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Effect of betaine supplementation on rumen fermentation pattern of Gir calves

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Abstract
The present study was carried out with the objective of investigating the effects of betaine supplementation on rumen fermentation performance of Gir calves. A total sixteen Gir calves were randomly divided into four groups, *viz.* T_1 (Control), T_2, T_3 and T_4. Calves in T_1 group were fed basal diets without betaine supplementation and treatment groups were fed basal diets plus betaine @ 10, 15 and 20 grams in T_2, T_3 and T_4 groups, respectively for a period of 90 days. rumen fermentation pattern revealed that strained rumen liquor pH was significantly ($p<0.05$) decreased in T_4 group than T_1, T_2 and T_3 groups. While there was significant ($p<0.01$) increase in Total volatile fatty acid (TVFA) concentration with betaine supplementation in T_3 and T_4 groups than control group (T_1) which also significantly differed from each other. Total nitrogen concentration was significantly ($p<0.01$) increased in T_4 groups than other (T_1 and T_2) groups and difference between T_1 and T_2 was non-significant ($p>0.05$). Non-protein nitrogen was significantly ($p<0.01$) increased in T_4 group than control (T_1) group. Betaine supplementation had non-significant effect on average concentration of trichloroacetic acid precipitable nitrogen (TCA-N). While, ammonia nitrogen (NH_3-N) was significantly ($p<0.01$) decreased with betaine-supplemented groups (T_2, T_3 and T_4) compared to control (T_1) group.

Keywords: Betaine, Gir calves, rumen fermentation pattern

Introduction
India is the fastest-growing country, in which the majority of the people are financially depend on agriculture and animal husbandry. Gir is a famous milch cattle breed of India from Gujarat. Calves play an important role in the development of dairy sector, as the future of the dairy herd merely depends upon the successful raising of young calves.
The first step in running a successful dairy is starts with proper nutritional care (Anonymous, 2012) [1]. The reduced growth rate during hot and cold seasons in 1 to 1.5 years age of calf. To achieve maximum growth of calf, feed must be balanced. Increasing the nutrient availability for better utilization of crop residue has been a primary focus of farmers for sustainable profit of farmers. Feed additives in diet used to improve the overall digestibility of feed have been tried over the years.
Betaine (BET) is a feed additive and trimethyl derivative of the amino acid glycine and is widely found in a variety of plants. Betaine is extracted from sugar beet molasses. It have positively effect on intestinal function and microbes in monogastric animals (Ratriyanto *et al.*, 3 Introduction 2009; Eklund *et al.*, 2005) [5] and increased performance, total-tract nutrient digestibility, rumen cellulase activity and microbial abundance in ruminants (Wang *et al.*, 2020) [8].

Materials and Methods
The study was conducted on Gir calves at Cattle Breeding Farm and Department of Animal Nutrition, College of Veterinary Science and A.H., Kamdhenu University, Junagadh, Gujarat. Junagadh is located at 70.5˚ east longitude and 21.4˚ north latitude and is about 60 meter above mean sea level. The annual temperature varies between a minimum of 6.5 °C and a maximum of 43.5 °C. After the collection of rumen liquor immediately pH of SRL (Strained Rumen Liqour) was determined using a pen type Digital pH meter (DPH-2,

ATAGO) at each interval. The TVFA was determined by Markham's steam distillation method. Ammonia nitrogen was estimated by Conway's micro-diffusion method. Total-N in SRL was analyzed using a semi-automatic nitrogen analyzer. Non-protein nitrogen was analyzed by digestion, distillation and titration. TCA precipitable nitrogen calculated by the difference of Total nitrogen and Non-protien nitrogen.

Experimental details

Sixteen Gir calves of around one year of age were randomly selected from cattle breeding farm. They were assured of their health and disease. Selected calves were randomly divided into four groups with four calves in each, considering their body weight. The duration of the experiment was 90 days. Experimental calves were randomly divided into four groups with four calves in each using a completely randomized design (CRD). The nutrient requirements of the calves in terms of DCP and TDN were met as per ICAR (2013) feeding standards. The roughage part consisted of seasonal green fodder (Green sorghum, Super Napier and sorghum sudan) and dry fodder (Groundnut haulms). The concentrate part consisted of compound cattle feed (BIS type-II) (Table 1). Feed additive as betaine powder. The experiment consisted of four dietary treatments: T_1 = Basal diet fed without supplementation of Betaine. T_2 = Basal diet fed with supplementation of Betaine with 10 gram/animal/day. T_3 = Basal diet fed with supplementation of Betaine with 15 gram/animal/day. T_4 = Basal diet fed with supplementation of Betaine with 20 gram/animal/day.

Table 1: Proximate composition and cell wall fractions of different feeds and fodders used in the experiment (% DM basis)

Ingredients/Attributes	Green Sorghum	Green Super napier	Green Sorghum sudan grass	Groundnut haulms	Compound cattle feed
DM	28.02	34.59	30.05	92.00	90.00
OM	90.30	92.66	90.24	88.28	93.37
CP	6.09	5.55	7.92	10.85	20.87
EE	2.46	2.31	1.51	2.16	2.98
CF	32.06	29.57	33.11	41.38	10.03
NFE	49.69	55.23	47.70	33.89	59.49
Total Ash	9.70	7.34	9.76	11.72	6.63
NDF	63.68	64.69	65.97	70.63	34.91
ADF	51.86	52.57	52.34	50.77	18.52
Hemicellulose	11.82	12.12	13.63	19.86	16.39
Cellulose	38.09	39.37	37.67	35.26	12.33
Lignin	2.96	2.52	2.77	3.54	2.62

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; NFE, nitrogen-free extract; NDF, neutral detergent fibre; ADF, acid detergent fibre.

Collection and analysis of rumen liquor

About 100 ml rumen liquor samples were collected from two calves in each treatment group at the end of the experiment with the help of a stomach tube against negative pressure created by a suction pump. The samples were collected at '0' hours before feeding and '3' hours and '6' hours post-feeding to study the changes in the pH, total volatile fatty acids (TVFA) and various nitrogenous fractions from strained rumen liquor (SRL). The rumen liquor was quickly brought to the laboratory and strained through four layers of a muslin cloth referred as strained rumen liquor (SRL). After collection, 0.1ml of 10N H_2SO_4 was added except in samples used for pH determination to stop microbial activity. The samples were then stored in a deep freezer for estimation of TVFA and various nitrogen fractions.

Results and Discussion

Rumen fermentation pattern

Average values for ruminal pH, total volatile fatty acid, ammonia nitrogen, TCA precipitable nitrogen, total nitrogen and non protein nitrogen of experimental Gir calves determined from the rumen liqour collectied before feeding (0 hour) and 3 and 6 hours post feeding have been presented in Table 2.

Ruminal pH

The average pH values of SRL are presented in Table 2. The average pH values in SRL of T_1, T_2 T_3 and T_4 groups were 7.18±0.04, 7.08±0.06, 6.95±0.10 and 6.88±0.06, respectively. Statistical analysis revealed significantly ($p<0.05$) decreased ruminal pH in 20 gram/d betaine supplemented (T_4) group as compared to T_1, T_2 and T_3 groups but between T_1, T_2 and T_3 were non-significant effect was found. This decline in pH value on betaine supplementation might be due to increased TVFA production because of increased digestibility of crude fibre. Regarding pH values at various hours irrespective of treatment significantly ($p<0.05$) decreased values of pH were found at 3 hours post feeding which then increased at 6 hours. This decreased in pH at 3 hours post feeding is because of increased production of TVFA at 3 hours and further increase in rumen pH values at 6 hours post feeding is due to the utilization of TVFA by microbes as well as buffering action of saliva.

In support of present findings, Liu *et al.* (2021) [3] reported betaine supplementation significantly ($p<0.05$) decreased ruminal pH. However, Shah *et al.* (2020) [6] found non-significant effect of betaine supplementation on rumen pH. Similarly, Wang *et al.* (2010) [7], Nakai *et al.* (2013) [4], Ghoneem and El-Tanany (2023) [2] and Wang *et al.* (2020) [8] also found statisticaly non-significant but numerically lower values of ruminal pH on betaine supplementation.

Total Volatile Fatty Acid (TVFA)

The average TVFA values of SRL are presented in Table 2. The values were 6.93±0.23, 7.31±0.13, 8.26±0.36 and 9.63±0.23 Mmol/dL in T_1, T_2, T_3 and T_4 groups, respectively. Statistical analysis revealed significantly ($p<0.01$) increased TVFA in T_3 and T_4 groups as compared to T_1 groups. Difference between T_3 and T_4 was also significantly higher in 20 gram/d betaine supplemented

group (T_4) as compared to 15 gram betaine supplemented group. Whereas, non significant (p>0.05) effect was found between T_1 and T_2 group. Increased TVFA production is because of increased digestibility of crude fibre. Regarding TVFA value at various hours irrespective of treatment it was observed numerically increased values of TVFA were found at 3 hours post feeding which then decreased at 6 hours. This increased in TVFA at 3 hours post feeding is because of increased production of TVFA at 3 hours and further decrease in rumen TVFA values at 6 hours post feeding is due to the utilization of TVFA by microbes as well as buffering action of saliva.

In accordance to the present findings, Wang *et al.* (2010) [7] found total volatile fatty acid (TVFA) concentration was increased with betaine supplementation. Likewise, Shah *et al.* (2020) [6], Liu *et al.* (2021) [3] and Ghoneem and El-Tanany (2023) [2] also found the concentration of VFA was significantly increased (p<0.05) with betaine supplementation. In contrast, to this study Nakai *et al.* (2013) [4] found no significant effect of betaine supplementation, incubation time or interaction between betaine supplementation and incubation time on the concentration of volatile fatty acid compared to control group.

The increase in ruminal total VFA concentration was due to the positive impact of betaine addition on activity of carboxymethyl cellulase, cellobiase, xylanase and pectinase as well as population of total protozoa, *Ruminococcus albus* and *Fibrobacter succinogenes*. Ruminal *Ruminococcus albus* and *Fibrobacter succinogenes* are dominant fibrolytic bacteria and protozoa is responsible for more than 30% of fibre degradation in the rumen.

Ammonia Nitrogen (NH_3-N)

The average NH_3-N values in SRL of T_1, T_2 and T_3 groups were 20.08±0.67, 18.66±0.00, 17.16±0.63 and 16.66±0.68 mg/dL, respectively Table 3. Statistical analysis revealed significantly (p<0.01) decreased in NH_3-N with betaine supplemetation in T_2, T_3 and T_4 groups than control group (T_1) but difference between T_2, T_3 and T_4 were non-significant to each other. Regarding NH_3-N value at various hours irrespective of treatment it was observed that significantly (p<0.05) increased values of NH_3-N at 3 hours post feeding which then decreased at 6 hours. This increased in NH_3-N at 3 hours post feeding is because of increase degradation of crude protein at 3 hours and further decreased in NH_3-N values at 6 hours post feeding is due to synthesis of microbial protein by various microbes as well as buffering action of saliva.

In support of present findings, Wang *et al.* (2010) [7], Nakai *et al.* (2013) [4], Ghoneem and El-Tanany (2023) [2] found that betaine supplementation significantly (p<0.05) decreased in ammonia nitrogen. Similarly, Shah *et al.* (2020) [6] also found betaine supplementation significantly (p<0.05) decreased in ammonia nitrogen.

In contrast to this study, Liu *et al.* (2021) [3] observed ammonia nitrogen concentration elevated with dietary inclusion of betaine supplementation

Total Nitrogen

The average values of total nitrogen concentration of SRL are presented in Table 3. The average total nitrogen values of T_1, T_2, T_3 and T_4 groups were 68.13±3.02, 69.76±2.91, 75.83±1.63 and 81.43±1.99 mg/dL, respectively. Statistical analysis revealed significantly (p<0.01) increased total nitrogen in 20 gram/d betaine treated (T_4) group than T_1 and T_2 groups. Difference between T_1 and T_2 was non-significant. Regarding total nitrogen value at various hours irrespective of treatment it was observed that numerically increased value of total nitrogen at 3 hours post feeding which then decreased at 6 hours. This increased in total nitrogen at 3 hours post feeding is because of increase degradation of crude protein at 3 hours and further decreased in total nitrogen values at 6 hours post feeding is due to synthesis of microbial protein by various microbes as well as buffering action of saliva.

TCA Precipitable Nitrogen (TCA-N)

The average TCA-N values of SRL are presented in Table 3. the values were 43.86±3.29, 43.86±2.49, 47.13±1.34 and 47.45±0.91 mg/dL in T_1, T_2, T_3 and T_4 groups, respectively. Statistical analysis revealed no-significant effect (p>0.05) of all levels of betaine supplemented group (T_2, T_3, and T_4) was observed over control (T_1) group. But there was numerically higher value of betaine treated groups than control group. The increased TCA-N concentration in present study is indicative of more synthesis of microbial protein.

Non Protein Nitrogen (NPN)

The average values for non protein nitrogen concentration of SRL are presented in Table 3. The average non protein nitrogen values of T_1, T_2 T_3 and T_4 groups were 24.26±0.76, 25.90±0.70, 28.70±0.44 and 30.56±0.96 mg/dL, respectively. Statistical analysis revealed significantly (p<0.01) increased with 20 gram/d betaine treated groups (T_4) over control groups (T_1) and difference between control and 15 gram betaine treated group was significantly (p<0.05) higher in T_3 compared to T_1 group and there was non-significant effect was found for 10 gram betaine treated (T_2) group as compared to control (T_1) group. Observed non significant (p>0.05) effect between 10 (T_2) and 15 gram (T_3) betaine supplemented group. Regarding non protein nitrogen value at various hours irrespective of treatment it was observed that significantly (p<0.05) increased value of non protein nitrogen at 3 hours post feeding which then decreased at 6 hours. This increased in non protein nitrogen at 3 hours post feeding is because of increase degradation of crude protein at 3 hours and further decrease in non protein nitrogen values at 6 hours post feeding is due to synthesis of microbial protein by various microbes as well as buffering action of saliva.

Table 2: Rumen fermentation parameters at different intervals in control and different treatment groups

Hours	Treatment				
	T_1	T_2	T_3	T_4	MEAN±SE
Rumen pH					
0	7.27±0.08	7.22±0.09	7.12±0.19	7.10±0.13	7.18^{B}±0.04
3	7.05^{b}±0.09	6.85^{ab}±0.08	6.67^{a}±0.08	6.57^{a}±0.07	6.78^{A}±0.10
6	7.22±0.11	7.17±0.06	7.07±0.12	6.98±0.20	7.11^{B}±0.05
Overall Mean±SE	7.18^{b}±0.04	7.08^{b}±0.06	6.95^{b}±0.10	6.88^{a}±0.06	
p(Treatment) value = 0.04, p(period) = <0.001					
TVFA (Mmol/dL)					
0	6.60^{a}±0.14	7.10^{a}±0.35	7.65^{a}±0.38	9.40^{b}±0.37	7.68±0.61
3	7.30^{a}±0.26	7.55^{a}±0.18	8.90^{b}±0.46	9.90^{b}±0.17	8.41±0.61
6	6.90^{a}±0.36	7.30^{ab}±0.12	8.25^{b}±0.26	9.60^{c}±0.38	8.01±0.60
Overall Mean±SE	6.93^{a}±0.23	7.31^{ab}±0.13	8.26^{b}±0.36	9.63^{c}±0.23	
p(Treatment) value = <0.001, p(period) = 0.008					
P<0.01 = Significant					

Table 3: Nitrogen Content parameters at different intervals in control and different treatment groups

Hours	Treatment				
	T_1	T_2	T_3	T_4	MEAN±SE
NH_3N (mg/dL)					
0	14.75±0.47	15.50±0.50	14.00±0.82	14.00±0.41	14.56^{A}±0.35
3	24.00^{b}±1.15	22.00^{ab}±0.82	20.50^{ab}±1.26	19.50^{a}±0.96	21.50^{B}±0.97
6	21.50^{b}±0.95	18.50^{ab}±0.50	17.00^{a}±0.58	16.50^{a}±0.96	18.37^{B}±1.12
Overall Mean±SE	20.08^{b}±0.67	18.66^{a}±0.00	17.16^{a}±0.63	16.66^{a}±0.68	
p(Treatment) value = <0.001, p(period) = <0.001					
Total nitrogen (mg/dL)					
0	64.40±5.11	66.50±4.62	71.40±3.33	76.30±3.85	69.65±2.65
3	72.10^{a}±2.39	74.20^{a}±2.91	82.60^{ab}±1.80	88.20^{b}±2.91	79.27±3.74
6	67.90^{a}±1.76	68.60^{a}±2.42	73.50^{ab}±1.76	79.80^{b}±3.70	72.45±2.74
Overall Mean±SE	68.13^{a}±3.02	$69.76^{a\pm}$2.91	75.83^{ab}±1.63	81.43^{b}±1.99	
p(Treatment) value = <0.001, p(period) = <0.001					
TCA-N (mg/dL)					
0	42.70±5.98	46.20±3.33	49.70±3.50	51.10±3.30	47.42±1.88
3	44.80±1.97	42.70±2.39	44.80±1.97	45.20±2.52	44.38±0.56
6	44.10±2.39	42.70±2.88	46.90±2.88	46.05±2.06	44.93±0.94
Overall Mean±SE	43.86±3.29	43.86±2.49	47.13±1.34	47.45±0.91	
p(Treatment) value = 0.21, p(period) = 0.48					
NPN (mg/dL)					
0	21.70±1.34	20.30±1.34	21.70±1.34	25.20±1.14	22.22^{A}±1.04
3	28.00^{a}±1.14	31.50^{ab}±1.34	35.70^{b}±1.34	$36.40^{b\pm}$1.61	32.90^{B}±1.95
6	23.10^{a}±1.34	25.90^{ab}±0.70	28.70^{b}±0.70	30.10^{b}±1.34	26.95^{A}±1.55
Overall Mean±SE	24.26^{a}±0.76	25.90^{ab}±0.70	28.70^{bc}±0.44	30.56^{c}±0.96	
p(Treatment) value = <0.001, p(period) = <0.001					
p<0.01 = Significant					

Conclusion

There was a reduction in rumen pH as well as NH_3-N upon betaine supplementation. While, Total-nitrogen and Non-protein nitrogen were increased. Based on overall results of present experiment it may be concluded that betaine supplemented @ 20 g/d in Gir calves improves rumen performance without any adverse effect on health status of Gir calves.

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