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EFFECTS OF VARYING LEVELS OF CALCIUM IN RATIONS FOR BUFFALO (*BUBALUS BUBALIS*) CALVES ON GROWTH RATE AND NUTRIENT DIGESTIBILITY

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ABSTRACT

Twelve Nili-Ravi buffalo calves of age between 8-10 month and average body weight 112 ± 3.30 kg were randomly divided into three treatments (4 calves per treatment). Three isonitrogenous (16.14% CP) and isocaloric (66% TDN) dietary treatments: A) Calcium (Ca) 0.46% + Phosphorus (P) 0.31%; B) Ca 0.59% + P 0.31% and C) Ca 0.75% + P 0.31% on dry matter basis with Ca : P ratios of 1.5:1, 2:1 and 2.5:1, respectively. Levels of Ca were adjusted by addition of varying amount of limestone powder and di-calcium phosphate in total mixed rations (TMR). Animals were fed on 2 kg oats green fodder and TMR on *ad-libitum*. Feed intake for each animal was recorded daily. Growth was monitored by weighing calves fortnightly. Blood samples were taken pre and 90 days post mineral supplementation approximately 3 hours after morning feeding. At the end of experiment, one week digestibility trial was also conducted. Results revealed that dry matter intake (DMI) was not different among the treatments; whereas, total weight gain, average daily weight gain and FCR were higher ($P<0.05$) in the treatment C compared with the treatment A. The digestibility of DM was similar among all treatments whereas, the digestibility of CP and CF was higher ($P<0.05$) in the treatment C compared to the treatment A. Serum P concentration increased ($P<0.05$) with dietary P; whereas, the serum Ca concentration remained unchanged. It is therefore, concluded that increasing the dietary Ca concentration improved feed intake, weight gain, FCR as well as nutrients digestibility in growing buffalo calves.

Key words: Buffalo calves, Growth rate, Digestibility, Ca and P ratios, Serum Ca and P levels

INTRODUCTION

Animals have specific requirements for minerals that vary with type, age, body weight and production status of animals (Sharma *et al*., 2002). For better growth, skeletal development and energy utilization, animals need minerals supplementation (Rasby *et al*., 1998; Mandal *et al*., 2003). Minerals are essential for normal growth and reproduction of animals and serve as intermediate role in the action of hormones and enzymes at cellular level in an integrated fashion (NRC, 2001). Usually forages are good source of Ca and P; but their concentrations vary with plant species, plant part, and maturity. Cereal grains are low in Ca content and oilseed meals have moderate to high levels of P (Rasby *et al.*, 1998; Lardy and Lardy, 2005). Usually in traditional feeding, animals are facing problem of improper Ca to P ratios and imbalance of minerals in their diets. Therefore, Ca deficiency mostly occurs in animals that are fed on low roughages and high grain diets.

Phosphorus is known as the master mineral, involved in many metabolic (Rasby *et al*., 1998), neurological and cellular functions. Underwood (1981) reported subnormal and depressed growth rate in young animals who consumed Ca and P deficient diets. Poor growth rate due to P deficiency may be linked with poor feed efficiency and improper energy metabolism (Long *et al.*, 1957; Ammerman and Goodrich, 1983). Phosphorus supplementation of ruminants' diets increases the protein degradability, VFAs concentrations and bacterial population in the rumen (Zain *et al.*, 2010). Minerals availability and digestibility in the gut needs proper combination of some minerals for normal body functions. Mandal *et al.* (2003) suggested that Ca to P ratios as 1:1 to 2:1 for young calves is more suitable in terms of growth rate and feed efficiency. Ferguson and Sklan (2005) found that 0.33% to 0.40% of dietary P concentration in dairy animals is safe level with no negative effects on either reproduction or milk production.

Previous studies in Holstein calves of 3-4 month age (Wise *et al.*, 1963) and Hereford calves of 10-12 months age (Tillman *et al.*, 1959) revealed that optimal growth rates and skeletal development can be achieved when fed whole diets containing P 0.20% to 0.22% of DM. In an other study, Wise *et al.* (1963) fed three levels of dietary Ca (0.27, 0.81 and 2.43%) and three levels of P (0.17, 0.34 and 0.68%) with nine Ca to P ratios (0.4:1, 0.8:1, 1.2:1, 1.6:1, 2.4:1, 3.6:1, 4.8:1, 7.1:1 and 14.3:1) calculated as Ca levels/P levels of 9 different combinations to the cattle young calves. They reported that dietary ratios of Ca to P as 1:1 upto 7:1 all gave satisfactory and similar results but growth rate and feed efficiency were depressed significantly ($P<0.05$) on diets with below 1:1 and over 7:1 ratios. Therefore, either excess or deficiency of Ca and P can affect nutrients availability and utilization. Further studies by Rasby *et al.*, (1998) reported that feeding minerals more than animal requirements resulted in higher mineral excretion

in soil and ground water as well increased feeding cost. The present study, therefore, was been planned to determine the appropriate Ca: P ratio for optimum growth performance and nutrients utilization in growing buffalo calves when fed on high concentrate diets.

MATERIALS AND METHODS

Animals and experimental diets: Twelve growing male buffalo calves of Nili Ravi breed (8 to 10 months old, 112±3.30 kg average body weights) were taken from Livestock Research Station, National Agricultural Research Center (NARC), Islamabad and assigned to three equal treatments (4 calves per treatment). Before the start of experiment, animals were de-wormed, vaccinated (Aftovaxpur® FMD and NARC HS Vaccine) and given an adaptability period of 15 days. The calves were housed in individual tie stalls (pens measuring 4 ft × 9 ft) in well ventilated, concrete floor and asbestos sheet roof shed. Daily sweeping and cleaning of floors and bathing the calves with fresh ground water was practiced to provide hygienic environment. Clean water was offered 4-5 times per day in one bucket.

Three isonitrogenous (16.14% CP) and isocaloric (66% TDN) total mixed rations containing three levels of calcium (Ca) and one level of P on DM basis: A) Ca 0.46% + 0.31% P; B) Ca 0.59% + 0.31 P and C) Ca 0.75% + 0.31 P on dry matter with Ca to P ratios of 1.5:1, 2:1 and 2.5:1, respectively. Levels of Ca were adjusted by addition of varying amount of limestone powder and di-calcium phosphate in rations (Table 1). Total mixed rations were offered on *ad-libitum* whereas; calves were also offered 2 kg/ calf fresh oat fodder daily during the entire experimental period. In oats fodder, concentrations of Ca and P level was very low, therefore was not included in mineral calculations of diets.

Growth performance and digestibility: Growth performance trial was conducted for 4 months from December 2009 to April, 2010. Actual amount of feed offered and refusal by individual animal was recorded on a daily basis. Animals were weighed fortnightly using an electronic scale (Avery Berkel L122) after restriction of feed and water intake for 16 hours (Anjum *et al*., 2012). Feed conversion ratio (FCR) was calculated as kilograms of feed intake per kilogram of weight gain.

Digestibility trial of 7 days duration involving daily collection of faeces and recording of feed intake. Feed and fodder samples were collected fortnightly while fecal samples on daily basis, composited by animal, dried at 60°C in air forced oven, ground and analyzed for crude protein, crude fibre, Ca and P according to AOAC (1990) methods. The brief details of analytical procedures are given below:

Crude protein: A known amount of the oven dried sample (W_1) was taken in Kjeldahl's flask. Five grams of catalyst mixture containing $CuSO_4$ and K_2SO_4 (9:1) and 25 ml of concentrated H_2SO_4 were added. The sample was boiled in a digestion rack, initially at low temperature and then with vigorous boiling till the content became clear. After cooling, the contents of the flask were diluted with distilled water in a 250 ml volumetric flask. A 10 ml aliquot of this solution was transferred to the Kjeldahl distillation apparatus and distilled in the presence of 10 ml of 40 percent NaOH solution. The ammonia so liberated was collected in a flask containing 10 ml of 2 percent boric acid solution having 2 drops of methyl red as an indicator. The distillate was titrated against standard 0.1N H_2SO_4 to light pink end point. The nitrogen and crude protein in percentages were calculated according to the following formulae: Nitrogen (%) = [{(ml 0.1N H_2SO_4 × 0.0014× 250 ml)/ (W_1×10)} ×100] and Crude protein = {Nitrogen (%) × 6.25}.

Crude fiber: A known weight (W_1) of the oven dried sample was taken in a 500 ml beaker. The sample was boiled in 200 ml 1.25% H_2SO_4 solution for 30 minutes. The contents were filtered and the residue was washed with distilled water. The residue was then transferred to a beaker and was digested with 200 ml 1.25% NaOH solution for 30 minutes; the contents were filtered and washed. The residue was then transferred to tare crucible and dried to a constant weight. The weight of the dried residue was recorded (W_2). The residue was then ignited in muffle furnace at 550^0C till white/gray ash was obtained. The crucible was cooled in desiccators and weight of ash (W_3) was recorded. The loss in weight during ignition was considered as crude fibre and its percentage was calculated by the following formula: Crude fibre (%) = [{(W_2-W_3)/W_1} ×100]

Ca and P: Analysis of feed and green fodder was done for Ca and P according to AOAC (1990**)** procedure**.** Two grams, finely ground sample was taken in a clean and dry porcelain crucible, ashed in muffle furnace at 650 ^{0}C, the ash dissolved in 3% HCl, gently boiled, cooled, then added few drops of concentrated HNO_3, heated, cooled, diluted with 30ml distilled water, heated again, cooled, filtered in 100 ml volumetric flask and made the volume up to mark with distilled water. Now these digested samples were analyzed for Ca by using atomic absorption spectrophotometer (M-Series Atomic Absorption Spectrophotometer By Thermo Electron Corporation, 150204) and P by using spectrophotometer (Spectronic-21UVD By Burch and Lamb) respectively.

Serum Ca and P: Blood samples from each animal were collected at 0 day and at 90 day of experiment at 3-4 hours post feeding from jugular vein in a 10 ml tube. Blood samples were allowed to clot at room temperature for one hour and serum was separated. Serum samples were frozen at -20˚C for subsequent Ca and P analysis

using Ca and P commercial Kits (AMP Medizintecnik Gmb, BR 7202, and BR3702). Absorbance of Ca and P was read at Spectrophotometer (UV-double Beam) at 650 and 340 nm, respectively.

Statistical analysis: The data were statistically analyzed by using analysis of variance (ANOVA) according to completely randomized design as described by Steel *et al*., (1997) by using Minitab 15 software. Variables means for treatments showing significant differences in the ANOVA were further separated by Duncan's Multiple Range Test. Data are given as means plus or minus the standard error of the mean.

RESULTS AND DISCUSSION

Dietary Ca to P ratios of 1.5:1, 2:1 and 2.5:1 are designated as A, B and C, respectively. Results revealed no difference (P>0.05) in DMI for growing buffalo calves (Table 2). Our results are in line the work by Kincaid *et al.* (1981) and Jackson and Hemken (1994) who reporting that feed intake was not affected by increasing Ca contents in the diets of lactating Holstein cows and young calves. Similar observations were documented in lactating dairy buffaloes when fed on 80, 100 and 120% of NRC recommended Ca and P supplemented diets (Begum *et al*., 2010). In the current experiment the average daily weight gain of calves was higher (P<0.05) in the C treatment than calves fed on A feed. Similarly, FCR was better (P<0.05) in calves of treatment C compared to those fed ration A (Table 2). Mandal *et al.* (2003) suggested that Ca to P ratios as 1:1 to 2:1 for young calves is more suitable for better feed efficiency and growth rate. Perhaps above mentioned ratios are for cow calves and we have used buffalo calves of 8-10 months age in our study which need 2:1 or 2.5:1 Ca to P ratio. Many factors like age of animal, dietary contents, body calcium status, Ca to P ratios, etc. influenced Ca absorption (NRC, 2001), so when dietary intake is adequate, then Ca is deposited in bones of animals due to the effect of calcitonin. The variation in the above results may be related to breed differences. Body weight gain and FCR were improved significantly (P<0.05) in calves fed ration C with elevated Ca to P ratio 2.5:1 compared to 1.5:1 ratio. This may be due to an interaction of higher Ca (0.75%) with lower P (0.30%) contents in calves' fed diet C. From the perusal of the Table 3, it is evident that the intake of DM, CP, and CF was similar among the treatments. Similarly, there was no difference (P>0.05) for DM digestibility among the treatments. However, digestibility of CP and CF was significantly (P<0.05) higher in group C than group A. But CF digestibility of group B was statistically similar to that of groups A and C. Results show that calves fed ration C with Ca to P ratio of 2.5:1 to be more effective to increase CP and CF digestibility of feed compared to those fed ration A with Ca to P ratio of 1.5:1. Minerals availability and digestibility in the gut needs proper combination of some minerals (like Ca and P) for normal body functions. The possible explanation for improvement in CP and CF digestibility of ration C may be due to limestone addition which buffers the intestinal pH, resultantly improves nutrient digestibility (Kincaid *et al.*, 1981). Wheeler and Noller, (1976) reported improved feed efficiency in dairy

Table 1. Ingredients and nutrient composition of experimental rations fed to buffalo calves

Ingredients	Treatments			Oats fodder
	A	B	C	
Constant ingredients*	91.75	91.75	91.75	-
Sugar cane molasses	6.25	5.80	5.30	-
Di-calcium phosphate	1.60	1.60	1.60	-
Limestone powder	0.40	0.85	1.35	-
Total	100	100	100	-
Chemical composition (% DM)				
Moisture	11.12	11.11	11.07	74.17
Crude protein	16.21	16.08	16.12	8.15
Crude fibre	19.34	18.85	19.10	21.85
Total Digestible Nutrient	66.00	66.00	66.00	53.55
Calcium (Ca)	0.46	0.59	0.75	0.16
Total phosphorous (P)	0.31	0.30	0.30	0.21
Ca to P ratios**	1.5:1	2:1	2.5:1	-

*Constant ingredients consisted of maize oil cake 20%, rapeseed cake 11%, canola meal 10%, rice polish 5%, wheat bran 10%, maize gluten feed 5%, maize grains 5%, wheat straw 25%, urea 0.5% and common salt 0.25%. Oats green fodder 2 kg/animal/day was also offered.

**Ca : P ratio was calculated as reported by Wise *et al.* (1963) = (Ca level/P level).

Table 2. Effect of varying levels of dietary calcium on feed intake, live weight gain and feed conversion ratio in buffalo calves

Parameters	Treatments[1]		
	A	B	C
Average DM intake (kg/head/day)	3.20±[*]0.09	3.32±0.05	3.25±0.04
Average initial weight (kg)	108.25±5.36	110.50±5.19	109.50±5.93
Average final weight (kg/head)	173.05^{b}±8.39	182.50^{ab}±8.44	183.90^{a}±5.38
Total weight gain (kg/head)	64.80^{b}±4.41	72.00^{ab}±4.22	74.40^{a}±3.39
ADG (kg/day)	0.54^{b}±0.04	0.60^{ab}±0.04	0.62^{a}±0.02
Feed conversion ratio (FCR)[**]	5.93^{b}±0.34	5.53^{ab}±0.28	5.24^{a}±0.25

Values in the same row with different superscripts differ significantly (P<0.05)
[1]Three dietary treatments: A) contained Ca 0.46% + P 0.31%; B) Ca 0.59% + P 0.31% and C) Ca 0.75% + 0.31% on dry matter basis with Ca: P ratios of 1.5:1, 2:1 and 2.5:1, respectively.
[*]±SEM = standard error of the mean
[**]FCR was calculated as kilograms of feed intake per kilogram of live weight gain

Table 3. Effect of varying levels of dietary calcium on nutrient intake and their digestibility in buffalo calves

Parameters	Treatments[1]		
	A	B	C
Average daily intake (kg/day)			
Dry matter	3.76±[*]0.07	3.85±0.04	3.53±0.14
Crude protein	0.42±0.01	0.44±0.01	0.39±0.02
Crude fiber	0.76±0.01	0.99±0.04	0.73±0.02
Nutrients digestibility (%)			
Dry matter	58.68±1.07	60.18±1.48	61.26±1.65
Crude protein	54.81^{c}±1.10	58.08^{b}±0.70	62.11^{a}±1.40
Crude fiber	55.31^{b}±1.20	58.64^{ab}±1.45	59.97^{a}±1.44

Values in the same row with different superscripts differ significantly (P<0.05)
[1]Three dietary treatments: A) contained Ca 0.46% + P 0.31%; B) Ca 0.59% + P 0.31% and C) Ca 0.75% + 0.31% on dry matter basis with Ca: P ratios of 1.5:1, 2:1 and 2.5:1, respectively.
[*]±SEM = standard error of the mean

Table 4. Effect of varying levels of dietary calcium on blood serum Ca and P concentration in buffalo calves

Parameters	Treatments[1]		
	A	B	C
Calcium (mg/dL)			
Pre-minerals supplementation	9.43±[*]0.34	9.55±0.34	9.47±0.33
90 days post-minerals supplementation	9.31±0.47	9.51±0.43	9.44±0.51
Phosphorus (mg/dL)			
Pre-minerals supplementation	6.65^{B}±0.18	6.48^{B}±0.23	6.45^{B}±0.50
90 days post-minerals supplementation	7.77^{A}±0.22	7.85^{A}±0.05	8.15^{A}±0.40

Values with different superscripts in the same row (small letters) and in the same column (capital letters) for each parameter differ significantly (P<0.05).
[1]Three dietary treatments: A) contained Ca 0.46% + P 0.31%; B) Ca 0.59% + P 0.31% and C) Ca 0.75% + 0.31% on dry matter basis with Ca: P ratios of 1.5:1, 2:1 and 2.5:1, respectively.
[*]±SEM = standard error of the mean

cattle when limestone was added to complete rations. Other scientists (Ha *et al.,* 1983; Froetschel *et al.,* 1991; Kinal and Pres 1995) reported that addition of limestone to the diets of ruminants increased (P<0.05) digestibility of fiber.

The results of blood serum Ca and P concentrations at the 0 day and 90 days post mineral supplementation are presented in Table 4. Serum Ca and P values at the 0 day and 90 days post mineral supplementation were almost within the range reported by Fraser (1991) in "The Merck Veterinary Manual". Pre

and post dietary Ca and P supplementation did not influence serum concentration of Ca (P>0.05) whereas, elevation of dietary Ca and P increased (P<0.05) serum P concentration in all treatment groups. These results are in line with the previous workers (Brodison *et al.*, 1989; Call *et al*., 1987; Forar *et al*, 1982) who reported that feeding higher level of P increased blood serum P. Our results are supported by Kincaid *et al*. (1981) who reported that dietary Ca and P concentration did not affect Ca concentration in plasma because Ca concentration in plasma is controlled by homeostatic mechanisms in which Ca deposited in bone. Wise *et al*. (1963) observed that dietary Ca intake by beef calves did not influence serum concentration of Ca. Steevens *et al.* (1971) reported no effect of dietary Ca concentration on blood calcium whereas increasing dietary P concentration linearly increase blood P in dairy animals.

Conclusion: Based on the results of present study it can be concluded that ration containing 0.75% Ca and 0.31% P on dry matter basis with Ca: P ratio 2.5:1 is more suitable for better weight gain, FCR and nutrients digestibility in growing Nili-Ravi buffalo calves. However, additional work with greater numbers of calves would be needed to establish relationship between dietary Ca and P.

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