

Influence of manipulated rumen fermentation using buffers on the ruminal environment in crossbred calves

Short Title: Buffers on the ruminal environment in crossbred calves

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Abstract

To study the effect of manipulation of rumen fermentation using buffers in crossbred calves on the rumen environment nine crossbred calves were divided into three groups based on different phenotypic traits (Age 131-221d, lightweight-LW 57.5-93.9 Kg) and were given grass mixture and wheat straw as green and dry roughages and concentrate mixture contained barley grain and mustard cake. One animal from each group was randomly allotted to one of the three treatments viz. T-1, T-2, and T-3. Buffer in the form of sodium bicarbonate and magnesium oxide in combination at the rate of 0.00 and 0.00, 0.20 and 0.10, and 0.40 and 0.20 per cent of LW were given in T-1, T-2, and T-3, respectively. The ruminal study trial was conducted 30 days after the start of the experiment and lasted for 5 consecutive days. During this period, the ruminal liquor was sampled after 0, 2, 4, 6, 8 and 10 hours of feeding and analyzed for pH, total volatile fatty acids, acetate, butyrate, iso-butyrate, Iso-valerate, propionate, valerate, acetate to propionate (A:P) ratio, total nitrogen, ammonia nitrogen, urea nitrogen and non-ammonia and urea nitrogen. The data were analyzed statistically using suitable procedures. The results of the rumen metabolism study indicated that the pH and content of acetate, propionate and ammonia nitrogen decreased and TVFA, BA, VA, and A:P ratio increased in the rumen liquor due to the supplementation of buffer in calves' nutrition. A declining pattern of pH up to 10 hours; concentration of IBA, AN, and urea nitrogen up to 8 hours and total volatile fatty acid up to 6 hours in rumen liquor after feeding were recorded. The overall conclusion can be made that the addition of buffer in ruminant nutrition (buffer feed technology) was responsible to change the ruminal environment in such a way that it became helpful to produce additional fat due to manipulated pH and A:P ratio.

Keywords:

A:P ratio, Ammonia nitrogen, Buffers, Crossbred calves, Magnesium oxide, Rumen fermentation, Ruminal environment, Sodium bicarbonate, TVFA.

Introduction

Buffer feed technology reveals to add buffers in animal nutrition with the object to keep pH constant in rumen fluid. At constant pH between 6.5 to 7.0 maximum potential can be explored from the animals but in general, after 4 to 6 hours of feeding, the acidity of rumen content goes down drastically (Kishore et al. 1996) due to the fermentation of feed and production of several volatile fatty acids and results to depress microbial activity in rumen ecosystem.

To check the fall of ruminal pH many chemicals have been tried but the use of sodium bicarbonate and magnesium oxide in combination showed better potential in terms of intake (Toro et al. 1982), digestibility of different nutrients (Pierce et al. 1983), higher volatile fatty acid concentration in rumen liquor (Rogers et al. 1985), milk yield (Elekelberger et al. 1985), yield of different milk constituents (Singh et al. 1996).

A significant effect on acetate and propionate production, however, was not observed but Acetate to propionate (A:P) ratio was increased in rumen liquor when sodium bicarbonate (Mees and Merchen 1985) or with magnesium oxide (Lee and Hsu 1985) was included in cattle nutrition. On the other hand, many research workers reported increased production of acetate in rumen fluid on buffer included diet (Kishore et al. 1996) in goats. Which is the main precursor to the synthesis of milk fat and thus the yield of milk fat is increased. Teh et al. (1985), Zhelev and Proferov (1987), Johnson et al. (1988), Solorzano et al. (1989), Kishore (1997), and Singh et al. (1996) worked out that buffers (in the form of sodium bicarbonate or with magnesium oxide) inclusion to the diet either increased milk fat yield or keeps it constant.

But before offering the buffer feed technology directly to the cow, a thorough

study of calves to fix the level suitable for cattle is needed. The present investigation is, therefore, an effort to study the effect of manipulation of rumen fermentation using buffers in crossbred calves on the rumen environment.

Materials and methods

Nine crossbred calves were selected from the herd of the college Dairy Farm. At the start of the experiment, the age (131-221d), live-weight (LW=57.5-93.9 Kg), heart girth (91-105 cm), body length (75-92 cm), height (78- 93 cm), collar length (52-71 cm) and tail length (42-57 cm) were recorded. All the animals were dewormed and housed in calf sheds having separate feeding mangers and water troughs for individual feeding. The large-sized open enclosure was used for exercise. All of the animals received grass mixture as green fodder and wheat straw as dry fodder. The concentrate mixture contained barley grain and mustard cake. The chemical composition of feeds and fodders offered to the animals is presented in Table 1. The amount of each feed ingredient for each animal was calculated based on the NRC feeding standard. Apart from this, each animal was also given 20 g of common salt and 25 g of mineral mixture daily.

Based on different phenotypic traits viz. age, weight, heart girth, and length, the animals were divided into three groups. One animal from each group was randomly allotted to one of the three treatments viz. T-1, T-2, and T-3. Buffer in the form of sodium bicarbonate and magnesium oxide in combination at the rate of 0.00 and 0.00, 0.50 and 0.25 and 1.00 and 0.50 per cent of assumed dry matter intake to be 4 per cent of LW or 0.00 and 0.00, 0.20 and 0.10, and 0.40 and 0.20 per cent of LW were given in T-1, T-2, and T-3, respectively.

The ruminal study trial was conducted on subjected animals 30 days after the start of the experiment and lasted for 5 consecutive

days. Rumen liquor was collected with the help of a rubber tube inserted into the rumen through the mouth of the animal and by creating a vacuum with the help of a hand-driven suction pump. The samples of rumen liquor were drawn after 0, 2, 4, 6, 8, and 10 hours of feeding. The liquor collected was filtered through muslin cloth and then used for analysis. The pH, ammonia nitrogen, and urea nitrogen were estimated immediately. A 1.0 ml sample was preserved for total nitrogen and 10 ml for volatile fatty acid determination. The

samples obtained during the ruminal study trial were analyzed for pH, total volatile fatty acid, acetate, butyrate, iso-butyrate, iso-valerate, propionate, valerate, total nitrogen, ammonia nitrogen, and urea nitrogen (Issiki et al. 1981, Oser 1965 and ISI 1975). A:P ratio and non-ammonia and urea-nitrogen were analyzed mathematically. The data recorded during the experiment were analyzed statistically using factorial design using the method discussed by Snedecor and Cochran (1967).

Table 1: Chemical composition of feeds (%DM)

Parameters	Straw	Grass	Barley grain	Mustard cake	Buffer
DM*	91.2	28.2	90.3	99.2	100
Ash	10.0	10.6	9.30	7.50	100
Organic matter	90.0	89.4	90.7	92.5	0.00
Crude protein	3.10	6.90	10.2	35.0	0.00
Ether extract	1.00	1.80	2.60	2.00	0.00
Total carbohydrates	85.9	80.9	77.9	55.5	0.00
Gross energy#	3.98	4.06	4.20	4.62	0.00
Crude fibre	35.2	44.6	10.1	7.90	0.00
Nitrogen free extract	50.7	36.1	67.8	47.6	0.00
Calcium	0.10	0.40	0.20	0.20	0.00
Phosphorus	0.10	0.20	0.20	0.20	0.00
Sodium	0.20	0.60	0.40	0.40	18.0
Magnesium	0.10	0.30	0.30	0.40	19.5
Potassium	5.80	0.90	0.50	1.20	0.00
Neutral detergent fibre	74.2	71.1	56.8	53.5	0.00
Acid detergent fibre	51.1	38.2	42.0	43.9	0.0
Hemicellulose	23.1	32.9	14.8	9.60	0.00
Cellulose	43.0	31.9	37.0	33.0	0.00
Lignin	2.80	2.70	2.30	2.00	0.00
Cell content	25.8	28.9	43.2	46.5	0.00

* Fresh basis; # M cal/Kg DM

Results and discussion

Voluntary intake of digestible dry matter, digestible organic matter, and digestible energy (Table 2) did not differ in T-1 and T-2 and T-2 and T-3, whereas intake of crude protein and digestible crude protein was lowest only in T-1 and remained equal in T-2 and T-3 and that remained unchanged among all the treatment in terms of per Kg $W^{0.75}$ but when these were

expressed in terms of per 100 Kg LW, intake of digestible dry matter and digestible organic matter became non-significant and digestible energy was noted to be lowest only in T-1 and remained non-significant in T-2 and T-3. Increased intake of digestible dry matter in T-3 was due to the effect of buffer addition in the diet on the digestibility coefficient of dry matter (Kishore 1997). A similar pattern of intake

of these nutrients is reviewed in the literature (Hemminger and Krichgassner 1972, Toro et al. 1982, Johnson et al. 1988, Solorzano et al. 1989, and Kishore et al. 1998). The reason could be that the addition of buffers increased liquid turnover rate, solid retention time (Stocks 1983), rate of dilution of ruminal fluid, outflow rate, and duodenal passage of digesta (Dewhurst et

al. 1972). Water intake is reported to be related directly proportionate to the level of buffers inclusion in the diet Kishore (1997). The reason for the same could be the addition of sodium ions (Fettman et al. 1981, Stocks 1983, Rogers et al. 1985, Johnson et al 1988, Kishore et al. 1996, and Kishore et al. (1998).

Table 2: Voluntary Intake (g)

Nutrients	Voluntary Intake (per Kg W ^{0.75})				Voluntary Intake (100Kg LW)			
	Treatments				Treatments			
	T-1	T-2	T-3	CD at 5%	T-1	T-2	T-3	CD at 5%
DM	78.1 ±9.0	84.3 ±4.6	85.1 ±1.5	11.2	2.59 ±0.07 [#]	2.69 ±0.05 [#]	2.73 ±0.06 [#]	0.39
DDM	42.8 ±0.7 ^B	49.4 ±3.4 ^{A,B}	52.1 ±1.5 ^A	7.9	1.42 ±0.03 [#]	1.57 ±0.04 [#]	1.67 ±0.06 [#]	0.21
CP	4.6 ±0.4 ^B	6.9 ±0.4 ^A	7.6 ±0.4 ^A	1.7	153 ±12 ^b	231 ±15 ^a	245 ±17 ^a	53
DCP	2.70 ±0.20 ^B	4.10 ±0.20 ^A	4.90 ±0.20 ^A	1.1	88.7 ±7.7 ^b	139 ±8 ^a	156 ±9 ^a	38
DE	183 ±2 ^{*B}	194 ±7 ^{*AB}	206 ±8 ^{*A}	17	6.05 ±0.13 ^{Sb}	6.45 ±0.15 ^{§a}	6.65 ±0.06 ^{§a}	0.35

^{*} In Kcal; [#] In Kg; [§] In M cal.

^{A,B} = Value bearing different subscripts among the row and group differ significantly i.e., P<0.05.

^{a,b} = Value bearing different subscripts among the row and group differ significantly i.e., P<0.05.

Increased level of buffers addition in the ruminant diet is inferred to increase total volatile fatty acid production in rumen liquor (P<0.05) (Table 3 A and B). A similar trend was also reported by Rogers et al. ((1985), The et al. (1985) and Awadalla and Raghieb, (1986), and Kishore et al. (1996).

Administration of buffers affected acetate production adversely i.e., an increased dose of buffers decreased production (P<0.05). A similar pattern was advocated by Hadjipanayiotou (1982) and Kishore et al. (1996). Mees and Merchen (1985), Armel et al. (1988), and Johnson et al. (1988) did not note any exact trend in this regard, while, Rogers et al. (1985) and Teh et al. (1985) reviewed a positive correlation between the dose of buffers and production of acetate in the rumen ecosystem. The

variation between the trends may be due to the various ratio of roughage and concentrate offered to the animals.

Buffer supplementation in calves' nutrition was responsible to increase butyrate concentration in rumen liquor with the enhancement in the level (P<0.05). Increased production of butyrate with an increased dose of buffer had been observed by Armel et al. (1988) and Kishore et al. (1996) whereas, Rogers et al. (1985) did not show any significant trend.

Production of iso-butyrate followed the same pattern as recorded in the case of butyrate production. A higher amount of buffer regime was significantly related to the production of iso-butyrate (P<0.05). The increasing pattern of production of iso-butyrate with the increased dose of sodium

bicarbonate and magnesium oxide separately (Armbel et al. 1988) or a combination of both chemicals (Kishore et al. 1996).

Iso-valerate production was unaffected by the level of buffer administration to the ruminant diet ($P>0.05$). Armbel et al. (1988) reported that the use of sodium

bicarbonate caused increased synthesis of this fatty acid salt but the use of magnesium oxide altered this trend. The combined effect of both chemicals appeared in the present study. Rogers et al. (1985) explored that the use of sodium bicarbonate had no specific effect on the production of iso-valerate during the buffer regime.

Table 3A: Characteristics of the ruminal environment

Parameter	T-1	T-2	T-3	CD at 1%
pH	61.5±0.08 ^b	6.67±0.07 ^a	6.71±0.09 ^a	0.08
TVFA	88.3±0.4 ^c	93.1±0.6 ^b	100.8±0.5 ^a	1.4
Acetate (meq/l)	62.8±0.0 ^a	62.7±0.1 ^b	62.5±0.0 ^c	0.2
Butyrate (mg/l)	11.6±0.1 ^c	12.7±0.1 ^b	14.0±0.1 ^a	0.3
Isobutyrate (mg/l)	1.06±0.01 ^a	1.26±0.02 ^b	1.40±0.01 ^a	0.04
Isovalerate (mg/l)	2.21±0.01	2.20±0.01	2.18±0.01	0.03
Propionate	26.0±0.00 ^a	25.1±0.0 ^b	24.4±0.00 ^c	0.1
Valerate (mg/l)	2.51±0.1 ^c	2.34±0.02 ^b	2.58±0.01 ^a	0.02
A:P ratio	2.47±0.00 ^c	2.50±0.00 ^b	2.56±0.00 ^a	0.02
Total-N (mg/l)	55.1±0.8	55.5±0.68	54.1±0.6	1.13
NH ₃ -N (mg/l)	7.76±0.03 ^a	7.55±0.03 ^b	7.43±0.04 ^c	0.06
Urea-N (mg/ml)	3.08±0.01 ^c	3.36±0.01 ^b	3.77±0.02 ^a	0.03
NAUN (mg/ml)	44.2±0.8	44.8±0.7	43.8±0.6	1.1

^{a,b,c} = Values bearing different superscripts differed significantly.

Data revealed that an increased dose of buffers in ruminant nutrition interrupted the synthesis of propionate in the rumen ($P<0.05$). Armbel et al. (1988) reported that the use of sodium bicarbonate and magnesium oxide stimulated the synthesis of propionate. Similar findings were observed by Hadnipanayiotou (1982), Rogers et al. (1985), The et al. (1985), Zelev and Profirov (1987), Johnson et al. (1988) and Kishore et al. (1996).

The pattern of valerate production in the rumen was increased with the increased level of buffers in ruminant nutrition. Similar findings were recorded by Armbel et al. (1988) and Kishore et al. (1996), whereas Teh et al. (1985) and Rogers et al. (1988) did not prove any trend in their study. It could be presumed that the addition of magnesium oxide which was given by Armbel et al. (1988), Kishore et al.

(1996), Rogers et al. (1985), and The et al. (1985) provided stability to the pattern.

The trend of the ratio between acetate and propionate (A:P) revealed that an increased level of buffers made the ratio wider. Similar findings were observed by Mees and Merchen (1985), Rogers et al. (1985), Teh et al. (1985), Armbel et al. (1988), Johnson et al. (1988), and Kishore et al. (1996). The main reason could be due to the low production of propionate and the higher production of acetate in the rumen ecosystem.

The trend of development of acidity in rumen liquor with the regime of buffer showed that it had a negative correlation with the level of dose of buffers i.e., an increased level of buffer caused decreased acidity or increased pH. Stocks (1983), Rogers et al. (1985), Teh et al. (1985), Newbold et al. (1991) and Kishore et al.

(1996) recorded similar findings i.e., increased level of buffer inclusion in the diet caused to increase in ruminal pH or to decreased ruminal acidity (Kishore et al. 1996). It is fact that sodium bicarbonate and magnesium oxide both are alkaline and became a factor to increase the pH of the rumen liquor immediately, but higher pH in rumen liquor recorded was responsible for

more fibre degradation in the rumen (Erdman et al. 1980 and Kishore et al. 1998) and resulted in higher production of acetate and propionate, which caused to neutralize increased pH or decreased acidity. A similar trend was observed by Mees and Merchen (1985), Zhelev and Profirov, (1987), lee and Hsu (1992), and Kishore (1997).

Table 3B: Characteristics of the ruminal environment

Parameter	P-1	P-2	P-3	P-4	P-5	P-6	CD at 1%
pH	7.00 ±0.08 ^a	6.65 ±0.08 ^b	6.57 ±0.11 ^c	6.39 ±0.19 ^d	6.12 ±0.25 ^f	6.24 ±0.04 ^c	0.02
TVFA	95.4 ±1.8 ^a	93.1 ±1.6 ^b	93.0 ±1.5 ^b	93.3 ±1.5 ^b	93.9 ±1.9 ^b	95.7 ±2.2 ^a	0.9
Acetate (meq/l)	62.7 ±0.1	62.6 ±0.1	62.5 ±0.1	62.7 ±0.1	62.6 ±0.1	62.6 ±0.1	0.2
Butyrate (mg/l)	12.9 ±0.3 ^{ab}	13.1 ±0.4 ^a	13.0 ±0.3 ^{ab}	12.5 ±0.3 ^c	12.4 ±0.3 ^c	12.8 ±0.4 ^b	0.2
Isobutyrate (mg/l)	1.20 ±0.04 ^d	1.20 ±0.04 ^d	1.27 ±0.05 ^b	1.31 ±0.06 ^a	1.24 ±0.05 ^c	1.22 ±0.04 ^{cd}	0.02
Isovalerate (mg/l)	2.20 ±0.02	2.18 ±0.02	2.20 ±0.02	2.20 ±0.02	2.18 ±0.01	2.19 ±0.02	0.02
Propionate	25.2 ±0.2	25.1 ±0.2	25.1 ±0.02	25.2 ±0.2	25.2 ±0.2	25.2 ±0.2	0.1
Valerate (mg/l)	2.34 ±0.06 ^b	2.34 ±0.06 ^b	2.33 ±0.07 ^b	2.38 ±0.05 ^a	2.35 ±0.05 ^b	2.39 ±0.07 ^a	0.02
A:P ratio	2.49 ±0.02	2.49 ±0.02	2.49 ±0.02	2.49 ±0.02	2.49 ±0.02	2.49 ±0.02	0.01
Total-N (mg/l)	54.7 ±0.92	54.8 ±1.0	54.9 ±1.0	55.6 ±1.0	55.6 ±1.0	54.4 ±1.1	1.4
NH ₃ -N (mg/l)	7.56 ±0.10 ^{cd}	7.60 ±0.1 ^{bc}	7.53 ±0.07 ^d	7.66 ±0.04 ^a	7.52 ±0.03 ^d	6.65 ±0.02 ^{ab}	0.05
Urea-N (mg/ml)	3.36 ±0.08 ^d	3.39 ±0.08 ^c	3.37 ±0.09 ^{cd}	3.43 ±0.11 ^{ab}	3.42 ±0.09 ^b	3.45 ±0.12 ^a	0.02
Non-Ammonia and urea nitrogen (mg/ml)	43.8 ±0.9	43.9 ±1.0	43.5 ±1.1	44.6 ±1.0	44.6 ±1.0	43.1 ±1.1	1.6

^{abc} - Values bearing different superscripts differed significantly.

The content of total nitrogen in rumen liquor remained non-significant with the buffer regime in animal feeding. The trend in literature concluded that the increased dose of buffers in caprine nutrition declined total nitrogen in rumen liquor (Kishore et al. 1996). The difference may be due to the different animal species used for the experiment.

Decreasing pattern of the content of ammonia nitrogen in rumen liquor with an increased level of buffers included in the diet was observed in the present study. It was presumed that during the proteolytic activity, deamination took place in the rumen liquor by which proteins braked down to peptides, amino acids, and finally ammonia. Carbon dioxide, produced during the metabolism of carbohydrates, combined

with the ammonia and produced urea, Due to the addition of buffers in the diet microbial population (Shimada et al. 1989), which resulted in increased production of urea nitrogen in the rumen liquor. Similar results were recorded in the present investigation. As far as the content of non-urea and ammonia-nitrogen concentration in rumen liquid was concerned it remained unchanged with the increased level of the buffer. It could be due to the faster recycling of nitrogen to form microbial protein. However, results in this connection are not available in the literature to confirm the results.

The results of the rumen metabolism study indicated that pH declined, the concentration of total volatile fatty acid, butyrate and valerate, and A:P ratio also increased, and the concentration of acetate, propionate and ammonia nitrogen decreased in rumen liquor due to supplementation of buffer in calves' nutrition. The pattern of pH showed decreasing up to 10 hours and total volatile fatty acid up to 6 hours, and iso-butyrate, ammonia nitrogen, and urea nitrogen concentration in rumen content followed an equal pattern. Thus, the overall conclusion can be made that the addition of buffer in ruminant nutrition (buffer feed technology) was responsible to change the ruminal environment and thus it became helpful to produce surplus fat due to the appropriate pH and A:P ratio.

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