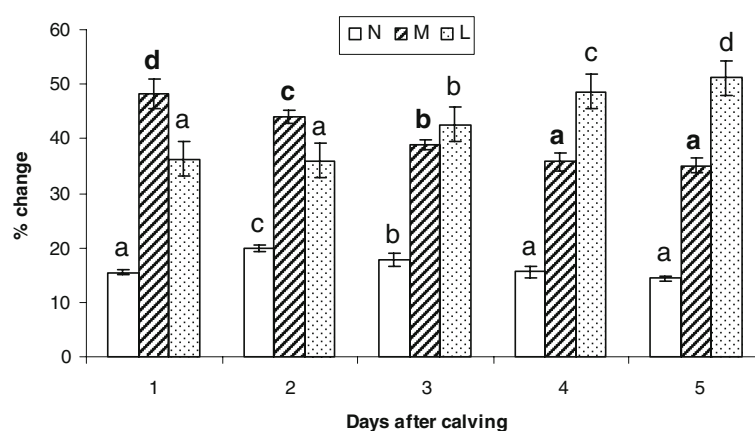
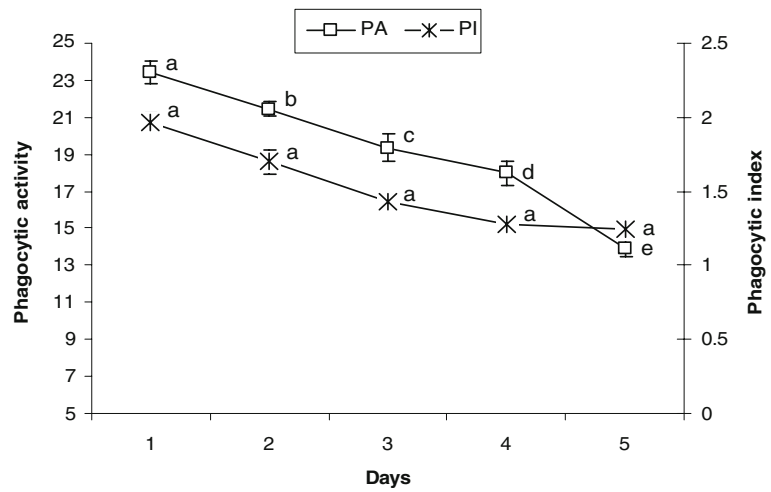


Fig. 5 Phagocytic activity and index in colostrum of Murrah buffaloes after calving



The mean level of IgG in Murrah buffaloes at calving was found to be 54.0 mg/ml by ELISA test. Butler (1973) reported an IgG level of 50.5 mg/ml in bovines by a radial immunodiffusion method; however, Zhang et al. (2001) reported 67.2 mg/ml on the first day. Ginel et al. (2003) indicated that ELISA should be used for estimating immunoglobulin levels as it has a much higher sensitivity than the single radial immunodiffusion assay but has a comparable specificity and precision.

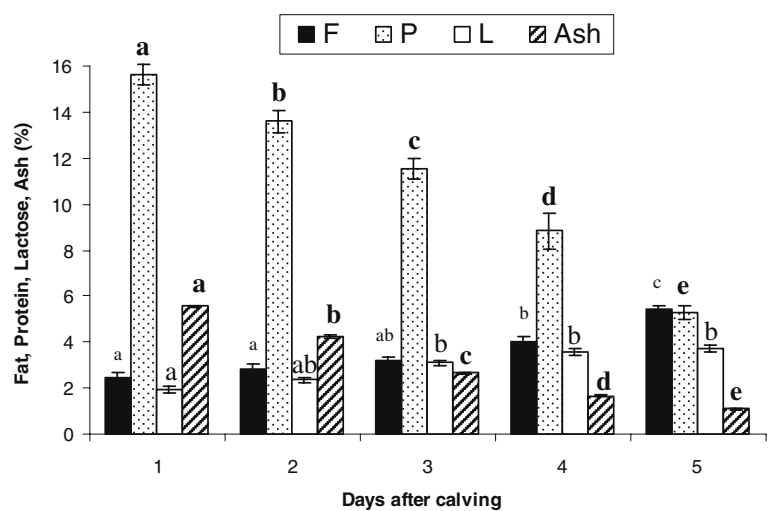
The mean levels of IgA and IgM were 3.22 mg/ml and 5.22 mg/ml respectively; these could not be compared with other estimates of IgA and IgM levels in buffaloes as there is no available literature. They

were, however, twice the values reported by Quigley et al. (1994) for cows.

The values of buffalo SCC were within range reported by Dang et al. (2007b). Similar values were also reported in dairy cows (Barkema et al. 1999). Macrophages were the most prominent cells followed by lymphocytes and neutrophils at calving. Jensen and Eberhart (1981), studying mammary gland secretions in pregnant dairy cows, reported an increase in neutrophils and a decrease in lymphocytes as parturition approached.

The highest PA was recorded in colostrum on day 1, further strengthening the recommendation that colostrum should be fed to calves within 6–

Fig. 6 Colostrum composition of Murrah buffaloes after calving



10 hrs of birth. Sugisawa et al. (2001) found that the very high levels of immunoglobulins in colostrum increased the PA by up to 25%, and was dose dependent.

PI was highest on day 1, indicating that the engulfment of foreign bodies was also highest at this time. However, phagocytosis by colostrum neutrophils in the present study was significantly lower than that reported by Dosogne et al. (2001) in which neutrophils were incubated with *S. aureus*. The reason for this may be the large size of yeast particles resulting in lower engulfment by neutrophils. Mehrzad et al. (2001) also found that the chemiluminescence response and viability of milk neutrophils were lowest between 3 and 11 days postpartum. Meglia et al. (2001) found that the weeks just before and after parturition were characterised by neutrophilia, eosinopenia, lymphopenia and monocytosis, but time had no effect on neutrophil phagocytosis and oxidative burst.

Conclusions

Our results present the levels of various immunoglobulin fractions in buffalo colostrum. Further, as the PA and PI of colostrum neutrophils are very high at parturition, this study reinforces the recommendation for timely feeding of colostrum to provide immunity to the calf and increase its chance of survival.

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