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Mineral Dynamics of Blood and Milk in Dairy Buffaloes Fed on Calcium and Phosphorus Supplementation

I. Begum, A. Azim, S. Akhter[1], M. I. Anjum* and M. Afzal

Animal Nutrition Program, Animal Sciences Institute, National Agriculture Research Centre, Islamabad; [1]Pir Mehr Ali Shah University of Arid Agriculture, Rawalpindi, Pakistan
*Corresponding author: mianjum_narc@yahoo.com

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ABSTRACT

A study was conducted to determine the effect of supplementation of calcium (Ca) and phosphorus (P) on nutrient utilization, milk production, its composition and reproductive performance of Nili-Ravi buffaloes. Twelve lactating buffaloes were randomly divided into three groups, with 4 animals in each group, fed diets containing 80, 100 and 120% NRC recommended Ca and P, respectively. Daily feed consumption and milk yield (MY) were recorded. Fortnightly collected milk samples were analyzed for total solids (TS), milk protein (MP), milk fat (MF), lactose, Ca and P contents. Calcium and P concentrations were also determined in blood serum. Milk yield was 10%, MF 4% and TS 3.7% higher in buffaloes fed on 120% Ca and P than those fed on 100% Ca and P of NRC recommendation but dry matter intake was similar among all groups. However, MP, lactose and solids not fat were not affected by mineral supplementation. In milk, slightly increased concentrations of Ca and P were noted during the last two weeks of experiment in all groups. Dietary protein digestibility was higher in buffaloes fed 120% Ca and P, crude fibre and dry matter digestibility was higher in 80 and 100% Ca and P supplemented groups respectively however, difference was non-significant among the groups. Serum P concentration was higher but Ca was lower in 120% Ca and P supplemented group compared to other two groups however, these values were within the normal range. Buffaloes fed 120% Ca and P conceived 100% but in buffaloes fed 100 and 80% Ca and P, conception rates were 75 and 50% respectively. Number of services per conception was lower in buffaloes fed 120% Ca and P supplemented diet. It may be concluded that 120% Ca and P supplementation increased milk production and reproductive performance of lactating buffaloes.

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INTRODUCTION

Calcium (Ca) and phosphorus (P) are essentially required for dairy animals in larger amounts than other minerals. Over 70% of the total minerals in the body are Ca and P, while about 99% of the Ca and 80% of P found in the animal are in bones and teeth. Along with other important physiological functions, Ca and P are important constituents of milk and blood plasma, ranging from 9 to 10 and 4 to 6 mg/dl, respectively (NRC, 2001). Calcium and P deficiency causes reduced appetite and milk yield, a decline in reproductive efficiency, poor feed utilization, lowered disease resistance, increased incidence of milk fever, reduce growth rate, osteoporosis and osteomalacia (NRC, 2001).

Large ruminants, like buffaloes and cattle mainly derive their nutritional input from crop residues, which constitute more than 50% of the total diet (Hanjra et *al.*, 1995). These crop residues are particularly deficient in minerals and crude proteins (Given and Moss, 1995). Mineral contents of forages are influenced by a variety of factors such as soil moisture, its pH and texture. Phosphorus deficiencies in plants are due the lack of P in parent soil material (Stoddart *et al*., 1975).

The soil of Pothohar region is generally deficient in macro and micro-minerals, particularly 70-80% in P, 15% in potassium, 60% in zinc and 45% in boron (Rafique, 2004). Therefore, it seems logical that livestock, particularly dairy animals, kept in these regions require mineral supplements for optimum productive efficiency.

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Generally, mineral sources include natural feeds (forages, grains and by-products) and mineral supplements to balance the minerals contents in the feed.

It has been observed that dietary minerals supplementation enhanced milk production, milk composition (Al-Nor *et al*., 2004) and fertility (Saghar, 2003) in Nili-Ravi buffaloes. Supplementation of crop residues with concentrates, good quality fresh legumes and minerals can significantly improve feed intake and digestibility, resulting in improved production (Ibrahim, 1983; Manidol, 1985; Saadullah, 1985). Kincaid *et al.* (1981) observed lowered milk production from cows fed rations containing 0.3% phosphorus than from cows fed rations with 0.55% phosphorus. According to Rekhis *et al.* (2001), supplementation of di-calcium phosphate to dairy cattle had a significant effect on milk fat content and milk quantity and quality (protein and density) were higher in the supplemented group compared to the control group. Wu *et al.* (2000) studied milk production by dairy cows fed three levels of phosphorus (0.31, 0.40 and 0.49%) and found lowest milk yield in 0.31% P group**.** Parsaad *et al*. (1984) studied mineral supplementation and their influence on nutrients digestibility in buffalo calves and observed that digestibility of dry matter, crude protein and crude fiber were 67.84, 59.34 and 50.35%, respectively. Qureshi *et al.* (2000) reported that increasing Ca and P contents in rations for dairy buffaloes had favorable effects on reproductive performance. Although information regarding mineral supplementation in cattle diets is available (Kincaid *et al.*, 1981; Rekhis *et al*., 2001; Wu *et al*., 2000), such information on Ca and P supplementation in lactating buffaloes is limited. Hence, this study was planned to determine the effect of Ca and P supplementation on nutrient utilization, milk production, composition, and reproductive performance of Nili-Ravi buffaloes.

MATERIALS AND METHODS

Animals and experimental design

The present study was conducted at the Livestock Research Station, Animal Sciences Institute, National Agricultural Research Centre (NARC), Islamabad, Pakistan. Twelve buffaloes of Nili-Ravi breed (4 to 5 years old, average body weight of 550 ± 50 Kg) at first and second lactation and producing 7-8 liters/day were randomly divided into three equal groups, following completely randomized design. Animals of three groups were fed mixed rations containing Ca and P at 80, 100 and 120% of NRC recommendations, respectively. Experiment lasted from 2nd month of calving up-to the next conception.

Feeds and feeding

Nutritional requirements of experimental buffaloes for maintenance were fulfilled by offering 20-25 Kg green fodder (Mott grass, oats and Rye grass), while production requirements were met by providing 12 Kg/animal mixed ration (25% wheat straw + 75% concentrate). The concentrate consisted of cottonseed cake (20%), maize oil cake (10%), rapeseed cake (12%), rice polishing (10%), corn gluten feed (9%), maize grains (10.5%), wheat bran (20%), molasses (8%), and common salt (0.5%). This mixed ration contained 14.15% CP, 16-17% CF, 4.11% EE, 7.20% total ash, 0.18% Ca and 0.35% P, while NFE was 58.24% and TDN was 66.00%. Di-calcium phosphate and calcium oxide were used as Ca and P sources, respectively. Animals were placed in individual pens equipped with cemented manger and fed ration twice daily. Before morning feeding, weighed amount of Ca and P were mixed in ration and then fed to the animals. Feed and green fodder samples were analyzed for proximate analysis (AOAC, 1990**)** at Animal Nutrition Laboratories, NARC, Islamabad, Pakistan.

Milk analysis

Buffaloes were milked twice daily at 0500 and 1800 hrs and milk production was recorded daily. Milk samples (200 ml) collected from morning and afternoon milkings were pooled and frozen for subsequent analysis at fortnightly intervals. Samples were thawed immediately before analysis, mixed thoroughly using vortex mixer and analyzed for total solids, protein and total ash (AOAC, 1990). Fat contents of milk were determined by Gerber method. Briefly, 10 ml H_2SO_4 (85%), 11 ml milk and 1 ml isoamyle alcohol were taken in Gerber tube, mixed well and centrifuged at 1000 rpm for 10 minutes. Fat corrected milk (FCM) was measured as: 4% FCM = 0.4 × Kg milk + 15 × Kg fat (Mandal *et al*., 2003). Solids not fat (SNF) was calculated as total solids minus fat, while lactose was calculated by the following equation:

Lactose (%) = 100- (% moisture + % fat + % crude protein + % ash).

Atomic Absorption Spectrophotometer (M-Series Atomic Absorption Spectrophotometer, 150204) and Spectrophotometer (Spectronic-21UVD) were used to determine Ca and P contents in milk, respectively as per Gaines *et al.* (1989).

Blood analysis

Blood samples were collected after 3-4 hours post feeding at fortnightly basis from jugular vein into 10 ml tube, allowed to clot at room temperature for one hour and serum was separated by centrifugation at 3000 rpm for 15 minutes. Serum samples were frozen at -20^{o}C for subsequent Ca and P analysis by using Ca and P commercial kits (AMP Medizintechnik GmbH, BR7202, and BR3702, 650 nm and 340 nm, respectively).

Nutrient digestibility

During the last 15 days of the experiment, nutrients digestibility trial was conducted. During digestibility trial, 10% less feed was offered to minimize the refusal. Feed and orts samples were collected daily, composited by animal and were stored for chemical analysis. Faeces were weighed daily and aliquots (10% of total faeces) were composited by animal, dried first at 60^{o}C and then at 100^{o}C in air forced oven, grinded and analyzed for DM, CP, CF, ash, Ca and P according to AOAC (1990).

Reproductive performance

Experimental animals were closely monitored for onset of estrous by exposure to a teaser bull round the clock. Number of services per conception was recorded. Conception and pregnancy were confirmed by ultrasonography, using a real time B-mode ultrasound scanner equipped with a 7.0 MHz rectal probe (Aloka SSD-500, Aloka Co., Ltd., Japan).

Statistical analysis

Data collected on different parameters were subjected to statistical analysis by using analysis of variance technique under completely randomized design. Means of different parameters were tested by using least significant difference (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

Dry matter intake, milk yield and composition

The results of dry matter intake (DMI), milk yield, 4% FCM and milk composition of experimental buffaloes are given in Table 1. Buffaloes fed on 120, 100 and 80% of Ca and P consumed 18.59 ± 0.29, 18.12 ± 0.22 and 18.17 ± 0.31 Kg DM, respectively, the difference among the groups was non-significant (P>0.10). Highest average milk yield and 4% FCM were observed in buffaloes fed on 120% Ca and P, followed by buffaloes fed on 100 and 80% Ca and P groups. There was non-significant difference (P>0.10) between 80 and 100% Ca and P supplemented groups but both of these groups significantly differed from 120% Ca and P supplemented group. Higher milk protein was observed in 120%, followed by 100 and 80% Ca and P supplemented groups but the difference was non-significant (P>0.10) among the groups. Higher milk fat was observed in buffaloes fed on 120% Ca and P, followed by buffaloes fed on 100% and 80% Ca and P, the difference was significant (P<0.01) between 120 and 80% Ca and P supplemented groups. An increase in milk total solids and total ash was observed with increasing level of Ca and P supplementation. Buffaloes fed 120% Ca and P level produced milk with maximum total solids and 80% fed group had minimum total solids and the difference was significant, but these values did not differ from 100% Ca and P fed group. Buffaloes fed on 100% Ca and P had highest milk lactose content, followed by those fed on 80 and 120% Ca and P supplementation but the difference was statistically non-significant (P>0.10) among the groups.

Kincaid *et al*. (1981) also observed higher milk production in cows fed ration containing 0.55% P than cows fed 0.30% P. Rekhis *et al.* (2001) observed higher milk yield and fat content in di-calcium phosphate supplemented group compared to non-supplemented group. Wu *et al.* (2000) fed three levels of P (0.31, 0.40 and 0.49%) and noted lowest milk yield at 0.31% P and there was no effect on reproductive performance with different P levels. Valk and Sebek (1999) reported lower milk yield and reduced DMI when supplemented P at the rate of 0.24% in dairy cattle diet. Diets containing 0.37 or 0.57% P fed to lactating cows increased milk production and composition. Average milk production was 35.1 and 34.9 Kg/day and milk fat was 3.92 and 3.98% in dairy cows fed diets supplemented with 0.37 or 0.57% P (Lopez *et al.,* 2004). In the present study, lactose content did not vary significantly throughout the lactation because the close relationship between lactose synthesis and the amount of water drawn into milk makes lactose a stable milk component (Pollot, 2004). Al-Nor *et al*. (2004) reported that by minerals supplementation, milk composition was enhanced in lactating Nili-Ravi buffaloes. Calcium and P concentrations in milk after mineral supplementation were slight higher than before mineral supplementation but the differences were non-significant (P>0.10) among the groups (Table 1). There was minor increase in Ca and P concentrations in milk during last days of experiment.

Ca and P concentrations in serum

Results of blood serum Ca and P concentrations are given in Table 2. There was significant (P<0.10) difference between 80 and 120% groups for both Ca and P levels in blood serum. Serum Ca was higher in 80% Ca + P supplemented group, while serum P was higher in 120% Ca + P supplemented group. These results are in line with the findings of Wu *et al*. (2000). Previous workers reported that feeding higher level of P increased blood serum P (Brodison *et al.,* 1989; Call *et a*l., 1987; Forar *et al*., 1982). The lower blood serum Ca concentration was observed in 120% group in our study which might be due to higher utilization of Ca in milk. Lactation tends to lower the level of blood Ca as a result of the transfer of blood Ca to the milk. Moreover, the dietary Ca may reduce the Ca mobilization from bones for metabolism, resulting in lower Ca level in blood serum (Boda and Cole, 1980).

Nutrients digestibility

Average values of daily dry matter intake (DMI), crude protein (CP), crude fiber (CF) and their digestibility are shown in Table 3. Buffaloes fed 80, 100 and 120% Ca and P supplemented feeds had similar DM, CP and CF consumption among all groups. Digestibility of DM was highest in animals fed on 100% Ca and P, followed by 120% and 80% Ca and P but the differences were non-significant among the groups. A similar trend was noted for CF digestibility. Similarly, differences in the digestibility of CP among all groups were non-significant. Buffaloes fed on 120% Ca and P supplementation had highest CP digestibility, followed by 100% and 80% groups.

Reproductive performance

Estrous cycle commenced early and number of services per conception were less in buffaloes supplemented with 120% Ca + P as compared to animals fed 80% and 100% Ca and P (Table 2). Conception rates were 100, 75 and 50% in buffaloes fed 120, 100 and 80% Ca and P supplemented diets, respectively. Parsaad *et al.* (1984**)** noted that low level of Ca could affect fertility and ovarian-pituitary axis. Pugh *et al*. (1985) observed that the altered Ca:P ratio affected the reproductive performance of animals. Kumar *et al*. (1992) concluded that optimum level of Ca is required for maintenance of normal reproductive cycle and it has sensitizing action on reproductive organs through various hormones which further substantiate the findings of deficient Ca level in anestrous buffaloes**.** Saghar (2003) also observed that dietary minerals supplementation enhanced fertility rate.

Economics of milk production

Table 4 shows the economics of milk production in lactating buffaloes fed diets with different levels of Ca

Table 1: Effect of Ca and P supplementation on dry matter intake, milk yield and its composition in lactating buffaloes

Parameters	Levels of Ca and P as % of NRC requirements		
	80	**100**	**120**
Av. Dry matter intake(Kg/day)	18.17 ± 0.31	18.12 ± 0.22	18.59 ± 0.29
Milk yield (liter/day)	10.14 ± 0.21^{b}	10.75 ± 0.31^{b}	11.80 ± 0.31^{a}
4% FCM (liter/day)	14.50 ± 0.09^{b}	15.75 ± 0.13^{b}	17.78 ± 0.13^{a}
Milk composition (%)			
Milk protein	4.08 ± 0.23	4.24 ± 0.20	4.61 ± 0.16
Fat	6.87 ± 0.12^{b}	7.10 ± 0.21^{ab}	7.38 ± 0.19^{a}
Total solids	16.34 ± 0.32^{b}	16.59 ± 0.27^{ab}	17.21 ± 0.26^{a}
Solids not fat	9.48 ± 0.32	9.49 ± 0.24	9.89 ± 0.34
Total ash	0.67 ± 0.01^{b}	0.69 ± 0.02^{b}	0.75 ± 0.02^{a}
Lactose	4.57 ± 0.19	4.73 ± 0.33	4.47 ± 0.11
Mineral Concentration in milk (mg/dl)			
Ca (before supplementation)	198.37 ± 3.65	201.62 ± 1.34	208.70 ± 4.91
Ca (75 days after supplementation)	211.57 ± 2.10	209.01 ± 5.04	221.07 ± 2.14
P (before supplementation)	203.67 ± 9.89	191.50 ± 6.35	207.25 ± 12.42
P(75 days after supplementation)	207.60 ± 4.48	200.00 ± 13.65	211.75 ± 18.52

$^{a,\,b}$ values in the same row with different superscripts differ significantly ($P<0.10$).

± = Standard error of the mean; Av =Average; FCM= Fat Correct Milk; NRC= National Research Council

Table 2: Effect of Ca and P supplementation on blood serum Ca and P concentrations and reproductive performance in lactating buffaloes

Parameters	Levels of Ca and P as % of NRC requirements		
(mg/dL)	**80**	**100**	**120**
Calcium (mg/dL)	8.61 ± 0.18^{a}	8.34 ± 0.11^{ab}	8.24 ± 0.10^{b}
Phosphorus (mg/dL)	7.14 ± 0.08^{b}	7.29 ± 0.11^{ab}	7.48 ± 0.11^{a}
Reproductive performance			
Estrous activity (after start of experiment days)	55 ± 3.52	50 ± 4.77	43 ± 2.80
Percent of animals in estrous	100	100	100
Conception rate (%)	50	75	100

^{a,b} values in the same row with different superscripts differ significantly ($P<0.10$); ± = Standard error of the mean.

Table 3: Effect of Ca and P supplementation on nutrients digestibility in lactating buffaloes

Parameters	Levels of Ca and P as % of NRC requirements		
	80	**100**	**120**
Average daily intake (Kg)			
Dry matter	16.35 ± 0.03	16.31 ± 0.05	16.73 ± 0.07
Crude protein	2.04 ± 0.02	2.03 ± 0.01	2.09 ± 0.03
Crude fiber	2.64 ± 0.01	2.63 ± 0.02	2.70 ± 0.02
Nutrients digestibility (%)			
Dry matter	51.60 ± 3.13	54.02 ± 0.93	53.11 ± 0.63
Crude protein	51.75 ± 0.77	53.65 ± 3.12	55.72 ± 1.90
Crude fiber	40.43 ± 1.52	38.77 ± 0.93	37.37 ± 0.66

± = Standard error of the mean

Table 4: Economics of milk production of lactating buffaloes fed on Ca and P supplementation

Parameters	Levels of Ca and P as % of NRC requirements		
	80	**100**	**120**
Concentrate (DM) intake (Kg/day)	13.60	13.69	13.80
Fodder (DM) intake (Kg/day)	4.57	4.43	4.80
Cost of concentrate @ Rs.12.50/Kg	170	171.13	172.5
Cost of fodder @ Rs.2.50/Kg	11.43	11.08	12.00
Cost of Ca and P supplementation/day	3.50	4.50	5.00
Total cost Rs/day (concentrate+fodder+Ca and P)	184.93	186.71	189.50
Daily milk production (L)	10.14	10.75	11.80
Cost of milk @ Rs.36 /L	365.04	387.00	424.80
Net income (Rs./day/animal)	180.11	201.00	235.30
Economic efficiency (feed cost/Kg milk production)	18.23	17.37	16.06
Net profit/Kg milk (Rupees)	17.77	18.63	19.94

and P supplementation. Results revealed that economic efficiency (feed cost/Kg milk production) of 120% Ca and P supplemented group was 13.5 and 8.15% less than the buffaloes fed diets supplemented with 80 and 100% Ca and P levels, respectively.

Conclusions

Results of the present study revealed that supplementation of 120% Ca and P in diets of lactating buffaloes increased milk production, milk fat, milk total solids and reproductive performance as compared to 80% and 100% Ca and P supplemented groups. This indicates that minerals supplementation is important to reduce the economic losses due to minerals deficiencies and helpful in increasing the income of farmers.

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