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Comparative rumen fermentation and microbial characteristics of Surti buffalo calves supplemented with different yeast derivatives

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Abstract
The present study was conducted on 21 apparently healthy Surti young buffalo female calves aged (6-12 months) divided into 3 groups of 7 each categorized as group I (control), group II (supplemented with rumen specific yeast *Saccharomyces cerevisiae* CNCM I-1077 @ $4x10^9$ cfu/animal/day and group III (supplemented with selenoyeast-inactivated yeast *Saccharomyces cerevisiae* containing selenomethionine ensuring dietary inclusion of selenium @ 0.3 ppm). Rumen liquor (5 ml) was collected at day 0 of study (beginning), day 45 and day 90 of study (end) and analysis for rumen parameters (pH, total volatile fatty acids concentration, concentration of ammonia-nitrogen concentration, protozoal motility, enumeration of ruminal protozoa (entodiniomorphs and holotrichs) and bacteria was done. At day 90 of study, group II had significantly ($P\leq0.01$) high pH as well as TVFA of rumen liquor both between as well as within the group. Within group II the ammonia-nitrogen content was significantly ($P\leq0.05$) low at day 90 with respect to day 0. Rumen liquor of group II animals at day 45 and 90 presented significantly ($P\leq0.01$) higher scores of protozoal motility within the group. Rumen liquor of group II animals contained significantly ($P\leq0.05$) high protozoa concentration at day 90 between groups. Within groups at day 45 and 90 protozoa concentration were similar however within group II it significantly ($P\leq0.01$) increased from day 45 to day 90 of study. Group II at day 90 also possessed significantly high entidiniomorph% both between ($P\leq0.01$) and within ($P\leq0.05$) the groups. Group II calves had significantly ($P\leq0.05$) low holotrichs % at day 90. Ruminal bacterial count in rumen liquor was significantly ($P\leq0.01$) highest in group II animals at day 45 between the groups. The effects of selenoyeast supplementation were moderate and better than control. Thus it was concluded from the present study that dietary supplementation of Rumen specific yeast (*Saccharomyces cerevisiae* CNCM I-1077) in female Surti buffalo calves improves rumen fermentation parameters, enhances production of favourable rumen metabolites and optimizes rumen microbial profile. Its effect is better than selenoyeast supplementation.

Keywords: Female surti buffalo calves, rumen fermentation, rumen specific yeast, selenoyeast

Introduction
India is an agrarian based economy. Animal husbandry is one of the major component of rural livelihood intertwined with agriculture activities. Dairying in one of the integral part of livestock's contribution to GDP. Buffaloes (*Bubalus bubalis*) comprise an integral component of Indian dairy sector. Reproduction and production performance of buffaloes depend on the foundation laid during young age. Apart from maintaining good health of young calves it is desirable especially for females to have rapid growth rate and attain early puberty that helps in maximizing production during its life cycle. It has been reported that only 60-65% of the dairy animal potential yield is realized possibly due to issues related to feeding, breeding, health and management (Birthal and Jha, 2005) [1]. Low productivity is attributed to feeding that plays a crucial role (Garg *et al.*, 2012) [2]. Young calves at an early age are pre-ruminant and approximately by 6 months are on the verge of completing the dynamic phase of rumen development. At this juncture the feed also must support the newly developed rumen in order to enhance the fermentative digestion. Supplementation of probiotic yeast has been practised mostly in adults but less commonly in calves. Yeast has properties of scavenging oxygen to provide anaerobic environment, promoting lactate utilizing bacteria to stabilize pH, increasing dry matter intake, total volatile fatty acid and improving ruminal microbial profile. Several derivatives have been tried in past and currently being explored.

Two such forms are rumen specific yeast and selenoyeast. Rumen specific yeast is expected to act on improving ruminal parameters specifically and selenoyeast has the characteristic of supplying selenium in organic form (selenomethionine). Selenium in organic as well as inorganic form has direct or indirect effects on rumen fermentation parameters (Xun *et al*., 2012; Galbraith *et al*., 2016 and Del *et al*., 2013) [3, 4, 5]

Thus the present study was planned with the objective to study comparative rumen fermentation and microbial characteristics of surti buffalo calves supplemented with different yeast derivatives

Materials and methods

Location of study

The study was conducted in the Department of Veterinary Physiology and Biochemistry, NAU, Navsari, Gujarat following ethical guidelines and was approved by IAEC vide NAU/NVC/IAEC/6/2015 01/08/2015.

Experimental design

21 apparently healthy Surti young buffalo female calves aged (6-12 months) were randomly selected and divided into 3 groups of 7 each categorized as group I (control), group II (supplemented with rumen specific yeast *Saccharomyces cerevisiae* CNCM I-1077 @ $4x10^9$ cfu/animal/day and group III (supplemented with selenoyeast-inactivated yeast *Saccharomyces cerevisiae* containing selenomethionine ensuring dietary inclusion of selenium @ 0.3 ppm). Calves were maintained at Livestock Research Station of university and standard conditions of feeding and management were adopted. The duration of study was 90 days during which supplementation of both yeast derivatives was done in group II and III calves. 5 ml of Rumen liquor was collected by ruminocentesis method at day 0 of study (beginning), 45 and 90 day of study (end) by piercing left paralumbar fossa using a sterile stainless steel needle (16-18 gauge) and plunger. Collection of rumen liquor was done 4 hours post-feeding. Maximum aseptic precautions were taken while rumenocentesis.

Rumen liquor analysis

The collected rumen liquor was analyzed for rumen liquor parameters (pH, total volatile fatty acids concentration, concentration of ammonia-nitrogen concentration, protozoal motility, enumeration of ruminal ciliate protozoa (entodiniomorphs and holotrichs) and bacteria. pH of ruminal fluid was determined using pH meter. Motility of rumen protozoa was determined by placing strained rumen fluid on clean glass slide under cover slip, observing under microscope (low power) and scoring the movement on a numerical scale of 0 (no movement of protozoa) to 4 (very rapid movement of protozoa, whole mass is moving). Total volatile fatty acid was determined by distillation of 1 ml strained rumen liquor along with 5% oxalic acid and 10% potassium oxalate and titrating the distillate against 0.01N Sodium hydroxide using phenolphthalein as indicator. Ammonia nitrogen was estimated with the help of 'Conway diffusion technique' wherein strained rumen liquor was mixed with saturated solution of potassium carbonate and after incubation at 38°C liberated ammonia absorbed by boric acid (2%) solution (having mixed indicator) followed by titration against 0.01N H_2SO_4. For determining the concentration of protozoa, strained rumen fluid was mixed with lugol's iodine in the ratio of 1:10 and 0.01 ml was spread on designated area of glass slide for observation under microscope (low power). All ciliate protozoa were counted and average number of protozoa per microscopic field was calculated to determine its total concentration in rumen liquor. Amongst ciliate protozoa, holotrichs and entodinomorphs were also separately counted for their relative proportion. Characterization of holotrichs was done by presence of cilia all over the body and of entodinomorphs by lack of cilia over body but congregated in tufts and restricted to mouth. For determining total concentration of ruminal bacteria, strained rumen fluid was centrifuged and supernatant was mixed with formalin. Formalin mixed rumen liquor was serially diluted with distilled water up to final dilution of $1x10^{-4}$. 0.01 ml of this dilution was taken on glass slide, stained with saturated nigrosine solution and was spread as thin smear within marked area. Smear was dried and counting bacteria was done under oil immersion lens of microscope to calculate average bacteria per microscopic field that was further used to determine the total concentration of bacteria in rumen fluid.

Statistical analysis

The collected data were compiled, tabulated and analyzed by using SAS 9.3 software. Statistical analysis was carried using repeated measure two-way ANOVA with PROC GLM procedure.

Results

The results for rumen fermentative and microbial characteristics are mentioned in table 1.

Effect of rumen specific yeast supplementation on rumen fermentation characteristics

The pH of rumen liquor on day 90 was significantly ($P \leq 0.01$) high in group II between the groups. Within groups, the values of pH increased with successive stages in all except group I. Within group II the pH at day 90 was significantly ($P \leq 0.01$) higher than earlier stages. In group II calves, total volatile fatty acids (TVFA) was significantly ($P \leq 0.01$) high between the groups at day 90. Within groups the level of TVFA increased from 0 to 90. The increase was significant ($P \leq 0.01$) at day 45 and at day 90 as compared to 0 day within group II. There was non-significant difference in the value of ammonia-nitrogen in rumen liquor between groups. Within groups the ammonia-nitrogen content decreased throughout the study period with advancing age and was significantly ($P \leq 0.05$) low at day 90 as compared to day 0 in the group II animals.

Effect of rumen specific yeast supplementation on ruminal microbial characteristics

Rumen liquor of group II animals at day 45 and 90 presented significantly ($P \leq 0.01$) higher scores of protozoal motility within the group. The general increase in protozoal motility was noticed with increasing age in calves. Rumen liquor of group II animals contained significantly ($P \leq 0.05$) high protozoal concentration at day 90 between groups. Within groups at day 45 and 90 protozoa concentration were similar however within group II it significantly ($P \leq 0.01$) increased from day 45 to day 90 of study. The entidiniomorph% in ruminal liquor was significantly ($P \leq 0.01$) high between groups at 90 day in group II. Within group II it increased significantly ($P \leq 0.05$) up to 90 day whereas in other groups it decreased non-significantly from 45 to 90 day. Rumen liquor of group II calves had significantly ($P \leq 0.05$) low holotrichs% at day 90 among the groups. Further, its values were lower than the other groups at almost all stages. Ruminal bacterial count in rumen liquor was significantly ($P \leq 0.01$) highest in group II animals at day 45 between the groups.

Effect of selenoyeast supplementation on rumen fermentation and microbial characteristics

The rumen fermentation parameters between groups for Surti buffalo calves in group III (selenium supplemented group) revealed slightly higher values than those in group I at day 45 and 90 for rumen pH, TVFA, holotrichs %, at day 45 for ammonia nitrogen and at day 90 for protozoal count. Observations were found to have slightly lower values for ammonia nitrogen at day 90, entidiniomorph % and bacterial count at day 45 and 90. Within group III the values of most of the parameters were higher at day 90 as compared to day 0 except for ammonia nitrogen, entodiniomorphs% and holotrichs %. The differences of parameters were mostly non-significant.

Table 1: Rumen fermentation parameters (LSM±SE) in Surti buffalo calves

pH			
	0 day	45th day	90th day
Group I	5.86±0.11	5.82±0.13	5.84^{B*}±0.10
Group II	5.74^{b*}±0.20	5.89^{b*}±0.09	6.62^{A*a*}±0.03
Group III	5.78±0.19	5.84±0.09	6.05^{B*}±0.10
Total volatile fatty acids (mEq/litre)			
	0 day	45th day	90th day
Group I	88.00±3.65	92.14±3.51	90.86^{B*}±4.73
Group II	85.86^{c*}±3.25	98.29^{b*}±3.40	109.57^{A*a*}±2.58
Group III	90.43±3.55	96.14±2.12	96.43^{B*}±2.52
Ammonia nitrogen (mg%)			
	0 day	45th day	90th day
Group I	12.00±0.62	10.86±0.59	10.86±0.59
Group II	12.29^{a} ±0.52	10.86^{ab} ±0.74	9.71^{b}±0.92
Group III	12.29±0.68	11.14±0.74	10.57±0.72
Protozoal motility			
	0 day	45th day	90th day
Group I	1.43±0.20	1.71±0.18	1.86±0.26
Group II	1.57^{b*}±0.20	1.86^{b*}±0.14	2.57^{a*}±0.20
Group III	1.57±0.20	1.71±0.18	2.00±0.31
Protozoal count (x10^6/ml)			
	0 day	45th day	90th day
Group I	4.85^{b*}±0.18	5.67^{a*}±0.18	5.45^{Ba*}±0.11
Group II	4.90^{b*}±0.26	5.37^{b*}±0.16	6.08^{Aa*}±0.07
Group III	4.51^{b*}±0.37	5.56^{a*}±0.26	5.73^{Ba*}±0.11
Entodiniomorphs (%)			
	0 day	45th day	90th day
Group I	73.68±0.99	76.12±0.99	73.72^{B*}±0.26
Group II	71.14^{b}±1.69	77.22^{a} ±1.83	77.71^{A*a}±0.68
Group III	71.03±1.74	74.47±1.18	73.60^{B*}±1.03
Holotrichs (%)			
	0 day	45th day	90th day
Group I	26.32±0.99	23.88±0.99	26.28^{A*}±0.26
Group II	28.86^{a}±1.69	22.78^{b}±1.83	22.29^{B*b}±0.68
Group III	28.97±1.74	25.53±1.18	26.40^{A*}±1.03
Bacterial count (x10^{11}/ml)			
	0 day	45th day	90th day
Group I	4.06^{AB}±0.29	4.09^{A*}±0.12	4.34±0.21
Group II	4.26^{A}±0.19	4.20^{A*}±0.17	3.74±0.21
Group III	3.47^{B}±0.22	3.46^{B*}±0.17	3.81±0.18

Group I (Control-no dietary supplement); Group II (diet supplemented with rumen specific yeast Saccharomyces cerevisiae CNCM I-1077 @4x109 cfu/animal/day and Group III (diet supplemented with selenoyeast-inactivated yeast Saccharomyces cerevisiae containing selenomethionine ensuring dietary inclusion of selenium @ 0.3 ppm)

Mean bearing different superscripts in upper case letters differ significantly between groups and in lower case letters differ significantly within groups; Mean bearing superscripts with '*' differ significantly at $P \leq 0.01$ and without '*' at $P \leq 0.05$

Discussion

Effect of rumen specific yeast supplementation on rumen fermentation characteristics

Group II supplemented by rumen specific yeast presented higher pH with increasing age that was also significant at day 90 of study. Ideally lower pH is undesirable due to adverse effects of ruminal acidosis. pH stabilisation caused by dietary yeast supplementation is generally associated with decreased levels of lactic acid in rumen. This is mostly due to increase in lactate-utilising bacteria induced by *Saccharomyces cerevisiae* thus decreasing lactic acid concentrations and increasing ruminal pH. Similar results were also obtained by supplementing *Saccharomyces cerevisiae* (NCDC-49) in kids by Kamal *et al*. (2013) [6] and preruminant dairy calves (Xiao *et al.*, 2016 and Kumar *et al.*, 1994) [7, 8]. Increase in rumen pH due to supplementation of probiotic containing *Saccahromyces cerevisiae* in diet of buffaloes has also been reported by Mohan *et al*., (2015) [9]. In the present study the age of the calves were slightly higher than that of pre-ruminant stage but the effect of rumen pH improvement towards stability was still observed. This probably suggests the pronounced effect of rumen specific yeast supplemented in the present study.

Group II calves also showed higher TVFA concentration in rumen liquor. This effect could be due to probiotic yeast induced increased dry matter intake leading to increased fermentation and production of volatile fatty acid. Such finding is also mentioned in review done by Robinson and Garret (1999) [10] stating increased pH as well as TVFA after such supplementation. Supplementation of yeast in diet causing an increase in TVFA has also been reported in other studies (Laborde, 2008, Lascano and Heinrichs, 2009 and Quigley *et al*., 1992, Zain *et al.*, 2011, Mohan *et al.* 2015) [11, 12, 13, 14, 9].

Effect of rumen specific yeast supplementation on ruminal microbial characteristics

Rumen specific yeast supplementation in Group II also led to increased motility of rumen protozoa, high levels for protozoal concentration, entidiniomorph percentage, lower holotrichs percentage as well as non-significant lower ammonia nitrogen. These finding are in coherence with study of Mohan *et al.* (2015) [9] wherein they supplemented buffaloes with probiotic containing *Saccahromyces cerevisiae* and found higher protozoal motility, higher protozoal count and lowered ammonia-nitrogen in rumen liquor. A decrease in ammonia-nitrogen in rumen liquor after supplementation with *Saccharomyces cerevisiae* has been reported by Zain *et al.* (2011) [14].

The results for increased protozoal motility and count also supported by studies done by Maurya *et al.* (1993) [15], Panda (1994) [16], Plata *et al.* (1994) [17] and Mathieu *et al.* (1996) [18] wherein they reported increased ciliate protozoal population due to feeding of yeast culture to the animals.

An increase in bacterial count during the present study was observed due to rumen specific yeast supplementation in group II at day 45 of study. Similarly Zain *et al.* (2011) [14] have also reported an increase in bacterial count in rumen liquor when they supplemented *Saccharomyces cerevisiae* in diet.

Brossard *et al.* (2006) [19] stated that *Saccharomyces cerevsiae* can stimulate entidiniomorh protozoa in rumen and this finding is also seen in the present study. The study of Wanapat *et al.* (2013) [20] based on supplementing *Saccharomyces cerevsiae* fermented product have resulted in

lower holotrich count which also is a finding of present study in calves. This is in coherence with the fact that growing calves in the present study have a developing rumen undergoing transition towards fibre digestion that too more prominently in yeast fed group II and since holotrichs have no significant effect on fibre digestion so dietary yeast supplementation is likely to lower holotrich count in rumen liqour.

Effect of selenoyeast supplementation on rumen fermentation and microbial characteristics

The effect of selenoyeast supplementation in group III was better than control group for few parameters. The values for rumen parameters were most favourable in case of rumen specific yeast supplementation in group II. Selenoyeast supplementation effects in group III calves were either slightly better than or similar to control.

Wang *et al.* (2009) [21] studied effects of selenium yeast on rumen fermentation in dairy cows and found an increase in TVFA and decrease in ammonia nitrogen in rumen liquor. The supportive evidences for present findings of improved rumen fermentation parameters can also be seen in the study of Xun *et al.* (2012) [3] in which they found improved rumen fermentation parameters and health in sheep while seeing the effect of high-dose nano-selenium and selenium-yeast on feed digestibility and rumen fermentation. Naziroglu *et al.* (1996) [22] conducted study to observe effects of vitamin E and selenium on some rumen parameters in lambs. They found that total volatile fatty acids, the total counts of protozoa and percentage proportion of diplodinium were found to be significantly high in the supplemented than in the control group.

Conclusion

It was thus concluded from the present study that dietary supplementation of Rumen specific yeast (*Saccharomyces cerevisiae* CNCM I-1077) in female Surti buffalo calves improves rumen fermentation parameters, enhances production of favourable rumen metabolites and optimizes rumen microbial profile. Its effect is better than selenoyeast supplementation.

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