

# Impact of manipulated rumen fermentation using buffers on the behavior of cross-bred calves

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### Abstract

Twelve cross-bred calves (131-221d; 57.5-93.9kg) were divided into three groups. One animal from each group was randomly allotted to one of the four treatments viz.  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$ . The animals were given grass mixture and wheat straw. The concentrate mixture contained barley grain and mustard cake. The amount of each feed ingredient for each animal was calculated based on the NRC feeding standard. Apart from this, each animal also received 20g of common salt and 25g of mineral mixture. 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, 0.40 and 0.20 and 0.60 and 0.30 per cent of LW were given in  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$  respectively. During the excretion behavioral trial frequency of urine and fecal excretion during the whole day was divided into four quarters viz. 0.00-6.00 hours  $(Q_1)$ , 6.00-12.00 hours  $(Q_2)$ , 12.00-18.00 hours  $(Q_3)$ , and 18.00-0.00 hours  $(Q_4)$  were recorded. During the trial animal intakes, ruminates, or rests in each quarter of the day were recorded after 15 minutes. Statistical analysis was done using factorial RBD. Results of this study focused that the frequency of fecal excretion reduced and urine excretion increased, the standing period became longer, rumination cycle and mastication decreased due to the addition of buffer in the diet. The rumination cycle was completed in a short period and the number of mastication involved in one rumination cycle was less in the last quarter. The time used to chew one bolus was longer in  $Q_2$  and  $Q_3$ . Time taken per mastication was shorter in the afternoon session ( $Q_3$ ). The overall conclusion can be made that the addition of buffer in ruminant nutrition (buffer feed technology) was responsible to reduce rumination in calves.

Keywords: Behaviour, Buffers, Cross-bred calves, Magnesium oxide, Rumination, Sodium bicarbonate.

## Introduction

Ingestion behavior, through both diet selection and food ingestion, is a major way that an animal attempts to fulfill its metabolic requirements and achieve homeostasis. In domestic herbivores across a wide range of production practices, voluntary feed intake is arguably the most important factor in animal production, and a better understanding of systems involved in intake regulation can have important practical implications in terms of performance, health, and welfare (Ginane et al 2015). The feeding behavior of animals reflects the current motivation consequence of the integration of an individual, over time of many factors including sensorial, metabolic, and physiological signals. Animals may resolve the challenge of obtaining adequate nutrients in different ways from each other which may partly be due to different set-points in regulatory pathways (Provenza et al 2003). Other factors influencing the feeding behavior of ruminants include grazing management practices, type of vegetation and season, breed and stage of production, group size, and properties of diets fed in confinement (Goetsch et al 2010).

Improving the monitoring of rumination in cattle could help in assessing the welfare status and their risk of acidosis and other physical disorders. This method is very much easy, quick, and accurate to find out abnormalities in the physical state of the animals. Accurate monitoring of the rumination behavior of cattle using IMU signals from a mobile device. Rumination represents 5 to 9h/d for cattle (Vallentine 2001). It is a cyclic process that completes the chewing of fibrous ingested feed after it underwent anaerobic fermentation by microbes in the rumen. A cycle begins with the regurgitation of a rumino-reticular bolus followed by semi-circular jaw movements and ends with the deglutition (Jarrige et al 1995).

Buffer feed technology reveals to add buffers in animal nutrition with the object to keep pH constant in rumen fluid. The use of sodium bicarbonate and magnesium oxide in combination showed better potential in terms of milk yield (Elckelberger et al 1985), the yield of milk constituents (Singh et al 1998), average daily gain (Kishore et al 1998), chevon quality (Chandra et al 1997) and biodiversification of different nutrients from feed to milk (Kishore et al 1997).

But before offering the buffer directly to the cow a thorough study on calves was needed to fix up the suitable level of the buffer. This investigation was, therefore, planned as an effort to study the effect of manipulation of rumen fermentation using buffers in cross-bred calves on their behavior viz. consumption of feed, rumination, excretion, and their general behavior.

# Materials and methods

Twelve cross-bred calves (age 131-221d; LW 57.5-93.9kg; heart girth 91-105cm; body length 75-92cm; height 78-93; collar length 52-71; tail length 42-57cm) were selected from the herd of college Dairy Farm and de-wormed. They were housed in a calf shed having separate feeding mangers and water troughs for individual feeding and large sized open enclosure for exercise. Those were given grass mixture and wheat straw. The concentrate mixture contained barley grain and mustard cake (Table-1). The amount of each feed ingredient for each animal was calculated based on the NRC feeding standard along with 20g common salt and 25g mineral mixture.

Based on age and weight the animals were divided into three groups. One animal from each group was randomly allotted to one of the four treatments viz.  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$ . 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, 0.40 and 0.20 and 0.60 and 0.30 per cent of LW were given in  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$ , respectively.

During the excretion behavior trial, (after 35 days of the experiment, for four consecutive days) frequency of urine and fecal excretion during the whole day was divided into 4 quarters vi. 0:00-6:00 (Q1), 6:00-12:00 (Q<sub>2</sub>), 12:00-18:00 (Q<sub>3</sub>) and 18:00-0:00 hours (Q<sub>4</sub>) were recorded. Excreted urine and feces were also collected and weighed individually after each quarter. During the rumination behavior trial (after 40 days of the experiment, for 4 consecutive days) animals were studied at the time when they were in ruminating condition strictly for 20 rumination cycles in each quarter of the day.

Parameter	Straw	Grass	<b>Barley</b> grain	Mustard cake	Buffer
DM*	91.20	28.20	90.30	88.20	100.00
ТА	10.00	10.00	9.30	7.50	100.00
OM	90.00	80.40	90.70	92.50	0.00
СР	3.10	6.90	10.20	35.00	0.00
EE	1.00	1.80	2.60	2.00	0.00
ТСНО	85.90	80.90	77.90	55.50	0.00
GE**	3.98	4.06	4.20	4.62	0.00
CF	35.20	44.60	10.10	7.90	0.00
NFE	50.70	36.10	67.80	47.60	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	18.00
Magnesium	0.10	0.30	0.30	0.40	19.50
Potassium	5.80	0.90	0.50	1.20	0.00
NDF	74.20	71.10	56.80	53.50	0.00
ADF	51.10	38.20	42.00	43.90	0.00
Hemicellulose	23.10	32.90	14.80	9.60	0.00
Cellulose	43.00	31.90	37.00	33.00	0.00
Lignin	2.80	2.70	2.30	2.00	0.00
Cell content	25.80	28.90	43.20	46.50	0.00

 Table 1: Chemical composition of feeds (%DM)

\* Fresh basis

\*\* Mcal/kg DM

During the period of total time in one rumination cycle, time used for mastication, pause between two cycles, and the number of mastication per cycle were noted in both the conditions either sitting or standing separately. During the feeding and general behavior trial (after 50 days of the experiment, for 4 consecutive days) animals were kept under observation (after 15 m intervals) to study their sitting and standing behavior. Those were also observed for eating, ruminating, or resting activities. Observed data were subjected to statistical analysis using standard procedures (Snedecor and Cochran 1994).

## **Results and Discussion**

It has been observed that after 48 to 72 hours of the first buffer regime, the animals suffered from bloat followed by diarrhea

for one day which was cured automatically. Diarrhea was frequent in the treatment in which buffer was offered at the highest level, the animals could complete just an adaptation period on the  $21^{st}$  and the  $22^{nd}$  day died because of severe diarrhea followed by acute bloat and sudden death. Animals in T<sub>3</sub> also showed similar symptoms and died within the 8<sup>th</sup> and 9<sup>th</sup> weeks of the experiment. Severe diarrhea followed by acute bloat had been recorded by Kishore (1997) with kids and lactating goats because of including buffers in the diet.

The DMI remained unaffected because of the addition of buffers in the diet (Table 2) but digestible nutrients of DM, CP, and DE increased with the increased level of buffers regime. These results could be registered because of the increased digestibility coefficients of the respective nutrients. 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, Kishore et al 1996, Kishore 1997, Kishore et al 1998). The reason could be that the addition of buffers increases liquid turnover rate, solid retention time (Stocks 1983), rate of dilution of ruminal fluid, and out flow rate of duodenal passage of digesta (Dewhurst and Webster, 1992). Water intake was observed to be related directly proportional to the level of buffers inclusion in the diet. The reason for the same could be the addition of sodium ions as observed by Fattman et al (1981), Stocks (1983), Rogers et al (1985), Johnson et al (1988), Kishore et al (1996), Kishore (1997) and Kishore et al (1998) with sodium carbonate and Fettman et al (1984) with sodium chloride regime.

Nutrient	Unit	$T_1$	$T_2$	T <sub>3</sub>	CD at 5%
DM	g per kgW <sup>0.75</sup>	78.1±0.9	84.3±4.6	85.1±1.5	11.20
	kg per 100 kgLW	2.59±0.07	$2.69{\pm}0.08$	$2.73 \pm 0.08$	0.39
אחת	g per kgW <sup>0.75</sup>	$42.8 \pm 0.7^{(B)}$	$49.4 \pm 3.4^{(A)(B)}$	521.±1.5 <sup>(A)</sup>	7.90
DDM	kg per 100 kgLW	$1.42 \pm 0.03$	$1.57{\pm}0.04$	$1.67 \pm 0.05$	0.21
СР	g per kgW <sup>0.75</sup>	$4.6 \pm 0.4^{(B)}$	$6.9{\pm}0.4^{(A)}$	$7.6 \pm 0.4^{(A)}$	1.70
	g per 100 kgLW	153.2±12.2 <sup>(B)</sup>	231.2±15.3 <sup>(A)</sup>	$244.9 \pm 17.1^{(A)}$	62.90
DCP	g per kgW <sup>0.75</sup>	$2.7 \pm 0.2^{(B)}$	$4.1 \pm 0.2^{(A)}$	$4.9{\pm}0.2^{(A)}$	1.10
	g per 100 kgLW	$88.7 \pm 7.7^{(B)}$	138.8±8.4 <sup>(A)</sup>	155.7±8.9 <sup>(A)</sup>	38.00
DE	kcal per kgW <sup>0.75</sup>	182.5±1.5 <sup>(B)</sup>	194.4±6.6 <sup>(A)(B)</sup>	208.4±8.4 <sup>(A)</sup>	16.90
	meal per 100 kW	6.05±0.13 <sup>(B)</sup>	6.45±0.15 <sup>(A)</sup>	$6.65 \pm 0.08^{(A)}$	0.35

Table 2: Voluntary intake

 $^{(A)(B)}$ -Values bearing different superscripts among the row differed significantly i.e. p<0.05.

The frequency of excretion was decreased but urine excretion increased (Table 3) due to the buffers regime. Decreased frequency of fecal excretion could be increased DM digestibility (Hemminger and Krichgassner 1972, Toro et al 1982, Johnson et al 1988, Solorzano et al 1989, Kishore et al 1996, Kishore 1997, Kishore et al 1998). Increased frequency of urine excretion may be due to increased water intake (Fattman et al 1981, Stocks 1983, Rogers et al 1985, Johnson et al 1988, Kishore et al 1996, Kishore 1997, Kishore et al 1998) with sodium carbonate. The eating period remained unaffected but the standing period was increased and the sitting period was decreased in the animals because of the administration of buffers in the diet. There was no evidence in the literature for

comparison of such findings. Increased intake of digestible energy could be a possible reason to increase standing and decreased sitting periods. The unaffected eating period could be due to nonsignificant DM intake in the animals fed on the diet without and with buffers (Table 2). The rumination period and the number of ruminations and mastication per day were reduced because of the buffers regime. The reason could be that pH of ruminal fluid which is regulating factor of rumination was less fluctuating because of the presence of buffer. Yang and Beauchemin, (2006) dietary suggested that NDF and fermentable OM intake are critical in regulating rumen pH, Increased NDFD is increased because of the addition of buffers in the diet (Kishore 1997). Dietary particle size, expressed as per NDF, was a reliable indication of chewing activity. Resting period during which the animals neither took food nor ruminated increased because

of the introduction of the treatments which was due to the interactive effect of DMI and buffering action in the rumen environment.

Denometers	Treatment						
Parameters	Ι	II	III	CD			
Frequency of fecal excretion (n/d)	16.17±0.36 <sup>(A)</sup>	$12.33 \pm 0.36^{(B)}$	$13.00 \pm 0.41^{(B)}$	0.80**			
Frequency of urine excretion (n/d)	$12.00 \pm 0.85^{(B)}$	14.33±0.36 <sup>(A)</sup>	15.33±0.36 <sup>(A)</sup>	2.28*			
Standing period (m/d)	923.33±10.63 <sup>(B)</sup>	886.67±4.91 <sup>(B)</sup>	1038.33±46.21 <sup>(A)</sup>	102.76*			
Sitting period (m/d)	516.67±10.63 <sup>(A)</sup>	553.33±4.91 <sup>(A)</sup>	$401.67{\pm}46.21^{(B)}$	102.76*			
Eating period (m/d)	405.00±18.17	426.67±42.97	448.33±52.30	48.42*			
Rumination period(m/d)	501.67±18.91 <sup>(A)</sup>	$300.00 \pm 12.47^{(B)}$	$321.67 \pm 51.69^{(B)}$	78.00**			
Resting period (m/d)	630.00±12.47 <sup>(B)</sup>	713.33±32.52 <sup>(A)</sup>	$670.00 \pm 37.93^{(A,B)}$	93.00*			
No rumination cycle(n/d)	549.83±56.34 <sup>(A)</sup>	$300.63 \pm 10.70^{(B)}$	299.12.76 <sup>(B)</sup>	87.86**			
No mastication (n/d)	26597±909 <sup>(A)</sup>	$14131 \pm 691^{(B)}$	15209±999 <sup>(B)</sup>	4008**			
Rumination cycle in standing (s)	$45.88{\pm}1.84^{(B)}$	59.53±2.15 <sup>(A)</sup>	64.02±2.11 <sup>(A)</sup>	4.81**			
Rumination cycle in sitting (s)	$50.50{\pm}2.38^{(B)}$	63.53±1.73 <sup>(A)</sup>	$67.03 \pm 1.70^{(A)}$	6.67**			
No mastication in one cycle in standing (n)	$40.20 \pm 1.60^{(B)}$	$43.86 \pm 1.76^{(B)}$	49.18±2.08 <sup>(A)</sup>	4.00**			
No. Mastication in one cycle in sitting (n)	$45.95 \pm 2.21^{(B)}$	51.65±1.84 <sup>(A)</sup>	53.85±2.00 <sup>(A)</sup>	5.54*			
Time per bolus in standing (s)	$42.38 \pm 2.08^{(C)}$	$55.04 \pm 2.15^{(B)}$	60.18±2.02 <sup>(A)</sup>	4.77**			
Time per bolus in sitting (s)	$46.80 \pm 2.44^{(B)}$	59.58±1.72 <sup>(A)</sup>	62.28±1.65 <sup>(A)</sup>	6.65**			
Time per mastication in standing (s)	$1.05 \pm 0.02^{(B)}$	1.27±0.01 <sup>(A)</sup>	1.23±0.04 <sup>(A)</sup>	0.07**			
Time per mastication in sitting (s)	1.02±0.01 <sup>(B)</sup>	1.17±0.02 <sup>(A)</sup>	1.17±0.02 <sup>(A)</sup>	0.09**			
Pause per cycle in standing (s)	4.16±0.13	4.49±0.11	4.10±0.23	0.55*			
Pause per cycle in sitting (s)	3.70±0.11 <sup>(B)</sup>	3.95±0.11 <sup>(B)</sup>	4.75±0.18 <sup>(A)</sup>	0.55**			

## Table 3: Animal behaviour

## Photoperiodic effect on animal behavior

Findings during the rumination behavior study trial resulted in the time taken to complete one rumination cycle, the number of mastication per cycle, time used to masticate one bolus, pause between two rumination cycles, and time per mastication were increased in both of the positions i.e. sitting and standing, due to inclusion of buffers in animal feed. The literature is almost scanty on this line to compare the observations.

During four quarters of the day frequency of fecal excretion (Table 4) was lower in the early morning hours  $(Q_1)$  because during this period the animals used more time for taking rest or sleeping. The frequency of urine excretion was not affected because of the photo-periodic effect. The animals were in sitting positions during the early morning session and standing positions in the morning session, the reason could be again that during  $Q_1$  they were using more time for taking rest or sleeping whereas in Q<sub>2</sub> they were using more time for taking food and exercise. Time used for taking food during late night hours (Q4) was lowest whereas the time for rumination in night hours (Q1 & Q4), was highest. This is a well-known fact that the regulating factor of rumination is the pH of rumen content i.e. reduced pH increases rumination. Increased rumination in the night hours was due to the pH of rumen liquor being decreased after 12 hrs of feeding due to continued fermentation and thus increased rumination. The resting period was lowest in the morning hours  $(Q_1)$ . The literature on this line is guite scanty and as such the findings could not be compared.

The number of rumination cycles and mastication was recorded to be lowest in the early morning hour which could be because of the reason that the animals were busy

taking food or exercise. During this period they used very less time for rumination because of the increased time interval after feeding which may be due to the fall in pH during this period due to the fermentation of feed (Kishore et al 1996). The length of rumination cycles in both the positions i.e. standing and sitting were shortest in the last half of the day  $(O_3 \& O_4)$ . No clear-cut trend could be observed in terms of the number of mastication per rumination in two positions. Time is taken to masticate one bolus was prolonged in the last two quarters of the day  $(Q_3 \& Q_4)$  in both positions. During the standing position, time per mastication was shortened during evening hours  $(O_3)$  whereas it remained similar round the day in the sitting position. The pauses between two rumination cycles in both positions were not affected because of the impact of photoperiodism. The findings could not be compared with the literature due to want of information in this respect.

## Conclusion

Results of this study focused that the frequency of fecal excretion reduced and urine excretion increased, the standing period became longer, rumination cycle and mastication decreased due to the addition of buffer in the diet. The rumination cycle was completed in a short period and the number of mastication involved in one rumination cycle was less in the last quarter. The time used to chew one bolus was longer in  $Q_2$ and Q<sub>3</sub>. Time taken per mastication was shorter in the afternoon session  $(O_3)$ . The overall conclusion can be made that the addition of buffer in ruminant nutrition (buffer feed technology) was responsible to reduce rumination in calves.

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#### **Table 4 Animal behavior**

D (	Period					
Parameters	Q1	Q2	Q3	Q4	CD	
Frequency of fecal excretion (n/d)	$2.89{\pm}0.23^{(C)}$	$3.44{\pm}0.12^{(B)}$	$4.06{\pm}0.29^{(A)}$	$3.44{\pm}0.28^{(B)}$	0.51	
Frequency of urine excretion (n/d)	3.44±0.24	3.17±0.21	3.44±0.12	3.83±0.32	0.77	
Standing period (m/d)	234.44±12.49 <sup>(B)</sup>	310.56±15.12 <sup>(A)</sup>	$242.78{\pm}10.78^{(B)}$	161.67±7.05 <sup>(C)</sup>	25.69	
Sitting period (m/d)	125.56±13.66 <sup>(B)</sup>	49.44±8.59 <sup>(C)</sup>	117.22±8.93 <sup>(B)</sup>	198.33±15.19 <sup>(A)</sup>	25.68	
Eating period (m/d)	98.89±6.18	163.89±9.90	99.44±3.14	64.44±12.21	24.51	
Rumination period(m/d)	125.00±20.40 <sup>(A)</sup>	47.22±6.39 <sup>(C)</sup>	88.89±4.83 <sup>(B)</sup>	113.88±7.97 <sup>(A)</sup>	14.34	
Resting period (m/d)	168.33±6.19 <sup>(A)(B)</sup>	148.89±12.29 <sup>(B)</sup>	171.67±8.43 <sup>(A)</sup>	182.22±8.06 <sup>(A)</sup>	22.45	
No rumination cycle(n/d)	125.58±23.96 <sup>(A)</sup>	47.29±8.02 <sup>(C)</sup>	98.17±7.75 <sup>(B)</sup>	136.49±16.38 <sup>(A)</sup>	16.15	
No mastication (n/d)	6148±1126 <sup>(A)</sup>	2345±371 <sup>(C)</sup>	4489±228 <sup>(B)</sup>	5663±510 <sup>(A)</sup>	737	
Rumination cycle in standing (s)	57.87±2.83 <sup>(B)</sup>	64.47±3.45 <sup>(A)</sup>	55.66±2.80 <sup>(B)</sup>	47.91±2.30 <sup>(C)</sup>	3.54	
Rumination cycle in sitting (s)	67.04±2.06 <sup>(A)</sup>	60.99±3.09 <sup>(A)(B)</sup>	56.38±3.17 <sup>(B)</sup>	57.01±3.33 <sup>(B)</sup>	4.10	
No mastication in one cycle in standing (n)	42.77±1.23 <sup>(B)</sup>	51.17±1.66 <sup>(A)</sup>	46.83±2.11 <sup>(A)(B)</sup>	36.89±1.67 <sup>(C)</sup>	2.94	
No. Mastication in one cycle in sitting (n)	563.37±2.00 <sup>(A)</sup>	$49.79 \pm 2.48^{(B)}$	47.01±2.01 <sup>(B)</sup>	$48.77 \pm 2.60^{(B)}$	5.54	
Time per bolus in standing (s)	53.59±2.83 <sup>(B)</sup>	61.08±3.16 <sup>(A)</sup>	51.80±2.81 <sup>(B)</sup>	43.68±2.34 <sup>(C)</sup>	3.51	
Time per bolus in sitting (s)	62.86±1.93 <sup>(A)</sup>	56.83±2.96 <sup>(A)(B)</sup>	52.39±3.16 <sup>(B)</sup>	52.81±3.26 <sup>(B)</sup>	4.89	
Time per mastication in standing (s)	1.23±0.05 <sup>(A)</sup>	1.19±0.04 <sup>(A)</sup>	1.12±0.02 <sup>(B)</sup>	1.19±0.05 <sup>(A)</sup>	0.00	
Time per mastication in sitting (s)	1.12±0.03	1.16±0.04	1.10±0.03	1.08±0.02	0.08	
Pause per cycle in standing (s)	4.63±0.17	4.28±0.17	3.86±0.16	4.23±0.21	0.49	
Pause per cycle in sitting (s)	4.19±0.25	4.16±2.25	3.99±0.12	4.20±0.22	0.41	

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