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Studies on Distribution and Disappearance Pattern of Calcium from Calcite Powder and Its Influence on Rumen Fermentation

By B. Abegaze
Jimma University, Ethiopia

Abstract - Mineral supplements differ in their bio-availability, which must be taken into consideration, before the use of any such supplement. Two crossbred fistulated calves housed in individual pens and fed Calcium carbonate (T1) and Calcite powder (T2) were used to study the distribution and disappearance pattern of calcium and its influence on rumen fermentation. The proportion of Ca distributed in soluble, particulate and solid phase of the rumen digesta, pH of the filtrate, Ammonia-N and VFA concentration, rate of disappearance of Ca and rumen flow rate, dry matter intake and other related measurements were used as evaluation parameters. The results obtained showed that there were no significant difference ($P<0.05$) between the treatments in NH3- N concentration in the rumen liquor of the experimental animals, indicating that replacing $CaCO_3$ with calcite powder did not affect NH_3-N concentration in the rumen. There was no significant difference between different sources of Ca fed to animals in total DMI or DMI per 100 kg body weight of animals, suggesting that calcite powder had any adverse effect on the palatability of the diet. Rumen pH and concentration of NH_3-N and total VFA in the rumen liquor of animals were similar, indicating that functions of cellulolytic organisms in the rumen were not affected by feeding calcite poweder.

Keywords : Calcium carbonate, Calcite poweder, Disappearance pattern, Solubility and Rumen fermentation.

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Keywords : Calcium carbonate, Calcite poweder, Disappearance pattern, Solubility and Rumen fermentation.

I. Introduction

Mineral supplements are nutritional devices to fortify the normal feeds and fodders in the areas to meet the mineral needs of livestock and poultry at specific levels of animal productivity. The effort to increase animal productivity manipulating by genetic potentials has further accentuated the problem of mineral nutrition and also of the mineral supplements. In case of high producing animals, there is a tremendous daily drainage of calcium (Ca) through milk and adequate mineral supplements need to be devised to replenish the daily loss, in spite of the fact that internal regulatory mechanism in animal can take care of the transient periods of enhanced needs. ISI (now BIS) has laid down the specifications of minerals supplements and the same has been reviewed a number of times (1960, 1961, 1968, 1982, 1992 and 2002). In certain countries, specific mineral supplement like high calcium, high magnesium and high phosphorus are commercially manufactured to meet the demands of specific areas in question, called region specific mineral supplements. Mineral supplements are available under various trade names in the market. The Bureau of Indian Standards (BIS) imposes compositional standards of feeds and mineral mixture for different categories of livestock and modifies the standards periodically. (12) recommended the use of bone meal, chalk powder and di-calcium phosphate as a source of Ca and P in mineral mixture. Quality specifications were also laid down (13). (14) allowed the use of calcined bone meal, in addition to steamed bone meal, chalk powder and di-calcium phosphate. In 1982, ISI recommended the use of ground limestone in the list of ingredients for use in formulation of mineral mixture. In 1992 specifications for Mg and S were laid down (4).

(5) withdrew the use of supplements of animal origin i.e. bone meal, di-calcium phosphate of animal origin, calcined bone meal, etc., and allowed the use of calcite powder in mineral mixture. Indian cattle feed industry is using calcite powder on wide scale on account of it's being easily available and cheaper source of calcium (26). (25) reported that various mineral supplements differ in their bio-availability, which must be taken into consideration, before the use of any such supplement it is necessary to comparatively scan them for availability of useful elements and also ensuring the absence of toxic levels of incriminating minerals in them. Unfortunately there is no literature on the availability/ utilization of Ca from calcite powder in livestock although; calcite has been used as a buffer in high milk producing cows (17). This being the case, the major objective of this research undertaking was to study/to determine ruminal distribution and

Author : Jimma University College of Agriculture and Veterinary Medicine, P.O.box 307, Jimma, Ethiopia.
E-mail : abegazebeyene@yahoo.com

disappearance of calcium from calcite powder and its influence on rumen fermentation.

II. Materials and Methods

a) Management of the Experimental animals

Two crossbred male rumen fistulated calves of 4 years of age housed in individual pens were used for this study. Based on the calcium and phosphorus content of $CaCO_3$ and calcite powder two mineral mixtures (Table 1) were prepared using Diammonium phosphate as the sole source of P.

Two concentrate mixtures were also prepared by mixing maize, barley, groundnut cake, wheat bran, mustard cake and the respective mineral mixtures shown in Table 2. The quantity of NPN supplied through DAP of mineral mixtures in concentrate mixture of group I and group II was adjusted with the addition of urea and the concentrate mixtures were made iso nitrogenous and iso caloric.

The experimental treatments differed only with respect to supplements of Ca from mineral mixture as the mineral mixtures of different treatment groups contained different sources of Ca supplements. Each animal was offered 3 kg concentrate mixture and 5 kg wheat straw/ day for study period of 21 days at the end of which the samples of rumen liquor were taken for the estimation of the parameters to be studied the animals were then switched over to the next treatment in a switch over design in which experimental pre-feeding was followed again for 21 days followed by sampling.

b) Sample Collection and Ca Determination

The rumen contents were mixed manually by inserting hand inside the rumen followed by collecting of about 500 ml of rumen digesta from each animal in stoppered measuring cylinder. The digista were filtered through four layers of cheeas cloth by pressing it hard. Finally, the proportion of Ca distributed in soluble, particulate and solid phase of the rumen digesta was determined.

III. Determination of Rumen Metabolites

The pH of the filtrate was immediately recorded with the help of ECIL digital pH meter. Ammonia-N was determined by Micro diffusion technique of (6) and calculated as Ammonia-N (mg/ 100 ml SRL) = ml of acid used x normality of acid x 14 x 100. The Total volatile fatty acids (TVFA), was estimated by the method of (2) and calculated as:

$$\text{TVFA (in meg/ 100 ml of SRL)} = \frac{\text{Vol. of NaOH used x Normality of NaOH x 100}}{2}$$

The Individual VFA fractions were partitioned and estimated with the help of Nucan gas chromatograph. A standard mixture of acetate, propionate and butyrate in the molar ratio of 60:30:10 was also run into the gas chromatograph under similar conditions, as those for the samples. Different proportions of acetate, propionate and butyrate in the samples were calculated as

Peak area = ½ height of the peak x width of the base line

a) Rumen disappearance pattern of Ca

The disappearance of Ca from the rumen was calculated based on the principles of disappearance pattern of N from rumen as described by(24) and calculated as.

Disappearance of Ca through rumen fluid = rumen fluid flow rate (1/ hr) x mean Ca concentration of rumen fluid (mg/ l ml)

The rumen fluid flow rate (1/ hr) of all the animals under different treatments was calculated by estimating first their respective rumen volumes. The concentration of Ca in the f rumen fluid was estimated after ashing known amount of aliquot.

b) Rumen fluid volume and flow rate

Rumen fluid volume was estimated with the help of polyethylene glycol (PEG, of M. W.4000) according to (11) as modified by (30). During rumen volume and flow rate determinations the feeding pattern was slightly changed. The animals were maintained in a steady state condition of rumen fermentation by offering wheat straw and water distributed at hourly intervals. About 100 ml of 25% solution of PEG was infused into the rumen just at the time of feeding of concentrate mixture as recommended by (30). Samples of the rumen liquor collected at 1, 2, 3, 4, 5, 6, 7 and 8 hrs after the infusion of PEG solution were stored in deep freezer for further analysis.Estimation of PEG concentration was done according to (30). A standard curve was drawn for different concentration of PEG against OD readings.

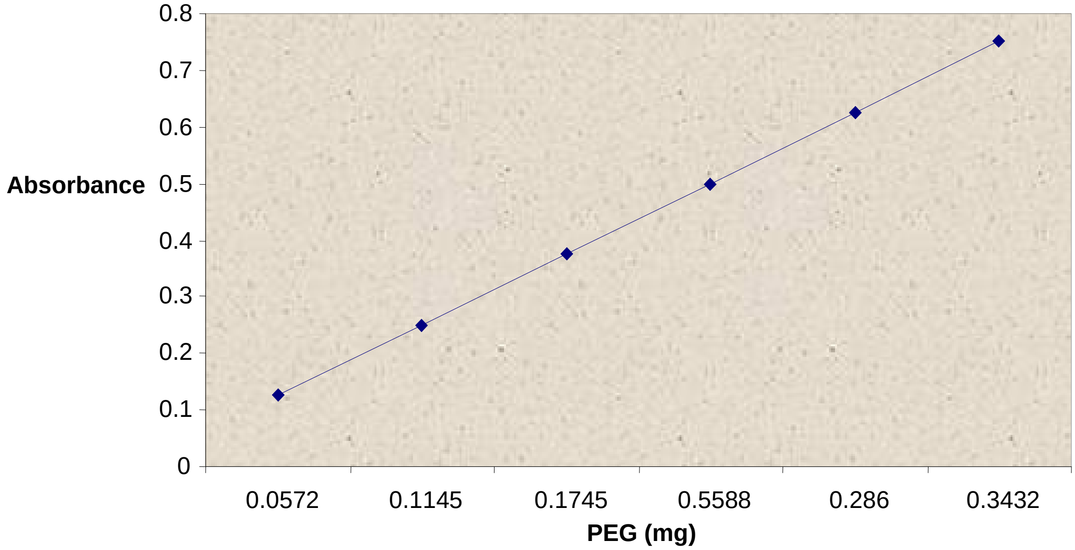

Fig 1 : Standard curve for estimation of PEG

Finally data on different parameters were subjected to t-test

IV. Results and Discussions

a) Mineral composition of experimental concentrate mixtures

The mineral composition of concentrate mixtures of treatment I (T_1) and treatment II (T_2) prepared from mineral mixtures containing $CaCO_3$ and calcite powder respectively is presented in Table 3. Although there was some variation in the chemical composition of $CaCO_3$ and calcite powder, quantities of Ca and P per 100 kg of concentrate mixture were so adjusted that the respective concentrate mixture supplied the same quantity of Ca and P. The Ca content of concentrate mixtures fed to animals in T_1 and T_2 was 1.2% whereas P content ranged from 0.64 to 0.65%. Wheat straw which was the sole source of roughage in both the treatments contained 0.22% Ca and 0.08% P. There was only little variation in Mg and other trace mineral contents in concentrate mixtures under the respective treatment groups.

b) Dry matter and Ca intake of animals

The average live weight of animals and their total dry matter intake have been presented in table 4. The mean value for DMI of animals in $CaCO_3$ and calcite powder fed treatments was 8.19±0.41 and 8.66±0.66kg per day respectively and differences were non-significant ($P>0.05$). Dry matter intake per 100 kg body weight of animals in respective treatment groups was 1.90 and 1.99 kg/ day, respectively. These difference were also non-significant ($P>0.05$). (Intake of Ca through concentrate mixture, wheat straw and total has been presented in Table 4. In the treatment groups T_1 and T_2 the total dietary Ca content ranged from 54.18 to 55.41 (g/d) indicating that about 27.00 and 35.72% of Ca in the respective concentrate was from externally added supplement. These different sources of Ca fed to animals in concentrate mixtures did not show any difference in the total DMI or DMI per 100 kg body weight of animals. The results suggested that neither the $CaCO_3$ nor the calcite powder had any adverse effect on the palatability of diet.

V. Effect of Different Sources of Casupplements on Rumen Metabolite

a) Rumen Metabolites

The average value of rumen pH, NH_3-N concentration and total and individual VFAs in SRL collected 4 hours after feeding have been presented in Table 5.

b) Rumen pH

Rumen pH in different treatment groups T_1 and T_2 were respectively 6.21 ± 0.11 and 6.99 ± 0.06 and there was no significant difference between the treatments. (7) observed that major minerals play an important role in maintaining the physico-chemical characteristics of rumen medium, the major buffering components in the rumen are Na, K, P and VFAs and any change in these components in rumen results in the change in rumen pH. In the present experiment no significant effect on rumen pH was observed as there was no difference in the supply of dietary P or Na and the supply of minerals was similar in both the groups. The results of the present study are in concurrence with the findings of (23),Who fed different sources of Ca to calves and observed that rumen pH in different treatment groups varied between 6.20 ± 0.13 to 6.56 ± 0.07 and that there was no treatment effect in the various groups.

c) Ammonia-N

NH_3-N concentration in the rumen liquor of animals in treatments T_1 and T_2 were 27.48 ± 3.23 and 25.13 ± 2.81 mg) 100 ml SRL (Table 5). These values were statistically similar, indicating that replacing $CaCO_3$ with calcite powder did not affect NH_3-N concentration in the rumen. The increased ammonia-N concentration in the rumen fluid during P deficiency has been reported *in vivo* (9) and *in vitro* (18, 19, 20, 21) as a result of reduced utilization by rumen microorganism. In the present investigation the diet was adequate in P in both the treatments and thus, there was no difference in the NH_3-N concentration among the various treatment groups.

d) Total volatile fatty acids

The level of total VFAs in respective treatment groups T_1 and T_2 were 8.02 ± 0.50 and 7.94 ± 0.21 meq/ 100 ml SRL. The ruminal TVFA showed no significant difference ($P>0.05$) indicating that functions of cellulolytic organisms in the rumen were not affected by feeding the different sources of Ca supplement in concentrate mixtures. The relative percentage of individual VFAs i.e. acetate, propionate and butyrate also showed no treatment effect (Table 5). It was evident from the data in Table 5 that the treatment did not influence the relative proportions of acetate, propionate and butyrate. Therefore, it can be concluded that different sources of Ca supplements (calcium carbonate and calcite powder) exerted no influence on energy metabolites. (23) used different sources of Ca and P such as dicalcium phosphate and chalk powder, marble powder and sodium phosphate, gypsum and sodium phosphate, rock phosphate and sodium phosphate and superphosphate and sodium phosphate and observed that neither TVFAs nor the proportion of acetate, propionate or butyrate differed in any of the treatments. (1) fed $CaCO_3$ and Ca propionate (prilled) to steers and observed that TVFA were not affected by Ca level or its source, however, the addition of Ca propionate decreased the molar percentage of acetate due to the increase in propionate being fed. In fact, the proportion of acetate was lower with supplemented Ca propionate compared with $CaCO_3$.

VI. Distribution of Calcium in RuminalContents

The distribution of Ca in soluble (centrifuged supernatant SRL), particulate (centrifugate mass) and solid phases (separated solid portion) of the rumen digesta collected 4 hrs after feeding, have been presented in Table 6.

The % Ca content ranged from 8.84 to 12.03 in soluble phase, 19.01 to 25.21 in particulate phase and 62.75 to 72.14 in solid phase of the rumen digesta respectively. The distribution of Ca was lowest in soluble phase of rumen content and was a little more than doubles the value of soluble phase in the particulate phase. However, Ca was mostly located in solid phase or rumen digesta in both the treatment groups. Data in Table 6 further indicates the distribution of Ca from $CaCO_3$ and calcite powder in different phases of rumen digesta did not vary significantly. It was observed earlier through; *in vitro* studies with ruminal buffer, that various Ca supplement sources did not have similar Ca solubility in the rumen. The Ca solubility was influenced by pH changes (phase-II). Such findings indicated that there may be variability of Ca distribution in soluble, particulate and solid phase in rumen liquor. The present studies suggested that Ca from different sources was soluble from 8-12%. These findings are in agreement with the findings of (22) who observed that Ca from various sources such as marble, gypsum, rock phosphate and superphosphate was soluble in the rumen fluid to the extent of 7-9%. The proportion in the solid phase was the resultant effect of its distribution in the soluble and particulate phase and was about 62-72% of total Ca present in the rumen contents which was lower than the observations of (22) who observed that the proportion of Ca from marble, gypsum, rock phosphate and superphosphate varied between 80.5 to 86.7% of total Ca present in the rumen content.

a) Calcium concentration in SRL

The calcium concentration in the ruminal fluid is influenced by the dietary levels of this element and its solubility in the rumen contents (33, 32, and 22). In the present experiment, Ca concentration in SRL samples collected at 0, 1, 2, 3, 4, 5, 6, 7, and 8 hours after feeding of concentrate mixtures prepared from $CaCO_3$ and calcite powder as a source of Ca is presented in Fig. 2.

The mean values of Ca concentration in T_1 and T_2 were 28.45 ± 1.40 and 14.88 ± 0.80 mg / 100 ml SRL (Table 7) which were statistically not similar. It was further evident that in both the treatments the maximum concentration of Ca (mg/ 100 SRL) was after 1 hour of feeding Calcium concentration in the ruminal fluid is known to be influenced by dietary level of Ca and its solubility in the rumen contents (33, 32). In the present experiment dietary intake of Ca was similar in both the treatment groups but the solubility of Ca from calcite powder was lower hence the concentration of Ca was lower in the SRL of T_2 group, indicating that the sources of Ca in the diet exerted significant effect on Ca concentration of SRL in contrast, (22) observed that there was no variation in the Ca concentration (mg/ 100 ml SRL) of calves fed similar quantity of Ca through various sources of Ca supplements i.e. marble, gypsum, rock phosphate and super phosphate, which might be due to the fact that in this studies solubility of Ca from different sources of Ca was similar supports the findings of the present study.

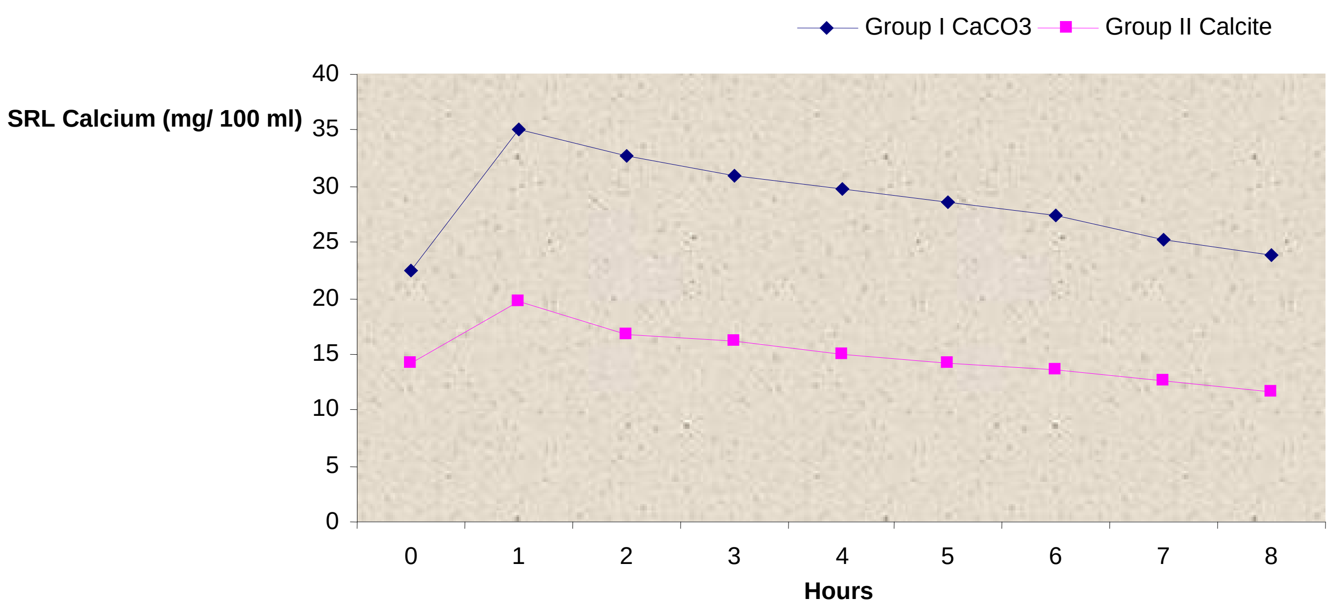

Fig. 2 : Calcium Concentration in SRL

VII. Ruminalfluid Volume and Its Flow Rate

The data on rumen fluid volume, rumen fluid flow rate and water intake of animals have been presented in Table 8.

The mean values of rumen fluid volume under various treatments (T_1 and T_2) were 56.52 ± 1 and 56.25 ± 1.26 L, respectively. Statistical analysis of the data did not reveal any significant variation in the rumen volume of calves fed diets containing $CaCO_3$ or calcite powder as source of Ca. The results revealed that rumen volume was not influenced by the source of Ca supplement used. The mean liquid outflow rate (L/h) in crossbred calves fed on diets containing $CaCO_3$ (T-1 1.67 ± 0.22) was lower than the diet containing calcite powder (T_2 – 2.04 ± 0.19) as a source of Ca. However, statistical analysis of the data revealed that $CaCo_3$ or calcite powder did not alter the liquid outflow rate in crossbred male caves. The findings of the present study are in agreement with the findings of many earlier workers (28) who reported that rumen fluid volume, that ruminal fluid out flow and ruminal fluid outflow per kg DMI were not altered significantly by the source of Ca included in the diet. (22) also demonstrated that source of Ca in the diet of crossbred calves did not influence the rumen volume and rate of passage of liquid digesta, however, he reported lower values of rumen volume and rate of passage of liquid digesta than that recorded in the present study, which could possibly be due to difference in the age of experimental animals affecting DMI and water intake.

Ingestion of certain inorganic salts like sodium chloride or sodium bicarbonate was found to increase water intake, rumen fluid volume and flow rate (8, 31) and decrease DMI and digestion of organic nutrients (28). In the present study the ingestion of sodium salt (sodium chloride) was similar in both the treatment groups which might also be reason that there was no significant variation in water intake or rumen fluid volume of both the treatment groups.

Rumen fluid volume as percent of body weight in calves fed different sources of Ca (T_1 and T_2) was 13.08±0.23 and 11.24±0.64 respectively and the variations among the treatments were not significant (Table 8). Percent volume was comparable with those reported by (27, 29, 3 and 10).

a) Rumen disappearance rate of calcium

The rumen disappearance rate of Ca through rumen fluid under various treatments has been presented in Table 8. These values were 475.12±23.54 and 303.55±6.39 mg/hr for the groups T_1 and T_2 respectively. The difference in disappearance rate of Ca through rumen fluid was statically significant, showing variability in rumen disappearance rate of Ca on using $CaCO_3$ and calcite powder as a source of Ca in the diet. This difference could be attributed to low solubility of Ca from calcite powder at rumen pH 7 (Phase-II) resulting in lower Ca concentration in soluble phase of SRL (Table 8). The availability of Ca to the animals from the lower gut may depend upon the rumen fluid concentration and the digesta flow rate, since the treatment groups in the present study indicate significant difference in the Ca concentration of SRL and ruminal disappearance rate of Ca in the availability of Ca from both the Ca supplements ($CaCO_3$ and calcite powder) to the animals *in vivo.* Thisneeds to be evaluated further.

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Table 1 : Composition of mineral mixtures used/ 100 kg of concentrate mixture*).

Treatments	Ca/ P sources used	Ca and P content		Qty. (kg/ 100 kg of conc. mixture	Total supply of Ca and P through supplement in 100 kg conc. mix.	
		Ca%	P%		Ca (g)	P (g)
Group I	Calcium carbonate	39.2	0.12	1.0113	396.73	1.21
	Diammonium phosphate		23.0	0.7000	--	161.00
	Total				396.73	162.42
Group II	Calcite powder	40.9	1.1	0.9779	399.96	10.76
	Diammonium phosphate		23.0	0.5800	--	133.4
	Total				399.96	144.16

* In addition to Ca and P sources used the following ingredients were added to the mineral mixture of group I and group II for the preparation of complete mineral mixture. sodium chloride 0.900 kg, trace mineral mixture 0.1185 kg containing magnesium carbonate 90 g, ferrous sulphate 15 g, copper sulphate 2.1 g, cobalt chloride 1.5 g, potassium iodide 0.3 g, zinc sulphate 7.5 g and manganese dioxide 2.1 g.

Table 2 : Composition of concentrate mixtures* (kg/ 100 kg).

	Group I	Group II
	Treatment-I	Treatment-II
Maize	20.0000	20.0000
Barley	20.5204	20.6486
Groundnut cake	10.0000	10.0000
Wheat bran	34.0000	34.0000
Mustard cake	12.0000	12.0000
Urea	0.7300	0.7750
Calcium carbonate	1.0113	--
Calcite powder	--	0.9770
Diamonium phosphate	0.7000	0.5800
Sodium chloride	0.9000	0.9000
Trace M. Mixture*	0.1185	0.1185

* Contained magnesium carbonate 90 g, ferrous sulphate 15 g, copper sulphate 2.1 g, cobalt chloride 1.5 g, potassium iodide 0.3 g, zinc sulphate 7.5 g and manganese dioxide 2.1 g. Vitamin supplement, having the strength of 82,500 IU of vitamin A, 50 mg of vitamin B, 12,000 IU of vitamin D and 10 mg of vitamin K per g of spectromix powder was mixed 10 g of concentrate mixtures.

Table 3 : Mineral composition of experimental concentrate mixtures and wheat straw (%DM basis).

Parameters	Wheat Straw	Conc. Mixture I	Conc. Mixture II
Total ash	11.20	5.17	4.59
AIA	6.44	0.36	0.37
Ca%	0.22	1.20	1.20
P %	0.08	0.65	0.64
Mg%	0.13	0.45	0.39
Fe (ppm)	525.00	710.00	699.00
Cu (ppm)	18.50	22.00	27.00
Zn (ppm)	32.00	61.00	57.00
Mn (ppm)	33.00	58.00	55.00

Table 4 : Average live weight, intake of DM using different sources of Ca.

Parameter	Treatment	
	T_1 ($CaCO_3$)	T_2 (Calcite powder)
Average live weight (kg)	432±0.91	435±0.91
DM intake		
Concentrate (kg)	3.69±0.01	3.71±0.01
Wheat straw	4.50±0.17	4.95±0.13
Total DM intake (kg)	8.19±0.41	8.66±0.62
DM intake/ 100kg BW	1.90	1.99
intake of Ca (g) through		
Concentrate	44.2	44.52
Wheat straw	9.9	10.89
Total	**54.18±17.19**	**55.41±16.82**

Similarly Lall (1987) used DCP, marble powder gypsum and super phosphate in the ratio/n of calves as a source of Ca and observed that there was no adverse effect of these Ca sources on the DMI of the calves.

Table 5 : Rumen metabolites in animals under various treatments.

	T_1	T_2	t (calculated value
Rumen pH	6.21 ± 0.11	6.99 ± 0.06	0.76
NH_3 – N (mg/ 100 ml SRL)	27.48 ± 3.23	25.13 ± 2.81	0.78
Rumen VFA			
i) Total Volatile fatty acids(meq/100 ml SRL)	8.02 ± 0.50	7.94 ± 0.21	
ii) Proportion of individual VFA's (%)			
Acetate	50.93 ± 0.62	49.64 ± 0.79	1.28
Propionate	27.47 ± 0.35	27.48 ± 0.30	0.03
Butyrate	21.58 ± 0.73	22.87 ± 0.90	1.11

Table 6 : Calcium distribution (%) in ruminal contents of animals under various treatments.

	Treatment-1 ($CaCO_3$)	Treatment-II (calcite powder)
Distribution of Ca%		
In soluble phase	12.03 ± 1.24	8.84 ± 1.32
In particulate phase	25.21 ± 6.28	19.01 ± 5.78
In solid phase	62.75 ± 16.21	72.14 ± 18.18

Table 7 : Mean calcium concentration (mg/ 100 ml) in SRL of animals under various treatments function of time.

Treatment-1 ($CaCO_3$ as Ca source)	Treatment-II (calcite powder as Ca source	t (calculated values)
28.45 ± 1.40	14.88 ± 0.80	8.42**

** Significant (P<0.01)

Table 8 : Effect of different dietary calcium supplements on rumen fluid volume, out flow rate and disappearance pattern of Ca through rumen fluid.

Parameters	Treatment-1 ($CaCO_3$)	Treatment-II (calcite powder)	(t)calculated value
Water intake (lit/ day)	51.74 ± 3.79	47.68 ± 3.06	0.84
Rumen fluid volume	56.52 ± 1	56.25 ± 1.26	0.18
Rumen fluid flow rate (l/ h)	1.67 ± 0.22	2.04 ± 0.19	1.025
Rumen fluid volume as % of B.W.	13.08 ± 0.23	12.93 ± 0.29	0.41
Rumen fluid flow rate per kg DM (Lit/ kg DM)	5.57±0.74	6.38± 0.60	0.85
Rumen disappearance rate of Ca through rumen fluid (mg/h)	475.12±23.54	303.55±16.39	6.02*

* Significant ($P<0.05$)