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Comparative Evaluation of Rumen Responses, Blood and Serum Indices in Murrah Buffaloes, Vrindavani and Tharparkar Cattle Fed on a Similar Diet

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ABSTRACT

Rumen microbial ecosystem is a complex, highly diversified microenvironment comprising of bacteria, protozoa, archaea, fungi and phages, which enable ruminants to utilize poor quality fibrous feedstuffs. Comparison between cattle and buffalo for the rumen metabolism is challenging and interesting to understand. With this background, the present study has been carried out by conducting a feeding trial of 120 days to compare rumen fermentation, microbial profile and blood indices in Murrah buffalo (n=6), Vrindavani (n=6) and Tharparkar (n=6) cattle on a similar diet. The daily dry matter intake (kg/d) was higher (P<0.001) in Murrah buffaloes and Vrindavani as compared to Tharparkar. The mean pH was significantly lower (P<0.01) in the Vrindavani cattle as compared to Murrah buffalo and Tharparkar cattle. The rumen ammonia nitrogen (NH_3-N) concentration was higher (P<0.001) in Murrah buffalo group than both cattle species, whereas the total volatile fatty acid (TVFA) and propionate production was higher (P<0.05) in Vrindavani as compared to remaining two groups The lactic acid concentration was similar among the species at 0 and 2 h post feeding; however, there was a significant increase in rumen lactic acid concentration at 2 h post feeding in all the groups compared to 0 h.. The activity of rumen microbial enzymes like carboxy methyl cellulose (CMCase) and avicelase was higher (P<0.001) in Murrah buffalo as compared to other two groups, however, amylase and protease activities were similar between Murrah buffalo and Vrindavani. The population of protozoa as assessed by microscopic counting and RT-PCR was lower (P<0.05) in Vrindavani. The number of total bacteria, total methanogens, total fungi and *P.ruminicola* were comparable (P>0.05) among the three groups i.e. Murrah buffalo, Vrindavani and Tharparkar cattle. The values of blood indices (bio-chemicals and enzymes) were similar in all the three groups except Hb, PCV which were higher in Murrah buffalo. It can be inferred that the better ruminal functioning might be responsible for the said higher efficiency in feed utilization in buffaloes than cattle

KEYWORDS: Blood indices, Murrah buffalo, Real time PCR, Rumen fermentation, Similar diet, Tharparkar, Vrindavani

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INTRODUCTION

In India, livestock farming is an integral part of the agriculture and India hosts around 302.79 million bovines (20th Livestock Census of India) and is the world's largest producer of milk with 187.7 million tonnes per year (NDDB, 2018-19).. Ruminants like buffalo, indigenous and crossbred cattle can not only utilize high fibrous roughage diet, but also convert it into usable nutrients for us. The above mentioned three species of ruminants have similarities; however there are also some differences such as fermentation processes, microbial population of rumen, capacities of the digestive system, metabolism and physiology (Wanapat et al., 1999; Wanapat et al., 2000; Chanthakhoun et al., 2012). For instance, the, ratio of propionate to acetate, protozoal numbers in the rumen digesta and fibrolytic activity were higher in buffalo (Kennedy et al., 1992). Cattle and buffalo have been reported to have differences in the microbial population of the rumen and also community composition under similar feeding

management (Iqbal et al., 2018). Wanapat et al. (2014) also found that ruminal cellulolytic, proteolytic and amylolytic bacteria were significantly developed and higher in swamp buffalo than those found in cattle fed similar diets. The overall population of bacteria, *F. succinogens* and *Ruminococcus* were higher in buffalo whereas *Prevotella* were higher in Jersey cattle by using qPCR (Iqbal et al., 2018). It was also reported that Firmicutes, a phylum associated with efficient feed utilization, were significantly higher in buffalo (Myer et al., 2015).

The comparison between cattle and buffalo for the various parameters like rumen metabolism, methane production is challenging, but at the same time interesting to understand the rumen functioning of these two ruminant species under the similar feeding regimen and environmental conditions. With this background, the present study has been carried out to compare Tharparkar and Vrindavani cattle and Murrah buffalo for effect on rumen fermentation, microbial profile and blood indices on a diet comprised of concentrate to roughage ratio of 70:30.

MATERIALS AND METHODS

The study was carried out to compare the rumen fermentation, microbial profile and blood indices in three species (6 each) of ruminants (approximately 9-10 months old), male growing Murrah buffalo (BW 255.5±7.84 kg), Vrindavani (crossbred cattle) (BW 273.5±5.51 kg) and Tharparkar (indigenous cattle) (BW 205±9.93 kg) cattle were fed 30% roughage (wheat straw) and 70% concentrate containing 16% CP to meet their nutrient requirement as per ICAR (2013). The animals were kept in well ventilated experimental sheds having facility for individual feeding. Fresh and clean drinking water was made available *ad libitum* thrice in a day. Animals were treated with ecto and endo parasites prior to initiation of the experimental trial. The animals were adapted for two weeks prior to the start of actual feeding trial, in which the concentrate feed was introduced gradually to required level (70%). The ratio of roughage and concentrate was maintained throughout the actual experimental period from day one to the end of the trial. The formulation of concentrate mixture was done as given in the Table 1.

Table 1. Physical composition (%) of concentrate mixture

Ingredient	Percentage in the ration
Maize	39
Wheat bran	43
Soybean meal	15
Mineral mixture	2
Common salt	1
Total	100

Nutrient composition of the experimental feed: The values of proximate principles and fibre fractions of the experimental feed i.e. wheat straw and concentrate mixture offered to the animals is presented in Table 2.

Table 2. Chemical composition of wheat straw and concentrate mixture

Attributes	Wheat Straw	Concentrate mixture
Dry matter	92.0	91.4
Organic matter	92.2	92.3
Total ash	7.8	7.66
Crude protein	3.1	15.8
Ether extract	1.15	4.1
Neutral detergent fibre	75.0	40.1
Acid detergent fibre	56.1	9.64
Hemicellulose	18.9	30.4

The samples of feed ingredients and residues were analysed for proximate principles as per the procedures of AOAC (1995) and fibre fractions by Van Soest et al. (1991). The collection of the rumen liquor was done with stomach tube after 0 and 2 h of feeding on day 30th of experimental feedin, and the pH was noted immediately with an electronic pH meter (Model: pH Spear, Eutech instruments, Malaysia, pH Range: - 1.00 to 15.00, Resolution: 0.01 pH, Accuracy: ±0.01 pH) calibrated against standard buffer solution. Volatile fatty acids (VFAs) were estimated by using Nucon-5765 gas chromatograph (AIMIL, New Delhi, India) equipped with a double flame ionization detector (FID) and the glass column (4 ft length and 1/8 inch diameter packed with chromosorb 101 which acts as a stationary phase in the column) as per the method described by Cottyn and Boucque (1968). the rumen liquor was used for estimation of ammonia nitrogen (Weatherburn, 1967) and lactic acid concentration (Barker and Summerson, 1941).The enzymes from the rumen contents were extracted as per the method described by Hristov et al. (1999) and Agarwal et al. (2000).The number of total protozoa, holotrichs and oligotrichs in the rumen liquor was counted under the microscope by using the haemocytometer as the procedure given by Kamra et al. (1991). Total bacteria, protozoa, methanogens, fungi and *Prevotellaruminicola* populations were quantified by RT-PCR using specific primers for prescribed microbes. The primers used were targeting specific 16SrRNA gene for bacteria and methanogens. For protozoa and fungi, the primers used were targeting 18SrRNA and internal transcribed spacer 1 (ITS1) region. The genomic DNA was extracted from the rumen liquor sample by using the method described by Yu and Morrison (1994). The premix was dispensed (20µl) in duplicate in each well for each sample in 96 well PCR plate. The plate was sealed and placed in real time thermal cycler (BioradCFX96 real time system, country?) programmed as follows (Primers used for different rumen microbes and their annealing temperature is mentioned in Table 3).

Table 3. Primers used for qPCR for absolute quantification of different rumen microbes

Target	Primer Sequence	Size	Annealing T (°C)
Bacteria	F- CGGCAACGAGCGCAACCC	130	60
Methanogens	F-TTCGGTGGATCDCARAGRGC R- GBARGTCGWAWCCGTAGAATCC	140	60
Protozoa	316f- GCTTTCGWTGGTAGTGTATT 539r- CTTGCCCTC6AATCGTWCT	223	55
Total fungi	F-GAAGGAAGTAAAAGTCGTAACAAGGTTTC R- CAAATTCACAAAGGGTAGGATGATT	110	60
Prevotellaruminicola	F-GGTTATCTTGAGTGAGTT R-CTGATGGCAACTAAAGAA	485	54

Blood samples were collected from all the groups at zero and final day of experimental feeding by jugular vein puncture in the tubes with/without anticoagulant for different analysis. The blood samples were transported to the laboratory by using ice bath. Serum was separated from whole blood after clotting and stored for further analysis. Haemoglobin and packed cell volume were analysed as per Oser, (1979) and Jain, (1986). Haemoglobin concentration was determined by Sahli's or acid hematin method and packed cell volume was analysed by microhaematocrit centrifugation

technique (Jain, 1986). All the biochemical parameters and enzymes were estimated by using commercial diagnostic kits (Coral clinical systems; Geno Biosciences Pvt. Ltd., India) as per the given procedure.

The statistical analyses were performed using SPSS computer package (SPSS version 20.0, SPSS Inc., Chicago, USA). Data obtained for rumen fermentation and blood indices were analysed using General Linear Model Multivariate ANOVA using the model, intercept + species + period + species × period to analyse the effect of species, period and their interaction. The means were compared using Duncan's multiple range test if the main effect was significant (i.e., $P<0.05$).

RESULTS AND DISCUSSION

Rumen fermentation profile

To compare the rumen fermentation pattern among the three species, rumen liquor was analysed for the various rumen metabolites, enzymes and microbial profile. The data on rumen pH, ammonia nitrogen and lactate are presented in the Table 4.

Table 4. Rumen pH and metabolites in Murrah buffalo, Vrindavani and Tharparkar cattle fed on similar diet

Attributes	Murrah	Vrindavani	Tharparkar	Mean	SEM	P-value		
						S	P	S*P
pH								
0 h	6.64^{a}	6.58^{a}	6.82^{a}	6.68^{q}	0.04	0.01	≤0.001	0.04
2 h	5.87^{b}	5.34^{a}	5.63^{ab}	5.61^{p}				
Mean	6.25^{B}	5.96^{A}	6.22^{B}					
NH_3N (mg/dl)								
0 h	6.36^{a}	5.93^{a}	6.1^{a}	6.12^{p}	0.21	≤0.001	≤0.001	0.02
2 h	11.31^{b}	8.47^{a}	7.87^{a}	9.21^{q}				
Mean	8.83^{B}	7.19^{A}	6.98^{A}					
Lactate (mg/dl)								
0 h	1.98	1.81	2.04	1.94p	0.14	0.77	≤0.001	0.71
2 h	3.18	2.91	2.71	2.93q				
Mean	2.57	2.35	2.37					

^{abc}Means bearing different superscripts in rows and columns differ significantly
^{ABC}Means bearing different superscripts in a row differ significantly ($P<0.05$)for a parameter
^{pq}Means bearing different superscripts in a column differ significantly ($P<0.05$)for a parameter
SEM standard error of the mean; S=Species; P=Period; S*P= Interaction between species and period

Rumen pH

The rumen pH was comparable at 0 h among the three groups. But at 2 h post feeding the rumen pH of Vrindavani cattle was significantly lower than Murrah buffalo, whereas it was similar to Tharparkar. The mean pH was significantly ($P<0.01$) lower in the Vrindavani cattle as compared to Murrah buffalo and Tharparkar cattle but it was comparable at 0 h among the different groups. There was an interaction between species and period of rumen liquor sampling as at 0 h the rumen pH was similar in all the groups, whereas, at 2 h post-feeding, the rumen pH differed significantly among the groups. The rumen pH has been reported to vary from 5.5 to 7.5 in roughage-fed animals and it might vary according to feed change (Dehority, 2003). Mean pH was reduced in all the groups post two h of feeding, which might be due to the postprandial effect. There was interaction between species and period of feeding and sampling of rumen liquor on the change in pH. It might be due to higher protozoal count found in Murrah buffalo than the crossbred cattle group and may be due to better rumen metabolic controlling mechanisms of buffalo than crossbred cattle and chewing activity of buffalo may partly explain rumen pH alteration as reported by Wanapat, (2009). Similarly, Franzolin et al. (2002) also found higher rumen pH in buffalo than cattle fed with increasing

levels of neutral detergent fibre. Buffaloes might have higher rumen pH due to saliva secretion is more intensive and higher buffering capacity of saliva flowing into the rumen as reported by Sivkova et al. (1997). In our study, it was found that the ruminal pH after 2h post feeding was lower in buffaloes than crossbred Vrindavani cattle, whereas the pH remained intermediate in indigenous Tharparkar cattle.

Ammonia nitrogen

The concentration of NH_3-N (mg/dL) in the rumen liquor of all the three groups was similar at 0 h feeding. However, there was significant increase ($P<0.001$) in the rumen NH_3-N level at 2 h post feeding with concentration being higher in Murrah buffalo as compared to other two cattle species. The interaction between species and period of sampling of rumen liquor on the change in NH_3-N concentration showed that the increase in ammonia nitrogen in buffaloes is only after two hours of feeding. Similar to the present results, many studies higher NH_3-N in buffaloes than the cattle. Budhi et al. (2003) reported higher rumen NH_3-N concentration in buffaloes (15.17 mg/dL) than Ongole cattle (5.17 mg/dL). Sinha et al. (2017) also reported higher ruminal NH_3-N in buffalo (9.6 mg/dL) than crossbred cattle (8.2 mg/dL) on 60 % concentrate and 40 % roughage based total mixed ration. The higher concentration of rumen ammonia nitrogen in buffalo indicates greater proteolytic activity in the rumen of buffalo than in the cattle as reported by Bhatia et al. (1995). Abdullah et al. (1992) also reported that cattle had lesser rumen ammonia than the buffalo when both of them offered similar diet. Wanapat and Pimpa (1999) reported that increased level of ruminal NH_3-N lead to increased protozoal population, which was in accordance with our study where Murrah buffalo presented with higher NH_3-N and higher number of total protozoa in the rumen as assessed by microscopic count and RT PCR. Buffaloes had higher concentration of ammonia. Bird, (1991) reported that buffaloes may have higher NH_3-N concentration, that may be due to the rapid and extensive biodegradation of protein (dietary and endogenous) and may be higher numbers of ciliate protozoa present in the rumen, which are thought to be involved in the ammonia production.

Lactic acid

There was no difference among the species neither at 0 nor at 2 h post feeding; however, there was a significant increase ($P<0.001$) in rumen lactic acid concentration at 2h as compared to 0h post feeding, irrespective of species. All the values for rumen lactic acid concentration were in normal range. Similarly, Sinha et al. (2017) reported no significant variation in rumen lactic acid concentration, neither between the treatment groups of different roughage: concentrate (40:60) ratios nor between the buffalo and crossbred cattle. Similarly Wanapat and Pimpa, (1999) reported that lactic acid (mg/ml) in the rumen liquor of animals increased significantly upon increase in proportion of concentrate mixture in the diet. In the present study, all the three groups had similar diet therefore the lactic acid concentration in the rumen liquor was also similar in all the three species.

Volatile fatty acids and their fractions

The mean values for TVFA concentration (mM/dl) were higher ($P<0.01$) in Vrindavani group (12.55) as compared to Murrah buffalo (10.82) and Tharparkar cattle (10.73) (Table 5). The VFA concentration at 0 h were similar in the three groups, however the values were elevated at post 2 h of feeding being highest in Vrindavani cattle group than other two groups. The mean acetic acid concentration was significantly higher ($P<0.01$) in Vrindavani cattle as compared to Tharparkar but was similar to Murrah buffalo. The concentration of propionate was also higher ($P<0.02$) in Vrindavani than Murrah buffalo but was similar to Tharparkar. The acetate to propionate ratio was significantly lower (Pd"0.001) in Vrindavani (2.83) and Tharparkar (2.85) than Murrah buffalo group (3.66).As the propionate production was higher in both Vrindavani and Tharparkar, a lower acetate to propionate ratio was observed in these groups than Murrah buffalo. It was evident from our findings that in both the cattle groups, the fermentation process was diverted

towards more propionate production leading to comparatively efficient energy utilization compared to Murrah buffalo. Similarly, Wang et al. (2020) reported that Holstein calves had lower acetate and higher propionate concentration than buffalo calves on similar diet.

Table 5. Rumen volatile fatty acids (mM/dL) profile in Murrah buffalo, Vrindavani and Tharparkar cattle

Attributes	Murrah	Vrindavani	Tharparkar	Mean	SEM	P-value		
						S	P	S*P
TVFA								
0 h	7.19^{a}	6.34^{a}	6.42^{a}	6.64^{p}	0.23	0.01	≤0.001	≤0.001
2 h	14.46^{a}	18.78^{b}	15.06^{a}	16.09^{q}				
Mean	10.82^{A}	12.55^{B}	10.73^{A}					
Acetate								
0 h	4.96^{a}	3.94^{a}	4.13^{a}	4.34^{p}	0.17	0.01	≤0.001	≤0.001
2 h	8.87^{a}	11.2^{b}	8.19^{a}	9.42^{q}				
Mean	6.91^{AB}	7.57^{B}	6.15^{A}					
Propionate								
0 h	1.24	1.42	1.35	1.33^{p}	0.10	0.02	≤0.001	0.10
2 h	2.62	3.94	3.36	3.30^{q}				
Mean	1.92^{A}	2.68^{B}	2.35^{AB}					
Isobutyrate								
0 h	0.01	0.01	0.02	0.01	0.01	0.43	0.42	0.59
2 h	0.03	0.00	0.03	0.02				
Mean	0.018	0.003	0.02					
Butyrate								
0 h	0.96	0.97	0.91	0.94	0.08	0.26	≤0.001	0.25
2 h	2.62	3.26	3.13	3.00				
Mean	1.78	2.11	2.02					
Isovalerate								
0 h	0.04a	0.00^{a}	0.02^{a}	0.02^{p}	0.01	0.03	≤0.001	0.01
2 h	0.12^{b}	0.13^{b}	0.03^{a}	0.09^{q}				
Mean	0.07^{B}	0.06^{B}	0.027^{A}					
Valerate								
0 h	-	-	-	-	0.03	0.58	≤0.001	0.58
2 h	0.2	0.25	0.33	0.25				
Mean	0.09	0.12	0.16					
Acetate/Propionate								
0 h	3.96	2.8	3.08	3.27	0.1	≤0.001	0.14	0.4
2 h	3.38	2.88	2.64	2.96				
Mean	3.66^{B}	2.83^{A}	2.85^{A}					

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^{pq}Means bearing different superscripts in a column differ significantly (P<0.05) for a parameter
SEM standard error of the mean; S= Species; P= Period; S*P= Interaction between species and period

Rumen microbial enzymes

The mean activity of CMCase and avicelase was higher in Murrah buffalo when compared to the remaining two ruminant groups at the two time periods i.e. 0 h and 2 h post feeding. Amylase activity was significantly higher in Murrah buffalo followed by Tharparkar and then Vrindavani. The activity of Protease were significantly (P<0.01) higher in Murrah buffalo and Vrindavani groups than Tharparkar group (Table 6). This might be the reason

for the higher proteolysis in rumen which led to the increased ammonia nitrogen concentration in Murrah buffalo and Vrindavani cattle. In a trial conducted by Wora-anu, (2006) it was found that ruminal proteolytic, cellulolytic and amylolytic bacteria of swamp buffalo were significantly higher than that in cattle fed similar diets. This might have the reason for the higher activity of CMCase, avicelase and Protease and Amylase in buffalo group as they harbour higher microflora when compared to other ruminant groups. Xylanase activity did differ neither among the species nor between the sampling times.

Table 6. Rumen microbial enzyme profile in Murrah buffalo, Vrindavani and Tharparkar

Attributes	Murrah	Vrindavani	Tharparkar	Mean	SEM	P-value		
						S	P	S*P
CMCase								
0 h	40.47^{b}	37.69^{a}	34.31^{a}	37.49^{p}	0.94	≤0.001	≤0.001	≤0.001
2 h	84.59^{b}	56.62^{a}	64.89^{a}	68.70^{q}				
Mean	62.52^{B}	47.15^{A}	49.59^{A}					
Xylanase								
0 h	48.49	57.54	48.31	51.45	1.74	0.13	0.32	0.91
2 h	46.98	52.26	44.37	47.87				
Mean	47.74	54.9	46.34					
Avicelase								
0 h	45.43^{b}	29.87^{a}	19.54^{a}	31.61^{p}	1.36	≤0.001	≤0.001	0.01
2 h	63.47^{b}	43.30^{a}	54.29^{b}	53.69^{q}				
Mean	54.45^{B}	36.58^{A}	36.91^{A}					
Amylase								
0 h	240.19^{c}	218.80^{b}	182.58^{a}	213.85^{p}	2.86	≤0.001	≤0.001	≤0.001
2 h	414.68^{c}	247.18^{a}	378.30^{b}	346.72^{q}				
Mean	327.43^{C}	232.98^{A}	280.43^{B}					
Protease								
0 hr	1027.05	1104.63	1012.25	1047.98^{p}	14.84	0.01	≤0.001	0.07
2 hr	1425.22	1395.88	1229.23	1350.11^{q}				
Mean	1226.13^{B}	1250.25^{B}	1120.73^{A}					

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^{ABC}Means bearing different superscripts in a row differ significantly (P<0.05) for a parameter
^{pq}Means bearing different superscripts in a column differ significantly (P<0.05) for a parameter
SEM: Standard error of the mean; S=Species; P=Period; S*P=Interaction between species and period. *Units: CMCase, avicelase and amylase: nmol glucose/hr/ml; xylanase: nmol xylose/hr/ml; protease: ng of azocasein hydrolysed/h/ml.

Microscopic counting of rumen protozoa

The population (log_{10}) of total protozoa, holotrichs and oligotrichs in the experimental animals are presented in the Table 7. The mean holotrichs population was higher (P<0.01) in Tharparkar cattle group followed by Vrindavani cattle and were lowest in the Murrah buffalo group. The number at 2 h post feeding increased significantly in all the groups, with higher population of oligotrichs in Murrah buffalo (5.24) followed by Tharparkar (5.14) and was lowest in Vrindavani (4.84). The population of total protozoa was found to be significantly higher in the Murrah buffalo and Tharparkar cattle as compared to Vrindavani cattle group. Rumen protozoa, with the population size of 10^5-10^6 cells per ml of rumen liquor comprise half of the ruminal microbial bio-mass of the rumen. Similarly, Wanapat et al. (2000) also observed lower

numbers of holotricha (*Isotricha* and *Dasytricha*) in buffalo compared to those in cattle raised under similar traditional village conditions. In contrast to above finding, the population of oligotrichs and total protozoa population was higher in Murrah buffalo and Tharparkar and were lowest in Vrindavani cattle. When compared between Murrah buffalo and Tharparkar, Murrah buffalo had higher numerical value for the total protozoa which coincided with the result of Kumar et al. (2002) in which they reported that higher number of total protozoa in buffalo (9×10^5 /ml) than those in cattle (6×10^5 /ml) when fed an oat-hay-concentrate based feed. Holotrich protozoa such as *Isotricha* and *Dasytricha* feeds on the soluble/easily digestible carbohydrates, which interns produces rumen acids, thus decreases rumen pH. Higher pH values were recorded for the buffalo which was coincided with the lower holotricha population in the same species. Water buffalo (Khuzestan) had significantly ($P<0.05$) higher rumen protozoal concentration (3.68×10^5/ ml of rumen content) than the cow (2.18×10^5) when fed with the roughage and concentrate in the ratio of 70:30 (Jabari et al., 2014).

Table 7. Microscopic counting of rumen protozoa ($\log_{10}$ cells/mL) in Murrah buffalo, Vrindavani and Tharparkar cattle

Attributes	Murrah	Vrindavani	Tharparkar	Mean	SEM	P- value		
						S	P	S*P
Holotrichs								
0 h	3.77^{a}	4.09^{b}	4.22^{b}	4.02^{p}	0.02	≤0.001	≤0.001	0.03
2 h	4.15^{a}	4.15^{a}	4.37^{b}	4.22^{q}				
Mean	3.95^{A}	4.12^{B}	4.29^{C}					
Oligotrichs								
0 h	5.21	4.79	5.08	5.03^{p}	0.02	≤0.001	0.04	0.95
2 h	5.29	4.9	5.21	5.13^{q}				
Mean	5.24^{B}	4.84^{A}	5.14^{B}					
Total Protozoa								
0 h	5.22	4.87	5.14	5.08^{p}	0.02	≤0.001	0.03	0.96
2 h	5.32	4.97	5.27	5.19^{q}				
Mean	5.27^{B}	4.91^{A}	5.20^{B}					

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^{ABC}Means bearing different superscripts in a row differ significantly ($P<0.05$) for a parameter
^{pq}Means bearing different superscripts in a column differ significantly ($P<0.05$) for a parameter
SEM-Standard error of the mean; S= Species; P= Period; S*P= Interaction between species and period

Quantification of rumen microbes using real time PCR

The number ($\log_{10}$) of total bacteria, total methanogens, total fungi and *P. ruminicola* were comparable ($P>0.05$) among the three groups (Table 7.). The values of total protozoa was found to be significantly ($P<0.01$) higher in the Murrah buffalo (8.83) than the Vrindavani (7.69) and Tharparkar (7.60), which was in agreement with the microscopic protozoal counting. Similar to our findings, Iqbal et al. (2018) found no difference in methanogen population between buffalo and jersey cattle (8.3 vs 8.1) on similar feeding regimen. Kamra, (2015) found that there was similar population density of total bacteria, total fungi and fibre degrading bacteria

in crossbred cattle and buffalo. Kumar et al. (2013) observed more population of protozoa, methanogens and cellulolytic bacteria in buffalo than cattle. Similar abundance of rumen microbial group have been reported in buffaloes with metagenomic studies also, with minor shifts within the group (Kala et al., 2020). Australian Holstein cattle harbour more methanogenic population among all other cattle breeds as reported by Parmar et al. (2017). Rumen microbiota is more related with the type of feed taken by the animal, therefore any shift in rumen microbes was not expected in the present study also because the animals of all the species were under same environment and on similar feed.

Table 8. Quantification of rumen microbes (log_{10}/ml of rumen liquor) at 2 hr post feeding in Murrah buffalo, Vrindavani and Tharparkar cattle

Attributes	Murrah	Vrindavani	Tharparkar	SEM	P-value
Total bacteria	10.73	10.95	11.00	0.11	0.64
Total methanogens	3.79	3.86	3.79	0.06	0.89
Total fungi	6.52	7.27	6.98	0.18	0.27
Total protozoa	8.83[b]	7.69[a]	7.60[a]	0.24	0.04
Prevotella ruminicola	6.51	6.7	6.1	0.28	0.71

[abc]Means bearing different superscripts in a row differ significantly (P<0.05); SEM- Standard error of the mean.

Blood metabolites of Murrah buffalo, Vrindavani and Tharparkar cattle

The mean concentration of Haemoglobin (g/dl) and packed cell volume (%) was significantly (P<0.01) higher in Murrah buffalo than the Vrindavani and Tharparkar cattle. The mean serum glucose (mg/dl) level was not differed among the groups. The total protein, albumin, globulin (g/dl) and A/G ratio was also comparable among the three ruminant groups. The blood urea concentration (mg/dl) was also similar among the experimental groups. The values pertaining to the general metabolism of the current experimental animals revealed that the blood parameters which were analysed were within the normal physiological range (Table 9). Values for all the blood indices increased with the age and were higher at 120 d as compared to 0 d of the trial. The concentration of Hb and PCV was higher in Murrah buffalo as compared to other groups but they were in normal physiological range. The values were within the normal range indicating that there was no major difference in metabolism in the three groups upon similar feed and it indicates good health condition of the animals during the experimental period. Murrah buffalo was presented with higher Hb and PCV concentration and it was in normal range which was in agreement with a study done by Agarwal et al. (2016) conducted a haematological profile of growing Indian cattle and Murrah buffalo on maintenance ration with 60% TDN and 12% CP and reported Hb (11-13.5 g/dl) and PCV (36-42) for Murrah buffalo. Abdullah et al. (1992) reported that there were no significant differences in terms of plasma urea concentration between these two ruminant species (51.3 in cattle vs 51.4 mg/L in buffalo in cattle) which was in agreement with our study where, there was no significant difference in the blood urea among the three groups.

Serum enzymes

The activity of serum enzymes i.e. AST, ALT, and ALP was similar in all the three experimental groups. There was no effect with period of collection as there was no change in values of both the periods. The serum enzymes such as AST, ALT, and ALP are used as biomarkers for the health status and are commonly measured to diagnose the illness of the patient. Many factors such as animal's physiological status, age, sex, season, nutrition and management etc. have impact on the normal range of these serum enzymes.

Table 9. Blood indices in Murrah buffalo, Vrindavani and Tharparkar cattle

Attributes	Murrah	Vrindavani	Tharparkar	Mean	SEM	P-value		
						S	P	S*P
Haemoglobin (g/dl)								
0 d	10.90	8.15	8.53	9.19^{p}	0.13	≤0.001	≤0.001	0.10
120 d	11.90	10.60	10.50	11.00^{q}				
Mean	11.40^{B}	9.37^{A}	9.51^{A}					
Packed cell volume (%)								
0 d	47.52^{b}	35.37^{a}	34.97^{a}	39.29^{q}	0.57	≤0.001	0.03	0.01
120 d	39.78^{a}	36.15^{a}	34.17^{a}	36.70^{p}				
Mean	43.65^{B}	35.76^{A}	34.57^{A}					
Glucose (mg/dl)								
0 d	61.67	53.90	58.27	57.94^{p}	0.93	0.34	≤0.001	0.01
120 d	67.52	67.37	62.75	65.88^{q}				
Mean	64.61	60.63	60.51					
Total Protein (g/dl)								
0 d	7.45	7.45	7.50	7.47^{p}	0.08	0.25	≤0.001	0.28
120 d	8.09	8.75	8.57	8.47^{q}				
Mean	7.77	8.10	8.04					
Albumin (g/dl)								
0 d	4.67	4.80	4.82	4.77^{q}	0.08	0.65	≤0.001	0.97
120 d	3.27	3.49	3.44	3.40^{p}				
Mean	3.98	4.15	4.13					
Globulin (g/dl)								
0 d	2.75	2.62	2.67	2.68^{p}	0.08	0.73	≤0.001	0.40
120 d	4.82	5.28	5.13	5.08^{q}				
Mean	3.79	3.95	3.90					
Albumin: Globulin ratio								
0 d	1.75	1.85	1.82	1.81^{q}	0.05	0.94	≤0.001	0.92
120 d	0.67	0.67	0.65	0.67^{p}				
Mean	1.21	1.26	1.24					
Blood Urea (mg/dl)								
0 d	22.70	22.02	20.00	21.58^{p}	0.69	0.93	≤0.001	0.39
120 d	30.69	30.38	32.26	31.12^{q}				
Mean	26.70	26.21	26.13					
AST (IU/L)								
0 d	75.45^{ab}	85.10^{b}	72.32^{a}	77.63^{p}	1.38	0.11	0.04	≤0.001
120 d	93.84^{b}	74.73^{a}	82.30^{a}	83.63^{q}				
Mean	84.65	79.92	77.31					
ALT (IU/L)								
0 d	31.51	35.99	29.55	32.35^{q}	0.76	0.12	0.01	0.31
120 d	29.31	28.59	27.01	28.31^{p}				
Mean	30.41	32.29	28.28					
ALP (KA/L)								
0 d	15.25	15.53	16.36	15.72^{p}	1.08	0.50	≤0.001	0.33
120 d	31.89	35.09	27.91	31.64^{q}				
Mean	23.58	25.31	22.14					

^{abc}Means bearing different superscripts in rows and columns differ significantly (P<0.05) for a parameter. ^{ABC}Means bearing different superscripts in a row differ significantly (P<0.05) for a parameter. ^{pq}Means bearing different superscripts in a column differ significantly (P<0.05) for a parameter. SEM- Standard error of the mean; S= Species; P= Period; S*P= Interaction between species and period.

CONCLUSIONS

From the above findings, we can conclude that buffalo and cattle differ in some rumen attributes with buffaloes having higher ammonia nitrogen concentration, protozoa count, acetate production, rumen enzymes CMCase, avicelase, protease than cattle. It was also observed that there was a higher TVFA, lower A:P ratio in Vrindavani than other two groups. The microbial profile including total bacteria, fungi, methanogens and *Prevotella rumicola* was similar among the three groups. All these observations indicate that the better ruminal functioning might be responsible for the said higher efficiency in feed utilization in buffaloes than cattle**.**

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