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Original Article

Advanced studies on leptin and some biochemical parameters in lactating Buffaloes

Ibrahim F. Hassan[1], Khaled A. Kahilo[1], Madeha M. Ateia[2], Azza M. EL-kattawy[1] and *Sabreen Ezzat Fadl[3]

[1]Biochemistry Department, Faculty of Vet. Medicine, Kafrelsheikh University, Egypt
[2]Animal Production Institute and [3]Animal Health Research Institute, Kafrelsheikh, Egypt

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ABSTRACT

The present work was designed to clarify the relationship between leptin, body weight and their metabolic effects in blood and milk of lactating buffaloes through determination of daily milk yield and some biochemical parameters. Biochemical parameters chosen were: serum and milk leptin, serum and milk Prolactin, serum insulin, serum insulin growth factor-I, serum T3 and serum and milk immunoglobulins (IgG, IgM and IgA). The results showed that, Serum leptin exhibited significant positive correlation with IgG and IgA and positive correlation but not significant with insulin, IGF-1 and IgM. Moreover, there was a negative correlation but not significant with body weight, Prolactin and T3. Whereas milk leptin showed a high significant positive correlation with IgG, IgM and IgA. This positive correlation was not significant with body weight and Prolactin and negatively correlated and not significant with daily milk yield. So, milk leptin has a very important role as an immunostimulant. Owing to the important role of leptin as an immunostimulant we advise buffalo cows' breeders to Provide balanced rations at the prepartum and postpartum periods for opposing the immunosuppressive effect induced by starvation and Provide milk at postpartum period for suckling of newly borne calves as a mean for transferring passive immunity.

Keywords: lactating buffaloes, serum and milk leptin, serum and milk Prolactin, serum insulin, serum insulin growth factor-I, serum T3, serum and milk immunoglobulins (IgG, IgM and IgA)

INTRODUCTION

Leptin appears to have a range of roles as a growth factor in a many cells: as a mediator of energy expenditure; as a permissive factor for puberty; as a signal of metabolic status and modulation between the foetus and the maternal metabolism. Moreover it is perhaps importantly in all of these interactions, to also interact with other hormonal mediators and regulators of energy status and metabolism such as insulin, glucagon, insulin-like growth factors, growth hormone and glucocorticoids [1]. Less attention has been paid so far to the metabolic effects of leptin and body weight on milk yield and biochemical parameters. Therefore, the objective of the present study was to clarify the effect of leptin on body weight, daily milk yield, prolactin, insulin, insulin like growth factor-I, triiodothyronine, and immunoglobulins in blood and milk of healthy buffalo cows. Leptin was discovered in 1994 as a 16-kDa non-glycosylated peptide hormone, the product of the ob gene [2]. Leptin is initially produced as 167-amino acid polypeptide with an amino-terminal secretory signal sequence of 21 amino acids, and then undergoes proteolytic cleavage of the signal peptide to produce a secretory form of 146-amino acid protein [3]. It is produced and secreted mainly by adipocyte in proportion to body fat mass as a satiety signal of body energy stores to the hypothalamus [4]. Leptin is multifunctional hormone. The main function is to regulate appetite, energy expenditure and body weight. Leptin, the product of the ob gene, is a pleiotropic hormone, secreted by adipocytes in humans and many other terrestrial mammal species, that regulates fat mass and energy expenditure [5]. In ruminants, it is reported that, circulating leptin concentrations are considerably altered by changes in nutritional and physiological conditions such as parturition and lactation, and also that the susceptibility to infection insults in cows increases during periparturient period [6]. It is well known that mammary differentiation and milk secretion are controlled by reproductive and

***Corresponding Author Address: Sabreen Ezzat Fadl,** *Animal Health Research Institute, Kafrelsheikh, Egypt; E-mail: abdob522011@yahoo.com*

metabolic hormones [7], and these hormones also affect the synthesis and secretion of leptin from white adipose tissue [8].

Leptin regulates energy balance through its impact on appetite and fat metabolism, and its concentration indicates the size of body fat reserves. It acts centrally to suppress appetite by inhibiting the production of orexigenic peptides in the hypothalamus [5]. These actions maintain fat stores within a narrow range in normal terrestrial mammals. Leptin is thought to have evolved as a protective mechanism to promote feeding and fat deposition when fat stores become depleted [9].

MATERIALS AND METHODS

Animals used: For performing the present study, a total of 30 healthy lactating buffaloes at fourth lactation period were used. The animals were belonging to large ruminant unit, Sakha Experimental farm, Animal Production Research Institute at Kafrelsheikh governorate. The animals under study were divided into three groups, 10 lactating buffaloes each according to the following table.

Methods: The body weight was obtained directly before collection of blood and milk samples. The daily milk yield was obtained from the archive at day of collection of blood and milk samples. All biochemical parameters measured by using enzyme linked immuno-sorbent assay (ELISA) kit. Where the serum and milk leptin levels were determined according to the method described by [10], the serum and milk Prolactin levels were determined according to the method described by [11], the serum insulin levels was determined according to the method described by [12], the serum IGF-I level was determined according to the method described by [13], the serum T3 level was determined following the method cited by [14], and the serum and milk immunoglobulins levels were determined following the method described by [15]. The obtained data were analyzed using student-t test and randomized complete block design for analysis of variance (ANOVA) according to [16].

RESULTS

Effect of stage of lactation on body weight, daily milk yield, serum leptin, milk leptin, serum prolactin, milk prolactin, serum insulin, serum IGF-I and serum T3 in lactating buffaloes: The present work indicated that, B. W. of lactating buffalo cows were significantly increased ($p \leq 0.05$) throughout period of lactation. The mean values of daily milk yield were significantly decreased ($p \leq 0.05$) with stage of lactation between mid and late lactation. But effect on the mean values of serum leptin, showed insignificant increase with stage of lactation but milk leptin exhibited a significant decrease with stage of lactation. The present study revealed that, the mean values of serum Prolactin was insignificantly increased then decreased with stage of lactation but the mean values of milk Prolactin was constant with stage of lactation. The present study revealed that, serum insulin was significantly increased with stage of lactation but serum insulin like growth factor-I increased with stage of lactation but not significant. Also serum T3 was significantly increased ($P<0.05$) with stage of lactation.

Effect of stage of lactation on serum and milk immunoglobulins concentration in lactating buffaloes: The present study revealed that, serum immunoglobulins were significantly increased ($P<0.05$) in IgG, IgM then significantly decreased ($P<0.05$) in the late stage whereas IgA was significantly increased ($P<0.05$) and not significantly changed in the late stage of lactation. But milk immunoglobulins were significantly decreased ($P<0.05$) in IgG, IgM and IgA in mid lactation and not significantly changed in the late stage of lactation.

Overall correlation coefficients between body weight, serum leptin, milk leptin and different biochemical parameters in serum and milk: The present study showed that, there was an indirect correlation between body weight and serum leptin. In the same time there were a direct correlation between body weight and serum Prolactin. Moreover, there were a direct correlation between body weight and serum insulin and insulin like growth factor-I and the correlation was significant in insulin. There was a direct highly significant correlation between body weight and serum T3. The study revealed an indirect highly significant correlation between body weight and serum IgG and IgA, whereas this correlation was indirectly significant between body weight and IgM. There was a direct significant correlation between body weight and daily milk yield. Moreover, there was a direct correlation between body weight and milk leptin. Furthermore, there was a direct correlation between body weight and milk Prolactin. Meanwhile there was a direct highly significant correlation between body weight and milk immunoglobulins. The study demonstrated that, there was an indirect correlation between serum leptin and Prolactin. Also, there was a positive correlation between serum leptin, insulin and insulin like growth factor-I. On the contrary there was an inverse correlation between serum leptin and T3. Also, it was clear from the present study that, there was a direct significant correlation coefficient between serum leptin and serum immunoglobulins. There was inverse correlation coefficient between milk leptin and daily milk yield. Also, there was direct correlation coefficient between milk leptin and milk Prolactin. The study showed that, there was a highly significant direct correlation coefficients between milk leptin and milk immunoglobulins.

DISCUSSION

Effect of stage of lactation on body weight, daily milk yield, serum leptin, milk leptin, serum prolactin, milk prolactin, serum insulin, serum IGF-I and serum T3 in lactating buffaloes: The results of the present work indicated that, B. W. of lactating buffaloes were significantly increased ($p \leq 0.05$) throughout the periods of lactation. This increment could be attributed to negative energy balance due to high milk production, where the decrement was clearly noticed in early lactation and at peak of lactation but the increment was noticed in late lactation period. This finding was in complete agreement with that reported by [17], who recorded that, the most important period is early and peak lactation when the demand for energy is highest. A cow producing 35 kg of milk daily requires three times more energy for production than for body maintenance. The energy requirements of a lactating cow are met through a combination of dietary intake and mobilization of body reserves. Dairy cattle, have been selected for high milk production, cannot maintain a positive dietary energy balance during early lactation and must mobilize body reserves.

It was clear from the present study that, the mean values of milk yield were significantly decreased ($p \leq 0.05$) at late lactation periods compared to mid lactation period. This decrement could be attributed to new pregnancy. This finding goes hand with hand with that reported by [18], who stated that, pregnancy has been reported to have a negative effect on daily milk yield of dairy cows due to hormonal changes, causing regression of the mammary gland, and nutrient requirements of the fetus, reducing available nutrients for milk production. Who also added that, the effect of pregnancy was little at the beginning of gestation and becomes greater at later stages of gestation when growth and nutrient requirements of the conceptus are larger. Also, the present study demonstrated that, the mean values of serum leptin exhibited an insignificant increase with the stage of lactation, this insignificant increment could be attributed to days postpartum. These results agreed with that cited by [19], Who stated that, blood serum leptin tended to be influenced by time. They also added that, leptin concentrations at day 14, 28, 35, 43, and 49 were greater than day-0, presumably resulting in the weak positive correlation between days postpartum and serum leptin**.** But concerning the effect of stage of lactation on serum prolactin, the present study revealed that, the mean values of serum Prolactin was insignificantly increased at mid and late lactation periods compared to early lactation period. This increment at both periods could be attributed to NEB. Moreover, the decrement at the late lactation period compared to mid lactation period might be attributed to the beginning of new pregnancy at this period. The obtained results came in accordance to that reported by [20] who stated that, the basal level of prolactin increases throughout the course of pregnancy, up to tenfold by term, and remains elevated during the postpartum period in humans and in cattle.

Concerning the effect of stage of lactation on the mean values of milk leptin, the present study revealed that, milk leptin exhibited a significant decrease with stage of lactation. This finding agreed with the findings of [19] who stated that, milk leptin was influenced by day postpartum, as the days postpartum increased, milk leptin decreased. It was clear from the present study that, the mean values of milk prolactin is constant with stage of lactation. This finding was in close agreement to the work of [21] who stated that, most of the hormones are transferred into milk by diffusion. However, evidence is available for active mechanisms like those for progesterone in goats and prolactin in cows. Most of the hormone profiles in milk are similar to the ones in blood plasma. Hormone concentrations in milk seem to be a good estimate of the average hormone content in plasma, especially for the measurement of longer-lasting secretory activities like progesterone and estrogen release during the estrous cycle or seasonal changes of prolactin in ruminants.

Concerning the effect of stage of lactation on the mean values of serum insulin, the present study revealed that, serum insulin significantly increased with stage of lactation. This increment in the mean values of serum insulin might be attributed to negative energy balance where lactating buffaloes under go negative energy balance after parturition. These results disagree with that reported by [22] who stated that, the onset of negative energy balance around parturition was associated with decreased plasma insulin and increased plasma growth hormone (GH), suggesting that both hormones could mediate a portion of the effect of energy balance on plasma leptin.

With respect to the effect of stage of lactation on the mean values of serum insulin like growth factor-I, the present study revealed that, serum insulin like growth factor-I was insignificantly increased with stage of lactation. Cows in NEB have greater concentrations of growth hormone (GH) and non-esterified fatty acids (NEFA), and lesser concentrations of insulin-like growth factor-I (IGF-I), insulin and glucose [23]. Studying the effect of stage of lactation on the mean values of serum T3, the present study revealed that, serum T3 was significantly increased ($P<0.05$) with stage of lactation. This increment could be attributed to increased daily milk yield at mid lactation and new pregnancy in late lactation periods. These results came in accordance to that reported by [24] who reported that, as compared to the end of gestation, the plasma T3 concentrations increased during lactation and this increase was not observed for T4.

Effect of stage of lactation on serum and milk immunoglobulins concentration in lactating buffaloes: The obtained data showing the effect of stage of lactation on the mean values of serum immunoglobulins, demonstrated that, the mean values of serum immunoglobulins were significantly increased ($P<0.05$) at early lactation period compared to mid and late lactation periods. There were a sharp decrement in the mean values of immunoglobulins which might be attributed to the secretion of immunoglobulins in colostrums directly after calving throughout the early lactation period. The previous finding agreed well with those reported by [25] who stated that, immunoglobulin-A in adult bovine serum was eluted predominantly in the second peak of the protein profile and only low concentrations were found. Little or no antibody activity was detected in the IgA class and the antibody was attributed mainly to IgM and IgG. IgG is the only isotype that can pass through the placenta, thereby providing protection to the fetus in its first weeks of life before its own immune system has developed, IgA represents about 15 percent to 20 percent of immunoglobulins in the blood; however, it is primarily found in external secretions of the body. It is secreted across the mucosal tract into the stomach and intestines and is also found in tears, saliva, and maternal milk, Because IgM is a large molecule, it cannot diffuse well and is found in very low quantities in the interstitium. IgM is primarily found in serum; however, because of the J chain, it is also important as a secretory immunoglobulin [26].

Studying the effect of stage of lactation on the mean values of milk immunoglobulins showed that, there were a significantly decreased milk immunoglobulins ($P<0.05$) in IgG, IgM and IgA at mid lactation period and not changed in late stage in IgG only. This results goes hand with those reported by [27] who stated that, in bovine mammary secretions there is a rapid fall in immunoglobulin content so that after a few days all immunoglobulins and antibody activity have declined to very low levels. The immunoglobulins in colostrums and early milks represent concentration of serum antibodies.

Overall correlation coefficients between body weight, serum leptrin and milk leptin with different serum and milk biochemical parameters: Studying overall correlation coefficients between body weight and different serum biochemical parameters revealed the presence of indirect correlation between body weight and serum leptin. This finding agreed with those reported by [28] who reported that, low body weight cows showed significantly lower leptin concentrations than high body weight cows. This study clearly showed that, plasma leptin concentrations are high during late pregnancy and decline to a nadir at parturition. On the contrary the results obtained by [22], showed that, the periparturient reduction in plasma leptin precedes significant depletion of lipid reserves. Concerning overall correlation coefficients between body weight and serum Prolactin there were a direct correlation between body weight and serum Prolactin, this finding agreed with that reported by [29] who reported that, Cows with greater nutrient intake had increased plasma Prolactin than cows with lower nutrient intake.

Concerning overall correlation coefficients between body weight and serum insulin and insulin like growth factor-I revealed the presence of direct correlation between body weight and serum insulin and insulin like growth factor-I which is significant in insulin. This finding came in accordance to that reported by [17] who reported that, under feeding caused a decrease in the plasma concentration of insulin ($P < 0.01$) and an elevation in the plasma concentration of GH. Concerning overall correlation coefficients between body weight and serum T3 revealed the presence of direct highly significant correlation between body weight and serum T3. This finding agreed with that observed by [29] who observed that, concentrations of T3 and T4 were greater in moderate-body condition cows than low- body condition cows. Moreover, overall correlation coefficients between body weight and serum immunoglobulins revealed the presence of indirect highly significant correlation between body weight and serum IgG and IgA. On the other side there were an indirect significant correlation between body weight and IgM. This finding agreed with that cited by [30] who cited that, obesity, a state of energy excess, has been associated with increased susceptibility to infection, bacteria, and poor wound healing.

Concerning overall correlation coefficients between body weight and daily milk yield showed that, there were a direct significant correlation between body weight and daily milk yield. This finding agreed with that observed by [31], who found that, mature cows produce about 25% more milk than 2-year-old heifers. Increased body weight accounts for about 1/5 of this increment. The remaining 4/5 results from increased udder development during recurring pregnancies. Large cows generally produce more milk than small cows, but milk yield does not vary in direct proportion to body weight. Rather, it varies by the 0.7 power of body weight, which is an approximation of the surface area of the cow (metabolic body size). A cow which is twice as large as another usually produces only about 70% instead of 100% more milk [32].

With respect to overall correlation coefficients between body weight and milk leptin showed that, there were a direct non significant correlation between body weight and milk leptin. This finding might be attributed to the development of the mammary gland. This finding agreed with that cited by [33] who stated that, most of the leptin in milk is synthesized by the mammary gland, milk leptin concentration is substantially greater than, and not correlated with, the plasma leptin concentration. Also concerning overall correlation coefficients between body weight and milk Prolactin our data revealed that, there were a direct non significant correlation between body weight and milk Prolactin. This finding might be attributed partly to the presence of leptin. This relationship was previously explained by [34] who reported that, Prolactin can regulate leptin and leptin receptor gene expression in the bovine mammary gland. Also concerning overall correlation coefficients between body weight and milk immunoglobulins the obtained data revealed that, there was a direct highly significant correlation between body weight and milk immunoglobulins. This finding might be attributed to development of the mammary gland and increased production of leptin which influence immune system. This fact agreed with [35] who stated that, circulating leptin concentrations are considerably altered by changes in nutritional and physiological conditions, such as feeding-fasting cycles, parturition and lactation, as well as immune activities. Concerning overall correlation coefficients between serum leptin and different serum biochemical parameters. Our results revealed that, there were an indirect correlation between serum leptin and Prolactin and this finding agreed with the work of [36] who reported that, during lactation, the level of Prolactin was high and that of serum leptin was low. Whereas [2] found that, serum leptin concentrations were positively correlated with serum Prolactin and estradiol.

It was clearly observed from the obtained results that, there were a positive non significant correlation between serum leptin, insulin and insulin like growth factor-I and these results agreed with that reported by [33] who stated that, insulin is a positive regulator of plasma leptin. Also, there was an agreement with that mentioned by [17] who stated that, insulin is an important positive regulator of leptin synthesis in mature dairy cattle, as shown by positive correlations between their plasma levels, and by elevated plasma leptin during hypoglycemic-hyperinsulinemic conditions. Also [37] found that, there were a direct correlation between insulin and insulin like growth factor-I as increased insulin stimulated the secretion of insulin like growth factor-I. Also, our results showed that, there were an inverse correlation between serum leptin and T3 concentrations. This fact agreed with [2] who stated that, postpartum serum leptin concentrations were inversely correlated with serum thyroxin. On the contrary our results disagreed with that reported by [1] who stated that, no correlation between serum leptin and thyroid hormone concentrations. It was clear from the present study that, there were a direct significant correlation coefficient between serum leptin and serum immunoglobulins but IgM is not significant. These results agreed with those of [38] who recorded that, leptin has also been shown to regulate the immune response. It was clear from the present study that, there were an inverse correlation coefficient between milk leptin and daily milk yield. Our results agreed with those observed by [35] who found that, daily milk yield influences leptin concentrations during lactation as higher daily milk yield were related to lower leptin production. On the contrary [39] observed that, there were no relationship between milk yield and leptin concentrations. Moreover, it was clearly observed from the present study that, there were a direct correlation coefficient between milk leptin and milk Prolactin. This finding came in accordance to that recorded by [34] they found that, Leptin up-regulated the lactogenic effect of prolactin in the bovine mammary gland. Concerning the correlation coefficient between milk leptin and milk immunoglobulins there were a highly significant direct correlation. These results agreed with those of [40] who stated that, as reported for other species, we found significant concentrations of immunoreactive leptin in bovine milk.

Table (1): Effect of stage of lactation on body weight, daily milk yield, serum leptin, milk leptin, serum Prolactin, milk prolactin, serum insulin, serum IGF-I and serum T3 concentration in lactating buffaloes.

Criterion / Stage of lactation	Body weight (kg)	Daily Milk Yield (kg)	Serum Leptin (ng/ml)	Milk Leptin (ng/ml)	Serum Prolactin (ng/ml)	Milk Prolactin (ng/ml)	Insulin (ng/ml)	IGF-I (ng/ml)	T3 (ng/ml)
Early	476.0± 11.6[b]	--	7.70 ± 0.54 [a]	12.58 ± 1.18a	7.59 ± 0.91 a	6.28 ± 0.46 a	12.61 ± 1.29b	167.6 ± 16.3[a]	3.23 ± 0.37 [c]
Mid	507.5 ±15.97	6.00 ± 0.33 [a]	9.67 ±1.35a	10.91 ± 0.55[a]	10.46 ± 2.17a	5.70 ± 0.26 a	22.84 ± 3.15a	187.14 ± 8.4a	6.50 ± 1.05 [b]
Late	645.0 ±24.69	4.10 ± 0.35 [b]	10.50 ±0.92[a]	8.41 ± 0.47 [b]	8.95 ± 2.23 [a]	6.43 ± 0.66 [a]	18.9 ± 2.69[ab]	199.84 ± 8.0 [a]	12.2 ± 0.6 [a]

Values are expressed as range & means ± standard errors.
Means in a column without a common letter differ significantly at P<0.05.

Table (2): Effect of stage of lactation on serum and milk immunoglobulins concentration in lactating buffaloes.

Criterion / Stage of lactation	Serum IgG (ng/dl)	Milk IgG (ng/dl)	Serum IgM (ng/dl)	Milk IgM (ng/dl)	Serum IgA (ng/dl)	Milk IgA (ng/dl)
Early	1474.78 ± 24.05 [c]	1033.87 ± 34.04 [a]	178.39 ± 8.89 [c]	738.65 ± 47.94 [a]	22.31 ± 1.98 [b]	623.39 ± 32.62 [a]
Mid	1808.38 ± 18.30 [b]	160. 74 ± 7.09 [b]	376.54 ± 36.05 [a]	24.62 ± 1.47 [b]	53.98 ± 2.04 [a]	51.79 ± 1.22 [b]
Late	1867.50 ± 12.29 [a]	171.32 ± 10.49 [b]	294.88 ± 22.34 [b]	17.80 ± 0.83 [b]	52.17 ± 1.76 [a]	30.23 ± 0.93 [b]

Values are expressed as range & means ± standard errors.
Means in a column without a common letter differ significantly at P<0.05.

Table 3 Overall correlation coefficients among serum and milk leptin concentration and body weight with different serum and milk biochemical parameters

	Serum Leptin	Milk Leptin	Body weight
Serum leptin	--	--	--
Milk leptin	--	--	--
Body weight	- 0.34	0.32	--
Serum Prolactin	- 0.19	--	0.21
Milk Prolactin	--	0.28	0.12
Serum T3	- 0.29	--	0.60***
Serum insulin	0.12	--	0.44*
Serum IGF1	0.12	--	0.21
Serum IgG	0.36*	--	- 0.79***
Milk IgG	--	0.51**	0.78***
Serum IgM	0.28	--	- 0.54**
Milk IgM	--	0.49**	0.76***
Serum IgA	0.45*	--	- 0.79***
Milk IgA	--	0.52**	0.73***
Daily Milk yield	0.00	- 0.36	0.50*

Fig (1) : Effect of stage of lactation on body weight of buffaloes

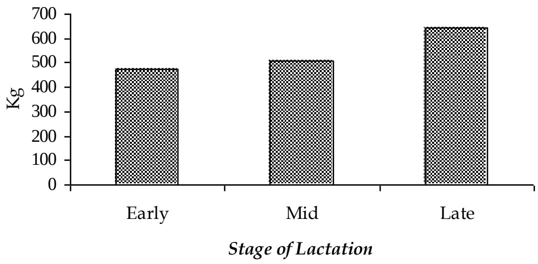


Fig (2) : Effect of stage of lactation on daily milk yield of lactating buffaloes

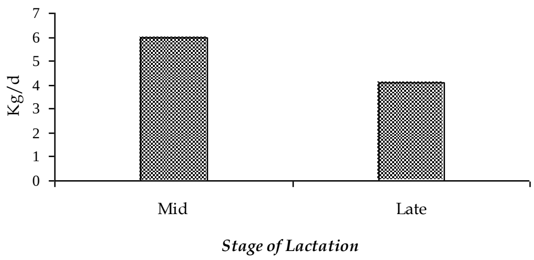


Fig (3) : Effect of stage of lactation on serum leptin concentration of lactating buffaloes

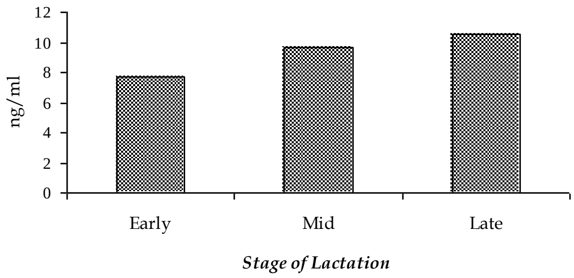
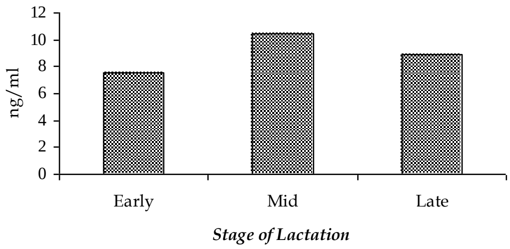


Fig (4) : Effect of stage of lactation on serum prolactin concentration of lactating buffaloes



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