International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 189-193

Received: 28-07-2019
Accepted: 30-08-2019

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Effect of supplementation of different yeast forms on rumen fermentation characteristics and microbial profile in postpartum Surti buffaloes

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Abstract
Twenty one postpartum Surti buffalo were selected and divided into 3 groups of 7 buffaloes each *viz*. group I (control), group II and group III. Diet of group II buffaloes was supplemented with rumen specific yeast *Saccharomyces cerevisiae* CNCM I-1077 @ $10x10^9$ cfu/animal/day of group III was supplemented with selenized yeast-inactivated yeast *Saccharomyces cerevisiae* containing selenomethionine ensuring dietary inclusion of selenium @ 0.3 ppm. Dietary supplementation was done for 90 days from parturition. Rumen liquor (5 ml) was collected at day 0 (parturition), 45 and 90 day postpartum. Rumen liquor was analyzed for rumen parameters (pH, total volatile fatty acids concentration, concentration of ammonia-nitrogen, protozoal motility, enumeration of ruminal protozoa (Entodiniomorphs and holotrichs) and bacteria. Group II that was supplemented with rumen specific yeast showed significant increase in rumen pH, TVFA, protozoal motility and its count. Remaining rumen parameters in group II were better but non-significant as compared to other two groups. Selenized yeast supplementation in group III led to significantly higher levels of TVFA, protozoal count and entodiniomorphs and significant decrease in holotrichs during postpartum stages as compared to control group. Observations for remaining parameters mostly had levels between control (group I) and group II (rumen specific yeast supplemented group) however the differences were non-significant. Thus it was concluded from the present study that supplementation of rumen specific yeast (*Saccharomyces cerevisiae* CNCM I-1077) in the diet of postpartum Surti buffaloes for a period of 3 months after parturition favours production of beneficial fermentation metabolites in rumen and improves rumen microbial profile. It was also concluded that supplementation of rumen specific yeast in postpartum Surti buffaloes was more beneficial than selenized yeast.

Keywords: Rumen specific yeast, Selenized yeast, dietary supplementation, rumen, postpartum Surti buffaloes

Introduction
Dairying in India is an integral component of livestock production that itself is a major contributor to the rural economy that primarily is based on agriculture. Amongst the dairy animals, buffaloes comprise a species that contributes sizeably. Buffaloes can efficiently utilize the roughages and crop by-product into high quality milk suitable for a wide range of dairy products. Postpartum followed by lactation is stressful due to intensive production pressure thus adversely affecting their performance. Issues related to feeding, breeding, health and management has been reported to be cause of only 60-65% realization of potential of the dairy animal (Birthal and Jha, 2005) [1]. Feeding has been a major constraint for low productivity that states qualitative or quantitative supply of nutrients or unscientific approach for feeding as a major reason (Garg *et al.*, 2012) [2]. Thus there is need to adopt the scientific feeding strategies for dairy animals such as improving feed efficiency. Feed efficiency can be improved by feeding probiotics. Yeast has been commonly used for supplementation in diet as probiotic. Several forms of yeast have been used for dietary supplementation. Two such forms are rumen specific yeast and selenized yeast. Rumen specific yeast is expected to act specifically on improving ruminal parameters and selenized yeast carries the virtue of supplying selenium in organic form (selenomethionine). Supplementation of yeast as probiotic impacts directly or indirectly rumen fermentation parameters (Xiao *et al*., 2016; Mohan *et al*., 2015) [3, 4]. Rumen specific yeast favours scavenging of oxygen to provide anaerobic environment, promotion of lactate utilizing bacteria to stabilize pH, increased dry matter

intake, total volatile fatty acid and improvement in ruminal microbial profile. Trace element selenium has been supplemented in dairy cows for various beneficial effects that are more pronounced when it is supplemented in organic form as compared to inorganic form (Ran, 2010 and Chorfi, 2011) [5, 6]. Initial effects of such organic form of selenium supplementation are observed on rumen fermentation and microbial health. Its supplemntation 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) [7, 8, 9].

Thus the present study was planned to compare the effects of supplementation of different yeast forms on rumen fermentation characteristics and microbial profile in postpartum Surti buffaloes.

Materials and Methods

Location of study and experimental design

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

Twenty one postpartum Surti buffalo were selected and divided into 3 groups of 7 buffaloes each *viz*. group I (control), group II and group III. Diet of group II buffaloes was supplemented with rumen specific yeast *Saccharomyces cerevisiae* CNCM I-1077 @ $10x10^9$ cfu/animal/day of group III was supplemented with selenized yeast-inactivated yeast *Saccharomyces cerevisiae* containing selenomethionine ensuring dietary inclusion of selenium @ 0.3 ppm. Dietary supplementation was done for 90 days from parturition. Rumen liquor (5 ml) was collected 4 hours post-feeding at day 0 (parturition), 45 and 90 day postpartum. Collection of rumen liquor was done by ruminocentesis method by piercing left paralumbar fossa using a sterile stainless steel needle (16-18 gauge) and plunger. Maximum aseptic precautions were taken during the procedure.

Rumen liquor analysis

Rumen liquor was subjected to analysis of parameters for rumen fermentation characteristics and microbial profile such as pH, total volatile fatty acids (TVFA) concentration, concentration of ammonia-nitrogen concentration, protozoal motility, enumeration of ruminal ciliate protozoa (entodiniomorphs and holotrichs) and bacteria. The pH of rumen liquor was measured using pH meter. For determination of rumen protozoal motility strained rumen fluid was placed on sterile glass slide and covered with cover slip, followed by observing the movement of protozoa under microscope (low power) and assigning the score based on a numerical scale of 0 (no movement of protozoa) to 4 (very rapid movement of protozoa, whole mass is moving). For measuring the concentration of TVFA in rumen liquor distillation of 1 ml strained rumen liquor along with 5% oxalic acid and 10% potassium oxalate was done followed by titrating the distillate against 0.01N Sodium hydroxide. Phenolphthalein was used as indicator. The concentration of ammonia nitrogen was measured using 'Conway diffusion technique'. Rumen liquor was mixed with saturated solution of potassium carbonate. This mixture was incubated at 38 °C to liberate ammonia that was absorbed by boric acid (2%) solution (having mixed indicator). This was titrated against 0.01N H_2SO_4. For enumeration of protozoa, strained rumen liquor was mixed with lugol's iodine in ratio of 1:10. 0.01 ml of this mixture was spread on marked area of glass slide and was observed under microscope (low power). Ciliate protozoa were counted and average number of protozoa per microscopic field was determined for calculating its total concentration in rumen liquor. Among the ciliate protozoa, holotrichs and entodinomorphs were counted for their relative proportion. Holotrichs were identified by presence of cilia all over the body and entodinomorphs were identified by lack of cilia on the body and found in tufts confined to mouth. For enumeration of bacteria in rumen liquor, after centrifugation of rumen liquor supernatant was mixed with formalin. Serial dilution of formalin mixed rumen liquor was done with distilled water to reach final dilution of $1x10^{-4}$. 0.01 ml of this dilution was put on glass slide followed by staining with saturated solution of nigrosine. After staining it was spread as thin smear within marked area. After drying of the smear bacteria was counted under oil immersion lens of microscope. Average bacteria per microscopic field were calculated to determine the total concentration of bacteria in rumen fluid. The data of results observed was analyzed using SAS 9.3 software. Statistical analysis was done using repeated measure two-way ANOVA with PROC GLM procedure.

Results and Discussion

The rumen liquor of postpartum Surti buffaloes was analysed for parameters related to rumen fermentation and its microbial profile and the results are presented in table 1.

Effect of supplementation of rumen specific yeast in group II

The pH of rumen liquor was significantly ($P \leq 0.01$) high between groups at day 45 postpartum and day 90 postpartum in group II. Within group II, pH of rumen liquor at day 45 and 90 was significantly ($P \leq 0.05$) higher than that at calving. Total volatile fatty acid (TVFA) levels of rumen liquor in group II at day 45 ($P \leq 0.05$) and 90 postpartum ($P \leq 0.01$) were significantly higher between the groups. Similarly within group II significant ($P \leq 0.01$) increase in TVFA was observed at day 45 and 90 postpartum as compared to that at calving. The ammonia nitrogen level of rumen liquor at day 45 and 90 postpartum was lowest in group II between the groups. Within group II, ammonia nitrogen levels decreased postpartum up to 90^{th} day. However ammonia-nitrogen differed non-significantly.

Low pH in rumen is not favourable for rumen health. It may cause lactic acidosis. Supplementation of dietary yeast such as *Saccharomyces cerevisiae* favour growth of lactate-utilizing bacterial population in rumen thus reducing the amount of lactic acid produced in rumen. Thus they stabilise the pH and cause an increase in pH of rumen liquor. Mohan *et al*., (2015) [4] and Kumar *et al*. (1994) [10] reported the rise in rumen pH after supplementation of probiotic *Saccahromyces cerevisiae* in diet of buffaloes. Group II buffaloes in the present study also showed higher TVFA concentration in rumen liquor. This can be attributed to the effect of dietary probiotic supplementation that may lead to increase in dry matter intake causing increased fermentation of feed and production of volatile fatty acid. Such finding has also been mentioned by Robinson and Garret (1999) [11] wherein they stated that increased pH as well as TVFA is observed after such type of supplementation.

Table 1: Rumen fermentation parameters (LSM±SE) in postpartum Surti buffaloes

pH			
	At parturition	45th day postpartum	90th day postpartum
Group I	5.63±0.10	5.86^{B} ±0.02	5.70^{B} ±0.12
Group II	5.64^{b*}±0.13	6.14^{Aa*}±0.14	6.27^{Aa*}±0.08
Group III	5.51±0.24	5.73^{B}±0.06	5.75^{B}±0.19
Total Volatile fatty acids (mEq/litre)			
	At parturition	45th day postpartum	90th day postpartum
Group I	99.43±4.43	96.00^{B} ±3.24	98.29^{C*}±2.19
Group II	94.57^{b*}±2.13	107.57^{Aa*}±2.66	113.43^{A*a*}±1.17
Group III	98.86±3.45	102.71^{AB} ±1.36	104.43^{B*}±2.52
Ammonia nitrogen (mg%)			
	At parturition	45th day postpartum	90th day postpartum
Group I	15.71±0.92	14.86±0.59	15.14±0.59
Group II	14.57±1.13	13.71±0.68	13.43±0.57
Group III	14.57±1.13	14.00±0.62	14.00±0.62
Protozoal motility			
	At parturition	45th day postpartum	90th day postpartum
Group I	2.57±0.20	2.71±0.18	2.86±0.26
Group II	2.57^{b}±0.20	3.14^{a}±0.14	3.29^{a}±0.18
Group III	2.57±0.20	2.71±0.18	3.00±0.31
Protozoal count (x10^{6}/ml)			
	At parturition	45th day postpartum	90th day postpartum
Group I	4.85^{b*}±0.18	5.67^{a*}±0.18	5.44^{Ca*}±0.08
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	5.70^{Ba} ±0.10
Entodiniomorphs (%)			
	At parturition	45th day postpartum	90th day postpartum
Group I	76.46±1.56	78.77±1.71	78.52±1.08
Group II	77.55±2.23	80.74±1.78	79.91±1.41
Group III	75.75^{b} ±1.97	79.83^{a} ±0.84	81.16^{a} ±0.84
Holotrichs (%)			
	At parturition	45th day postpartum	90th day postpartum
Group I	23.54±1.56	21.23±1.71	21.48±1.08
Group II	22.45±2.23	19.26±1.78	20.09±1.41
Group III	24.25^{a} ±1.97	20.17^{b} ±0.84	18.84^{b} ±0.84
Bacterial count (x10^{11}/ml)			
	At parturition	45th day postpartum	90th day postpartum
Group I	4.16±0.15	3.86±0.19	3.93±0.25
Group II	4.13±0.19	4.21±0.23	4.41±0.34
Group III	3.83±0.32	3.57±0.28	3.97±0.28

Group I (Control-no dietary supplement); Group II (diet supplemented with rumen specific yeast Saccharomyces cerevisiae CNCM I-1077 @10x109 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 $P \leq 0.05$

Several studies (Laborde, 2008, Lascano and Heinrichs, 2009 and Quigley *et al*., 1992, Zain *et al.,* 2011, Mohan *et al.* 2015) [12, 13, 14, 15, 4] have also reported an increase in TVFA concentration in rumen liquor due to supplementation of yeast in diet. Similarly increased pH and TVFA as an effect of *Saccharomyces cerevisiae* on ruminal fermentation has also been reported by Zain *et al.* (2011) [15] and Doležal *et al.* (2005) [16]. Lowered ammonia-nitrogen in rumen liquor after supplementing buffaloes with probiotic containing *Saccahromyces cerevisiae* has been reported by Mohan *et al.* (2015) [4]. Similarly a decrease in ammonia-nitrogen in rumen liquor after supplementation with *Saccharomyces cerevisiae* has been reported by Zain *et al.* (2011) [15].

Protozoal motility within group II increased significantly ($P \leq 0.05$) at day 45 and 90 postpartum than at calving. Protozoal count between the groups was significantly ($P \leq 0.05$) high at day 90 in group II and within the same group it was significantly ($P \leq 0.0$) high both at day 45 and 90 postpartum than at calving. Even though bacterial count of rumen liquor between and within groups was highest at day 45 and 90 postpartum in group II, it was non-significant. The percentage of entodiniomorphs and holotrichs among protozoal population between groups was highest at day 45 in group II but the difference was not significant.

The study of Mohan *et al.* (2015) [4] had similar conclusions to the present findings wherein they observed higher protozoal motility as well as protozoal count in rumen liquor after supplementing buffaloes with probiotic containing *Saccahromyces cerevisiae*. The present findings of increased protozoal motility and count after supplementation with rumen specific yeast are also supported by studies done by Panda (1994) [17], Plata *et al.* (1994) [18], Maurya *et al.* (1993) [19], and Mathieu *et al.* (1996) [20] wherein they also reported increased ciliate protozoal population after feeding of yeast culture. 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. Similar increase in bacterial count in rumen liquor was reported by

Zain *et al.* (2011) [15] after dietary supplementation of *Saccharomyces cerevisiae*. Zain *et al.* (2011) [15] and Doležal *et al.* (2005) [16] also found increased protozoal count due to effect of *Saccharomyces cerevisiae* supplementation on ruminal fermentation.

Effect of Selenized yeast supplementation in Group III

Selenized yeast supplementation between the groups in group III led to significant ($P \leq 0.05$) higher levels of TVFA and protozoal count at day 90 as compared to control group. Observations for remaining parameters mostly had values between control (group I) and group II (rumen specific yeast supplemented group). Between the group pH at day 90, TVFA at day 45, protozoal motility at day 90, protozoal count at day 90, entodiniomorphs at day 45 and 90 and bacterial count at day 90 were higher than that of control group. Lower values for pH at day 45, ammonia nitrogen at day 45 and 90, protozoal count at day 45, holotrichs at day 45 and 90 and bacterial count at day 45 were observed between the groups as compared to control group. Significant ($P \leq 0.05$) increase in protozoal count and entodiniomorphs and significant decrease in holotrichs were noticed in group III at stages of post calving due to selenized yeast supplementation.

Similar to the findings of present study Wang *et al.* (2009) [21] found an increased TVFA and decreased ammonia nitrogen in rumen liquor while studying effects of selenium yeast on rumen fermentation in dairy cows. Xun *et al.* (2012) [7] also reported improved rumen fermentation parameters in study wherein they studied the effect of high-dose nano-selenium and selenium-yeast on feed digestibility and rumen fermentation in sheep. Naziroglu *et al.* (1996) [22] studied the effects of vitamin E and selenium on some rumen parameters in lambs and observed significant rise in total volatile fatty acids, the total counts of protozoa and percentage proportion of diplodinium in the supplemented group. It has been reported that incorporation of organic selenium as selenomethionine was to a greater extent into rumen microorganism than inorganic selenium (Galbraith *et al.*, 2016) [8]. Similar to the present findings, in a study to observe effects of dietary level of selenium and grain on digestive metabolism in lambs, Del *et al.* (2013) [9] reported an increase in protozoal count as well as volatile fatty acid (acetate and propionate). Enhanced incorporation of selenomethionine in microflora owing to its high bioavailability in the form of selenoyeast can be the reason for improved rumen fermentation characteristics. Shi *et al.* (2011) [23] also reported an increase in TVFA in rumen liquor in their study to observe effect of elemental nano-selenium on feed digestibility, rumen fermentation, and purine derivatives in sheep.

Conclusion

Supplementation of rumen specific yeast (*Saccharomyces cerevisiae* CNCM I-1077) in the diet of postpartum Surti buffaloes for a period of 3 months after parturition favours production of beneficial fermentation metabolites in rumen and improves rumen microbial profile. It was also concluded that supplementation of rumen specific yeast in postpartum surti buffaloes was more beneficial than selenized yeast.

Acknowledgement

Authors are thankful to Dean, Veterinary College, NAU, Navsari for financial assistance and research facilities and to Research Scientist, LRS, NAU, Navsari for availability of experimental animals.

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