Milk Production, Blood Metabolites and Circulatory Levels of Hormones in Crossbred Goats

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ABSTRACT : Eighteen crossbred goats were selected from the Institute's goat herd to determine the changes in hormones, blood metabolites and yield and composition of milk during lactation. The blood and milk samples were collected from each goat in a heparinized vacutainer tubes at fortnightly interval for a period of 150 days. In milk samples, fat, protein and lactose contents were estimated while in blood plasma hormones viz., prolactin, GH, cortisol, insulin, T_4 and T_3 were measured using radioimmunoassay methods. The plasma concentration of prolactin, GH and cortisol were high during early lactation when the goats acquired peak milk yield. During remainder of lactation their concentration varied. The high NEFA concentration during early lactation indicated mobilization of body reserves as the body weights also decrease during early lactation. However, with the advancement of lactation, the body weights of the goats and the concentration of NEFA declined which indicated utilization of NEFA for energy yielding purposes in addition to fatty acid synthesis. The ambient temperatures did not influence plasma concentration of prolactin, GH, insulin, T_3 and T_4 during the lactation cycle. The fat content of milk varied significantly ($p<0.01$) but protein and lactose content of milk remains unchanged during different stages of lactation. Growth hormone was positively correlated with insulin ($p<0.05$) during lactation while prolactin had a positive correlation with lactose and plasma NEFA ($p<0.01$) and negative correlation with T_3 ($p<0.05$). ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 7 : 963-967)***

Key Words : Hormones, Blood Metabolites, Milk Yield and Composition, Lactation, Crossbred Goats

INTRODUCTION

After parturition, the maintenance of lactation is regulated by inter-play of number of hormones like prolactin, GH, insulin, T_4 and T_3, which not only affect circulatory levels of blood metabolites but also the yield and composition of milk in cattle, buffaloes and goats (Knight, 1993; Knight and Flint, 1995). The role of various hormones during different stages of lactation is very well documented in cows and buffaloes (Yousef and Johnson, 1966, 1987; Johke et al., 1971; Koprowski and Tucker, 1973; Malven et al., 1987; Prema et al., 1989; Singh and Ludri, 1994; Singh and Ludri, 1999a). Though goat is a ruminant like cow but the differences in hormonal requirement of lactation can not be ruled out. The information on the hormonal changes in relation to blood metabolites during lactation in goats under Indian condition of tropical climates is not available. The present study was therefore, undertaken i) to study the changes in hormonal levels, blood metabolites, milk production and composition and, ii) to find out correlations, if any, among levels of various hormones, milk yield and composition and also the blood glucose and plasma non-esterified fatty acids, which are important energy yielding substrate during lactation.

MATERIALS AND METHODS

Selection and management of goats

Eighteen crossbred goats, twelve Alpine×Beetal (AB) and six Saanen×Beetal were selected from the institute's goat herd immediately after the parturition. All the goats were in their second or third lactation, and were kept in a goat pen with brick flooring for the experimental period of 150 days. Those were offered *ad lib* green fodder, which consisted of Berseem (*trifolium alexandrinum*) and Mustard (*brassica compestris*) while water was available all the time of the day. The concentrate mixture having 20% CP and 70% TDN was fed based on milk production, at the level of 400 g per kg milk, by the time of milking. Goats were hand milked twice daily at 05:00 AM and 04:00 PM and the quantities of milk from individual goat at each milking were recorded.

Collection and analysis of milk and blood samples

At fortnightly intervals (periods), milk samples were collected from individual goat throughout the experimental period of 150 days duration. Aliquots of milk in proportion to yield from each milking of individual goat were composited for analysis of milk constituents. In milk samples, fat was determined volumetrically by Gerber's method (I.S.I. 1958), protein by the method of Singhal and Des Raj (1989) and lactose by picric acid method (Perry and Doon, 1950). At 8:00 AM, jugular blood samples were collected in heparinized vacutainer tubes at fortnightly intervals from each goat. The blood samples were centrifuged and plasma was stored frozen in a freezer (-21°C) till analysis. For glucose estimation blood samples were collected in the tubes containing sodium fluoride and were immediately transferred into ice bucket. Blood glucose was estimated by Nelson-Somogyi method as described by

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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).

Hormone assays

Plasma prolactin, growth hormone (GH) and cortisol were measured using Radioimmunoassay methods (Saha and Singh, 1998; Singh and Ludri, 1999). The iodination of prolactin and growth hormone (10 ug) was done using 10 ul (2.0 mCi) carrier free iodine Na I^{125} in a reaction vial. The reaction was initiated with 10 ul chloramine-T (1 mg/ml, 0.5 M PO_4 buffer) for a period of 30 seconds and was stopped by addition of 10 ul sodium metabisulfite (3 mg/ml, 0.5 M PO_4 buffer). The contents were transferred to the top of column packed with Sephadex G-75 and 10 drops of elution were collected in the tubes containing 0.5 ml of PO_4 /BSA buffer. The first peak of the labeled hormone was used for assay of prolactin and GH. Ovine growth hormone (oGH-AFB-8758C), ovine prolactin (oPRL-AFP-4328C) and their antiserum (NIDDK-oGH, NIDDK-oPRL-1L) were obtained gratis from Dr. Philip 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, USA. The radioactive iodine for the iodination was supplied by BRIT, Mumbai, India. Cortisol was estimated by the method supplied with highly specific antiserum for cortisol (Lot. No. 89 F-4801, Sigma, USA) as described by Saha and Singh (1998). Thyroxine (T_4), tri-iodothyronine (T_3) and insulin were estimated by Radioimmunoassay kits supplied by Board of Radiation and Isotope Technology, Mumbai, India. The intra- and inter-assay variations of coefficients were 6.3 and 8.5, 5.5 and 8.7, 5.77 and 10.49, 6.50 and 9.00, 5.53 and 9.97 and 5.24 and 8.90% for GH, Prolactin, cortisol, insulin, T_4 and T_3, respectively. Sensitivity of the assay of GH, prolactin and cortisol was 5, 10 and 5 pg/tube, respectively. The analysis of data was carried out using Least Square Mean with interactions to find out the significant changes in hormones, blood metabolites and milk production and composition during lactation. The data analysis was carried out using least square analysis (2- way ANOVA) with interactions. Mean and standard errors and the correlation coefficients were also found out for the different parameters studied (Snedecor and Cochran, 1980). The breed difference for the various parameters studied were also compared.

RESULTS

The mean milk yield and composition, blood glucose and plasma NEFA concentration have been presented in table 1 and the mean levels of plasma hormone in table 2. During the experimental period the maximum ambient temperature declined steadily from 11.53°C to 6.09°C upto 5th period of lactation due to the onset of the winter season. The increase in maximum temperature from 6th period was due to onset of spring season. The minimum temperature also exhibited similar pattern of change during different periods of lactation. The mean body weight of the goats was high during early lactation and declined thereafter but during late lactation period of 9 and 10, the body weights were high. Mean milk yield of the crossbred goats was low during first fortnight of lactation and thereafter increased to peak yield of 1.51 kg during the 3rd period of lactation, after that a continuous decrease was observed till the end of lactation. The changes in milk yield during different periods of lactation were significant ($p<0.01$). The fat percent declined upto period 3 of lactation and increased thereafter when the milk yield decreased. The fat percent of milk varied significantly ($p<0.01$) during different periods of lactation without any effect of breed (table 3). Lactose percent was high during early lactation and thereafter fluctuated; however, changes in protein and lactose content of milk were not significant during lactation. Blood glucose though varied significantly during different period of lactation ($p<0.01$) but there was no distinct pattern of change in blood glucose levels during different periods of lactation. Plasma NEFA concentration declined gradually from 0.85 to 0.07 mM/l till the end of lactation ($p<0.01$). Blood glucose and plasma NEFA varied in both the breeds of goats ($p<0.01$) but their concentration were not significant during different periods of lactation.

Plasma prolactin concentration were high ($p<0.05$) in period 1 and 2 of lactation and thereafter fluctuated during different periods of lactation ($p<0.01$). During late lactation plasma prolactin levels were low. The initial high level of GH and prolactin were concomitant to the high milk yield during first two periods of lactation. However, GH level did not significantly varied with different period of lactation and also between the two breeds of goat. Mean plasma level of GH was high during 3rd period of early lactation. Plasma cortisol levels were also high when milk yield and prolactin concentrations were highest and were low during 5th and 6th period of lactation and again rise during late lactation ($p<0.01$). Plasma T_4 and T_3 levels significantly changed during different periods of lactation but no distinct pattern of change was observed with periods of lactation. Plasma concentration of all the hormones viz., prolactin, GH, cortisol, T_4 and T_3 and insulin were not significantly different between the two breeds of goat. Insulin concentrations were highest during the 3rd period of lactation conconitant to the peak yield and varied significantly during different periods of lactation ($p<0.01$).

During early lactation, prolactin was positively correlated with lactose and negatively with fat content of milk ($p<0.05$). Plasma cortisol was positively correlated

Table 1. Mean Blood metabolite, yield and composition of milk during different periods of lactation

Attributes	Periods of lactation									
	Early lactation			Mid-lactation				Late-lactation		
	15	30	45	60	75	90	105	120	135	150
Body weight	48.28	50.08	46.97	46.91	46.97	45.50	43.72	43.50	47.22	47.33
	±0.43	±0.44	±0.64	±0.39	±0.40	±0.44	±0.67	±0.60	±0.65	±0.60
Blood glucose (mg %)	55.00	49.15	52.35	54.41	48.46	57.21	54.00	53.89	45.78	49.74
	±1.11	±1.38	±1.41	±1.96	±1.85	±2.26	±1.54	±1.53	±1.47	±1.70
NEFA (mM/l)	0.85	0.71	0.23	0.16	0.18	0.14	0.12	0.06	0.06	0.07
	±0.09	±0.10	±0.03	±0.02	±0.03	±0.01	±0.02	±0.00	±0.00	±0.00
Milk yield (kg)	1.09	1.44	1.51	1.44	1.18	1.31	1.24	1.58	1.12	1.00
	±0.10	±0.09	±0.07	±0.09	±0.06	±0.09	±0.11	±0.10	±0.11	±0.10
Fat (%)	4.53	4.41	4.05	4.38	4.21	4.68	4.28	4.32	4.07	3.99
	±0.22	±0.13	±0.11	±0.13	±0.10	±0.17	±0.07	±0.13	±0.07	±0.15
Protein (%)	2.86	2.80	2.82	2.69	2.83	2.71	2.85	2.77	2.73	2.76
	±0.07	±0.08	±0.05	±0.03	±0.03	±0.03	±0.04	±0.03	±0.02	±0.01
Lactose (%)	5.04	4.80	4.64	4.46	4.26	4.00	3.93	4.63	4.89	4.75
	±0.17	±0.08	±0.09	±0.07	±0.03	±0.09	±0.03	±0.07	±0.09	±0.10

Table 2. Mean plasma concentration of hormones and ambient temperature during different periods of lactation

Hormone	Periods of lactation									
	Early lactation			Mid-lactation				Late-lactation		
	15	30	45	60	75	90	105	120	135	150
Prolactin (ng/ml)	18.55	13.06	9.89	7.59	11.17	10.30	8.11	4.71	7.33	5.88
	±4.68	±2.52	±1.03	±1.48	±1.50	±1.22	±0.99	±0.56	±1.37	±0.88
GH (ng/ml)	0.87	0.96	1.26	1.19	0.86	0.92	0.80	0.81	0.93	0.88
	±0.12	±0.06	±0.22	±0.43	±0.05	±0.11	±0.05	±0.05	±0.14	±0.06
Cortisol (ng/ml)	4.06	7.06	7.40	5.92	1.62	3.80	5.94	7.26	10.65	11.57
	±0.73	±1.88	±2.23	±1.65	±0.38	±0.86	±1.82	±1.95	±2.52	±3.26
T_4 (ng/ml)	53.59	39.37	46.42	43.62	40.72	46.83	50.45	41.53	39.30	34.44
	±4.72	±3.71	±4.58	±2.66	±3.98	±4.47	±5.97	±4.18	±3.58	±3.48
T_3 (ng/ml)	1.42	1.54	1.73	1.61	1.65	1.62	1.74	1.86	1.67	1.74
	±0.09	±0.09	±0.15	±0.09	±0.09	±0.05	±0.09	±0.12	±0.10	±0.08
Insulin (μU/ml)	18.86	18.89	31.58	19.74	16.50	17.50	16.84	14.04	14.13	17.34
	±1.97	±1.95	±3.78	±2.22	±1.97	±2.76	±2.46	±1.59	±2.26	±2.97
Minimum Temp. (°C)	11.53 (10.0-13.1)	9.60 (8.40-10.90)	8.60 (3.40-14.00)	7.60 (3.90-12.50)	6.09 (1.20-11.0)	6.38 (2.60-10.2)	8.17 (4.70-15.60)	9.30 (4.80-15.0)	7.44 (5.50-11.20)	14.62 (11.40-18.30)
Maximum Temp. (°C)	28.90 (27.0-30.5)	26.14 (25.3-27.2)	23.60 (19.3-27.0)	22.40 (17.50-26.3)	17.80 (13.8-21.3)	18.28 (14.7-21.5)	21.53 (19.8-24.2)	21.29 (15.0-24.5)	23.64 (20.6-25.7)	28.67 (24.3-32.0)

Table 3. Summary of ANOVA of whole data on plasma hormones, blood metabolites, yield and composition of milk

Source of variation	d.f.	Mean sum of squares					
		Prolactin	GH	Cortisol	Insulin	T_4	T_3
Between periods	9	289.203**	0.429	163.990**	442.049**	596.09*	0.260
Between breeds	1	162.530	0.279	19.583	0.200	51.686	1.021*
Error	169	70.180	0.540	66.790	110.668	322.333	0.186
		Blood glucose	NEFA	Milk yield	Fat	Protein	Lactose
Between periods	9	227.280**	1.429**	0.515**	0.891**	0.617	2.499
Between breeds	1	66.466	0.001	1.374**	0.864	0.122	0.412
Error	169	48.516	0.041	0.162	0.341	0.401	0.155

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

with blood glucose ($p<0.01$) and plasma NEFA ($p<0.05$). Further, blood glucose was positively correlated with milk yield ($p<0.05$) and had a negative correlation with protein. Plasma NEFA was negatively correlated with lactose ($p<0.05$). Plasma T_3 had a negative correlation with prolactin and a positive with lactose ($p<0.05$). At peak of

lactation during 3rd period. T_3 was positively correlated with GH ($p<0.01$), insulin, NEFA and lactose ($p<0.05$) and negatively with T_4. The milk yield was negatively correlated with blood glucose ($p<0.05$). During mid-lactation, prolactin was positively correlated with NEFA and lactose ($p<0.05$) while the blood glucose had a negative correlation with NEFA ($p<0.01$) and protein ($p<0.05$). During late-lactation, NEFA was negatively correlated with milk yield and blood glucose and GH had a negative correlation with milk and NEFA ($p<0.05$). The overall correlation throughout the lactation indicated a negative correlation of plasma NEFA with milk yield and blood glucose. GH has a positive correlation with milk yield and insulin ($p<0.05$) and negative correlation with NEFA. Lactose was negatively correlated with milk yield and fat and the plasma NEFA ($p<0.05$). Plasma prolactin was correlated with lactose, and NEFA ($p<0.01$) and negatively with T_3 ($p<0.01$).

DISCUSSION

In the present study, an attempt was made to find out the relationship of hormones with yield and composition of milk and the metabolites during a lactation cycle of 150 days in goats. Plasma NEFA concentration exhibited a continuous decline in the concentration as observed for the milk yield changes, which indicated mobilization of the body reserves during early lactation. Further, low levels of NEFA after acquiring the peak of lactation indicate decreased energy requirement due to low milk yield. However, glucose levels did not conclusively indicate any role during lactation probably due to the fact that all the goats were at the similar level of milk production. Fluctuations in the body weight of goats during period 3 of early lactation indicated metabolic adjustments as the milk yields during different periods also increased and NEFA decreased. After 3rd period of lactation, the body weight of goats declined continuously upto 8th period of lactation. However, NEFA concentration did not increase due to the fact that this period fall in months of January and February when minimum and maximum ambient temperature remained low and the energy requirement of the goats for maintenance increased. This fact is also confirmed by the continuous fall in the NEFA concentration. Since NEFA is utilized for fatty acid synthesis by the mammary gland as well as for energy yielding purpose, which was reflected by the decline of NEFA levels to meet the energy requirement of the goats. The fat content of milk varied with the changes in the milk yield during lactation, whereas protein and lactose content of milk remain unchanged. The low percent of lactose during 5, 6 and 7th period of lactation indicated an effect of low ambient temperature during these periods of lactation when the energy requirement for maintenance increased. During 5th and 6th periods of lactation cortisol levels were low in goats when the maximum and minimum ambient temperatures were also low which indicated a definite role of low temperature on the levels of cortisol. But the effect of low temperature was not evident on plasma levels of GH, prolactin, T_3, T_4 and insulin. Dalvi et al. (1995) reported low levels of T_3 and T_4 during early lactation due to secretion of these hormones in milk during the process of biosynthesis of milk by the mammary gland as observed in this study. The plasma concentrations of T_3 and T_4 were not affected by low ambient temperatures during 5th and 6th period of lactation (Kloren et al., 1993) though earlier reports indicate a depressive effect of winter season on T_3 and T_4 levels in goats (Prakash and Rathore, 1991). Katti et al. (1991) reported a decrease in concentrations of T_3 at high temperature in African Pygamy goats. The high levels of GH during early lactation indicated the possible role of GH in peak lactation in goats. However, mean GH levels observed in this experiment were low compared to the earlier reports which was due to lower milk yield of the goats in the present study (Martal, 1976; Forsyth and Lee, 1993). The GH release is not dependent on changes in the levels of either NEFA or glucose in the circulation of normal fed British Saanen goats. Further, no relationship between the apparently spontaneous episodic release of GH and behavior, stages of sleep, air temperature, time of day or night or levels of prolactin, insulin, glucose or FFA in the blood has been reported (Tindal et al., 1978). The plasma prolactin levels observed in the study were similar with those reported earlier in goats (Johke, 1970). The high levels of plasma prolactin and cortisol during this period of lactation also suggest that their circulatory levels are also important at the peak of lactation. The suppression of prolactin with bromocryptine during early lactation result in decreased milk yield and low protein and lactose content of milk (Singh and Ludri, 1999a). The positive correlation of prolactin with lactose and of cortisol with blood glucose and plasma NEFA further supports this fact. Low cortisol concentrations during late lactation were associated with low milk yields and also with summer season as its level is also affected by environmental temperature (Ludri and Sharma, 1985). Further, the positive correlation between prolactin and GH concentration with the milk yield during early lactation indicated requirement of these hormones for establishment of lactation in goats.

CONCLUSION

On the basis of long term study of 150 day duration on lactating crossbred goats it was revealed that the composition of milk including fat, protein and lactose fluctuated due to differential behavior of different goats with proceeding lactation. All the goats lost their body weights and there was a concomitant decrease in plasma

NEFA levels as the lactation progressed, thereby indicated utilization of NEFA for energy yielding purposes, in addition to the fatty acid synthesis by the mammary gland. However, the concentrations of hormone viz., prolactin, GH, cortisol, insulin, T_4 and T_3 varied during lactation due to season and lactation stage.

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