



Bovine Ruminal Acidosis: Rumens Liquor Profile and its Therapeutic Management

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ABSTRACT

Ruminal acidosis is one of the common condition that oftenly found on account of ingestion of excessive amounts of readily fermented carbohydrate by ruminants. The present study was planned to characterize ruminal fluid and its therapeutic efficacy of suitable probiotics in the management of ruminal acidosis. A total of 265 animals were screened from different areas of Jammu region including the cases that were presented in Teaching Veterinary Clinical Complex, Faculty of Veterinary Sciences and Animal Husbandry, R.S. Pura. Forty three (43) animals were found positive for acidosis. The overall prevalence of ruminal acidosis including sub-acute form in Jammu region was 16.22%. The mean value of all acidotic animals was found 5.11 ±0.06. The odour of ruminal fluid of acidotic animals was pungent, sour and fishy whereas the consistency was watery. The mean Methylene blue reduction test time and the protozoal count in rumen liquor in acidotic animal were found to be of 9.50±.53 min and 0.66×10⁵/ml respectively. The microscopic evaluation of Gram-stained rumen fluid of acidotic animals was increased Gram +ve. The altered ruminal fluid parameters were returned to normal following fortnight probiotics treatment.

Keywords: Ruminal acidosis, Therapeutic management, Probiotics

Ruminal acidosis occurs when there is a sudden excess intake of highly fermentable carbohydrates, primarily starches and sugars. This normally manifests when feedlot cattle without proper adaptation are rapidly transitioned from roughage to high concentrate diets, or when dairy cow intake during transition pre- and post-partum is erratic (Beauchemin & Penner, 2009). It is also a exhibition of the total load of readily fermentable carbohydrates present in the rumen (Oetzel, 2003), since dairy cows often experience sub acute ruminal acidosis at the time of maximum carbohydrate consumption (Penner *et al.*, 2007). Bovine ruminal acidosis is an important alimentary tract disease which results from feeding of highly fermentable feeds to increase productivity. Introduction of highly fermentable starch into rumen stimulates the growth of most ruminal bacteria, thereby increasing production of volatile fatty acids if the transition from a forage to cereal grain diet is too abrupt or if the particle size of concentrate ration is too small, microbial population becomes unstable, leading to

production of lactic acid and causing acidosis (Khafipour *et al.*, 2009). Ruminal pH fluctuates considerably in one day period and is influenced by the intake of total fermentable carbohydrate, inherent capacity of the animal to provide bufferic condition, and rates of utilization and absorption of volatile fatty acids. If the absorptive capacity of the ruminal wall is impaired by abnormal ruminal papillae or rumenitis, then there is animal's ability to maintain a stable ruminal pH is affected. However, when ruminal pH drops below 5.6, VFA absorption is enhanced because VFA become more protonated or undissociated which increases absorption rate. Ruminants, regardless of age, breed, and sex are susceptible to overeating with grains and carbohydrates (Radostitis *et al.*, 2007).

Ruminal pH is a critical factor in the normal and stable function of the rumen because of its profound effect on microbial populations and fermentation products, and on physiological functions of the rumen, mainly motility

and absorptive function. Therefore, nonphysiological accumulation of organic acids and consequent reduction in pH below the normal (<5.6) has significant impact on microbial activity, rumen and animal productivity and health. When the ruminant abruptly ingests large amounts of readily fermentable carbohydrates (RFCs) or when the period of adaptation to RFCs is insufficient, RFCs are suddenly fermented and volatile fatty acids (VFAs) are accumulated, resulting in the rumen pH to drop below 5.5 for a prolonged period and creating the condition of fermentative acidosis (Ding and Xu, 2003). In this condition, lactic acid-producing bacteria, such as *Streptococcus bovis* and *Lactobacillus spp.*, are proliferated, leading to the accumulation of lactic acid, which is known as lactic acidosis (Gozho *et al.*, 2007). It is a common Alimentary tract disorder causing severe economic losses to the dairy industry due to significant reduction in milk yield with high mortality rate in acute cases (Patil *et al.*, 2008).

A little rather not much work has been carried out in Jammu region of J&K state, India on this aspect. Keeping in view importance of ruminal acidosis in bovines, the present study was designed to evaluate the therapeutic efficacy of suitable probiotics in reducing the risk of lactic acidosis in the high yielding cross-bred dairy.

MATERIALS AND METHODS

The aim of the present study was to characterize ruminal fluid of ruminal acidosis in dairy animals of Jammu region that included organized as well as unorganized dairy farms.

Evaluating therapeutic efficacy of suitable probiotics in the management of clinical cases of acidosis in cattle was another important objective of my study that offers the possibility to recommend probiotics as an alternative to conventional treatment of acidosis. A total of 265 animals were screened from different sources (Table 1).

Rumen liquor analysis

Before rumen liquor examination, all animals were examined clinically that included general assessment of the health status by the inspection of mucous membrane and auscultation of the heart and lungs. Rumen liquor was collected by a standard protocol i.e. rumenocentesis technique. Rumen liquor was examined for pH (using pH paper strips) immediately after sample was drawn, and physical characteristics such as (colour, odour and viscosity) were also recorded. Other parameters of ruminal fluid like MBRT, No. of protozoa and protozoal motility were also examined as per the method described Brar *et al.* (2014).

Evaluating the therapeutic efficacy of suitable probiotics

Acidotic animals were treated by feeding a probiotic containing *Saccharomyces cerevisiae* with dose @ 5000 million CFU/animal/day and *Aspergillus oryzae* with dose @ 50 million CFU/animal/day for consecutive 14 days. Changes in ruminal fluid parameters were recorded before and after feeding probiotics to affected animals and

Table 1: Details of Screened animals from different areas of Jammu region

Sl. No	Source(s)	No. of animals screened	Status		
			Lactating		Non-lactating
			Early	Mid	
01	Teaching Veterinary Clinics Campus, F.V.Sc &A.H, R.S.Pura	27	07	05	15
02	Areas in and around R.S.Pura (Kirpind, Simbal, Dablehar etc.)	70	13	14	43
03	Govt. Sub-district Veterinary Hospital ,R.S. Pura.	25	06	05	14
04	Dairy unit ILFC, F.V.Sc & AH, R.S Pura Campus.	28	10	08	10
05	Dairy animals in different farms of Jammu district as well as at farmers houses	115	28	26	61
			64	58	
	Total no. screened	265	122	143	

were compared with normal profile of control groups. In severely acute cases routine (conventional) and supportive treatment was carried out as per the clinical status of the animal. Data was statistically analyzed by standard method, using SPSS software.

RESULTS AND DISCUSSION

Ruminal acidosis is the consequence of feeding high grain diets to ruminant animals, who are adapted to digest and metabolize predominantly forage diet. Feeding diet that is progressively higher in grain tends to increase milk production. However, short-term gains in milk production are often substantially or completely negated by long-term compromises in cow health when high grain diets are fed. Compromises in dairy cow health due to ruminal acidosis are a concern not only for economic reasons, but also for animal welfare.

Rumen liquor profile

The minimum pH values of rumen liquor was found 5.1, whereas maximum pH was 7.0. Eight animals showed

a ruminal pH value of ≤ 5 , seventeen showed pH 5-5.5, eighteen showed pH 5.5-6.2 and two hundred twenty two animals showed pH > 6.2. The mean value of ruminal pH of all acidotic animal was found 5.11 ± 0.06 . Rumen liquor parameters are shown in Table 2.

Rumen liquor pH

The pH of rumen liquor of animals suffering with ruminal acidosis (i.e. acidotic animals) under study group was 5.11 ± 0.06 compared to healthy control group i.e. 6.65 ± 0.12 . Animals treated with probiotics showed significant ($p < .05$) increase in rumen pH at 7th day i.e. 5.95 ± 0.04 and it reached to 6.67 ± 0.09 on 14th day. The examination of the rumen fluid pH was the core-point of the investigation since it determined the presence or absence of acidosis in the screened animals. In the present study, there was significant ($p < 0.05$) decrease in pH of ruminal fluid (5.08) which might be due to increased fermentation of carbohydrates and accumulation of volatile fatty acids in the rumen. This is in accordance with that of (Ding and Xu, 2003) who reported pH of ruminal fluid below (5.5) in ruminal acidosis. The rumen pH was comparatively higher

Table 2: Rumen liquor profile in different trial groups at 0, 7th and 14th days

Parameters	Healthy Group (pH>6.8) (n=6)	Pretreated Group (pH 5.1-6) (n=6)	Posttreatment Group (n=6)	
			7 th day	14 th day
			Rumen pH	6.65 ± 0.12^c
Colour	Green - 4(66%) Yellow B-2(34%)	Milky -3(50%) Milky grey - 3(50%)	Milky grey-2(34%) Yellow Brown-4(66%)	Green -5(84%) Yellow Brown -1(16%)
Odour	Aromatic -6(100%)	Pungent -5(84%) Fishy - 1(16%)	Aromatic -3(50%) Pungent -3(50%)	Aromatic -6(100%)
Consistency	SV -6(100%)	Watery -6(100%)	SV -2(34%) Watery -4(66%)	SV -6(100%)
MBRT(min)	3.58 ± 0.15^a	9.75 ± 0.53^c	6.58 ± 0.14^b	3.63 ± 0.16^a
No. of Protozoa (Average Protozoa/field) $\times 10^3$ / ml	5.17 ± 0.31^c	$.66 \pm .00^a$	3.50 ± 0.22^b	5.50 ± 0.22^c
Protozoa motility	4.17 ± 0.31^c	1.33 ± 0.21^a	2.50 ± 0.22^b	3.33 ± 0.33^b
Microbial Composition	G -ve > G +ve in (100%)	G +ve > G -ve in (100%)	G -ve > G +ve - in (50%) G +ve > G -ve - in (50%)	G -ve > G +ve in (100%)

* Mean \pm S.E bearing different superscript differ significantly ($P < 0.05$); (SV-Slightly viscous), (G-ve & G +ve-Gram negative & Gram Positive respectively).

than reported by (Desai *et al.* 1999). It could be due to the time lag between the time of induction of acidosis to the time of bringing the animal to the hospital.

Colour of rumen liquor

Subsequently colour of rumen liquor colour of animals suffering with ruminal acidosis (i.e. acidotic animals) was noted down. It was observed that 50% showed milky colour and 50% showed milky grey colour when compared to healthy group the colour of rumen liquor was found in green to yellow brown. Rumen liquor colour was restored to normal colour after probiotics treatment in the affected animals.

Odour of rumen liquor

It was observed that odour of most of animals suffering with ruminal acidosis under study group were pungent, sour and fishy when compared to healthy group which was aromatic in nature. The odour of rumen liquor was restored to normal following post probiotics treatment.

Consistency of rumen liquor

The rumen liquor was watery in consistency in most of the animals suffering with ruminal acidosis and the consistency of rumen liquor was restored to normal i.e. slightly viscous following post probiotics treatment.

Methylene blue reduction test (MBRT)

Animals with a ruminal pH of 5.1-6 in the study group showed mean MBRT time of $9.50 \pm .53$ min whereas in healthy control group it was $3.58 \pm .15$ min. The MBRT time in animals treated with probiotics showed significant decrease on 14th day i.e. 3.63 ± 0.16 min. There was a significant ($p < 0.05$) increase in methylene blue reduction test (MBRT) time in acidotic animals i.e. 9.75 min. The increase in MBRT time could be attributed to the destruction of normal microflora and their decreased oxidation reduction potential in acidosis. Our results were in concurrence with that of (Patil *et al.* 2008) who also reported the MBRT time increase in cattle suffering with ruminal acidosis.

Protozoal activity

There was significant ($p < 0.05$) increase in the protozoal count in rumen liquor of animals suffering with ruminal acidosis (i.e. acidotic animals) under study group from 0 day i.e. 0.66×10^5 /ml to $5.50 \pm .22 \times 10^5$ /ml on 14th day following post probiotics treatment. Protozoal motility has improved in acidotic animals from sluggish movement (+) to rapid movement (+++) following post probiotics treatment. There was significant decrease ($p < 0.05$) in total protozoal (0.66×10^5 /ml) count and protozoal motility (sluggish) in the affected animals which might be due to high concentration of rumen lactate and low rumen pH because rumen protozoa are sensitive to changes in pH as multiplication and motility of the rumen protozoa are dependent on hydrogen ion concentration. The findings were in agreement with the results of (Patil *et al.* 2008) who reported decreased total protozoal count (0.36×10^5 /ml) in animals during acidosis. The results in the present study were also justified by other workers (Mohan *et al.* 2015).

Microbial composition

The microscopic evaluation of Gram-stained rumen fluid of animals suffering with ruminal acidosis (i.e. acidotic animals) under study showed significant increase in Gram positive bacteria as compared to healthy control group where there was more number of Gram negative bacteria. In post probiotics treatment rumen fluid showed significant increase in Gram negative than Gram positive bacteria. Microscopic evaluation of Gram-stained rumen fluid of animals in this study having rumen pH in the range of 5.2-6 showed absolute increase in Gram positive bacteria. The increase in Gram positive bacteria could probably due to resistant nature of these bacteria at low pH environment and secondly due to removal of inhibitory products in rumen fluid at low rumen pH. These results were in concurrence with that of (Patil *et al.*, 2008) who concluded the significant increase in Gram positive bacteria and decrease in gram negative bacteria in acidotic cattle, also the results were in consistent as reported by (Shukla *et al.*, 2004; Chehreh *et al.*, 2014).

Therapeutic evaluation

Acidotic animals revealed gradual improvement and recovered following post probiotics treatment after 14th

day. There was significant increase in rumen pH and gradual reduction in MBRT, moreover hematological and biochemical parameters were restored to normal. The gradual improvement and recovery of acidotic animals could be attributed that *Aspergillus oryzae* extracts and *Saccharomyces cerevisiae* culture might have stimulated lactate uptake by ruminal bacteria such as *Megasphaera elsdenii* and *Selenomonas ruminatum*. Our results were in concurrence with that observed by (Callaway and Martin, 1997) who demonstrated that soluble components in *Aspergillus oryzae* and another *Saccharomyces cerevisiae* culture filtrate had stimulated lactate uptake by *Selenomonas ruminatum* and *Megasphaera elsdenii*.

Our results were in conformity with the results of Guedes *et al.* (2008) who studied the effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation in non-lactating cows. They reported that *Saccharomyces cerevisiae* has the potential to reduce the risk of rumen acidosis in commercial cattle fed on maize silage based diet and, if used at the highest level, could be of further benefit due to increased fibre degradation of low quality maize silages. Similarly, Vyas *et al.* (2014) demonstrated the importance of yeast (*Saccharomyces cerevisiae*) and its positive effects, irrespective of its viability, in reducing the severity of sub acute ruminal acidosis. Goto *et al.* (2016) suggested that on administration of 20 to 50 g of a multi-strain bacterial probiotic (BP) for 7 days has improved the low pH and high lactic acid level of the ruminal fluid in cattle with sub acute ruminal acidosis. Our results were also supported by Waldrip *et al.* (1993) who reported that *Aspergillus oryzae* could stimulate lactate utilization by rumen bacteria *Megasphaera elsdenii*.

CONCLUSION

Out of 265 screened animals from different areas of Jammu region only forty three (43) animals were found positive for ruminal acidosis. The mean value of all acidotic animals was found 5.11 ± 0.06 . The odour of ruminal fluid of acidotic animals was pungent, sour and fishy whereas the consistency was watery. The mean MBRT time and the protozoal count in rumen liquor in acidotic animal was found to be of 9.50 ± 0.53 min and 0.66×10^5 /ml respectively. The microscopic evaluation of Gram-stained rumen fluid of acidotic animals showed increased Gram +ve bacteria. The combination of probiotics used *Saccharomyces*

cerevisiae with dose @ 5000 million CFU/animal/day and *Aspergillus oryzae* with dose @ 50 million CFU/animal/day is sufficient enough to correct clinical cases of ruminal acidosis with pH in the range of (5.1-6) in fortnight period. Further the prescribed probiotics (*Saccharomyces cerevisiae* and *Aspergillus oryzae*) can be recommended as an alternative to conventional treatment of ruminal acidosis.

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