

Plasma Protein Profile of Neonatal Buffalo Calves in Relation to the Protein Profile of Colostrum/Milk during First Week Following Parturition

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ABSTRACT : An investigation was made into the protein profile of colostrum/milk of ten Murrah buffaloes and of their ten buffalo calves during their first week of neonatal life to study the materno-neonatal transfer of immunoglobulins (Ig). Calves were pail fed 3.5 liter of colostrum and/or milk per calf/day exclusively from their dam. First blood sample from newborn calves was collected before colostrum feeding on the day of birth (day zero) and the sampling continued daily for seven days after colostrum/milk feeding. Colostrum/milk Ig and IgG values were 4.82 ± 2.60 , 2.19 ± 1.90 , 1.12 ± 0.82 , 0.69 ± 0.44 , 0.59 ± 0.31 , 0.47 ± 0.20 , 0.40 ± 0.22 , 0.40 ± 0.25 and 3.58 ± 1.90 , 1.08 ± 0.92 , 0.52 ± 0.40 , 0.31 ± 0.20 , 0.27 ± 0.14 , 0.22 ± 0.08 , 0.18 ± 0.09 , 0.14 ± 0.08 respectively during 0-7 days post partum. The concentration of total colostrum/milk proteins, Ig, IgG and albumin were highest within 12 h post-partum. Thereafter, the concentrations followed a declining trend which may be attributed to the reduced transfer of proteins from the maternal blood, declining synthesis by the mammary glands and/or depletion of stored proteins. The concentrations of plasma Ig and IgG before colostrum feeding on day zero were 0.42 ± 0.09 and 0.08 ± 0.03 respectively. The levels of plasma Ig were 1.90 ± 0.37 , 1.80 ± 0.31 , 1.80 ± 0.26 , 1.81 ± 0.28 , 1.78 ± 0.31 , 1.79 ± 0.21 , 1.80 ± 0.32 and of IgG were 1.57 ± 0.41 , 1.30 ± 0.29 , 1.31 ± 0.21 , 1.27 ± 0.18 , 1.23 ± 0.21 , 1.23 ± 0.16 , 1.26 ± 0.21 on days 1-7 after birth after colostrum/milk feeding. The concentrations of total plasma proteins, Ig, IgG were lowest before colostrum feeding and increased significantly ($p < 0.05$) after colostrum feeding in buffalo neonates. The results suggest that the highest amounts of colostrum Ig and IgG were available on the day of parturition and thus the calves should receive colostrum as early after birth as possible. Colostrum Ig and IgG concentrations were not correlated to plasma Ig and IgG concentrations in the post-suckle buffalo calves and therefore, colostrum Ig and IgG concentrations were probably not the principle determinants of calf post-suckle plasma Ig and IgG concentrations. (*Asian-Aust. J. Anim. Sci.* 2003, Vol 16, No. 3 : 348-352)

Key Words : Protein Profile, Plasma, Colostrum/Milk, Neonatal Buffalo Calves

INTRODUCTION

Various immunological and biochemical adjustments take place in the dam to meet the metabolic needs of foetus and neonates for growth and development. During foetal life there is maternal protection, and in the post-natal life the neonates are protected and nourished by the colostrum and milk from mother. This is a unique mammalian adaptation for the survival of species. The neonates need both pre- and post-natal immunological assistance, which is rendered passively through the placental and/or colostrum transfer of antibodies. The transmission of immunity is predominantly post-natal in ruminants (Brambell, 1970). Colostrum represents the accumulated secretions of the mammary glands over the last few weeks of pregnancy. Therefore the knowledge of changes in protein profile of colostrum/milk is of paramount importance in understanding the materno-neonatal transfer of immunoglobulins. Thus the present investigation was undertaken with the objectives of monitoring the changes in protein profile of colostrum/milk during first week following parturition and to study the protein profile of buffalo neonates before and after colostrum feeding and to

investigate the nature of relationship, if any, between colostrum and neonatal immunoglobulin profiles.

MATERIALS AND METHODS

The study was conducted on ten pregnant healthy Murrah buffaloes and ten calves (8 male and 2 females) delivered by the same buffaloes. These buffaloes were maintained on the standard feeding schedule recommended by the National Research Council (NRC) and management conditions at the dairy farm of Punjab Agricultural University, Ludhiana. Calves were pail fed 3.5 liters of colostrum and/or milk per calf/day, half of which was provided to them in the morning and half in the evening at the dairy farm. Each calf was fed colostrum/milk collected exclusively from the dam of that calf. First blood sample from new born calves was collected before colostrum feeding on the day of birth, and the day was designated as day zero (0-12 h after birth); immediately after this sampling, colostrum was fed to the calves. Thereafter blood was collected daily in the morning for seven days, and colostrum/milk was fed to the calves as scheduled. Blood samples collected under aseptic conditions, by jugular venipuncture in dried heparinised vials, were shifted to the laboratory under cold conditions. There the blood was immediately centrifuged for the separation of the plasma.

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Colostrum/milk samples were collected on the day of parturition, designated as day zero, and further sampling was done every morning till seven days post partum.

Total plasma proteins were estimated by the method of Reinhold (1953). Plasma albumin was determined by chemistry analyzer (RA-50, Miles India Ltd., Baroda) using albumin kit supplied by M/s Qualigens Fine Chemicals, Dr. Annie Besant Road, Bombay-400025, India. It is based on the principle that albumin in a buffered solution reacts with the anionic bromocresolgreen (BCG) with a dye binding reaction to give a proportionate green colour which is measured at 628 nm wavelength. The concentration of plasma globulins were arrived at by subtracting plasma albumin from total plasma protein concentration. Albumin: Globulin (A/G) ratios were calculated thereafter. Immunoglobulins were separated by precipitating plasma with saturated ammonium sulfate solution and then precipitates of immunoglobulins were dissolved in a known quantity of phosphate buffer saline (Oser, 1965). The immunoglobulins concentration was then estimated by the method of Lowry et al. (1951). The plasma IgG was estimated by polyacrylamide gel electrophoresis (PAGE) technique (Laemmli, 1970). The IgG band was identified on the basis of Rf values obtained by running standard IgG preparations alongside the samples. The quantification of different bands in the gels was undertaken on Beckman Spectrophotometer Gel Scanner (DU-64).

Total colostrum and milk protein concentrations were estimated by the method of Lowry et al. (1951). For determination of colostrum/milk immunoglobulins (Ig), 5 ml colostrum or 10 ml milk was centrifuged at 3,000 rpm for 15 min at 5°C and the cream layer was punctured to drain skim milk. Sodium acetate buffer, 0.2 M, was added drop wise to the skim milk with constant stirring until the pH was 4.8 and casein precipitated, centrifuged at 5,000 rpm for 10 min to remove the casein; the whey was treated the same way as plasma for separation (Oser, 1965) and estimation (Lowry et al., 1951) of immunoglobulins. After separation, immunoglobulins were subjected to PAGE in

the same way as plasma for determining colostrum/milk IgG. For estimation of colostrum/milk albumin, the whey was subjected to PAGE and albumin was determined in the same way as IgG.

The results were subjected to one-way analysis of variance. Significance was tested at 5% level by calculating critical difference for comparison of means. For statistical work, the methods detailed by Snedecor and Cochran (1976) were followed.

RESULTS AND DISCUSSION

Protein profile of colostrum/milk

Total colostrum/milk protein concentration, albumin, Ig, IgG values are presented in Table 1.

Total colostrum/milk protein and albumin concentrations declined significantly ($p < 0.05$) on the day one post partum and a non-significant decline in the contents was observed from day two post partum. This is in consonance with the findings of Maria et al. (1990) in buffaloes. Kholand et al. (1985) also observed a decreasing trend in colostrum proteins over a period seven days. On day zero total colostrum protein concentration was highest. This indicates that the highest concentration of colostrum proteins is available to neonates on that day.

Total colostrum/milk Ig and IgG concentrations declined significantly ($p < 0.05$) on day one post partum and declined non significantly still further as the post partum period increased. These findings are in agreement with Singh et al. (1982), Geene (1986), Abraham (1988) and Maria et al. (1990) in bovines.

Singh et al. (1982) observed that most of the milk proteins get stabilized about a week after calving, during which period significant changes occur in the Ig levels which contribute significantly to the total proteins during that time. Abraham (1988) observed a speedy decline in the total plasma proteins and Ig concentrations within 24 h post partum in buffaloes. Geene (1986) also reported a rapid decrease in the total Ig content in colostrum within 24 h

Table 1. Changes in the distribution of total milk protein (g/dL), albumin (g/dL), Ig (g/dL), IgG (%) and IgG (g/dL) concentrations up to 7 days post partum in Murrah buffaloes

Post partum (days)	Total protein	Albumin	Total Ig	IgG (%) of total Ig	IgG
0	13.27±4.37 ^a	5.58±0.34 ^a	4.82±2.60 ^a	74.39±3.50 ^a	3.58±1.90 ^a
1	8.56±3.59 ^c	2.48±1.04 ^b	2.19±1.90 ^b	49.33±2.88 ^b	1.08±0.92 ^b
2	7.15±2.33 ^{bc}	2.35±0.75 ^{bc}	1.12±0.82 ^{bc}	47.06±7.50 ^b	0.52±0.40 ^c
3	6.62±1.95 ^{bc}	1.98±0.58 ^{bc}	0.69±0.44 ^{bc}	45.46±3.24 ^b	0.31±0.20 ^c
4	6.18±1.90 ^b	2.22±0.67 ^c	0.59±0.31 ^{bc}	40.30±9.70 ^b	0.27±0.14 ^c
5	5.71±1.08 ^b	1.02±0.21 ^d	0.47±0.20 ^{bc}	38.86±5.32 ^b	0.22±0.08 ^c
6	5.62±1.35 ^b	1.12±0.25 ^d	0.40±0.22 ^c	38.86±5.34 ^b	0.18±0.09 ^c
7	5.18±1.25 ^b	1.03±0.24 ^d	0.40±0.25 ^c	36.21±4.23 ^b	0.14±0.08 ^c

Mean±SD within column bearing at least one common superscript do not differ significantly ($p < 0.05$).

No. of animals (n)=10.

after parturition. Maria et al. (1990) observed that colostral Ig levels decreased significantly from 1st to 5th milking (first milking within 4 h post partum and the rest every 12 h in succession). Vann et al. (1995) also reported that total colostral Ig along with IgG decreased over time.

It is obvious that the highest colostral total Ig and IgG concentrations are available on the day of parturition. The subsequent decline may be in part because of the reduction in IgG concentrations. This may be due to the reduced transfer of Ig from the maternal blood, declining synthesis by the mammary glands and/or depletion of stored Ig with the subsequent increased number of milkings.

Total plasma proteins, albumin, globulins and A/G ratios in neonates

These values are presented in Table 2. Total plasma protein concentration was lowest on day zero (before colostrum feeding). A significant ($p < 0.05$) increase after colostrum feeding on day one and thereafter a non-significant decrease was observed in the present study. These results are comparable with those reported by Joshi (1990) and Manhas (1993) in buffalo calves and Kurz and Willet (1991) in cow calves. The lowest concentration of plasma total protein before colostrum feeding on day zero may be due to the absence of transplacental transfer of maternal Ig. The significant ($p < 0.05$) increase after colostrum feeding on day one is due to the absorption of colostral Ig (i.e. from the colostrum fed to calves on day zero) by the neonates (Bush and Staley, 1980; Geene, 1986; Abraham, 1988). The non-significant decline in total plasma proteins from day two may be due to a decrease in the intestinal absorption of colostral proteins or reduced endogenous synthesis of globulins by the neonates.

Albumin concentration was lowest on day zero and increased significantly ($p < 0.05$) after colostrum feeding on day 1 and day 2 after birth. The overall range of mean values of plasma albumin observed in the present study are in agreement with reported by Kishtwaria et al. (1983) in Murrah buffalo calves. Nazki (1995) observed a positive

and significant ($p < 0.01$) correlation between plasma albumin concentration and neonatal age in buffalo calves.

The mean plasma globulin concentration was lowest on day zero and increased significantly ($p < 0.05$) on day one followed by a significant decrease on day two after birth. These findings are similar to those reported by Sridhar et al. (1988), Manhas (1993) and Nazki (1995). The low levels of globulins before colostrum feeding in the present investigation may be because the newborn calves are agammaglobulinemic or hypogammaglobulinemic (Ishikawa and Konishi, 1982; O'Kelley, 1991). A rapid and significant increase on day one may be due to the gamma globulins absorbed from the colostrum fed to calves on day zero. This is in accordance with the findings of Stott et al. (1979), Larson et al. (1980) and Vann et al. (1995). A significant decrease ($p < 0.05$) was noticed on day two. This decrease was comparable to that reported by Kurz and Willet (1991) in cow calves and may be attributed primarily to a decline in Ig fraction of globulins (Manhas, 1993). The reason for the decreasing trend in globulins over time may be due to reduced concentration and/or absorption of colostral globulins, catabolism of absorbed colostral globulins, and/or reduced endogenous synthesis of globulins by the neonates.

Higher A/G ratio on day zero and a rapid and significant ($p < 0.05$) decrease on day one are in agreement with the results reported by Sridhar et al. (1988) in cow calves. The increased levels of plasma globulins after colostrum feeding may be responsible for the rapid fall in A/G value (Pierce, 1955). This suggests that very high amounts of globulins are absorbed from colostrum by the neonates. An increase in A/G value on day two was mainly due to an increase in the albumin and decrease in the globulin concentrations. This is an indicative of an earlier synthesis of albumin as compared to globulins.

Total immunoglobulins (Ig) and IgG concentrations in neonates

These values are presented in Table 2. Total Ig

Table 2. Mean plasma total protein, albumin and globulin concentrations (g/dL) and A/G ratio, immunoglobulin (g/dL), IgG (%) and IgG (g/dL) before and after colostrum feeding in neonatal buffalo calves

Days after birth	Total protein	Albumin	Globulins	A/G ratio	Total Ig	IgG % of total Ig	IgG
Before colostrum feeding							
0	3.22±0.45 ^b	1.66±0.13 ^d	1.56±0.36 ^c	1.17±0.23 ^a	0.42±0.09 ^a	19.24±3.56 ^d	0.08±0.03 ^c
After colostrum feeding							
1	5.89±0.70 ^a	2.32±0.14 ^c	3.57±0.63 ^a	0.66±0.10 ^c	1.90±0.37 ^b	82.94±5.58 ^a	1.57±0.41 ^a
2	5.70±1.06 ^a	2.58±0.31 ^b	3.12±0.79 ^b	0.85±0.13 ^b	1.80±0.31 ^b	75.59±3.86 ^b	1.30±0.29 ^b
3	5.69±0.50 ^a	2.60±0.38 ^b	3.09±0.33 ^b	0.84±0.15 ^b	1.80±0.26 ^b	72.86±1.70 ^{bc}	1.31±0.21 ^b
4	5.72±0.64 ^a	2.70±0.32 ^{ab}	3.02±0.33 ^b	0.89±0.15 ^b	1.81±0.28 ^b	70.34±1.21 ^{bc}	1.27±0.18 ^b
5	5.78±0.22 ^a	2.78±0.33 ^a	3.00±0.23 ^b	0.92±0.14 ^b	1.78±0.31 ^b	69.25±1.31 ^c	1.23±0.21 ^b
6	5.82±0.52 ^a	2.83±0.29 ^a	2.99±0.24 ^b	0.94±0.15 ^b	1.79±0.21 ^b	69.25±2.56 ^c	1.23±0.16 ^b
7	5.92±0.72 ^a	2.83±0.38 ^a	3.09±0.37 ^b	0.91±0.11 ^b	1.80±0.32 ^b	70.52±2.13 ^c	1.26±0.21 ^b

Mean±SD with in column bearing at least one common superscript do not differ significantly ($p < 0.05$).

No. of animals (n)=10.

concentration was lowest on day zero. A significant ($p < 0.05$) four fold increase on day one (due to absorption of Ig from colostrum fed to calves on day zero) and a non-significant decrease thereafter, observed in present study, are comparable with those of Larson et al. (1980), Kulkarni (1981) and Manhas (1993). However, study conducted by Logan et al. (1974) in cow calves suggested that newborn calves were essentially free of gammaglobulin. In the present study, Ig was detected on day zero and this may be attributed to the fetal Ig synthesized by its lymphoid tissue in response to environmental/or maternal antigenic stimuli.

Although the new born calf is immunological competent at birth (McEvan et al., 1970), it is quite likely that the active antibody synthesis gets masked because of higher initial levels of plasma gamma globulins absorbed from colostrum (Sapre and Ramadwar, 1977) and significant endogenous production of major Ig starts only by 8-10 days after birth (Husband et al., 1972). The abrupt increase on day one, observed in this study, was due to a high intestinal absorption of colostral gamma globulins from the colostrum fed to calves on day zero. A similar finding was observed by Kulkarni et al. (1974), Kulkarni (1981) and Larson et al. (1980)

The concentrations of IgG reported in this study are in agreement with those of Naylor and Kronfeld (1977), Joshi (1990) and Manhas (1993). The presence of IgG though in small quantity, has been observed in the present study in pre colostral buffalo calves. This is in consonance with the findings of Jalnapurkar et al. (1976). The concentration of IgG was significantly ($p < 0.05$) increased (twenty fold) on day one and decreased on day two. The abrupt increase after colostrum feeding on day one may be attributed to absorbed colostral IgG, which is predominant in it. The non-significant decrease from day two onwards may be due to slowing down of absorption of colostral IgG from the intestine (Logan et al., 1974) and catabolism of absorbed Ig.

Figure 1 reveals that there is a sharp decline in colostral total Ig and IgG levels from day zero till day three post partum. This suggests that the highest amounts of colostral Ig and IgG are available on the day of parturition, therefore calves should receive colostrum as soon after birth as possible to improve their survivability and disease resistance. Plasma Ig and IgG levels increased sharply, four fold and twenty fold respectively, in post suckle buffalo calves on day one and these levels are more or less maintained up to seven days of age. Total Ig levels of colostrum on day zero and that of plasma on day one points towards the fact that relative to the amounts of immunoglobulins available in colostrum, the calf absorbs only a small amount that actually appears in plasma Ig concentrations. Thus, there is an abrupt rise in the concentrations of immunoglobulins in the blood plasma of newborn calves from negligible concentration at birth.

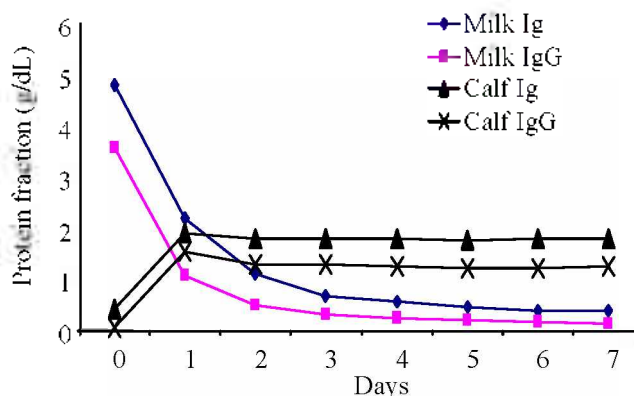


Figure 1. Colostral and milk Ig and IgG level in comparison to pre-(day zero) and post suckle plasma Ig and IgG levels in buffalo calves.

These findings are in accordance with those of Vann et al. (1995). Statistical analysis of the data reveals that no definite correlation exists between colostral total Ig and plasma Ig concentration of post suckle calves of the present study. Same is the case with IgG concentrations. Though the Ig in post suckle calves is mainly because of intestinal absorption of colostral Ig (Stott et al., 1979; Larson et al., 1980), the absence of relationship between colostral Ig concentration and plasma Ig concentration of post suckle calves could be ascribed to the amount of colostral intake and absorption, distribution and metabolism of Ig. The present observation supports the contention of Swecker et al. (1995) that the colostral Ig concentration is not the principle determinant of the post suckle plasma Ig concentration.

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