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Impact of ground flaxseed supplementation in feed on rumen profile in male buffalo calves

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Abstract
The objective of the study was to investigate the impact of ground flaxseed on rumen microbiology and fermentation pattern in male buffalo calves. In all eight apparently healthy, rumen fistulated male buffalo calves of 2.0-2.5 years age, were divided into two groups (control and treatment) of four animals in each group. The experimental animals were kept on complete ration consisting of green fodder, wheat straw, concentrate and mineral mixture. The animals of group II were supplemented with ground flaxseed @ 15% on dry matter basis (in concentrate replacing oilseed cakes) in TMR for 21 days. Rumen liquor samples were collected for 3 consecutive days at different time intervals *viz.* 0, 1, 3, and 6 hrs post prandial after the period of microbial adaptation. The results revealed that there was significant increase in total bacterial count, total protozoal count, TVFA, total nitrogen and molar percentage of propionate following supplementation of ground flaxseed with significant decrease in ammonia nitrogen, molar percentage acetate and butyrate. However, following supplementation of ground flaxseed, there was no significant change in percentage of holotrichs and entodiniomorphs as compared to control group. It can be concluded from the investigation that that oral administration of ground flaxseed in feed improved the digestive efficiency of animals by enhancing rumen microbial population and improving the concentration of rumen metabolites.

Keywords: Buffalo calves, ground flaxseed, rumen microbes, rumen metabolites

Introduction
Ruminants are unique in their ability to utilize feeds rich in cellulose, most likely due to the great diversity of microorganisms that break down feed in the rumen of the host animal. Microorganisms such as bacteria, fungi and protozoa break down complex compounds by hydrolysis to produce volatile fatty acids, mainly acetate, propionate and butyrate. At the same time, varying amounts of formic acid, hydrogen (H_2) and carbon dioxide (CO_2) are produced as end products in fermentation (Hook *et al.* 2010) [12]. Most of the methanogenic archaea in the rumen use H_2 to reduce CO_2 to produce methane (CH_4). The CH_4 produced is not used by the animal itself, but instead represents an energy loss (2–12% of gross energy) to the atmosphere, mainly by eructation, where it has a negative impact on the climate (Johnson and Johnson 1995) [17].

Flaxseed is an oilseed which can be used as a source of high-quality protein and fat for ruminants (Neveu *et al.* 2014) [21]. Flaxseed contains high levels of linolenic acid, averaging 18% of the total seed weight and 53% of the total fatty acids (Mustafa *et al.* 2002) [19]. Intake of flaxseed or oil by dairy ruminants has also been shown to mitigate methane production (Chilliard *et al.* 2009) [5], improve reproductive performance (Santos *et al* 2008, Zachut *et al.* 2010) [23, 31] and modulate immune functions (Caroprese *et al.* 2009) [4]. The reason for reduced CH_4 production after flaxseed feeding is due to high content of essential dietary Poly Unsaturated Fatty acid (PUFA) linoleic acid in flaxseed (Beauchemin *et al.* 2009) [3].

Added flaxseed decrease CH_4 emissions because they lower the amount of organic matter that is fermented in the rumen, the activity of the ruminal methanogens, and protozoal numbers (Beauchemin *et al.* 2009) [3].

Besides reducing methane emission, the target of rumen manipulation is to improve fiber degradation, prevent energy losses and improve the health of dairy animals. The systematic study on the effect of flaxseed supplementation on rumen microbes, their activity and rumen fermentation pattern is not available therefore keeping in view the importance of rumen microbial population in improving digestibility and health of ruminants, the present study was planned to evaluate the effect of ground flaxseed supplementation on rumen microbiology and fermentation status of male buffalo calves.

Materials and Methods

Eight apparently healthy fistulated male buffalo calves of 2-2.5years of age and weighing between 200-250 kg were used in the present study. The animals were offered conventional diet with roughage to concentrate ratio of 60:40 on DM basis as TMR, only once a day at 9.00 hours as per ICAR guidelines (2013). The total fat content in the control and the flaxseed-supplemented diets did not differ. Energy and protein content of the two diets were also equivalent.

The experimental animals were divided into control (goup1) and treatment (group 2) groups. The animals of group 1 were kept on control diet for 21 days. The animals in the treatment group were supplemented with ground flaxseed in TMR @ 15% on dry matter basis in concentrate (replacing oil seed cakes) per animal for 21 days. Rumen liquor samples were collected through rumen fistula at 0 hour (before feeding) and at 1, 3, and 6 hours after feeding on 22nd, 23rd and 24th day. Rumen liquor samples were preserved in equal volume of 10% formalin and few drops of saturated mercuric chloride solution for studying rumen microbiology and rumen metabolites, respectively. The samples were stored at -20 °C until analyzed. Total bacterial count and total protozoal count were determined according to the methods of Gall *et al.* (1949) [9] and Naga and El-Shazly (1969) [20], respectively. The differential counting of holotrichs and entodiniomorphs was carried out (Hungate, 1966) [13]. Total volatile fatty acids (Barnett and Reid, 1957) [2], ammonia nitrogen (Conway, 1957) [6] and total nitrogen (AOAC, 2000) [1] levels were estimated. The individual volatile fatty acids were determined as described by Cottyn and Boucque (1968) [7]. The feeds were analyzed for proximate principles (AOAC, 2000) [1] and cell wall constituents (Van Soest *et al.*, 1991) [2]. Data were analyzed by simple ANOVA, and factorial design (Snedecor and Cochran 1994) [27], by using SPSS (2012) version 20 and the differences in means were tested by Tukey's test.

Results and Discussion

The results of ground flaxseed supplementation on rumen microbial population in rumen liquor of male buffalo have been presented in Table 1. Total bacterial count in rumen liquor at different hrs. Pre and post feeding in group I and II varied from 6.54±0.094 to 5.5±0.114 and 9.46±0.308 to 8.10±0.114 (x10^9/ml), respectively.

Table 1: Effect of Ground flaxseed supplementation on rumen microbial population in buffalo calves

Sampling time after feeding (h)	0 (Before feeding)	1	3	6	Over Mean ± SE
Total bacterial count (x 10^9/ml)					
Group I (Control)	5.55±0.11^{aA}	5.85±0.12^{baA}	6.54±0.09^{cA}	5.89±0.08^{daA}	5.96± 0.07^{A}
Group II (Treatment)	8.10±0.11^{aB}	8.63±0.26^{bacB}	9.46±0.31^{cbB}	8.43±0.15^{dabB}	8.66± 0.13^{B}
Total protozoal count (x 10^5/mL)					
Group I (Control)	1.45±0.06^{aA}	1.60±0.08^{baA}	2.61±0.10^{cA}	1.60±0.05^{dabA}	1.81± 0.08^{A}
Group II (Treatment)	1.68±0.06^{aA}	1.91±0.07^{baA}	3.20±0.01^{cB}	1.82±0.07^{dabA}	2.15± 0.10^{B}
Holotrichs (%)					
Group I (Control)	26.02±1.09	27.28±1.21	26.87±1.20	27.13±0.65	26.82±0.52^{A}
Group II (Treatment)	25.26±1.62	26.78±1.32	25.59±1.43	24.99±0.84	25.65±0.65^{A}
Entodiniomorphs (%)					
Group I (Control)	73.98±1.09	72.68±1.22	72.72±1.13	72.87±0.65	73.06±0.51^{A}
Group II (Treatment)	74.74±1.62	73.22±1.32	74.41±1.43	75.264±0.86	74.41±0.66 ^{A}

$^{a, b, c}$ Mean values with different superscripts in a row differ significant (p<0.05); ^{A,B,C} Mean values with different superscripts in a column differ significantly (p<0.05)

The results indicated that there was initial drop in bacterial count which was followed by gradual increase and attained peak at 3 hrs. post feeding in both the groups. Initial drop in microbial population could be due to dilution effect of feed, water, saliva and attached of ruminal bacteria to incoming food particles. Subsequent increase in total bacterial count may be attributed to growth of rumen micro flora, dislodgment of bacteria from the plant fiber and availability of substrate for synthesis of microbial protein (Gill 1993) [10]. However the overall mean exhibited significant increase in total bacterial count in group II (treatment) when compared to group I (control). The increase in total bacterial count in flaxseed supplemented group as compared to non-supplemented group could be due to enhanced availability of proteins and energy from flaxseeds. Similar results were found by Ikwuegbu and Sutton (1982) [15] who observed that in sheep, the supplementation of linseed oil at increasing doses (13, 26 or 40 ml/day) increased the duodenal flow of total and bacterial nitrogen.

The total protozoal count at different time intervals ranged from 1.45±0.061 to 2.61±0.103 and 1.68±0.064 to 3.20±0.010 (x10^5/ml) in group I and II, respectively. It is evident from table, that there was initial decline in total protozoal count, which was followed by gradual increase at 3 hr. post feeding in both the groups. Initial decline in protozoal count could be due to dilution effect of feed, water, saliva and subsequent increase in protozoal count may be attributed to availability of substrate (Sharma *et al.* 2009) [24]. However the overall mean of total protozoal count in rumen liquor was significantly higher in group II supplemented with ground flaxseed as compared control.

The higher protozoal count in group II as compared to group I could be due to improved nitrogen and energy availability in flaxseed supplementation group. Similarly Purser and Moir (1966) [22] noticed that greater protozoal concentrations were associated with high concentration of ammonia in rumen liquor.

The results of administration of ground flaxseed on holotrich and entodinomorphs percentage in buffalo calves revealed that there was no significant change in percentage of holotrichs and entodiniomorphs in treatment group II following flaxseed supplementation when compared to control. Similarly, Ueda *et al.* (2003) [29] examined that, a 3% LO (linseed oil) supplementation to a concentrate-rich diet (65%) fed to dairy cows did not alter the numbers of holotrich protozoa *Dasytricha* spp. and *Isotricha* spp.

The data of administration of ground flaxseed on rumen metabolites and molar percentage of different volatile fatty acids in rumen liquor of male buffalo calves have been presented in Table 2 & 3 respectively.

Table 2: Effect of Ground flaxseed supplementation on rumen metabolites in buffalo calves

Sampling time after feeding (h)	0 (Before feeding)	1	3	6	Over Mean ± SE
Total Volatile Fatty acids (mEq/l)					
Group I (Control)	61.50±4.17^{aA}	80.33±3.23^{bA}	96.17±4.09^{cA}	85.83±3.29^{dbcA}	80.96±2.66^{A}
Group II (Treatment)	73.83±3.53^{adB}	86.67±2.03^{baA}	98.50±8.47^{cbdA}	96.08±8.52^{dbA}	88.77±3.37^{B}
Ammonia N (mg/dL)					
Group I (Control)	21.00±0.72^{aA}	26.58±0.94^{bA}	22.50±0.99C^{Aa}	15.67±0.69^{dA}	21.44±0.70^{A}
Group II (Treatment)	13.25±0.73^{aB}	17.83±1.06^{bA}	16.75±0.71^{cbB}	11.00±0.71^{daB}	14.71±0.56^{B}
Total N (mg/dL)					
Group I (Control)	97.68±3.91^{aA}	160.14±6.35^{bA}	103.18±4.32^{caA}	75.10±4.64^{dA}	109.02±5.15^{A}
Group II (Treatment)	169.50±10.00^{aB}	188.00±15.92^{baA}	150.69±11.85^{cabB}	124.60±5.98^{dcB}	158.20±6.54^{B}

$^{a, b, c}$ Mean values with different superscripts in a row differ significant (p<0.05); $^{A, B, C}$ Mean values with different superscripts in a column differ significantly (p<0.05)

Table 3: Effect of Ground flaxseed supplementation on molar percent of volatile fatty acids in buffalo calves

Sampling time after feeding (h)	0 (Before feeding)	1	3	6	Over Mean ± SE
Acetic Acid					
Group I (Control)	68.64±0.27^{aA}	68.57±0.21^{baA}	70.06±0.28^{cA}	69.65±0.28^{dcA}	69.23±0.16^{A}
Group II (Treatment)	60.64±0.29^{aB}	60.37±0.26^{baB}	61.81±0.28^{cB}	61.76±0.31^{dabcB}	60.90±0.16^{B}
Propionic Acid					
Group I (Control)	18.18±0.26^{aA}	18.30±0.28^{baA}	20.01±0.22^{cA}	18.47±0.30^{daA}	18.74±0.17^{A}
Group II (Treatment)	27.97±0.29^{aB}	27.95±0.21^{baB}	29.19±0.17^{cB}	28.35±0.28^{dabcB}	28.36±0.14^{B}
Butyric Acid					
Group I (Control)	9.60±0.00^{aA}	10.05±0.00^{bA}	10.21±0.00^{cA}	9.60±0.01d^{aC}	9.87±0.04^{A}
Group II (Treatment)	9.62±0.01^{aB}	9.76±0.01^{bB}	10.05±0.01^{cB}	9.84±0.01^{dB}	9.82±0.02^{B}

$^{a, b, c}$ Mean values with different superscripts in a row differ significant (p<0.05); $^{A, B, C}$ Mean values with different superscripts in a column differ significantly (p<0.05)

Total volatile fatty acids (TVFA) in rumen liquor in group I and II ranged between 61.50±4.166 to 96.17±4.086 and 73.83±3.533 to 98.50 ± 8.436 mEq/L respectively. It appears from table that there was progressive increase in levels of volatile fatty acids from 0-3 hrs. post feeding in both the groups and decline at 6 hr. Similar results have been reported by Singh *et al.* (2016) [26]. An initial increase in concentration of TVFA may be attributed to increase in fermentation rate due to increased availability of nutrients (Jain *et al.* 2005, Wanapat *et al.* 2013) [16, 30].

Maximum concentration of total volatile fatty acids at 3 hrs post feeding could be ascribed to maximum microbial fermentation of carbohydrates and catabolism of amino acids leading to formation of volatile fatty acids. However, decline in total volatile fatty acids at 6 hr post-prandial may be due to absorption of total volatile fatty acids through rumen wall into blood stream and decrease in availability of nutrients for microbial fermentation (Sharma *et al.* 2009) [24].

Present study revealed significantly higher levels of total volatile fatty acids following supplementation of ground flaxseed as compared to control. However this rise in TVFA is within the normal physiological range of 80-120 m. Eq/L.

The ammonia nitrogen concentration in group I and II at different time intervals ranged between 15.67±0.689 to 26.58±0.941 and 11.00±0.707 to 17.83±1.058 mg/dl, respectively. It is reflected from the table that there was an increase in level of ammonia nitrogen after feeding and attained the peak at 3 hrs. post feeding in both the groups followed by decrease at 6 hr. However overall mean depicted a significant decrease in ammonia nitrogen concentration in group II as compared to group I.

Post-prandial decline in NH3-N level in both the groups from 3 to 6 hr might be due to direct absorption of NH3-N through ruminal wall or the onward passage along with digesta from rumen or incorporation of nitrogen in the synthesis of microbial proteins (El-Galil *et al.* 2011; Singh and Bhatia 2012) [8, 25].

The decrease in ammonia nitrogen in the rumen may be due to improved utilization of ammonia for microbial protein synthesis following flaxseed supplemented diet as shown by enhanced bacterial and protozoal growth (Table 2).

The total nitrogen in rumen liquor at different hrs. post feeding in group I and II varied from 75.10±4.640 to 160.14±6.347 and 124.60±5.976 to 188.000±15.924 mg/dl respectively. It appears from the table that there was significant increase in level of total nitrogen and attained the peak levels at 1hr. post prandial which is followed by gradually decrease at 6 hr. post feeding in both the groups. However overall mean indicated significant rise in total nitrogen concentration during treatment group compared to control. Similar results were obtained by Ikwuegbu and Sutton (1982) [15] who observed that supplementation of linseed oil at increasing doses (13, 26 or 40 ml/day) in sheep increased the duodenal flow of total N and bacterial N. This rise in total nitrogen concentration in treatment group may be attributed to increase availability of protein in flaxseed supplemented group resulting in higher concentration of peptides amino acids and ammonia in the rumen (Neveu *et al.* 2014) [21].

The overall mean of acetic acid and butyric acid was significantly lowered in the treatment group when compared with control group whereas over all mean of propionic acid increased. Similarly, Gonthier *et al.* (2004) [11] observed that feeding of flaxseed at a higher level (12.5% of the diet DM) reduced the molar proportion of acetate and increased that of propionate, resulting in a lower acetate: propionate ratio. The rise in propionate concentration in the rumen following flaxseed supplementation may be due increased utilization of hydrogen for propionate formation and decrease in methanogen gene abundance (Li *et al.* 2012) [18].

Soder *et al.* (2013) [28] reported a decrease in the acetate: propionate ratio when 10% FS was supplemented in a forage-

based diet in a continuous culture system and Neveu *et al.* (2014) [21] reported a decrease in the acetate: propionate ratio when extruded FS was fed at 12.5% of the diet to dairy cows.

Conclusion

It can be concluded from the present investigation that oral administration of ground flaxseed in feed improved the digestive efficiency of animals by enhancing rumen microbial population and improving the concentration of rumen metabolites.

References

1. AOAC. Official Methods of Analysis, 17th Edition, Association of Official Analytical Chemists, Washington DC: AOAC International; c2000.
2. Barnett AJG, Reid RL. Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen. The volatile acid production from fresh grass. J Agric. Sci. 1957;48:315-321.
3. Beauchemin KA, McGinn SM, Benchaar C, Holtshausen L. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. J Dairy Sci. 2009;92:2118-2127.
4. Caroprese M, Marzano A, Entrican G, Wattegedera S, Albenzio, Sevi A. Immune response of cows fed polyunsaturated fatty acids under high ambient temperatures. J Dairy Sci. 2009;92:2796-2803.
5. Chilliard Y, Martin C, Rouel J, Doreau M. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, linseed oil or flaxseed and their relationship with methane output. J Dairy Sci. 2009;95:5199-5211.
6. Conway HJ. Micro diffusion Analysis and Volumetric Error. 4th Edn. Crossby Lockwood and Sons Ltd, London; c1957.
7. Cottyn BG, Boucque CV. Rapid methods for the gas chromatographic determination of volatile acids in rumen fluid. J Agric. Food Chem. 1968;16:105-107.
8. El-Galil A, Etab R, Ebtehag I, Elenin AM. Role of bacterial treatments for upgrading nutritive value of bean straw and native goats performance. J Am. Sci. 2011;7:502-510.
9. Gall LS, Wise Burroughs, Paul Gerlaugh and Edgington BH. Special methods for rumen bacterial studies in the field. J Anim. Sci. 1949;8: 433-440.
10. Gill SS. Profile of rumen bacteria in rumen liquor under different dietary regimes in buffalo calves (*Bubalus bubalis*). M.V.Sc. Thesis, Punjab Agricultural University, Ludhiana, India; c1993.
11. Gonthier C, Mustafa AF, Berthiaume R, Petit HV, Martineau R, Ouellet DR. Effects of feeding micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. J Dairy Sci. 2004;87:1854-1863.
12. Hook SE, Wright AD, McBride BW. Methanogens: methane producers of the rumen and mitigation strategies. Archaea, 2010, 945785.
13. Hungate RE. The Rumen and its Microbes. Academic Press, New York and London; c1966.
14. ICAR. Nutrient requirements of cattle and buffalo. Indian Council of Agricultural Research, New Delhi; c2013.
15. Ikwuegbu OA, Sutton JD. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Brit. J Nutr. 1982;48:365-375.
16. Jain N, Tiwari SP and Singh P. Effect of urea molasses mineral granules (UMMG) on rumen fermentation pattern and blood biochemical constituents in goat kids fed sola (*Aeschynomene indica* Linn) grass-based diet. Vet. Arh. 2005;75:521-530.
17. Johnson KA and Johnson DE. Methane emissions from cattle. J Anim. Sci. 1995;73:2483-2492.
18. Li L, Schoenhals KE, Brady PA, Estill CT, Perumbakkam S, Craig AM. Flaxseed supplementation decreases methanogenic gene abundance in the rumen of dairy cows. Anim. 2012;6:1784-1787.
19. Mustafa A, McKinnon JJ, Christensen DA, He T. Effects of micronization of flaxseed on nutrient disappearance in the gastrointestinal tract of steers. Anim. Feed Sci. Tech. 2002;95:123-132.
20. Naga MA, El-Shazly K. Activities of rumen micro-organisms in water buffalo (Bos bubalis L.) and in zebu cattle. J Dairy Res. 1969;36:1-10.
21. Neveu C, Baurhoo B, Mustafa A. Effect of feeding extruded flaxseed with different grains on the performance of dairy cows and milk fatty acid profile. J. Dairy Sci. 2014;97:1543-1551.
22. Purser DB, Moir RJ. Dietary effects upon concentrations of protozoa in the rumen. Indian J Anim. Sci. 1966;25:668-674.
23. Santos JEP, Bilby TR, Thatcher WW, Staples CR, Silvestre FT. Long chain fatty acids of diet as factors influencing reproduction in cattle. Reprod. Domest. Anim. 2008;43:23-30.
24. Sharma M, Singh DV, Singh R. Effect exclusive feeding of rice straw on rumen microbiology in buffalo calves (*Bubalus bubalis*). Himachal J Agric. Res. 2009;35:121-124.
25. Singh DV, Bhatia H. Effect of herbal formulation AV/DAC-16 supplementation on rumen profiles in buffalo calves (*Bubalus bubalis*). J Buffalo Sci. 2012;1:27-31.
26. Singh G, Jindal R, Singh R. Effect of HB Strong on metabolites of rumen liquor in buffalo calves with experimentally induced rumen dysfunction. Indian Vet. J. 2016;93:11-13
27. Snedecor GW, Crochan WG. Statistical Methods.7th Edn. Oxford and IBH Publications, New Delhi; c1994.
28. Soder KJ, Brito AF, Rubano MD. Effect of supplementing orchard grass herbage with a total mixed ration or flaxseed on fermentation profile and bacterial protein synthesis in continuous culture. J Dairy Sci. 2013;96:3228-3237.
29. Ueda K, Ferlay A, Chabrot J, Loor JJ, Chilliard Y, Doreau M. Effect of linseed oil supplementation on ruminal digestion in dairy cows fed diets with different forage: concentrate ratios. J Dairy Sci. 2003;86:3999-4007.
30. Wanapat M, Kang S, Hankla N, Phesatcha K. Effect of rice straw treatment on feed intake, rumen fermentation and milk production in lactating dairy cows. Afr. J Agric. Res. 2013;8:1677-1687.
31. Zachut M, Arieli A, Lehrer H, Livshitz L, Yakoby S, Moallem U. Effects of increased supplementation of n-3 fatty acids to transition dairy cows on performance and fatty acid profile in plasma, adipose tissue, and milk fat. J Dairy Sci. 2010;93:5877-5889.

32. Van Soest PV, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of dairy science. 1991 Oct 1;74(10):3583-97.