Replacement of dicalcium phosphate by rock phosphate in cattle diets: nutrients digestibility, plasma parameters, ruminal fermentation and microbial synthesis efficiency

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ABSTRACT - This study was conducted in order to evaluate the effects of replacing dicalcium phosphate by rock phosphate in diets of growing steers. It was determined the following aspects: apparent digestibility of diets; phosphorus (P), calcium and fluorine apparent absorption; ruminal pH; ruminal ammonia concentration; microbial efficiency and plasma P. Five rumen fistulated Holstein steers weighting 275 - 283 kg were used. Experimental design was a 5×5 Latin square and diets consisted of 0, 25, 50, 75 and 100% of dicalcium phosphate replacement by rock phosphate in the mineral supplement. Addition of rock phosphate in the diets produced a linear increase on intake, rumen outflow and fecal output and total disappearance of fluorine. Diets did not differ to apparent absorption of calcium as well as dry matter, organic matter, crude protein, neutral detergent fiber and non-fiber carbohydrates intake, excretion, digestion and partial and total apparent digestibility. Plasma phosphorus was not influenced by diets and the average was 5.93 mg/dL. There was no difference for ruminal pH and ruminal ammonia concentration. Dicalcium phosphate replacement did not affect apparent and true protein microbial synthesis. Total replacement of dicalcium phosphate by rock phosphate in growing steer mineral supplement did not affect ruminal environment and protein synthesis in rumen. Considering this, dicalcium phosphate replacement in growing steer diets decreases P absorption and depending on its requirements it should be carefully seen.

Key Words: phosphorus sources, plasma phosphorus, rumen microbial protein, ruminal fermentation

Substituição de fosfato bicálcico pelo fosfato de rocha em dieta para bovinos: digestibilidade dos nutrientes, parâmetros plasmáticos, fermentação ruminal e eficiência de síntese microbiana

RESUMO - Este estudo foi conduzido para avaliar os efeitos da substituição do fosfato bicálcico pelo fosfato de rocha na dieta de bovinos em crescimento. Foram determinados a digestibilidade aparente das dietas, a absorção aparente do fósforo, cálcio e flúor, o pH ruminal, a concentração de amônia ruminal, a eficiência microbiana e o fósforo no plasma utilizando-se cinco bovinos da raça Holandesa Preto-e-Branco, fistulados, pesando entre 275 e 283 kg. O delineamento estatístico foi um quadrado latino 5×5 e as dietas consistiram de 0, 25, 50, 75 e 100% de substituição do fosfato bicálcico pelo fosfato de rocha no suplemento mineral. A adição de fosfato de rocha nas dietas ocasionou aumento linear na ingestão, no fluxo omasal, no fluxo fecal e no desaparecimento total do flúor. As dietas não diferiram quanto à absorção aparente do cálcio, assim como em relação à ingestão, excreção, digestão e digestibilidades aparentes parcial e total da matéria seca, matéria orgânica, proteína bruta, fibra em detergente neutro e carboidratos não-fibrosos. O fósforo no plasma não foi influenciado pelos tratamentos e a média foi de 5,93 mg/dL. Não houve diferença para o pH ruminal e concentração de amônia ruminal. A substituição do fosfato bicálcico não afetou a síntese microbiana aparente e verdadeira de proteína. A total substituição do fosfato bicálcico pelo fosfato de rocha em suplementos minerais em bovinos em crescimento não afetou o ambiente ruminal e a síntese de proteína no rúmen. Assim, a substituição do fosfato bicálcico em dietas para bovinos em crescimento diminui a absorção de fósforo e deveria ser vista com cuidado dependendo dos requerimentos.

Palavras-chave: fermentação ruminal, fontes de fósforo, fósforo plasmático, proteína microbiana

Introduction

Ruminants and their ruminal microorganisms should receive the correct amount of energy and nutrients. Among these nutrients are the minerals which need to be offered according to animals requirements in right amounts and proportions using available sources.

Daily phosphorus supplementation is necessary to match requirements of ruminants even in the maintenance state. Actually, phosphorus is also considered an important source of environment contamination. Concerning about environment problems, animal nutritionists are trying to feed animals with the right amount of phosphorus, avoiding as much as possible fecal losses. In that case, it is important to use high quality phosphorus sources that offer a good combination of high total phosphorus content and high phosphorus apparent absorption coefficient.

Currently, some countries, farmers and industries are trying to replace low fluorine phosphorus sources as dicalcium phosphate by other sources as rock phosphate which has a high amount of fluorine. About 90% of ingested fluorine is stored in teeth and bones as calcium fluoride and fluorine apatite forms. According to Suttie (1980) high fluorine ingestion by cattle causes generalized hyperostosis and in some cases it is possible to observe bone lesions.

Considering the importance of phosphorus in ruminant nutrition and its availability in different feeds, this study was conducted to evaluate the effect of dicalcium phosphate replacement by rock phosphate over partial and total nutrient apparent digestibility, partial and total phosphorus, calcium and fluorine absorption, ruminal pH, ruminal ammonia concentration, microbial efficiency synthesis and plasma phosphorus in cattle.

Material and Methods

Five Holstein steers with 275 to 283 kg of body weight on average, implanted with ruminal cannula were used. Animals were individually housed in pens provided with feeders and automatic waterers. During experimental period they received meals twice a day (8:30 am and 4:30 pm). They were also weighted at the beginning of each experimental period to adjust dry matter intake that was set at 2.5% of body weight.

Chromic oxide was used as the external marker to calculate rumen outflow and fecal output. Marker was added to rumen, during the morning meal, using a daily amount of 10.0 g from the seventh day in each period.

Experimental period consisted of 21 days with 17 days of diet adaptation followed by four days of sampling (orts, rumen outflow, feces and blood).

The diets were as it follows: 100D = 100% dicalcium phosphate and 0% rock phosphate; 75D/25R = 75% dicalcium phosphate and 25% rock phosphate; 50D/50R= 50% dicalcium phosphate and 50% rock phosphate and 25D/75R= 25% dicalcium phosphate and 75% rock phosphate; 100R= 0% dicalcium phosphate and 100% rock phosphate. Diets also had different phosphorus/fluorine ratio which were the following: 100D= 441.58; 75D/25R= 119.20; 50D/50R= 68.90; 25D/75R= 48.45 and 100R= 37.36. Diets consisted of 2.4 g/kg of phosphorus. Feeds supplied 1.8 g/kg of total phosphorus, and then additional phosphorus (0.6 g/d) was supplied as dicalcium phosphate (DP; Foscálcio[®], Serrana Nutrição Animal – Bunge, São Paulo/BRA) and/or rock phosphate (RP) straight into the rumen trough ruminal cannula.

Rumen outflow (500 mL) and feces samples (50 g) were collected to determine partial and total apparent digestibility

Table 1 - Chemical composition of experimental ingredients and diets (%DM)

Ingredient (g/kg)	Chemical composition of ingredients					Proportion of diets				
	TDN	Crude protein	Calcium	Phosphorus	Fluorine	100D	75D/25R	50D/50R	25D/75R	100R
Corn silage	680	77	3.1	2.3	0	498.0	497.6	497.5	497.2	496.9
Soybean hulls	800	125	5.3	1.8	0	341.6	341.4	341.3	341.1	340.9
Cottonseed hulls	450	50	1.5	0.9	0	139.5	139.4	139.3	139.2	139.1
Urea	0	2,820	0	0	0	11.5	11.6	11.4	11.4	11.4
Dicalcium phosphate[2]	0	0	210	190	1.9	3.2	2.4	1.6	0.8	0
Rock phosphate[2]	0	0	210	110	13	0	1.4	2.7	4.1	5.5
Mineral supplement[1]	0	0	184	0	0	6.2	6.2	6.2	6.2	6.2
Chemical composition of diets										
Total digestible nutrients						674.8	674.8	674.8	674.8	674.8
Crude protein						120.3	120.3	120.3	120.3	120.3
Calcium						5.0	5.1	5.2	5.4	5.5
Phosphorus						2.6	2.6	2.6	2.6	2.6

[1] The composition of mineral supplement was: S = 12g/kg; Mg = 12 g/kg; Na = 100 g/kg; Ca = 120 g/kg; Co = 30 ppm; Cu = 800 ppm; I = 50 ppm; Mn = 1.200 ppm; Se = 12 ppm; Zn = 3.200 ppm.
[2] Supplied through ruminal cannulas during all experimental period.
D = dicalcium phosphate; R = rock phosphate.

of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), ether extract (EE), non-fiber carbohydrate (NFC) and phosphorus, calcium and fluorine apparent absorption. Rumen outflow samples were taken as described by Huhtanen et al. (1997). Samples were stored in plastic bags, labeled and frozen (-20ºC) until processing and analysis were carried out. During four days of each sampling period, a total of four rumen outflow and feces samples by animal/treatment were collected and composed. Samples were collected as the following: day 1 (8 a.m.), day 2 (12 p.m.), day 3 (4 p.m.) and day 4 (8 p.m.). Samples were dried in an oven at 55ºC for 96 hours, and ground through a 1 mm screen, using a hammer mill. Then, they were mixed, using the same amount and considering the dry weight, to make up animal/treatment/period pooled samples.

Orts were daily collected, weighted, sampled, and frozen until analysis. Concentrate and silage samples were weekly collected and pooled to the entire experimental period. Orts and silage samples were submitted to the same procedures as rumen outflow and feces samples were.

On day 18 of each experimental period, blood samples were collected 3 hours after feeding in heparinized vials. Then, plasma extraction was done in refrigerated centrifuges (4ºC) at 2500 x g for 15 minutes.

Ruminal fluid (100 mL) was collect on day 21 at 0, 2, 4, 6 and 8 hours after morning feeding in each experimental period. The 0 corresponded to the sample collected immediately before morning feeding while 8 was immediately before afternoon feeding. The pH was measured immediately after sampling, and then 1 mL of 1:1 sulfuric acid (v,v) was added to only 50 mL of ruminal fluid sample. After that, samples were stored (-20ºC) until performing of ammonia analysis.

Also, on day 21, a total of 1.5 kg of ruminal content was collected and mixed with 500 mL of saline solution (0.9%). The mixture was homogenized in a blender, strained through four layers of cheesecloth and then frozen (-20ºC).

Feeds, orts, rumen outflow and feces samples were analyzed for dry matter, crude protein, ether extract, calcium (AOAC, 1990), neutral detergent fiber (Van Soest et al., 1991), phosphorus (Fiske & Subbarow, 1925) and fluorine (Singer & Armstrong, 1968). Plasma was analyzed for inorganic phosphorus (Little et al., 1971).

The chromic concentration in rumen outflow and feces samples was determined using an atomic absorption spectrophotometer (Shimadzu®) according to the technique described by William & David (1962). These data were used together with nutrient concentration to calculate rumen outflow and fecal output of nutrients.

Ammonia concentration in ruminal fluid was measured by Ferner's method (1965). Ruminal content samples were analyzed as described by Cecava et al. (1990). Purine concentration in ruminal bacteria and in rumen outflow was determined by using the procedure described by Ushida et al. (1985), with modifications proposed by Bohnert et al. (1998).

Non-fiber carbohydrates were calculated using the following equation (Sniffen et al., 1992): NFC = 100 – (%NDF$_n$ + %CP + %EE + %ASH), where: NDF$_n$ = neutral detergent acid corrected for nitrogen. To obtain total digestible nutrients calculated (TDN$_c$) of diets, it was used the equation of Sniffen et al. (1992): TDN = DP + DNDF + (DEE x 2.25) + DNFC, where: DP = digestible protein; DNDF = digestible neutral detergent fiber; DEE = digestible ether extract; DNFC = digestible non-fiber carbohydrates. An estimative of total digestible nutrients (TDN) values were also done using the equations proposed by Weiss et al. (1999).

The ruminal and intestinal apparent nutrient digestibility coefficient were calculated using marker intake and fecal marker concentrations.

Total N microbial flow to duodenum (g/day) was estimated by dividing ruminal microbial N: microbial purine ration by total rumen outflow N: rumen outflow purine ratio then multiplying this by N individual total flow. True microbial efficiency synthesis was expressed in g of microbial N/kg of organic matter (OM) truly fermented in the rumen. The corrected organic matter digested in rumen (COMDR) was calculated using the equation:

COMDR= OM ingested (g/d) – [rumen outflow OM – microbial OM (g/d)]

Data were analyzed as a 5×5 Latin square using the GLM procedure of SAS (1998), and differences were considered significant at P<0.05. The pH and ammonia data were analyzed using the proc mixed statement in the same program.

The model used to the variance analysis was: $Y_{ijk} = \mu + A_i + P_j + T_k + e_{ijk}$ where: μ = overall means; A_i = animal effect; P_j = period effect; T_k = diet effect; e_{ijk} = error effect.

Results and Discussion

Different proportion among dicalcium phosphate and rock phosphate evaluated in this experiment did not affect (P>0.05) phosphorus intake, rumen outflow and ruminal apparent absorption as well as plasma phosphorus. Replacement of dicalcium phosphate by rock phosphate linearly increased (P<0.05) phosphorus fecal output while intestinal and total apparent absorption decreased linearly (P<0.05).

Phosphorus requirements suggested for growing steers are 14 g/day (NRC, 1984), 11 g/day (AFRC, 1991) and also 12 g/day (NRC, 1996). It is interesting to notice that AFRC (1991) value was calculated by using concentrated based diets, so, it increases to about 17 g/day to diets that are made up with more than 50% of forage. Considering this, phosphorus intake in this experiment was appropriated (13.7 g), with basal diet supplying 89% of total phosphorus.

Phosphorus fecal output observed in this experiment was similar to that observed by Vitti et al. (2000) in which steers were fed with dicalcium phosphate and two rock phosphate (Patos de Minas and Tapira) and reported high phosphorus fecal output values to diets using rock phosphate. Peeler (1972) stated that phosphorus lost is unavoidable and dependent on animal physiologic status, age, gender, besides phosphorus source bioavailability.

Phosphorus fecal output was 450 and 710 g/kg of ingested phosphorus for 100D and 100R diets, respectively. Phosphorus flow through gastrointestinal tract was composed not only by diet phosphorus but also by endogenous phosphorus, so, negative values (Table 2) observed for phosphorus ruminal apparent absorption coefficient indicate a significant salivary phosphorus secretion. Ruminants are able to recycle endogenous phosphorus efficiently through saliva, secreting concentrations from 496 to 1,240 mg/L, therefore increasing its ruminal concentration (Rosol & Carpem, 1997).

Phosphorus total apparent absorption value (0.543) observed for 100D diet agrees with Lofgreen (1960), Arrington et al. (1963) and Barreto (2009), that reported values from 0.50 to 0.70. On the other hand, considering the use of rock phosphate, Vitti et al. (1991), fed sheep and found phosphorus total absorption values of 0.310 and 0.367 for Patos de Minas and Tapira rock phosphate, respectively, which are higher than the one found in this experiment (0.289) for 100R treatment.

Diets had similar rumen apparent digestibility of phosphorus (-0.23), but phosphorus apparent absorption showed linear decrease when rock phosphate was included in the supplement (Table 2) as a result of intestinal phosphorus absorption decrease. This result could be attributed to the chemical structure of dicalcium phosphate and tricalcium phosphate (rock phosphate) that influence abomasal phosphorus solubility and absorption in the small intestine.

There were no diet effects (P<0.05) on evaluated calcium parameters (Table 2), and it was not affected

Table 2 - Intake, flow and apparent absorption of phosphorus, calcium and fluorine and phosphorus plasma concentration

	Diets[1]					Regression	R^2	SE
	100D	75D/25R	50D/50R	25D/75R	100R			
Phosphorus								
Intake (g/day)	13.6	13.7	13.6	13.9	13.9	Y = 13.73	NS[2]	0.18
Omasal flow (g/day)	16.8	17.1	16.6	17.2	17.0	Y = 16.94	NS	0.19
Fecal flow (g/day)	6.2	7.1	7.2	8.5	9.9	Y = 5.15 + 0.86 X	0.57	0.23
Ruminal apparent absorption	-0.23	-0.25	-0.22	-0.24	-0.23	Y = - 0.23	NS	3.45
Intestinal apparent absorption	0.63	0.58	0.57	0.51	0.42	Y = 0.68– 0.05 X	0.57	2.91
Total apparent absorption coefficient	0.54	0.48	0.47	0.39	0.29	Y = 0.61 – 0.06 X	0.43	3.62
Calcium								
Intake (g/day)	36.5	36.3	36.4	37.3	37.5	Y = 36.80	NS	0.58
Omasal flow (g/day)	28.8	28.6	28.9	29.9	30.3	Y = 29.30	NS	0.45
Fecal flow (g/day)	16.3	16.5	16.9	17.3	17.7	Y = 16.94	NS	0.44
Ruminal apparent absorption	0.21	0.21	0.21	0.20	0.19	Y = 0.20	NS	1.45
Intestinal apparent absorption	0.43	0.42	0.42	0.42	0.42	Y = 0.42	NS	1.66
Total apparent absorption	0.54	0.55	0.54	0.54	0.53	Y = 0.54	NS	1.15
Fluorine								
Intake (g/day)	30.1	140.1	220.3	370.6	480.2	Y = 32. 30+ 4.13 X	0.70	3.20
Omasal flow (g/day)	20.2	100.1	160.5	330.4	440.4	Y = 25.06 + 3.89 X	0.57	3.20
Fecal flow (g/day)	10.4	70.5	120.1	250.5	350.7	Y = 8.40 +2.87 X	0.44	2.70
Ruminal apparent absorption	0.33	0.29	0.27	0.11	0.08	Y = 0.32 – 0.002 X	0.54	3.62
Intestinal apparent absorption	0.49	0.30	0.25	0.24	0.20	Y = 0.39 – 0.002 X	0.45	3.83
Total apparent absorption	0.65	0.50	0.46	0.32	0.27	Y = 0.60 – 0.003 X	0.76	3.97
Phosphorus plasma concentration								
P (mg/dL)	5.896	5.958	5.956	5.788	6.038	Y = 5.927	NS	0.067

[1] 100D = 100% dicalcium phosphate and 0% rock phosphate; 75D/25R = 75% dicalcium phosphate and 25% rock phosphate; 50D/50R = 50% dicalcium phosphate and 50% rock phosphate, 25D/75R= 25% dicalcium phosphate and 75% rock phosphate; 100R= 0% dicalcium phosphate and 100% rock phosphate.
[2] NS: No significance (P>0.05).

(P>0.05) by phosphorus source in supplement. Calcium absorption is dependent on the availability of calcium from the feedstuffs and inorganic calcium sources in the diet, and the efficiency of intestinal calcium absorption in the animal being fed. The amount of calcium absorbed from the diet will generally equal the requirement of the body for calcium if the diet contains enough available calcium (NRC, 2001). According to Challa et al. (1989), this element is strongly influenced by level of phosphorus in the diet. Since diet phosphorus concentration was the same for all diets, absence of this treatment effect in calcium parameters is reasonable.

Inclusion of rock phosphate in supplement increased linearly (P<0.05) intake, rumen outflow and fecal excretion of fluorine. Ruminal fluorine disappearance was quadratic (P<0.05) and the highest calculated disappearance was observed for 67% of dicalcium phosphate replacement. Increasing rock phosphate in supplement lead to a linear decrease on ruminal and intestinal fluorine, and total apparent absorption (P<0.05).

Fluorine ingestion increase with the inclusion of rock phosphates in the supplement and occured as result of its higher fluorine concentration. Cattle are less tolerant to fluorine toxicity than other livestock animals (Phillips & Suttie, 1960). In long-term, experiments with beef cattle above 30 mg of dietary fluorine per g of dry matter diet caused excessive wear and staining of teeth (Hobbs & Merriman, 1959). In another study, beginning with young calves and lasting for 7 years, the tolerance for soluble florine was also 30 mg/g of the dry matter diet (Shupe et al., 1963).

Suttie et al. (1957) stated that fluorine ingestion limits were 40 mg/kg of DM/day and fluorosis signs started in 3 to 5 years when 50 mg of F/kg of dry matter were daily ingested. In this experiment fluorine daily ingestion (F/kg of DM) was: 5.24 mg; 24.54 mg; 38.49 mg; 64.65 mg and 83.92 mg to 100D, 75D/25R, 50D/50R, 25D/75R and 100R, respectively.

Fluorine ingestion for 25D/75R and 100R diets was 132.4 and 171.5 µg of F/g of LW, respectively. According to NRC (1974), fluorine ingestions higher than 100 µg of F/g of body weight was harmful to most animals. Linear increase of rumen outflow and fecal excretion of fluorine (P<0.05) could be partly as a consequence of a higher fluorine ingestion from rock phosphate.

Phosphorus plasma levels observed in this experiment were within the limits considered normal (4 to 8 mg/dL) by NRC (2001). In growing cattle, phosphorus plasma concentration range from 6 to 8 mg/dL (NRC, 2001).

Correlations between ingested and plasmatic phosphorus were also evaluated. While Braithwaite (1985) and Ternouth & Sevilla (1990) reported a correlation between ingested and plasmatic phosphorus; other authors state that for sheep and cattle this correlation does not exist or it is extremely low (Braithwaite, 1984; Challa & Braithwaite, 1988). In this experiment it was also observed a low correlation between ingested and plasmatic phosphorus (0.30, $R^2 = 0.65$) and between absorbed and plasmatic phosphorus (0.20, $R^2 = 0.63$).

Dry matter, crude protein, neutral detergent fiber, ether extract and non-fiber carbohydrate intake, rumen outflow and fecal output as well as ruminal, intestinal and total apparent digestibility coefficients were not affected (P>0.05) when rock phosphate replaced dicalcium phosphate (Table 3). Corrected total digestible nutrients (TDN_c) values (Table 3) was not influenced (P>0.05) by dicalcium phosphate replacement.

Ruminal dry matter apparent digestibility ranged from 0.55 to 0.57, and total dry matter apparent digestibility ranged from 0.69 to 0.73. Digestion of crude protein was not affected by replacement of dicalcium phosphate by rock phosphate.

Bacterial composition (dry matter, organic matter and nitrogen organic matter) was not affected (P>0.05) by diets (Table 4). Bacterial OM average value (863.8 g/kg) was lower than Clark et al. (1992) who reported high variation of bacterial organic matter values. The authors state that, a saline solution contamination during isolation bacterial procedure could be responsible for this variation.

Bacterial organic matter and nitrogen average values were lower than values found at Cornell Net Carbohydrate and Protein System (CNCPS), which recommends values of 95.6 and 10% respectively (Russell et al., 1992).

Replacing dicalcium phosphate by rock phosphate in supplement did not influence (P>0.05) rumen outflow of bacterial and nonbacterial nitrogen (Table 5).

There was no effect (P>0.05) of phosphorus source on true and apparent microbial synthesis efficiency. Apparent microbial synthesis efficiency, expressed as grams of bacterial nitrogen per kilogram of organic matter digested in rumen (g of N bac./kg of OMDR), ranged from 37.21 to 40.22. True microbial synthesis efficiency, also expressed as g of N bac./kg of COMDR, ranged from 17.30 to 18.78 (Table 4).

MacDowell (1992) also stated that when low digestibility sources, as rock phosphate, are used in cattle diets they reduce phosphorus concentration in gastrointestinal tract, affecting negatively the microbial protein synthesis.

Table 3 - Intake, omasal flow, feces output, ruminal apparent digestibility coefficient, intestinal apparent digestibility coefficient, total apparent digestibility coefficient and TDN in cattle receiving diets with inclusion of rock phosphate in replacement of dicalcium phosphate

	Diet[1]					Regression	R^2	SE
	100D	75D/25R	50D/50R	25D/75R	100R			
Dry matter								
Intake (g/day)	5746.6	5709.2	5724.2	5732.3	5722.0	Y = 5726.86	NS	13.50
Omasal flow (g/day)	2597.1	2462.6	2461.9	2429.3	2444.2	Y = 2479.02	NS	4.23
Feces output (g/day)	1770.8	1625.3	1546.1	1559.3	1557.4	Y = 1611.78	NS	4.91
Ruminal apparent digestibility coefficient	0.55	0.57	0.57	0.58	0.57	Y = 0.57	NS	1.28
Intestinal apparent digestibility coefficient	0.32	0.34	0.37	0.36	0.36	Y = 0.35	NS	0.56
Total apparent digestibility coefficient	0.69	0.72	0.73	0.73	0.73	Y = 0.72	NS	0.86
Crude protein								
Intake (g/day)	716.0	711.9	714.0	714.7	713.5	Y = 714.02	NS	5.49
Omasal flow (g/day)	619.2	619.3	625.8	632.0	621.3	Y = 623.52	NS	8.80
Feces output (g/day)	240.3	230.5	234.0	230.0	240.1	Y = 234.98	NS	2.51
Ruminal apparent digestibility coefficient	0.14	0.13	0.12	0.12	0.13	Y =0. 13	NS	2.11
Intestinal apparent digestibility coefficient	0.61	0.63	0.63	0.64	0.61	Y =0. 62	NS	1.36
Total apparent digestibility coefficient	0.66	0.68	0.67	0.68	0.66	Y = 0.67	NS	0.71
Neutral detergent fiber								
Intake (g/day)	3423.1	3613.3	3614.0	3622.6	3615.4	Y = 3577.68	NS	3.85
Omasal flow (g/day)	1331.2	1385.8	1325.7	1322.3	1378.0	Y = 1348.60	NS	4.68
Feces output (g/day)	1016.1	1075.3	977.8	980.6	1006.4	Y = 1011.24	NS	16.14
Ruminal apparent digestibility coefficient	0.61	0.62	0.63	0.64	0.62	Y =0. 62	NS	2.41
Intestinal apparent digestibility coefficient	0.24	0.22	0.26	0.26	0.27	Y = 0.25	NS	2.58
Total apparent digestibility coefficient	0.70	0.70	0.73	0.73	0.72	Y = 0.72	NS	1.01
Ether extract								
Intake (g/day)	84.0	83.5	83.8	83.1	83.6	Y = 83.60	NS	0.69
Omasal flow (g/day)	31.0	30.0	33.4	31.5	33.5	Y = 31.88	NS	0.82
Feces output (g/day)	8.3	8.2	10.0	10.8	10.1	Y = 9.48	NS	0.77
Ruminal apparent digestibility coefficient	0.63	0.64	0.60	0.62	0.60	Y =0. 62	NS	1.94
Intestinal apparent digestibility coefficient	0.73	0.73	0.70	0.66	0.70	Y = 0.70	NS	4.91
Total apparent digestibility coefficient	0.90	0.90	0.88	0.87	0.88	Y = 0.89	NS	1.77
Non fiber carbohydrates								
Intake (g/day)	1225.8	1002.3	1015.4	1011.0	1009.2	Y = 1052.74	NS	10.40
Omasal flow (g/day)	390.9	206.0	237.2	217.2	194.9	Y = 249.24	NS	18.68
Feces output (g/day)	316.4	122.0	133.9	145.8	106.2	Y = 164.86	NS	80.07
Ruminal apparent digestibility coefficient	0.68	0.79	0.77	0.79	0.81	Y =0. 77	NS	3.23
Intestinal apparent digestibility coefficient	0.19	0.41	0.44	0.33	0.46	Y = 0.36	NS	8.79
Total apparent digestibility coefficient	0.74	0.88	0.87	0.86	0.90	Y = 0.85	NS	1.46
Total digestible nutrients (g/kg)	726	753	760	759	759	Y = 751.4	NS	1.40

[1] 100D= 100% dicalcium phosphate and 0% rock phosphate; 75D/25R= 75% dicalcium phosphate and 25% rock phosphate; 50D/50R= 50% dicalcium phosphate and 50% rock phosphate, 25D/75R= 25% dicalcium phosphate and 75% rock phosphate; 100R= 0% dicalcium phosphate and 100% rock phosphate.
NS = No significance (P>0.05). SE = Standard error.

Table 4 - Dry matter, organic matter and N of mixed ruminal bacteria in cattle receiving diets with inclusion of rock phosphate in replacement of dicalcium phosphate

Item (g/kg)	Diet[1]					Regression	R^2	SE
	100D	75D/25R	50D/50R	25D/75R	100R			
Dry matter	904.1	919.8	897.0	923.6	928.1	Y = 914.5	NS	0.5
Organic Matter	849.6	872.2	851.0	872.7	873.4	Y = 863.8	NS	2.3
Nitrogen	64.5	64.9	60.5	65.2	64.1	Y = 63.9	NS	0.3

[1] 100D= 100% dicalcium phosphate and 0% rock phosphate; 75D/25R= 75% dicalcium phosphate and 25% rock phosphate; 50D/50R= 50% dicalcium phosphate and 50% rock phosphate, 25D/75R= 25% dicalcium phosphate and 75% rock phosphate; 100R= 0% dicalcium phosphate and 100% rock phosphate.
SE = Standard error.
NS = No significance (P>0.05).

Table 5 - Intake and flow of nitrogen and microbial synthesis

	Diets[1]					Regression	R^2	SE
	100D	75D/25R	50D/50R	25D/75R	100R			
			Intake (g/d)					
Total nitrogen	114.6	113.9	114.2	114.3	114.2	Y = 114.3	NS	5.5
			Omasal flow (g/d)					
Total nitrogen	99.1	99.1	100.1	101.1	99.4	Y = 99.8	NS	8.8
Bacterial nitrogen	96.1	96.1	97.1	98.1	96.4	Y = 96.8	NS	2.7
Non bacterial nitrogen	3.0	3.0	3.0	3.0	3.0	Y = 3.0	NS	0.03
			Microbial efficiency					
Apparent (g N/kg OMDR)	39.7	40.2	37.2	38.5	40.0	Y = 39.1	NS	2.2
True (g N/kg COMDR)	18.6	18.4	18.5	17.3	18.8	Y = 18.3	NS	0.9

[1] 100D= 100% dicalcium phosphate and 0% rock phosphate; 75D/25R= 75% dicalcium phosphate and 25% rock phosphate; 50D/50R= 50% dicalcium phosphate and 50% rock phosphate; 25D/75R= 25% dicalcium phosphate and 75% rock phosphate; 100R= 0% dicalcium phosphate and 100% rock phosphate.
SE = Standard error.
NS = No significance (P>0.05).

Ruminal pH variation as function of time and diets ranged from 6.44 to 6.91 and it was not affected (P>0.05) by dicalcium phosphate replacement. Ruminal pH response was quadratic (P<0.05) when considering the time after feeding (Figure 1). Minimum values, observed two hours post-feed time, were 6.44; 6.50; 6.44; 6.45 and 6.53, to the 100D, 75D/25R, 50D/50R, 25D/75R and 100R diets, respectively.

Ruminal pH showed a quadratic response (P<0.05) regarded to the time after feeding and all pH values for all diets were higher than 6.2, which is considered by Orskov & Fraser (1975) as a minimal limit to an appropriate cell wall degradation without harming celullotic microorganism. The unique significant source of starch in experimental diets was corn silage, which participated in less than 50% of dry matter. This fact contributed to maintain a favorable pH for microbial activity because quickly ruminal degradable carbohidrates concentration in the diets was low.

Replacement of dicalcium phosphate by rock phosphate did not affect (P>0.05) ruminal ammonia concentration that was also quadratic; however, regression equation had a low determination coefficient (r^2) showing that the curve had a variation in their data.

Ruminal ammonia concentration also showed a quadratic response in relation to time. Ruminal ammonia values ranged from 3.51 to 36.10 mg/100 mL of ruminal fluid and diets with higher rock phosphate addition showed higher ruminal ammonia amounts, but there was no diet effect. The highest ammonia values were found two hours after feeding while the lowest ones were immediately before feeding times (8:30 am and 4:30 pm).

In all diets, most of the time, ruminal ammonia concentration were higher than 5 mg/dL, considered as a minimum value for adequate microbial growth (Satter & Slyter, 1974). Nevertheless, considering the value preconized by Leng (1990), which is of 20 mg/dL of ruminal fluid as the one proper to an ideal voluntary intake in tropical conditions, it is possible to observe that in all diets, and all the time, concentrations were lower (11.17, 12.23, 12.32, 11.44, 9.59 mg/100 mL of ruminal fluid for 0, 2, 4, 6 and 8 hours post morning feeding, respectively) than the suggested value.
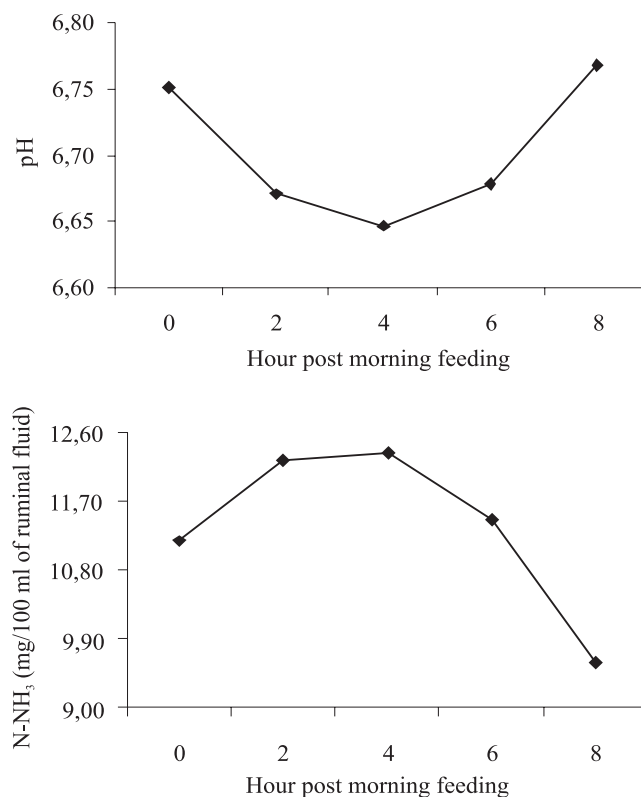

Figure 1 - pH and N-NH$_3$ variation as function of post-feeding time
Equation pH=6.75048 -0.05353X + 0.00708X^2; r^2=69.15
Equation N-NH$_3$= 11.17222 + 0.77373X - 0.12142X^2; r^2=58.23

Conclusions

Replacement of dicalcium phosphate with rock phosphate does not influence dry matter, crude protein, neutral fiber detergent, and non-fiber carbohydrates intake, fecal output and digestibility as well as total digestible nutrients values. There is an increase in phosphorous excretion when dicalcium phosphate is replaced by rock phosphate in cattle diets. Intake and flow of fluorine increase while its apparent absorption decreases with inclusion of rock phosphate. Plasma phosphorus is not influenced by phosphorus: fluorine ratio in diets. Ruminal pH and ruminal ammonia concentration is not influenced by dicalcium phosphate replacement. The same response is observed for microbial efficiency synthesis considering this experimental condition.

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