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Research Paper

CALCIUM AND PHOSPHORUS IN DIFFERENT LACTATIONAL AND REPRODUCTIVE STATUS OF BUFFALOES IN PLASMA AND MILK

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The present investigation was undertaken to assess the levels of Calcium and Phosphorus of blood and milk in different physiological status. The experimental animals were categorized into different groups according to phase of lactation (Buffaloes in Early, Mid and Late Lactation) and according to reproductive status (Prepubertal buffalo heifers, cyclic buffaloes, true anestrus buffaloes, buffaloes in early pregnancy, buffaloes in mid pregnancy and buffaloes in late pregnancy) comprising 12 animals in each group. The blood plasma and milk samples were collected from these animals and analyzed for Calcium and Phosphorus which were within normal physiological range. Statistical analysis of present data revealed that there was a significant difference ($P < 0.01$) in the values of plasma and milk calcium among different reproductive states in buffaloes. The reverse trends in the levels of plasma and milk Ca in both non-pregnant and pregnant buffaloes of different reproductive states in the present study supports the view that the Ca drain through milk reduces the level of plasma Ca which is reflected by higher concentration of milk Ca in corresponding groups. Statistical analysis of present data revealed that there was a significant difference ($P < 0.01$) in the values of plasma and milk P among different reproductive states in buffaloes. Milk Ca and P levels were influenced by different lactational status. Plasma P was influenced and Plasma Ca was not influenced by lactational status. Plasma Calcium and Phosphorus were significantly negatively correlated with that of milk in Buffalo.

Keywords: Calcium, Phosphorus, Lactational, Reproductive, Status, Buffaloes

INTRODUCTION

Major elements like calcium (Ca) and phosphorus (P), apart from being general components of

animal body are involved in other functions such as growth, maintenance of ionic, osmotic and acid–base balance and stability of cell

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membranes and thus indirectly affect reproductive functions.

Similar to soil and plants, animal tissue or fluid Ca and P concentrations are influenced by many factors, including kind and levels of production, level and chemical form of elements, mineral intake, interrelationships with other nutrients and animal adaptation (Patodkar *et al.*, 2016; and Waghmare *et al.*, 2016). Other factors such as animal physiological status, seasons, region, year and forage species affect the mineral status of animal tissues or fluids (Panda *et al.*, 2015).

Milk and milk products are important sources of nutrients for the human population in many countries. Milk is rich source of Ca, P. Milk yield is affected by low dietary levels of Ca and P has been suggested by Underwood (1977).

Much less information is known on the status of Ca and P in blood and milk during different lactational and reproductive status in buffaloes. Hence, the present investigation was undertaken to assess the Ca and P composition of blood and milk in different physiological status viz., lactational and reproductive.

MATERIALS AND METHODS

Buffaloes maintained under standard managemental practices at well organized "Takawane Private Buffalo Farm", Pargaon, Taluka: Shirur, District: Pune, Maharashtra, INDIA, were used for the present investigation. The experimental animals were categorized into different groups according to phase of lactation (Buffaloes in Early (Gr.I), Mid (G.II) and Late (Gr.III) Lactation) and according to reproductive status (Prepubertal buffalo heifers (Gr.I), cyclic buffaloes (Gr.II), true anestrus buffaloes (Gr. III), buffaloes in early pregnancy (Gr.IV), buffaloes in mid pregnancy (Gr.V) and buffaloes in late pregnancy

(Gr.VI)) comprising 12 animals in each group. All the selected experimental animals were apparently healthy except true anestrus buffaloes, which were confirmed by per-rectal examination having smooth ovaries without any activity.

Blood and milk samples, as the case may be, from experimental animals were collected at the same time for estimation of calcium and phosphorus content.

From each experimental animal 20 ml of whole blood samples, with heparin as an anticoagulant, were collected by jugular vein puncture using 16 gauge needles in morning cool hours, under aseptic precautions and blood plasma was separated and stored at -20 °C in deep fridge until further analyses. Milk samples from lactating animals were collected in 50 ml capacity sterile plastic containers with lid and milk whey was prepared by following method.

Whey Preparation: Two ml 1% citric acid (W/V) was added drop by drop to 5 ml of milk in a beaker so that the milk was completely coagulated. The contents were then filtered through quantitative ashless filter paper, Filtrum-120, Mfd. by FILTRUM FIBERTECHNOLOGIES Pvt. Ltd., Nandur, tal. Daund Dist: Pune, Maharashtra, India.

Samples were analyzed using UV-Visible Spectrophotometer (Systronics, 117). Plasma and milk (whey) were estimated for calcium by using Modified Arsenazo method of Biggs and Moorehead (1974) and for phosphorus by UV-molybdate method of Goodwin (1970) using a kits supplied by Pathozyme Diagnostics, Kagal, Dist: Kolhapur, Maharashtra, INDIA.

RESULTS AND DISCUSSION

The results with respect to mean \pm SE values of plasma as well as milk calcium and phosphorus

(mg/dl) in different reproductive status in buffaloes are presented in Table 1. Whereas, the results with respect to mean \pm SE values of plasma as well as milk calcium and phosphorus (mg/dl) in different lactational status in buffaloes are presented in Table 2.

Plasma Calcium (Ca)

Statistical analysis of present data revealed that there was a significant difference ($P < 0.01$) in the values of plasma calcium among different reproductive states in buffaloes. Among the first three groups (i.e., non pregnant buffaloes) the value for Group II was significantly higher than

Group III. These findings in present study are in accordance with the reports of Chaurasia *et al.* (2010) in buffaloes. However, present findings are not in agreement with Sharad Kumar *et al.* (2010) and Jayachandran *et al.* (2013) in either cows or buffaloes.

In the groups of pregnant buffaloes the level of plasma Ca during different periods of pregnancy differed significantly ($P < 0.01$) and show decreasing trend. Present findings were in agreement with Dodamani *et al.* (2009) and Padodara *et al.* (2012) in cows and were closely related with Abd Ellah *et al.* (2013) who also

Table 1: Mean \pm S.E. Values (mg/dl) of Plasma and Milk Calcium and Phosphorus in Different Reproductive Status in Buffaloes

Reproductive State	Gr	Calcium (mg/dl)		Phosphorus (mg/dl)	
		Plasma	Milk	Plasma	Milk
Prepubertal buffalo heifers	I	10.44 ^b \pm 0.13	-----	5.56 ^a \pm 0.11	-----
Cyclic buffaloes	II	11.00 ^a \pm 0.15	202.69 ^c \pm 2.30	5.41 ^a \pm 0.19	95.69 ^{bc} \pm 1.54
True anestrus buffaloes	III	09.96 ^c \pm 0.20	214.94 ^a \pm 1.32	3.98 ^c \pm 0.09	101.83 ^a \pm 1.08
Buffaloes in early pregnancy	IV	10.95 ^a \pm 0.12	197.18 ^c \pm 2.19	5.54 ^a \pm 0.10	89.77 ^d \pm 1.87
Buffaloes in mid pregnancy	V	10.69 ^{ab} \pm 0.13	201.66 ^c \pm 2.26	4.71 ^b \pm 0.19	95.15 ^c \pm 1.91
Buffaloes in advance pregnancy	VI	09.88 ^c \pm 0.11	208.29 ^b \pm 1.60	4.20 ^c \pm 0.16	99.62 ^{ab} \pm 1.12
Average value during pregnancy		10.51 \pm 0.10	202.38 \pm 1.38	4.82 \pm 0.13	94.84 \pm 1.16

Note: In the last column, similar super scripts indicates Non Significant difference & dissimilar super scripts indicates significant difference at 1% level of significance.

Table 2: Mean \pm S.E. Values (mg/dl) of Plasma and Milk Calcium and Phosphorus in Different Reproductive Status in Buffaloes

Lactational State	Calcium (mg/dl)		Phosphorus (mg/dl)	
	Plasma	Milk	Plasma	Milk
Buffaloes in early lactation	09.74 ^a \pm 0.17	197.98 ^c \pm 1.67	04.63 ^b \pm 0.19	99.93 ^a \pm 1.09
Buffaloes in mid lactation	09.57 ^a \pm 0.14	207.69 ^b \pm 1.62	05.32 ^a \pm 0.13	93.72 ^b \pm 1.57
Buffaloes in late lactation	09.45 ^a \pm 0.20	212.71 ^a \pm 1.31	04.80 ^b \pm 0.14	94.57 ^b \pm 1.64
Average	9.58 \pm 0.10	206.12 \pm 0.135	04.92 \pm 0.10	96.07 \pm 0.94

Note: In the last column, similar super scripts indicates Non Significant difference & dissimilar super scripts indicates significant difference at 1% level of significance.

reported significantly higher serum Ca in pregnant buffaloes in first half of pregnancy than in second half. Gradual decrease in the level of plasma Ca with the advancement of pregnancy in present study in buffaloes was similar to those recorded by Ahmed and Abdalla (2012) in both summer and winter season. Significant decrease in plasma Ca during late pregnancy in present study was in accordance with Hussain *et al.* (2001) in Buffaloes and Pal and Bhatta (2013) in cows. But it was not in accordance with Yokus and Cakir (2006) in cows. However, Deshpande (1983) in cows reported no significant effect of pregnancy on the level of blood Ca. Thus, the decline in plasma Ca level during pregnancy in present study may be due to competitive effect of foetal demand for Ca.

The placenta has a significant role in foetal Ca metabolism by transporting Ca ions from the dam to the foetus against the concentration gradient. It is also believed that increased level of alkaline phosphatase due to parathyroid stimulation during pregnancy, which is related to maternal mobilization of Ca from bones to foetus (Kahlon *et al.*, 1996). It might also be due to increased estrogenic concentration in late pregnancy, which decreases the appetite in animal leading to diminished Ca intake and its absorption Sahukar *et al.* (1984) and lowering effect of estrogen on blood Ca favouring its deposition in bones Manzoor *et al.* (1994). From the overall findings it can be concluded that the changes in the level of plasma Ca during various phases of reproduction are biophysiological adjustments to maintain normal homeostasis and homeorhesis. These biophysiological adjustments help the dam during pregnancy to ensure the integrity of various maternal functions in accordance with foetal demands Kahlon *et al.* (1996).

Statistical analysis of present data revealed that the level of plasma Ca during different periods of lactation did not differ significantly. However, there is a decreasing trend in the values of plasma Ca. The findings of gradual decrease in the level of plasma Ca with the advancement of lactation was in close agreement with Pasha *et al.* (2012) who also reported a significant decreasing trend in serum Ca in buffaloes in both winter and summer seasons. However, the findings are not in agreement with Piccione *et al.* (2012) in cows and Hagawane *et al.* (2009) in buffaloes.

Milk Calcium (Ca)

Statistical analysis of the present data revealed that the level of milk Ca (mg/dl) during different reproductive states in buffaloes differed significantly among the groups.

The reverse trends in the levels of plasma and milk Ca in both non-pregnant buffaloes (Groups II and III) and pregnant buffaloes (Groups IV to VI) in the present study supports the view that the Ca drain through milk reduces the level of plasma Ca which is reflected by higher concentration of milkCa. No reports are available in the literature to compare the milk values in different reproductive states, either in buffaloes or in cows.

Statistical analysis of present data reveals that the level of milk Ca (mg/dl) during different periods of lactation differed significantly ($P < 0.01$) and the values showed an increasing trend among the three group from early to late lactation. These findings were closely corroborated with the findings of Mathapati and Bhat (1988) in cows during three phases of lactation and with Anilkumar *et al.* (2003) in buffaloes from early to mid lactation in first five months. This is further supported by findings of Phukan *et al.* (2000b)

who also recorded in cow milk an increasing trend from early (from 15 to 90 days) to mid-late (105 to 300 days) lactation and Chauhan *et al.* (2008) who recorded apparent increasing trend in Jersey X Red Sindhi cows. However, present findings were not in agreement with the findings of Merkel *et al.* (1991) in buffaloes and cows and Patino *et al.* (2007) in buffaloes. Also, the value of milk Ca (mg/dl) in present study during early lactation was higher than those reported by Mahendra Singh and Anjuli Aggarwal (2001) and Ahmad *et al.* (2007) in buffaloes.

Gradual decrease with the advancement of lactation in the level of plasma Calcium although with no significant difference in buffaloes could be due to increasing loss of Calcium through milk with the progress of lactation and increased physiological demand of Ca by the udder. This is supported by levels of milk Ca during lactation in present study which indicates more and more loss of Ca through milk with the advancement of lactation. Ramberg *et al.* (1970) reported an outflow of Calcium into milk at the onset of lactation accompanied by a reduction in the pool size of Ca in the plasma. Secondly, Beigle (1999) reported that during the lactation period animals were storing cortical bone Ca up to 30 days, but for the rest of the lactation period cows were mobilizing Ca from the bone. Further, he opined that this mobilization of Ca must have been for milk production, as there was significantly ($P < 0.05$) less cortical bone Ca with the increased milk production.

Regarding decreased plasma concentration of Ca during early lactation Pickard (1978) opined that one of the major factors influencing the incidence of milk fever was the intake of Ca around the time of parturition. When the intake of Ca has been kept constant at a low level during

the dry period and increased just prior to calving, the fall in plasma Ca is minimized. Further, the low Ca level are not confined to the few days around parturition, but the symptoms identical to parturient hypocalcaemia can be seen in cows several weeks postpartum. It is often related to time when estrus is expected, because the estrogens favor the deposition of calcium in blood. In these events, involvement of calcitonin has been suggested. In milk fever, the high levels of nor-adrenalin in the plasma of cows are observed and this is known to stimulate the release of calcitonin. A sequence of events may be i) increase drain of Ca by the udder, a fall in plasma Ca, the cow feels unsteady, there is a release of nor-adrenalin which, causes calcitonin to be released and this further depresses plasma Ca with the initial impetus being the drain of Ca from the blood by the udder (Manzoor *et al.*, 1994).

The observation of milk Ca was extremely consistent with the above explanation, as the Ca content of the milk in present study; varied significantly during the three stages of lactation.

Plasma Phosphorus (P)

Statistical analysis of present data revealed that there was a significant difference ($P < 0.01$) in the values of plasma phosphorus among different reproductive states in buffaloes. Significantly lower value in Group III than II in the present study was in agreement with the findings of Chaurasia *et al.* (2010), Sharad Kumar *et al.* (2010) and Jayachandran *et al.* (2013) in buffaloes and Ramakrishna (1997) in cows. However, present findings were not in agreement with the findings of Shrivastava and Kharche (1986) who reported significantly higher value in anestrus than normal cyclic buffaloes. The involvement of phosphorus in phospholipids and cAMP synthesis may be a key factor to its effect on reproduction. A close

correlation between the reproductive hormones and inorganic phosphorus exists and marginal phosphorus deficiency could lead to anoestrus condition (Hurley, 1987). Further, Phosphorus is essential for transfer of biological energy, particularly through ATP, and deficiency of it may arrest the phenomenon of fertilization, and, this in turn, may cause early embryonic death resulting in the repeat breeder and anoestrus conditions of animals (Chaurasia *et al.*, 2010).

The level of plasma P during different periods of pregnancy differed significantly ($P < 0.01$). Further, there was a significant decreasing trend in the level of plasma P with the advancement of pregnancy in present study. This was in accordance with findings of Akhtar *et al.* (2012) in buffaloes with no significant difference and Sahukar *et al.* (1984) in cows who reported significantly increased serum P from first month of pregnancy to seventh month and then decreased in ninth month but with apparently decreasing trend. Apparently decreasing trend of the plasma P with advancement of pregnancy was also reported by Yokus and Cakir (2006) when values in mated and non mated cows were considered collectively and by Ahmed and Abdalla (2012) in cows during winter. However, increasing trend of plasma P during pregnancy was reported by Jacob *et al.* (2002) in cows. Significant decrease in plasma P during late pregnancy in present study was in accordance with reports of Hussain *et al.* (2001) in Buffaloes; Pal and Bhatta, (2013) in cows. But it was not in accordance with Contreras *et al.* (1990). Whereas, Siviah *et al.* (1986) and Deshpande (1983) reported no significant effect of gestation on the level of blood P in cows.

Thus the Significant decrease in the P level during the late pregnancy in present study might

be due to increased utilization of "P" at this stage and to enhance Carbohydrate metabolism of pregnancy (Sahukar *et al.*, 1984) or to meet requirement of the P for the secretion of colostrum (Rook and Thomas, 1983) and also for the requirement for fetal growth (Jacob *et al.*, 2002). It was also opined that increase in the rate of mobilization of Pi out of maternal circulation into fetus which could not be balanced by the increase in the rate of absorption of P from the gut or in the rate of resorption of Pi from the bone of the dam in pregnant sheep (Braithwaite, 1983).

From the present findings it can be concluded that the changes in the level of plasma phosphorus (along with plasma Calcium) during various phases of reproduction are indicative of biophysiological adjustments to maintain normal homeostasis and homeorhesis. Such biophysiological adjustments help the dam during pregnancy to ensure the integrity of various maternal functions in accordance with foetal demands (Kahlon *et al.*, 1996).

Statistical analysis of present data revealed that the level of plasma P during different periods of lactation differed significantly ($P < 0.01$). There was more or less apparent increase in the level of plasma P with the advancement of lactation, since it significantly increased from early to mid lactation and thereafter decreased significantly in late lactation but with the higher value in late lactation than in early lactation. Exactly similar trend but with no significant difference in the values of serum P during three stages of lactation in buffaloes was found in reports of Hagawane *et al.* (2009) and Paikrao (2001) in crossbred cows. However, partly closer observations with the present findings in buffaloes were seen with significant increase in the value of serum P from early to mid lactation in reports of Ali *et al.* (1983).

Piccione *et al.* (2012) in cows reported inconsistent trend in the values of serum P from postpartum period to second and fifteen weeks of lactation and end of lactation with apparent higher values in mid lactation. However, these findings are not in agreement with those of Pasha *et al.* (2012) who reported a non significant decreasing trend in serum P in buffaloes in both winter and summer seasons suggesting little or no effect of lactation on serum P level and also with Stodola *et al.* (1981) who reported decrease in the value of serum P from first to fourth month of lactation.

More or less apparent increase in the level of plasma P with the advancement of lactation in the present findings could be due to apparently decreasing loss of phosphorus through milk with the progress of lactation and decreased physiological demand of P by the udder. This is supported by concomitant change in levels of milk P during lactation in three lactating groups in present study.

Milk Phosphorus (P)

Statistical analysis of the present data revealed that the level of milk P (mg/dl) during different reproductive states in buffaloes differed significantly among the groups.

It was interesting to note that the level of milk P (mg/dl) in Group III was significantly higher than Group II and was exactly reverse to that of plasma P level between Groups III and II of the present research work. Further, the milk P levels during pregnancy differed significantly among the three groups of pregnant animals with a definite increasing trend which was concomitant with reverse trend recorded in plasma P. This plethora of change in plasma and milk P in pregnant buffaloes supports the view that the P drain

through milk reduces the level of plasma P and is reflected in higher concentration of milk P during the same periods. No reports are available in the literature to compare the milk values in different reproductive states, either in buffaloes or in cows.

Statistical analysis of present data revealed that the level of milk Phosphorus (mg/dl) during different periods of lactation differed significantly ($P < 0.01$). It was observed that the values showed apparently decreasing trend among the three groups Viz. early to mid to late lactation. These findings were corroborated with the findings of Merkel *et al.* (1991) in buffaloes and Chauhan *et al.* (2008) in Jersey X Red Sindhi cows with apparent decreasing trend. However, these were not corroborated with those of Anilkumar *et al.* (2003) in buffaloes who reported increase in the values from early to mid lactation when they observed the values month wise during lactation from first to fifth month, Patino *et al.* (2007) in buffaloes with apparently increasing trend, Merkel *et al.* (1991) in cows with no diff in early and Mid lactation, Chauhan *et al.* (2008) in Jersey cows and Phukan *et al.* (2000b) in Cow milk who recorded an increasing trend from early (from 15 to 90 days) to mid- late (105 to 300 days) lactation in the values of milk phosphorus.

Thus, the present findings of decreasing level of milk phosphorus confirms the earlier discussion regarding gradual increase in plasma phosphorus levels with the progress of lactation and is also very well supported by Forar *et al.* (1982) who reported that the inorganic phosphorus in plasma increased as milk yields decreased. Dietary phosphorus affected inorganic phosphorus in plasma but not in milk. They further opined that the inorganic phosphorus in milk and plasma was negatively correlated. First lactation

cows have higher plasma inorganic phosphorus than multifarious cows.

CONCLUSION

The macro minerals like Calcium and Phosphorus in blood and milk in different lactational and reproductive status of Buffalo were within normal physiological limits. Plasma P level was influenced by different lactational as well as reproductive status. However, plasma Ca was influenced by only different reproductive status. Milk Ca and P levels were influenced by different lactational as well as reproductive status. Levels of Ca and P were higher in milk than that of in plasma. Plasma Calcium and Phosphorus were significantly negatively correlated with that of milk in Buffalo. 🌐

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