Plasma Hormones, Blood Metabolites, Milk Yield and Composition in Early Lactation of Buffaloes Treated with Bromocryptine

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ABSTRACT : The study was conducted on six multiparous Murrah buffaloes which were earlier artificially induced into lactation. During the experimental period of 15 days, buffaloes were managed in a loose housing system. All the buffaloes were administered a single injection of bromocryptine (@ 100 μg/kg body weight) subcutaneously in the neck region at 08:30 A.M., 50 days postpartum (early lactation). Blood samples were collected from four buffaloes for a period of 5 days before the administration of bromocryptine i.e. on days -5, -4, -3, -2, -1, on day of treatment (day 0) and thereafter daily for a period of 9 days i.e 1, 2, 3, 4, 5, 6, 7, 8 and 9 to determine the hormones and blood metabolites. Homogeneous milk samples from all the buffaloes were collected at morning and evening milkings on days coinciding with the days of blood sampling for analysis of milk constituents. Administration of bromocryptine resulted in a significant inhibition of plasma prolactin within 24 hrs of treatment, but the response in all the buffaloes was not uniform. The effect of bromocryptine on plasma prolactin hormone lasted for 1-4 days but cortisol concentration were not altered. Administration of bromocryptine neither affected blood glucose nor plasma non-esterified fatty acids concentration.

Irrespective of level of milk production from different buffaloes, there was no effect of bromocryptine on milk yield which indicated that prolactin is not required for milk secretion during early lactation in buffaloes. Milk constituents like fat, protein and lactose were not affected by bromocryptine may be due to no effect of bromocryptine of milk yield.

(**Key Words**: Hormones, Blood Metabolites, Milk Yield and Composition, Bromocryptine, Early Lactation)

INTRODUCTION

After the parturition further maintenance of lactation is regulated by a number of hormones. However, hormonal requirement among mammalian species differ considerably, for example, in rabbit prolactin alone can maintain lactation whereas in cows prolactin is not a rate limiting hormone in established lactation, in place growth hormone becomes relatively more important (Hart 1973; Knight 1993). In biological studies, the requirement of the hormone's particularly prolactin and growth hormone during particular stage of lactation have been characterized either by supplementing growth hormone or by reducing prolactin concentration with bromocryptine (Knight and Flint, 1995).

Similarly, the requirement of prolactin during lactogenesis around parturition has been widely studied using bromocryptine, an anti-prolactin drug which not only suppresses prolactin but also delays onset of lactogenesis without affecting any other hormones (Schams et al., 1972; Forsyth and Lee, 1993). In small ruminants, bromocryptine treatment during lactation reduces milk secretion (Gabai et al., 1992; Buys et al., 1995; Singh, 1996). In lactating cows bromocryptine similarly reduces prolactin and milk yield by 10 to 20 percent (Karg et al., 1972). In buffaloes, information on the requirement of prolactin during different stages of lactation is lacking. Moreover, in artificially induced lactating buffaloes, the milk production varies due to variable response. In such animals, plasma prolactin levels may be critical or rate limiting. The present study was, therefore, undertaken to find out the effect of bromocryptine on plasma hormones, blood metabolites, yield and composition of milk, if any, during early lactation.

MATERIALS AND METHODS

Selection and management of animals

Six healthy multiparous Murrah buffaloes which were repeat breeders and were artificially induced into lactation using estrogen and progesterone hormone in the ratio of 1 : 1 (@ 0.1 mg/kg body wt./day) for a period of 7 days were selected for the study. The buffaloes were 6-10 years of age and had average body weight 581 ± 17.72 kg at the beginning of the experiment. The experiment was conducted during the month of January-February, 1997. All the buffaloes were managed in a loose housing system

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during the experimental period of 15 days. The buffaloes received a diet of concentrate mixture based on milk production only at the time of milking (1 kg concentrate upto 5 kg milk production). The concentrate mixture contained total crude protein 20% and total digestible nutrient as 70%. The green fodder was *ad libitum* which consisted of maize (*Zea mays*) and berseem (*Trifolium alexandrinum*). They had free accesses to fresh water. The buffaloes were hand milked twice a day at 06:00 in the morning and 06:00 after noon and milk production were recorded. The minimum and maximum ambient temperatures, dry and wet bulb temperatures and vapour pressure were also recorded during the study period.

Experimental treatment and collection of samples

2-Bromo-α-ergocryptine, a dopamine D-2 receptor agonist (Bromocryptine methane sulfonate, M/s Sigma Chemical Co., USA) was dissolved in ethanol and 0.9 percent NaCl solution (40:60 v/v), @ 100 μg/kg body weight. One ml of this solution was administered subcutaneously in the neck region at 08:30 A.M. when the buffaloes were in early lactation (50 days postpartum). Jugular blood samples were collected in heparinized tubes (20 IU heparin) daily at 09:00 A.M. from four of the buffaloes before feeding while in two buffaloes blood sampling could not be done due to some reason. The blood samples were collected from 45th day of lactation for a period of 5 day, before bromocryptine treatment, on the day of treatment, and thereafter daily for 9 days post-initiation of treatment. For glucose estimation, blood samples were also collected in the tubes containing sodium fluoride and were immediately transferred into ice-bucket. Plasma were separated by centrifugation at 3,000 r.p.m. for 25 minutes and stored at -20℃ till analysis for hormones and plasma NEFA. Milk samples were collected daily in the morning and evening from individual buffaloes throught the experimental period. Aliquots of milk samples, from each milking were composited in proportion to milk production for analysis of milk constituents.

Analytical methods

For the analysis of milk samples, fat was estimated by Gerber's Method (ISI, 1958), milk protein by formaldehyde titration (Singhal and Des Raj, 1989) and lactose in milk by Picric Acid Method (Perry and Doon, 1950). Blood glucose was estimated by Nelson-Somogyi Method as described by Oser (1965). Non-esterified fatty acid (NEFA) in plasma samples were estimated by extraction method (Chloroform: Haptane: Methanol, 49: 49:2) of Shipe et al. (1980). Plasma prolactin and cortisol hormone were estimated by RIA methods of Singh and Ludri (1997). For iodination, 10 μg bovine prolactin was iodinated with 10 μl (2.0 mCi) ^{125}I in a reaction vial. The reaction was initiated with 10 μl of chloramine T (1 mg/ml, 0.5 M PO_4 buffer) for a period of 35 seconds and was stopped by adding 10 μl sodium metabisulfite (3 mg/ml, 0.5 M PO_4 buffer). The contents of reaction vial were transferred to the top of the freshly prepared column with Sephadex G-75 and 10 drops fraction were collected in the tubes (12 $\times$ 100 mm) containing 0.5 ml of PO_4/BSA buffer. Fraction of first large peak was used for running the assay.

In the assay procedure, all the reagents were added to RIA tubes at a single sitting in the sequence of (a) buffer, (b) standards (1 to 64 ng/ml) or unknown samples, (c) antiserum (1:2,000,000), (d) radioiodinated PRL (15,000 cpm). The tubes were then vortexed and incubated at room temperature for 24 h. Next day, 0.1 ml of second antibody [GARGG (1:5) in PO_4 buffer] and NRS (1:200 in PO_4/BSA) was added to all the tubes except the total count tubes. One ml of polyethylene glycol (6% in 0.01 M PO_4 buffer) was added to stop the reaction and tube contents were vortexed. After 15 minutes, the tubes were centrifuged at 3,000 r.p.m. for 30 min at 4℃. The supernatant was decanted, tubes were dried and the radioactivity was counted for 1 minute in a Cobra make gamma counter. Bovine prolactin and its antiserum (anti-bPRL, AFP # 753180) were obtained gratis from Dr. Phillip F. Smith, National Hormone and Pituitary Program, Bethesda, MD, USA. Second antibody, goat antibody to rabbit gamma globulin (GARGG) was purchased from Calbiochem-Novabiochem Corporation, La Jolla, CA 92039, USA. Highly specific antiserum for cortisol (Lot No. 89F4801) and cortisol hormone was purchased from M/s Sigma Chemical Co. St. Louis, USA. The sensitivity of the prolactin assay was 12.5 pg/tube and for cortisol 6 pg/tube. Within assay (intra) coefficient of variations for prolactin and cortisol hormone were 6.50 and 6.47 percent (n = 3), respectively. Statistical analysis of data was done according to Snedecor and Cochran (1968). The experimental data was divided into 3 periods of 5 day each and analysis of variance, 2-way ANOVA without interaction were calculated to find out the significant changes in hormones, metabolites and milk production and composition in the 3 periods of study. Mean and standard error for each day were also calculated and correlation were found out for different parameters studied. The average values were also compared using paired "t" test for significant difference.

RESULTS

The average values of hormone, metabolites and yield and composition of milk during the experimental period has been presented in table 1 and the summary of analysis in tables 2 and 3. The effect of bromocryptine on plasma PRL in individual buffalo is being depicted

Table 1. Mean ± SE plasma hormones, blood metabolites and yield and composition of milk before and after administration of bromocryptime

Attributes	Days of Experiment														
	Before Treatment					After Treatment									
	−5	−4	−3	−2	−1	0*	1	2	3	4	5	6	7	8	9
Prolactin (ng/ml)	26.52 ± 4.47	25.21 ± 3.69	19.33 ± 3.40	15.96 ± 3.76	26.23 ± 7.23	25.90 ± 6.84	6.06# ± 1.84	10.47 ± 6.37	25.08 ± 7.10	20.74 ± 6.66	25.84 ± 9.51	21.15 ± 4.53	18.48 ± 5.37	19.40 ± 4.77	16.67 ± 5.70
Cortisol (ng/ml)	13.02 ± 3.48	26.00 ± 7.18	24.25 ± 9.57	19.02 ± 2.01	9.56 ± 4.77	23.25 ± 5.99	7.20 ± 0.29	11.31 ± 4.41	20.62 ± 6.52	19.25 ± 6.37	12.38 ± 3.44	8.82 ± 2.20	13.00 ± 4.44	9.62 ± 1.90	8.62 ± 1.46
Blood glucose (mg %)	48.77 ± 1.47	52.74 ± 3.12	42.83 ± 4.12	52.14 ± 3.30	41.40 ± 2.54	50.17 ± 2.65	62.69 ± 4.58	49.22 ± 6.53	54.48 ± 2.75	53.10 ± 5.83	40.86 ± 3.72	53.61 ± 3.90	61.67 ± 4.52	65.86 ± 2.24	63.70 ± 1.56
NEFA (μmol/L)	114.38 ±10.32	154.38 ±28.26	120.63 ±13.82	133.75 ±13.51	135.62 ±23.02	119.38 ±14.37	233.75 ±67.90	118.13 ±9.58	236.25 ±52.60	248.75 ±33.37	188.75 ±61.63	238.75 ±54.84	347.50 ±39.11	173.75 ±11.78	197.50 ±18.16
Milk yield (kg)	2.97 ± 0.63	3.00 ± 0.64	2.44 ± 0.54	3.02 ± 0.66	2.88 ± 0.61	3.22 ± 0.69	3.53 ± 0.73	3.56 ± 0.83	3.57 ± 0.77	3.37 ± 0.85	3.06 ± 0.73	2.90 ± 0.69	3.57 ± 0.73	2.45 ± 0.55	2.93 ± 0.82
Fat (%)	5.50 ± 0.16	6.17 ± 0.05	7.38 ± 0.35	6.68 ± 0.22	6.95 ± 0.20	6.68 ± 0.13	6.65 ± 0.13	7.30 ± 0.27	6.83 ± 0.23	6.87 ± 0.12	6.73 ± 0.17	6.85 ± 0.09	6.90 ± 0.16	6.87 ± 0.20	6.85 ± 0.18
Protein (%)	4.31 ± 0.35	4.51 ± 0.36	4.31 ± 0.24	4.65 ± 0.24	4.62 ± 0.09	4.70 ± 0.21	4.70 ± 0.20	4.87 ± 0.18	5.07 ± 0.10	4.62 ± 0.29	4.68 ± 0.22	4.90 ± 0.15	4.99 ± 0.20	4.53 ± 0.27	4.65 ± 0.27
Lactose (%)	5.18 ± 0.15	4.86 ± 0.15	4.83 ± 0.30	5.05 ± 0.20	4.84 ± 0.22	5.00 ± 0.20	5.02 ± 0.20	5.07 ± 0.17	5.18 ± 0.23	5.09 ± 0.18	4.97 ± 0.20	4.85 ± 0.23	4.93 ± 0.25	4.86 ± 0.21	4.83 ± 0.24

* Day of injection. # $p < 0.05$

graphically (Figures 1 and 2). During the experimental period of 15 days, the minimum and the maximum temperatures were 3.0 and 21.5℃. The values of temperature humidity index (THI) varied from 46.86 to 58.24 and 59.39 to 66.45 during the morning and evening hours, respectively.

Table 2. Summary of ANOVA of complete data on prolactin, cortisol, blood glucose and NEFA before and after administration of bromocryptine

Source of Variation	d.f	Mean Sum of Squares			
		Prolactin	Cortisol	Glucose	NEFA
Between animals	3	1,231.045**	49.959	318.067*	20,831.530*
Between days	4	180.093	36.697	226.355*	8,169.948
Between periods	2	125.292	334.541	473.696**	48,301.670**
Error	50	105.779	142.044	82.919	7,377.688

* $p < 0.05$, ** $p < 0.01$.

Table 3. Summary of ANOVA of complete data on yield and composition of milk before and after administration of bromocryptine

Source of Variation	d.f	Mean Sum of Squares			
		Milk Yield	Fat	Protein	Lactose
Between animals	5	44.615**	1.591**	4.547**	4.125**
Between days	4	0.088	2.043**	0.105	0.073
Between periods	2	2.882*	1.008*	0.878**	0.270**
Error	78	0.635	0.231	0.123	0.052

* $p < 0.05$, ** $p < 0.01$.

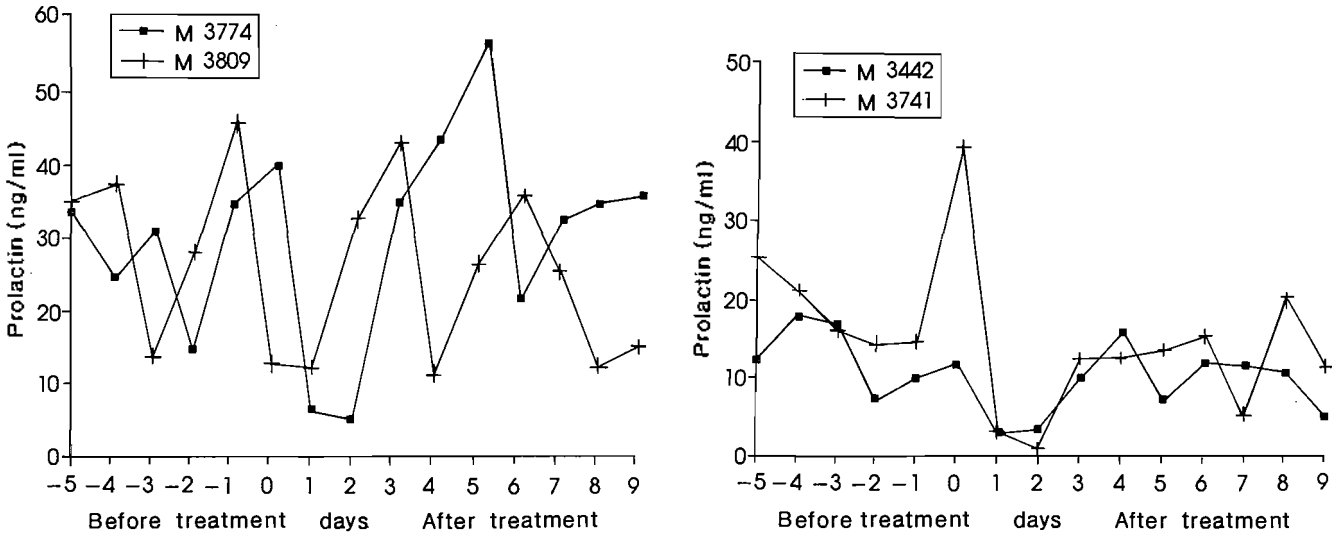

Figure 1. Plasma prolactin of individual buffaloes before and after administration of bromocryptine during early lactation.

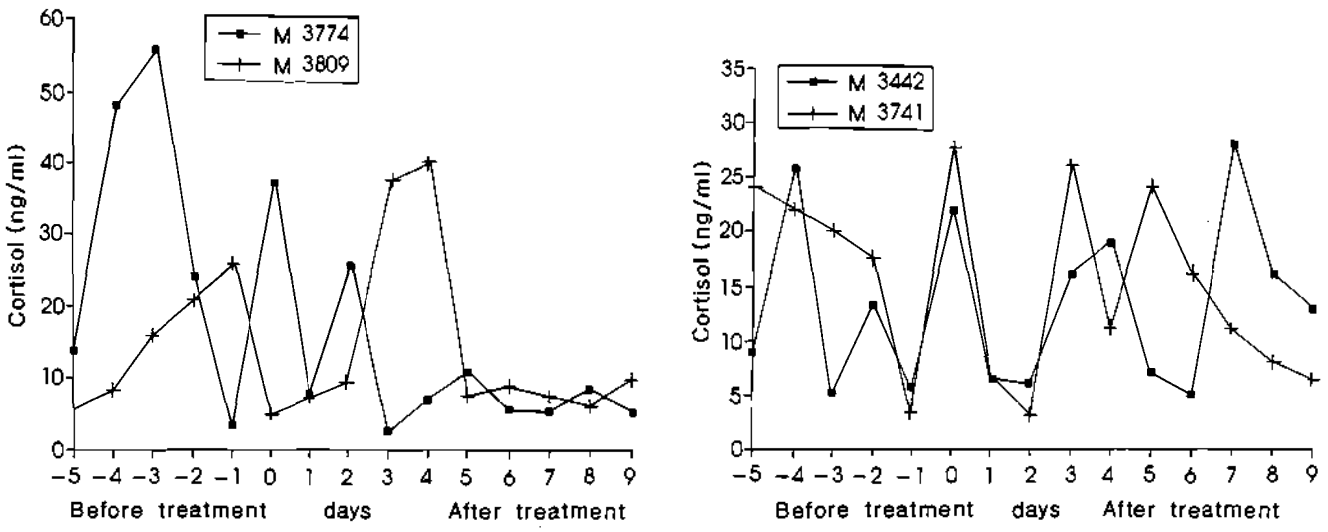

Figure 2. Plasma cortisol of individual buffaloes before and after administration of bromocryptine during early lactation.

Effect of bromocrytpine on plasma hormones

The average plasma PRL concentration varied from 15.96 to 26.52 ng/ml before the administration of bromocryptine. However, in individual buffaloes, plasma PRL concentration were low on day 3 and 2 before the start of treatment. Plasma PRL concentration remained similar on day 0 (day of injection) because blood samples were collected prior to administration of bromocryptine. Average plasma PRL declined significantly ($p < 0.05$; $t = 5.47$) on day 1 of bromocryptine administration, and remained low on day 2 and thereafter became normal as observed before. From day 3 to 9 the levels were variable. The individual buffalo responded differently to the suppressive effect of bromocryptine. In buffaloes, M 3442 and M 3774, PRL levels declined significantly on day 1 and 2 thereafter, effect of bromocryptine was not found. In case of M 3741, the basal levels of PRL remained low till day 9 post-injection period. In case of M 3809, the suppressive effect of bromocryptine on plasma PRL concentration was not evident. The average inhibition of PRL by bromocryptine on day 1 and 2 were 76.61 and 59.57%, respectively. But in individual buffalo inhibition % of prolactin varied from 4.38 to 96.74. Variation in plasma PRL on different days of experiment were non-significant. Plasma PRL varied significantly ($p < 0.01$) between the animals, is indicative of the fact that bromocryptine suppressed plasma PRL in a different way in different buffaloes and that the effect of bromocryptine was not uniform. Plasma cortisol concentration, before the start of treatment, varied between 9.56 to 26.00 ng/ml. The effect of bromocryptine on plasma cortisol level was not uniform in all the buffaloes. The apparent decline in cortisol levels on day 1 and 2 after administration of bromocryptine in M 3442, 3741 and 3774 was due to the higher levels of cortisol on day of injection. Plasma cortisol concentration varied in different animals on different day of experiment, but the changes in conceentration were non-significant.

Effect of bromocryptine on blood metabolites, milk production and composition

Average basal levels of glucose varied between 41.40 to 52.74 mg % before the administration of bromocryptine. Thereafter, average glucose levels varied between 40.86 to 65.86 mg %, respectively. Blood glucose varied significantly ($p < 0.05$) between the animals and on different days of experiment. The effect of bromocryptine on blood glucose levels was not evident due to day to day variation in blood glucose concentration ($p < 0.05$). Average plasma NEFA concentrations were highly variable and the values were between 114.38 to 154.38 and 118.13 to 347.50 μmol/L before and after the administration of bromocryptine, respectively. Due to the greater variability between the animals, changes in NEFA concentration were significant in different animals ($p < 0.05$) and between the periods of study ($p < 0.01$). There was no apparent effect of bromocryptine which is further supported by non-significant changes in NEFA concentration during different days of experiment. Milk yield of different buffaloes varied from 0.9 to 5.8 kg before the administration of bromocryptine, the average milk production being 2.44 ± 0.54 to 3.02 ± 0.66 kg during this period. Neither average milk production nor the milk production of individual buffalo was affected by bromocryptine. There was a greater variability in milk production (0.9 to 5.8 kg) between the animals ($p < 0.01$), therefore, the changes in milk production between periods were also significant ($p < 0.05$). The average values of milk fat before bromocryptine administration varied from 5.50 ± 0.16 to 7.38 ± 0.35% which remained almost

similar on different days after the administration of bromocryptine also. Average protein content of milk varied between 4.31 ± 0.35 to 5.07 ± 0.10% before and after the administration of bromocryptine. However, in one buffalo (M 3627), the protein content were lower ranging from 3.74 to 4.76% during the experiment. Average lactose content ranged from 4.83 to 5.18% during different days of experiment. In buffalo M 3442, since the milk yield was very low, the lactose % were also low and varied from 3.69 to 4.69%. There was no effect of bromocryptine on fat, protein and lactose content of milk. But the changes in fat, protein and lactose content of milk between the animals during different periods of study were significant ($p < 0.01$). Since there was no effect of bromocryptine on milk production and composition, the average values of these have been presented in the table. During the period of study the milk yield was significantly ($p < 0.01$) correlated with lactose content of milk while the fat was positively correlated with protein ($p < 0.01$) and negatively ($p < 0.05$) with lactose content of milk. Plasma prolactin was positively correlated ($p < 0.01$) with protein content of milk. Blood glucose was correlated positively with NEFA and negatively with milk yield and lactose ($p < 0.01$). The cortisol was neither correlated to prolactin and blood metabolites nor with yield and composition of milk.

DISCUSSION

In this experiment, plasma prolactin concentrations before bromocryptine administration were within the normal range reported as earlier in buffaloes (Singh, 1990; Yash Pal, 1996), but in some of the buffaloes higher basal values were observed. Bromocryptine treatment resulted in varying response in different buffaloes probably due to the differences in their intrinsic level of prolactin. The suppressive effect of bromocryptine was more pronounced in one of the buffaloes (M 3741). In this buffalo, basal level of prolactin could not become normal even after the period of experiment while in M 3774 and M 3442, the effect of bromocryptine lasted only for two days.

Since, release of prolactin and cortisol is affected by milking stimulus in ruminants, and the milking stimulus effect on prolactin release could not be controlled in this study, therefore, the levels of prolactin could not reach the minimum value and between animal differences existed significantly ($p < 0.01$). In buffaloes, administration of similar doses of bromocryptine around parturition inhibited plasma PRL for several days (Yash Pal, 1996) but in the present study, the inhibitory effect were of short duration. This fact is also evident from the percent inhibition of prolactin on day 1 of treatment which varied from as low as 4.38% and as high as 96.74%. The extent of percent inhibition of prolactin by bromocryptine was similar as reported by Akers et al. (1981). Previous reports in cattle and buffaloes given bromocryptine to suppress the periparturient surge of prolactin had indicated that inhibitory effect of bromocryptine on PRL last for a period between 4 to 6 days after the last injection (Smith et al., 1973, 1974; Schams et al., 1972; Karg et al., 1972; Yash Pal, 1996). Cortisol concentration remained unaffected due to bromocryptine treatment. Also, there was no effect of bromocryptine on blood metabolites viz., glucose and NEFA but the variations between the animals ($p < 0.05$) and periods ($p < 0.01$) were significant probably due to the differences in milk yield of buffaloes during different days of study. Yash Pal (1996) reported no adverse effect of bromocryptine on blood glucose and NEFA level in buffaloes as reported also by Singh (1996) in crossbred goats treated with bromocryptine (5 mg/d) during early lactation of 15-20 days.

In this study, bromocryptine treatment did not affected the milk production and composition of milk. Initially, it was thought that in artificially induced buffaloes producing different quantities of milk, the prolactin requirement of different buffaloes might be different and therefore the effect of bromocryptine on milk yield may be different. But irrespective of level of milk production, the effect of bromocryptine was not found. Though PRL levels were inhibited significantly in this study but the act of hand milking stimulus further stimulated release of PRL during milkings leading to variable response in different buffaloes and the effect of bromocryptine on milk yield could not be determined. The significant changes in milk yield and fat % ($p < 0.05$) and the protein and lactose ($p < 0.01$) between periods of study were due to animal to animal variation and not due to bromocryptine administration as reflected by the average values (table 1). The role of prolactin during early lactation in different species is questionable. In Hariana cows, administration of bromocryptine reduced milk yield by 14.87% over the control (Misra et al., 1984). In lactating Holstein and Brown Swiss cows bromocryptine (150 mg/day) administration also reduced plasma prolactin and the milk yields were reduced by 10 to 20% (Karg et al., 1972). Due to variable responses and small number of animals, they reported that prolactin is not a main galactopoietic hormone, rather in this species a complex hormones are necessary for galactopoiesis. But in lactating goats convincing evidences are now available to indicate that PRL depression dose reduces milk yield significantly and consistently by 10 to 20% (Knight, 1993) and to the extent of 16.80 to 28.5% (Singh, 1996) during early lactation.

In case of sheep, prolactin inhibition during lactation also depresses milk yield markedly (Kann et al., 1978).

But small amount of prolactin left in the blood circulation in bromocryptine treated cattle may be galactopoietic, the fact which remains to be investigated or perhaps the mammary secretory cell produces its own prolactin and remain in consequently immune to changes in circulating PRL concentration (Knight, 1993; Knight and Flint, 1995). The bromocryptine treatment did not alter milk composition due to the fact that there was no effect on milk production. Similar observation was also reported by Misra et al. (1984) in Hariana cows.

CONCLUSIONS

The plasma prolactin levels were effectively suppressed by bromocryptine within 24 h of administration but the response in different buffaloes was not similar. There was no effect of bromocryptine on plasma cortisol and blood metabolites viz., glucose and NEFA. The bromocryptine treatment did not influence milk production and composition which indicated that prolactin is not required for milk production during early lactation in buffaloes.

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