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Effect of Various Levels of Exogenous Fibrolytic Enzymes and Fumaric Acid on the Digestibility, Methane Emission and Performance of Growing Sahiwal Calves

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ABSTRACT

This study was aimed to test whether combination of exogenous fibrolytic enzymes and fumarate as fumaric acid, a hydrogen sink may result in any complementary effects *in vivo*. Growing Sahiwal calves (18; 6–12 month-old; average body weight, 132 kg; range 79–192 kg) were arranged into 3 groups in a randomized complete block design. Treatments were: Control (no additives), T1 (control + 0.75 g/kg DM of EFE + Fumaric acid (FA) @ 2% of DM and T2 (control + 1.5 g/kg DM of EFE + FA @ 2% of DM). Exogenous fibrolytic enzymes (EFE) (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w). During growth trial of 120 days, calves were fed with a basal diet containing sorghum stover, green grass (freshly cut) and concentrate mixture (50: 10: 40) *ad lib.* (15% in excess of the previous day's intake). Various levels or doses of EFE in combination with FA had no effect on final body weight, average daily gain and daily feed intake. There was no effect of supplementation of additives on apparent digestibility of DM, OM, CF, EE, NDF, and ADF but CP digestibility increased as a result of T1 treatment. Nitrogen intake and nitrogen retention was similar in three groups. Methane energy estimated as loss of GE intake decreased by 13.54 and 10.76 per cent, for T1 and T2 respectively, compared to the control and methane expressed as g/kg digestible dry matter intake was 12.29% lower on T1 treatment and 5.03 % with T2 compared to the control. These reductions were not reflected in growth performance of calves. It can be concluded that reduction of methane production caused by feed additives to the basal diet in the present study was though quantifiable it could not improve animal performance and nutrients utilization in contrast to the previous *in vitro* findings observed during the use of EFE and organic acids combination on roughage based diet.

KEY WORDS: Exogenous fibrolytic enzymes, Methane, Fumaric acid, organic acid, Sahiwal calves

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INTRODUCTION

Methane production is an inescapable consequence of the fermentation of carbohydrates in the rumen. About 2–12% of the ingested feed energy is lost as CH_4 (Johnson and Johnson, 1995). India has a livestock population of 536.76 million (20th Livestock Census), producing 12.74 Tg (Anuja Samal et al., 2024) of methane every year which contributes to 10.63 % of global methane emission from livestock. The radiative force of methane relative to CO_2 (global warming potential) is 25 kg CO_2/kg CH_4 (Forster et al., 2007). Abatement of enteric CH_4 emission is required to minimize the liability of livestock production for green house gases (GHG) emission (Patra, 2016). Traditionally ruminants are managed on grazing, but in view of continuous depletion of grazing lands and scarcity of either green or dry forages to feed livestock, crop residues like straws, stovers and other agro-industrial byproducts must be supplied through stall feeding. Using a multi-dimensional approach for improving the quality and thereby the utilization of food-feed crops, animal productivity can be enhanced, which can also lead to reduction in animal numbers, feed requirement and the emission of GHG (Blummel et al., 2009).

A comprehensive approach involving dietary interventions through the use of stover based balanced diet along with exogenous enzymes and organic acids are evaluated for feed efficiency and methane mitigation potential. Exogenous fibrolytic enzymes work in synergy with the endogenous rumen microbiological enzymes to enhance the digestibility and nutritive value of high fibrous diet (Morgavi et al. 2000). Earlier studies reported that on enzyme supplementation (Dong et al., 1999; Zhou et al., 2011; Chung et al., 2012), there was an increase of methane production and energy loss as CH_4 during ruminal fermentation while Giraldo et al., 2007, recorded no change. Arriola et al., (2011) observed reduced CH_4 production in dairy cows supplemented with fibrolytic enzyme while Beauchemin et al., (2008) proposed that dietary enzyme as feed additives may help mitigate enteric CH_4 emissions. Bayaru et al. (2001) reported a 23% decrease in methane production when fumarate (@ 2% of DM) was added to an all-forage (sorghum silage) diet fed to steers. Fumarate, an organic acid is classified as ruminal hydrogen sink and can divert the hydrogen formed during the anaerobic rumen fermentation from forming into methane. It is unclear whether the addition of fumarate in combination with EFE would improve fibre digestion and mitigate methane production.

Hence, there is a wide scope for evaluating the synergistic treatment effects on stover based diet with exogenous fibrolytic enzymes and fumarate with regard to their effect on growth, nutrient utilization and level of methane production in Indian conditions, systematically.

MATERIALS AND METHODS

Animals, treatments and experimental procedures

The experimental protocol was approved by Institute Ethics committee of Animal Experiments, Indian council for agricultural research (ICAR)-National Dairy Research Institute (NDRI), Karnal, India. Sahiwal male calves (18; 6–12 month-old; average body weight, 132 kg; range 79–192 kg), were selected from the Cattle Yard of NDRI, Karnal, and allotted to 3 treatments in a randomized complete block design (RCBD). The treatments were: control (no additives), T1 (control + 0.75 g/kg DM of EFE + Fumaric acid (FA) @ 2% of DM and T3 (control + 1.5 g/kg DM of EFE + FA @ 2% of DM). Exogenous fibrolytic enzymes (EFE) (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w) Enzymes were procured from M/s. Rossari Pvt. Ltd., India. The calves were housed in a well-ventilated stall having facilities for individual feeding and watering. Animals were dewormed using Albendazole @ 10 mg/ kg body weight before starting the experimental feeding. During the experiment (120 d), calves were fed with a basal diet (Table 1) containing sorghum stover, green grass (freshly cut) and concentrate mixture (50: 10: 40) *ad lib.* (15% in excess of the previous day's intake) once daily at 09: 00, and had access to freshwater all time. Additives were pre-mixed with concentrate at the time of feeding. The dose of added FA is in percent expressed on dietary DM content (Kolver et al., 2004; Kolver and Aspin, 2006). The concentrations of EFE and FA were selected based on the results of an *in vitro* experiment (Sudheer et al., 2017). Before starting the growth trial, the actual feed intake of each animal was measured to assess the EFE and FA doses. At the end of every fortnight doses were adjusted according to new average body weight (BW) and dry matter intake (DMI) for each animal in the treatment group.

BW and DMI

The animals were weighed before feeding and watering in the morning on two consecutive days at the start of experimental feeding and thereafter at fortnightly intervals during the experimental period of 120 days. Sorghum stover, green grass and concentrates were sampled weekly to determine their DM content, and diets were adjusted weekly for changes in DM content. DMI was recorded daily by subtracting the residual DM from the quantity of DM offered.

Growth performance

Growth rate was calculated on the basis of increase in BW at fortnightly intervals and feed conversion efficiency (FCE) was expressed as the ratio of feed intake on gain (kg/kg).

Metabolism trial

On day 60, a metabolism trial was conducted with 7 days collection period to determine nutrient digestibility and nitrogen balance. Animals were housed in metabolism shed (equipped for quantitative collection of faeces and urine separately) 1 week prior to the metabolism trial for their adaptation to the surroundings. Animals were weighed before and after trial consecutively for 2 days. Allotted rations were fed to each animal as explained earlier. Feeds and their respective residues were collected in separate polythene bags daily for DM estimation. These samples were pooled at the end of the collection period and ground to pass through 1 mm screen and preserved in air tight polythene bags until analyzed for proximate principles. Faeces voided during 24 h, was collected and weighed at 9.30 AM daily. After thorough mixing, an aliquot (1%) in duplicate dried at 80°C in oven for DM estimation and pooled for 7 days. Another aliquot (1/500) was mixed thoroughly with 5 ml of 25% H_2SO_4 and stored in pre weighed air tight plastic container. At the end of collection period, the plastic container was weighed and contents were emptied in a tray and mixed thoroughly and an aliquot (10 g) was analyzed for total N. Dried dung samples were ground to pass through 1 mm sieve size and analyzed for proximate principles and cell wall constituents (NDF and ADF) as per standard procedure described below. Total urine output was collected from each calf. Calves were fitted with urine collection bag, attached to the big plastic container through a plastic tube. Each container was added 100 ml of 10% H_2SO_4 daily to prevent bacterial destruction of purine derivatives in the collected urine. Total urine output for 24 h was measured daily at 9.30 AM and an aliquot (1/500 of total output) was taken for the nitrogen estimation. This aliquot was stored in plastic container containing 2 ml of 25% H_2SO_4. For urinary purine derivatives estimation, 1% of urine excreted/ day by each animal was taken and diluted five times with water and 20 ml of diluted urine was stored in the plastic container at – 20°C daily throughout the collection period.

Enteric methane emission

Enteric CH_4 emissions were measured for 5 days starting on day 80 of the trial, using the SF_6 tracer gas technique described by Johnson et al. (1994). A permeation tube containing SF_6, an inert gas tracer, was placed into the rumen of each animal approximately 2 week before CH_4 measurements commenced. The permeation tubes were manufactured at Environmental lab, ICAR-NDRI, India and were filled with excess of 1 g of SF_6 per bolus. The average release rate was 2.2±0.09 mg/ day, which was predetermined over the preceding 8 week period by weighing each permeation tube at the same time point once weekly. After collection of a sample, the canisters were pressurized with nitrogen, and the concentration of SF_6 in the canisters was analyzed by gas chromatography (GC), fitted with an electron capture detector (ECD) (250°C) and 3.3 m molecular sieve column with an i.d. of 0.32 mm to determine SF_6 concentrations. Another GC was fitted with a flame-ionization detector (FID) (100°C) and stainless steel column packed with Porapak-Q (length 1.5m; o.d. 3.2 mm; i.d. 2 mm; mesh range 80–100) to determine CH_4 concentration. The column and injector temperatures were 50 and 40°C in both gas chromatographs. All samples were analyzed in duplicate except standards, which were analyzed in triplicate. Nitrogen was used as the carrier gas at a pressure of 1kg/ cm^2. The standards were run at the beginning and end of each day with the medium standard run every 10 samples throughout the day. Gas concentrations (SF_6 and CH_4) were determined from peak areas and identified from their different retention times relative to the known standards. The methane output calculated using following formula:

$$CH_4(g/d) = \left(\frac{S_{CH_4} - B_{CH_4}}{S_{SF_6} - B_{SF_6}}\right) \times \left(\frac{M_{CH_4}}{M_{SF_6}}\right) \times Q_{SF_6} \times 1000$$

Where S_{CH4} and B_{CH4} are methane concentrations in sample and background's canisters (ppm), S_{SF6} and B_{SF6} represent the concentrations of SF_6 in sample and background's canisters (ppt), M_{CH4}

andM_{SF6} are molecular weight of methane and SF_6 (g), respectively and Q_{SF6} represents release rate of SF_6 (mg/d).

Laboratory analysis

The analytical DM content of samples was determined by oven drying at 105ºC for 24 h. Representative samples dietary ingredients were dried by oven at 65ºC for 72 h ground to pass a 1 mm screen and stored for subsequent analyses. Organic matter (OM) was determined by ashing at 550ºC for 5 h, Neutral detergent fibre (NDF) and acid detergent fibre (ADF), both expressed inclusive of residual ash, were determined by the method of Van Soest et al. (1991) and AOAC method (973.18, A-D, 2006), respectively. Crude protein (CP) content (N×6.25) was determined using KEL PLUS - N analyzer, which is based on the method described by AOAC (method 984.13 A-D, 2006). Ether extract (EE) was estimated using AOAC (method 920.39 A, 2006). Allantoin and uric acid in urine were determined by an ultraviolet visible spectrophotometer UV-2100 and intestinal flow of microbial-N was calculated using the procedure of IAEA (1997).

Statistical analysis

Data was subjected to one way analysis of variance (ANOVA) using statistical analysis system (SAS Inst. Inc., Cary, NC, USA) software. The differences among means were considered statistically significant at 5 % level of probability ($p \leq 0.05$). When a significant F value ($P < 0.05$) was detected, means were compared by the Duncan test.

RESULTS AND DISCUSSION

Chemical composition

Chemical compositions of ingredients in the basal diet are presented in Table 1. The feeding of additives on DMI during 120 days growth trial indicated no significant ($P>0.05$) effect of treatments throughout the trial (Table 2 & Fig 1). Previous studies have also reported that enzymatic treatment did not affect nutrient intake (Alvarez et al., 2009; Giraldo et al., 2008; Patel et al., 2024). Over all DMI expressed as (kg/day) though nonsignificant (Dean et al., 2013) an increase of 4.73 per cent in T1 treated group compared to control was recorded and on further dose increase of EFE (@1.5 g/ kg DM) in T2 showed no positive response over the control or T1 level of supplementation indicating that addition of fibrolytic enzymes at a particular level is effective in increasing the feed intake. Beauchemin et al. (2003) reported that application of higher doses of enzymes could be less effective than low rates of application in increasing feed intake. It can also be observed that, level of fumaric acid (@ 2% of diet DM) addition being constant in T1 and T2, the slight increase augmented in DMI in T1 is by the EFE alone and hence, it can be inferred that FA has no complimentary or synergistic effect on the intake of the diet (Mohini et al., 2009).

Table 1. Chemical analysis of diet ingredients (% of DM)

Item	Sorghum stover	Green grass	Concentrate mixture[1]
OM	92.7	85.4	88.4
CP	2.46	4.12	19.6
CF	34.2	38.1	17.3
EE	1.09	1.84	2.52
NFE	54.9	41.2	49.0
NDF	73.1	62.2	42.1
ADF	44.3	42.3	20.5

OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre. [1]Concentrate contained: maize grain, ground nut cake, de oiled mustard cake, wheat bran, rice bran, mineral mixture and common salt (33, 21,12,20,11, 2 and 1% of DM, respectively)

Growth rate and performance

Growth rate and performance of calves are given in Tables 3 and 4. Animals on T1 or T2 treatment compared to the control had similar BW during growth trial (Table 3), final BW, ADG and FCE (Table 4). The change in BW compared to control on feeding EFE and FA did not show any positive effect. It can be inferred that the beneficial effects as observed in T1, on combining EFE and FA for the above measure variables were of EFE alone, as T2 treated animals showed slightly lower values, compared to the T1 with FA level being constant in both treatments. In contrast, use of organic acids resulted in improved ADG (Martin et al., 1999), live weight gain (Wood et al., 2009) but in other reports no significant effects were observed in ADG (Carro et al., 2006), carcass traits (Martin et al., 1999), carcass yield (Carro et al., 2006). Eun et al. (2008) inferred that the variations in the growth performance of ruminants consuming forages treated or supplemented with fibrolytic enzymes may be due to factors such as specificity and enzyme activity as well as the manner and time of application and the nature of the cell wall of the forage. Earlier studies reported consequent to EFE supplementation enhanced rumen fiber digestion leading to improved FCE of ruminants (Holtshausen et al., 2011).

Table 2. Effect of feeding exogenous fibrolytic enzymes and fumaric acid on fortnightly DMI (kg/day) of Sahiwal male calves during 120 days of growth trial

Fortnight	Control	T1	T2
1st	2.78±0.37	2.84±0.30	2.74±0.28
2nd	3.46±0.44	3.60±0.28	3.32±0.32
3rd	3.81±0.47	4.02±0.32	3.75±0.35
4th	3.78±0.46	4.01±0.31	3.69±0.37
5th	3.31±0.48	3.50±0.36	3.25±0.36
6th	3.35±0.47	3.49±0.34	3.22±0.35
7th	3.22±0.45	3.40±0.35	3.18±0.36
8th	3.34±0.46	3.48±0.35	3.09±0.37
Overall mean ± SEM	3.38±0.45	3.54±0.32	3.28±0.34

Control (no additives), T1 (control + 0.75 g/kg DM of EFE + FA @ 2% of DM and T3 (control + 1.5 g/kg DM of EFE + FA @ 2% of DM). Exogenous fibrolytic enzymes (EFE) (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w).

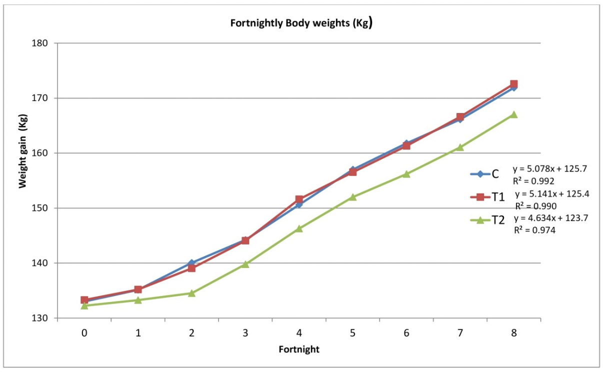

Fig 1 Fortnightly body weights (kg) of growing Sahiwal male calves fed with diet having various levels of additives

Table 3. Effect of feeding exogenous fibrolytic enzymes and fumaric acid on change of BW (kg) of Sahiwal male calves during 120 days of growth trial

Fortnight	Control	T1	T2
Initial	133.01±14.8	133.28±13.8	132.24±12.8
1st	135.13±16.7	135.18±15.0	133.26±13.0
2nd	140.05±17.9	139.03±14.8	134.52±13.7
3rd	144.22±18.8	144.08±15.7	139.77±14.2
4th	150.60±19.2	151.60±15.9	146.26±14.6
5th	157.02±18.9	156.53±16.7	152.00±14.4
6th	161.79±19.2	161.30±17.1	156.19±14.5
7th	166.11±19.3	166.61±16.5	161.04±14.4
8th	171.88±19.6	172.58±16.9	167.03±14.6
Weight gain (Kg)	38.88±4.90	39.30±3.49	34.78±2.07

Control (no additives), T1 (control + 0.75 g/kg DM of EFE + FA @ 2% of DM and T3 (control + 1.5 g/kg DM of EFE + FA @ 2% of DM). Exogenous fibrolytic enzymes (EFE) (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w).

Table 4. Effect of feeding exogenous fibrolytic enzymes and fumaric acid on growth performance in Sahiwal male calves

Parameter	Control	T1	T2
Initial BW (kg)	133.01±14.8	133.28±13.8	132.24±12.8
Final BW (kg)	171.88±19.6	172.58±16.9	167.03±14.6
ADG (Kg)	0.32±0.04	0.33±0.03	0.29±0.02
Daily feed intake (kg)	3.38±0.45	3.54±0.32	3.28±0.34
Feed conversion ratio (FCR) (kg intake / kg gain)	10.44±0.26	10.84±0.41	11.19±0.63

Control (no additives), T1 (control + 0.75 g/kg DM of EFE + FA @ 2% of DM and T3 (control + 1.5 g/kg DM of EFE + FA @ 2% of DM). Exogenous fibrolytic enzymes (EFE) (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w).

Metabolism trial

Results of metabolism trial are presented in Tables 5 and 6. There was no effect of supplementation of additives on apparent digestibility coefficient of DM, OM, CF, NDF, and ADF but CP and EE digestibility among the groups were significant (P<0.05) with higher DCP in group with T1 treatment followed by T2 and Control while EE digestibility coefficient was significantly higher in control group followed by T1 and T2 treatment groups. Sandeep et al., 2023 also reported similar results. In contrast to the results in this study, (Yang et al., 2000) reported that the application of fibrolytic enzymes to the concentrate instead of the TMR increased total tract DM digestion and milk yield while (Sutton et al., 2003) reported decreased digestibility and no effect on the milk yield. Animals on T1 showed significant (P<0.05) improvement of CP digestibility with an increase of 8.96 per cent compared to the control while T2 group had an increase of 1.02 per cent CP digestibility compared to the control. There was no effect of organic acids on apparent nutrients digestibility (Sniffen et al., 2006; Foley et al., 2009a; Mohini et al., 2009). Positive affects have been reported for increasing of CP (Bayaru et al., 2001), DM, OM, NDF and ADF (Liu et al., 2009; Nipane et al., 2023) digestibility because of feeding organic acid. The numerically higher DCP content in T1 and T2 than that observed in the control might be due to significantly (P<0.05) higher digestibility of CP in T1 and numerically higher digestibility observed in T2 (Table 5). The TDN per cent recorded nonsignificant variation and was due to the lack of statistical differences observed with the OM, CP, EE and NFE digestibilities. The nitrogen balance was positive in all groups (Table 6), indicating that there was retention of protein in the experimental animals. A significant increase in CP digestibility in group T1 compared to that of control was accompanied with lower nitrogen losses through faeces, which resulted in nonsignificant improvement in nitrogen retention in animals on T1 treatment. In the present study, Microbial N yield (g/CP/day) though statistically non significant, increased by 0.94 and decreased by 0.17 per cent respectively, over the control for T1 and T2 groups respectively (Table 7). Microbial protein synthesis in rumen depends upon supply of ammonia, energy and carbon skeleton for amino acid synthesis (Tomar et al., 2010). In general, intake of balanced nutrients along with supply of essential minerals improve microbial protein synthesis as is the case with the present study and it can be inferred that the supplementation of various levels of enzymes and addition of fumarate had no complimentary or synergistic effect in furthering the positive effect compared to the control.

Table 5. Final BW (kg), Intake (kg/day) and apparent digestibility of nutrients (%), digestible nutrient intake (kg/day), nutritive value (%), urinary excretion of purine derivatives (mM/d) and intestinal flow of microbial-N (g/d) of Sahiwal males fed with exogenous fibrolytic enzymes and fumaric acid

Items	Control	T1	T2
BW	150.37±19.2	151.35±15.9	145.35±14.2
DMI (kg/d)	3.86±0.45	3.88±0.34	3.67±0.35
Digestibility			
DM	55.20±1.09	54.64±1.12	52.47±1.91
OM	57.75±1.03	57.57±1.04	54.89±1.80
CP	62.60^{a}±0.80	68.21^{b}±0.99	63.24^{a}±1.72
CF	44.10±1.49	43.71±1.33	42.31±2.20
EE	73.75^{b}±0.61	68.99^{a}±0.88	66.65^{a}±1.47
NFE	64.88±0.91	64.03±0.98	61.56±1.64
NDF	47.08±1.38	45.95±1.30	42.86±2.23
ADF	42.56±1.55	41.50±1.38	40.94±2.26
Digestible nutrient intake			
DCPI	0.26±0.03	0.29±0.03	0.26±0.02
TDNI	2.18±0.27	2.18±0.21	1.98±0.20
Nutritive value			
DCP	6.85±0.10	7.40±0.18	7.02±0.27
TDN	56.01±1.02	56.01±1.13	54.03±1.82

$^{a, b}$ superscripts in same row differ significantly (P<0.05); Control (no additives), T1 (control + 0.75 g/kg DM of EFE + FA @ 2% of DM and T3 (control + 1.5 g/kg DM of EFE + FA @ 2% of DM). Exogenous fibrolytic enzymes (EFE) (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w).

Table 6. Effect of feeding exogenous fibrolytic enzymes and fumaric acid on nitrogen intake, losses and retention (g/day) of growing Sahiwal male calves

Items	Control	T1	T2
Intake	67.34±7.23	67.53±6.11	64.80±5.49
Losses through:			
Faeces	25.08±2.69	24.20±4.42	23.91±2.33
Urine	20.88±1.37	22.65±4.36	20.81±1.22
Retention	21.38±3.73	20.67±4.67	20.09±3.72

Control (no additives), T1 (control + 0.75 g/kg DM of EFE + FA @ 2% of DM and T3 (control + 1.5 g/kg DM of EFE + FA @ 2% of DM). Exogenous fibrolytic enzymes (EFE) (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w).

Table 7. Urinary excretion of purine derivatives (PD) and intestinal flow of microbial-N in Sahiwal male calves fed with diet having various levels of additives during metabolic trial

Parameter	Control	T1	T2	SEM	P
Urinary excretion (mM/d)					
Allantoin	39.51	39.89	39.45	0.44	0.92
Uric acid	10.74	9.72	10.42	0.30	0.38
Total PD	50.25	49.60	49.87	0.59	0.92
Intestinal flow					
Microbial N (g/d)	28.73	29.00	28.68	0.32	0.92

SEM: Standard error of means; Control (no additives), T1 (control + 0.75 g/kg DM of EFE + FA @ 2% of DM and T3 (control + 1.5 g/kg DM of EFE + FA @ 2% of DM). Exogenous fibrolytic enzymes (EFE) (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w).PD=purine derivative; N=nitrogen

Table 8. Enteric methane emissions in Sahiwal male calves fed with diet having various levels of additives

Parameter	Control	T1	T2	SEM	P
DMI (kg/day)	3.86	3.88	3.67	0.21	0.91
Methane (g/day)	74.33	64.5	63.03	4.45	0.52
Methane (g/kg DMI)	19.26	16.6	17.1	1.17	0.62
CH_4 energy loss (%)					
GE intake	8.27	7.15	7.38	0.50	0.62
DE intake	10.4	8.99	9.62	0.64	0.67
ME intake	12.7	10.9	11.7	0.78	0.67

SEM: Standard error of means; Control (no additives), T1 (control + 0.75 g/kg DM of EFE + FA @ 2% of DM and T3 (control + 1.5 g/kg DM of EFE + FA @ 2% of DM). Exogenous fibrolytic enzymes (EFE) (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w). DMI= dry matter intake, GE=gross energy, DE=digestible energy, ME=metabolisable energy

Recent studies have reported the effects on rumen methanogenesis of FA in combination with other substances such as bentonite (Abdl-Rahman 2010), safflower oil (Li et al., 2011) and essential oil (Lin et al., 2013), but information on methane mitigation with EFE and in particular *in vivo* remains limited. During methane estimation trial, DMI was also measured simultaneously and results have been summarized in Table 8. DMI in group fed T1 was higher and in group T2 was lesser than control but the differences were not significant ($P > 0.91$). The methane emitted by T1 and T2 groups though statistically non significant ($P>0.05$) they have recorded a decrease of 13.12 and 15.20 per cent compared to Control. Similarly, methane energy as loss of gross energy intake was decreased by 13.54 and 10.76 per cent for T1 and T2 respectively, compared to the control. The methane production (g/kg DDMI) was 12.29% lower on T1 treatment and 5.03 % with T2 compared to the control. These reductions were not reflected in growth performance of calves during 120 days. It can be inferred that the energy saved by lower methane emission was not high enough to any significant extent, to effect the productive parameters positively and also the diet without additives might not be having high methane emissions to mitigate, nor the lactate induced sub-optimal ruminal pH to ameliorate, hence mitigation of methane and improvement in production is not observed as expected.

CONCLUSION

It can be concluded from the results of the current study that the reduction of methane production observed with Exogenous fibrolytic enzymes (cellulase with activity >4500 – 5000 µM glucose/g/min and Xylanase with activity >7500 – 8000 µM xylose/g/min, mixed in 50:50 w/w) used at the level of 0.75 g/kg DM or 1.5 g/kg DM along with Fumaric acid @ 2% of DM) in the present study was not effective and could not improve productivity and nutrient utilization in contrast to the previous *in vitro* findings observed during the use of EFE and organic acids combination on roughage based diet and further investigation is required.

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