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RESEARCH ARTICLE

Effect of Feeding Time on Rumen Microbial Protein Synthesis in Cattle Fed with Sugarcane Tops as Staple Roughage

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ABSTRACT

A survey followed by *in vitro* and *in vivo* experiments were carried out to enhance rumen microbial protein synthesis in low milk yielding cattle under sugarcane tops based feeding system without altering the quantity of forage / feed ingredients. The survey revealed that majority of farmers fed the sugarcane tops twice daily during morning and evening and supplemented with groundnut cake at forenoon and evening along with de-oiled rice bran in drinking water. The half time ($t_{1/2}$) of organic matter and nitrogen determined by *in vitro* for sugarcane tops were 29 ± 2 and 40 ± 6 hour, respectively. The respective $t_{1/2}$ time of organic matter and nitrogen for the commonly fed supplemental feeds *viz*., groundnut cake and de-oiled rice bran were 7 ± 1 and 9 ± 1 and 8 ± 0 and 7 ± 1 hour, respectively. Altering the feeding strategy based on $t_{1/2}$ value of organic matter and nitrogen sources did not increased the rumen microbial protein yield by *in vitro* / *in vivo* experiments. Hence, it was recommended to adopt farmers feeding strategy in sugarcane tops based feeding system.

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INTRODUCTION

The current system of feed formulation for ruminants is mainly based upon the daily supply of nitrogen and energy to the rumen. The rate and time of energy release from the roughages in the rumen after consumption and feeding time of nitrogen source are important to increase the efficiency of nutrient utilization. Therefore, carving out a strategy for improving production of microbial biomass in the rumen, without altering the feed ingredients is of practical and economical significance to maximize animal productivity. Hence, this research work was undertaken to modify the existing feeding practices for enhancing microbial biomass production by feeding locally available feed ingredients without any additional input but only by altering the time and order of feeding the respective ingredients.

MATERIALS AND METHODS

A survey on farmer feeding practices for low milk yielding dairy cattle (5-8 kg / day) was undertaken in parts of TamilNadu state, India, wherever sugarcane tops was customarily fed to dairy cattle with supplementation of cereal grains, protein cakes, rice bran etc. A total of 30 farmers located in six agro climatic zones of TamilNadu were interviewed. The *in vitro* degradability characteristics of the sugarcane tops and supplemental feeds *viz*., groundnut cake, sesame cake, coconut cake and de-oiled rice bran were studied by Rumen Simulation Technique (RUSITEC) as described by Czerkawski and Brackenridge (1977). The half time ($t_{1/2}$) required for the respective roughage or supplemental feeds to deliver half organic matter were calculated as, ln 2/c = 0.693/c, where, c is the degradation rate of organic matter (Orskov *et al*., 1980). A new feeding strategy was evolved based on the $t_{1/2}$ of organic matter and nitrogen sources of the feed ingredients that were fed to cow by farmer. The difference between $t_{1/2}$ of organic matter sources and $t_{1/2}$ of nitrogen sources was calculated and the difference was used to fix the feeding time of ingredients. Thus, in this experiment, two feeding strategies were tested in semi continuous culture system. The feeding regimen followed by farmers will be hither to mention as farmer's feeding strategy

(FFS). The feeding time and order of feeding ingredients altered based on the $t_{1/2}$ time of roughage or de-oiled rice bran organic matter and the $t_{1/2}$ time of nitrogen source (protein cake) will be hither to refer as evolved feeding strategy (EFS). These two treatment groups were evaluated in the RUSITEC for their efficacy of microbial protein synthesis.

At 24 and 48 h of incubations, the dry matter and organic matter degradability of the rations were measured. The pH, ammonical nitrogen (NH_3-N) and microbial protein concentrations in the ruminal fluid were determined at nitrogen $t_{1/2}$ of oil cake. The concentration of NH_3-N was estimated colorimetrically as per the method of Weatherburn (1967). Microbial protein in terms of bacterial protein was estimated as described by Makkar *et al.* (1982). The efficiency of microbial protein synthesis (EMPS) was measured by calculating quantity of microbial crude protein synthesized per day to kilogram of apparently degraded organic matter (ADOM). The results of *in vitro* studies on influence of feeding strategies on rumen microbial protein synthesis was validated in crossbred cattle male calves with comparable mean weight of 101.2 ± 6.0 kg and were randomly distributed into two groups for the switch over design. There were two periods of study and the treatments *viz.*, FFS and EFS lasted for four weeks which consisted of 3 weeks of adaptation period and one week of collection period. Prior to the start of the experiment, all the calves were dewormed and uniformly fed with concentrate mixture and green fodder for 10 days.

During the metabolic trial, dung voided were collected at regular interval and weighed. Feed and dung samples were dried at 60 ^oC to a constant weight. The dried dung samples were ground through 1 mm sieve and preserved for analyzing their total ash (AOAC, 2006). For nitrogen estimation, $1/1000^{th}$ of weight of dung voided by individual animal was weighed into 250 ml of wide mouth polypropylene bottles to which was added 20 % sulfuric acid as preservative. The calves were harnessed with specially made urinary collection device for collecting urine. The total urine excreted for 24 h by each animal was collected in plastic cans containing 100 ml of 10 per cent sulphuric acid (v/v) in order to maintain an acidic condition of the urine. The urine voided was measured every day. The samples were preserved at -20 ^oC until analysis for nitrogen (AOAC, 2006) and purine derivatives (IAEA, 1997). The amount of microbial nitrogen supplied to the animal was calculated using the formula described by Chen and Gomes (1995). The efficiency of microbial nitrogen synthesis was expressed as grams of microbial N supply per kg apparently digestible organic matter intake (ADOMI) or grams of microbial N supply per kilogram of apparently digested organic matter in the rumen (ADOMR). The data obtained in different parameters of the study were subjected to statistical analysis as per the procedure of Snedecor and Cochran (1980) and SPSS (2001).

RESULTS

Sugarcane tops was fed twice daily by 50.0 per cent of the farmers at the rate of 2.50 ± 0.10 and 2.04 ± 0.12 kg on DM basis during morning (07:30 - 09:30 h) and in the evening (16:00 - 18:00 h), respectively to dairy cows. Even though 86.7 per cent farmers practiced feeding of paddy straw, only 36.7 per cent of farmers fed it in the forenoon (10:00 h) at the rate of 2.2 ± 0.11 kg on DM basis to their cows. Among the sugarcane tops feeding farmers, 60 per cent of them did not practice grazing their cattle, 73.3 percent of them did not practice feeding of cereal grains and 40.0 and 83.3 per cent of them supplemented groundnut cake and de-oiled rice bran, respectively to their cows. Only half of the groundnut cake feeding farmers (20 per cent) practiced feeding of groundnut cake twice daily in the forenoon (10:00-12:00 h) and in the evening (17:00-18:00 h) at the rate of 0.37 ± 0.04 and 0.34 ± 0.05 kg, respectively. Among the de-oiled rice bran feeding farmers, 52.0 per cent of them fed de-oiled rice bran at the rate of 0.70 ± 0.10 and 0.59 ± 0.07 kg, respectively during forenoon and evening along with groundnut cake mixed in drinking water. Majority of the farmers do not feed sugarcane tops alone as a sole source of roughage. Crop residues were fed along with sugarcane tops during morning. The survey results revealed that the sugarcane tops, crop residues, groundnut cake and de-oiled rice bran were being fed by majority of the farmers to their dairy cows in the proportions of 51.9, 25.2, 8.1 and 14.8 per cent, respectively. The half time required to deliver half of organic matter by sugarcane tops was more than a day (27 h). Therefore, feeding of sugarcane tops in the previous day resulted in maximum energy release within 3 h of feeding in the next day. However, $t_{1/2}$ of N from groundnut cake was 9 h. Hence, groundnut cake was fed 6 h before feeding of sugarcane tops in EFS. The evolved feeding strategy for sugarcane tops was determined based on $t_{1/2}$ of sugarcane tops organic matter and $t_{1/2}$ of groundnut cake nitrogen. The $t_{1/2}$ of organic matter from sugarcane tops was 27 h (1 day + 3 h). Therefore, feeding of sugarcane tops in the previous day resulted in maximum energy release within 3 h of feeding in the next day (Table 1).

The influence of feeding strategy in sugarcane tops based feeding system on *in vitro* ration degradability and rumen parameters is presented in Table 2. The per cent dry matter degradability or organic matter degradability of the ration between FFS and EFS at 24 h of incubation did not differ significantly. However, at 48 h of incubation, significantly higher dry matter degradability (P=0.028) and organic matter degradability (P=0.021) was observed in EFS compared to FFS in sugarcane tops based feeding system which was in line with the findings of Ayyappan *et al*. (2007). The pH of ruminal fluid and the concentration of NH_3-N at nitrogen $t_{1/2}$ of groundnut cake in the reaction vessels between the two feeding strategies did not vary significantly and concur with the report of Carro *et al*. (2009). Microbial protein concentration at nitrogen $t_{1/2}$ of groundnut cake and the microbial protein synthesized per day between the FFS and EFS did not vary significantly which simulate the findings of Henning *et al*. (1993) and contrary to the report of Ayyappan *et al*. (2007). The efficiency of microbial protein synthesized per kg apparently degraded organic matter observed in the EFS was comparable to FFS which concur with the findings of Newbold and Rust (1992) and Henning *et al*. (1993) and contrary to the observation of Ayyappan *et al*. (2007).

Table 1: Ingredients feeding time, quantity and proportion of feed ingredients used for *in vitro* and *in vivo* experiments

Farmer's feeding strategy (FFS)				Evolved feeding strategy (EFS)			
Feeding time (h)	Feed ingredient	Quantity (g)	Proportion (%)	Feeding time (h)	Feed ingredient	Quantity (g)	Proportion (%)
08:30	Sugarcane tops	5.7	28.6	08:00	Groundnut cake	0.8	4.2
10:00	Paddy straw	5.0	25.2		De-oiled rice bran	1.6	8.0
11:30	Groundnut cake	0.8	4.2	12:00	Paddy straw	5.0	25.2
	De-oiled rice bran	1.6	8.0		Sugarcane tops	5.7	28.6
16:00	Sugarcane tops	4.7	23.3	14:00	Groundnut cake	0.8	3.9
17:30	Groundnut cake	0.8	3.9		De-oiled rice bran	1.4	6.8
	De-oiled rice bran	1.4	6.8	20:00	Sugarcane tops	4.7	23.3
	Total	20	100		Total	20	100

Table 2: Influence of feeding strategy in sugarcane tops based feeding system on the *in vitro* ration degradability and rumen parameters (Mean* ± SE)

Parameter	Farmer's feeding strategy (FFS)	Evolved feeding strategy (EFS)	P value
Apparent DM degradability			
at 24 h, %	34.38 ± 0.37	35.82 ± 0.50	0.085
at 48 h, %	40.36 ± 0.58	42.29 ± 0.60	0.028
Apparent OM degradability			
at 24 h, %	32.89 ± 0.44	34.26 ± 0.52	0.135
at 48 h, %	38.89 ± 0.44	40.93 ± 0.55	0.021
Rumen parameters at N $t_{1/2}$ of groundnut cake			
pH	6.90 ± 0.02	6.93 ± 0.01	0.209
Rumen NH_3-N, mg %	7.60 ± 0.38	7.46 ± 0.32	0.727
MP concentration, mg %	44.26 ± 3.08	47.66 ± 3.01	0.240
Microbial protein synthesized, mg / d	299.09 ± 14.15	313.44 ± 10.05	0.409
EMPS, g Microbial protein / kg ADOM	50.89 ± 2.48	51.23 ± 1.82	0.917

*Mean of 8 observations; P> 0.05 do not differ significantly

The influence of feeding strategies on nutrient intake and its digestibility, nitrogen balance, purine derivatives excretion, microbial protein synthesized and the performance of calves is presented in Table 3. The intakes of digestible DM, digestible OM and digestible N between the feeding strategies did not vary significantly. The effect of changing the feeding time did not influence the digestibility of nutrients which was not in line with the findings of Chumpawadee *et al*. (2006). The quantity of nitrogen retained in the EFS was comparable to FFS. The excretions of total purine derivatives did not vary significantly between the feeding strategies. There was no improvement in microbial nitrogen synthesis due to synchronization, which concurred with the observation of Henning *et al*. (1993). Even though the efficiency of microbial nitrogen (MN) synthesized in terms of per kg ADOMI or per kg ADOMR in FFS and in EFS was comparable, there was 6.39 per cent lower efficiency was observed in EFS which was in line with the reports of Kim *et al*. (1999) and Valkeners *et al*. (2004). Comparable average daily body weight gain of calves and feed efficiency between the feeding strategies is in agreement with the observation of Vaughan *et al.* (2002) who found no improvement in milk yield of dairy cows fed synchronized energy source with nitrogen from grazing herbage and also with the report of Richardson *et al*. (2003) in growing lambs.

DISCUSSION

The significantly higher *in vitro* dry matter and organic matter degradability of the ration at 48 h of incubation in EFS might be due to supply of energy from the limited non structural carbohydrates of sugarcane tops during the initial 24 h for the synthesis of microbial protein and in later stages more microbial digestion of structural carbohydrates might have taken place and therefore the response in degradability was evident only at 48 h of incubation. The long time taken to start degradation of sugarcane tops may be another reason. The result of *in vitro* studies might have resulted in feed processing methods like drying and grinding of substrates and may alter the characteristics and, thus, the availability of dietary components (Davies *et al*., 1998). The similar pH and NH_3-N concentration between feeding strategies observed in this study was due to the stable pH maintained throughout the day in the ruminal fluid of the reaction vessels as a consequence of high buffering capacity of artificial saliva (Carro *et al*., 2009). The similar microbial protein synthesis per day and the efficiency of microbial protein synthesis observed between the feeding strategies was due to the similar level of NH_3-N for optimum microbial N synthesis (Trevaskis *et al*., 2001). The low efficiency of microbial protein synthesis might due to deficiency of some other microbial growth factors in both the strategies (Clark *et al*., 1992) and nitrogen. The slowly degradable organic matter from the sugarcane tops was not available for rapidly degradable nitrogen from groundnut cake which resulted asynchrony. However, in FFS, sugarcane tops which were fed twice in the morning and evening, the released energy might be available for a longer time to capture released nitrogen from groundnut cake. So, the microbial nitrogen synthesis per day per metabolic body weight and efficiency of microbial nitrogen synthesis were higher by 3.1 and 6.3 per cent respectively in FFS compared to EFS. The average quantity of apparent digestible organic matter and nitrogen were 1.55 kg and 22.78 g respectively in

Table 3: Influence of feeding strategy on digestibility of nutrients and performance of crossbred cattle male calves in sugarcane tops based feeding system (Mean* ± SE)

Parameter	Farmer's feeding strategy (FFS)	Evolved feeding strategy (EFS)	P value
Total dry matter intake, g / day	2744 ± 221	2861 ± 190	0.748
Organic matter intake, g / day	2502 ± 201	2609 ± 173	0.747
Nitrogen intake, g / day	40.89 ± 3.33	42.47 ± 2.82	0.771
Nitrogen retained, g / day	6.49 ± 0.91	6.63 ± 0.86	0.910
Dry matter digested, g / day	1622 ± 166	1610 ± 104	0.995
Organic matter digested, g / day	1553 ± 153	1554 ± 98	0.995
Nitrogen digested, g / day	22.69 ± 2.06	22.86 ± 2.18	0.958
Total PD excretion, mmol / day	34.58 ± 2.05	34.05 ± 1.51	0.878
Microbial nitrogen supplied, g / day	18.93 ± 1.55	18.46 ± 0.84	0.839
MN synthesized / kg ADOMI	12.90 ± 1.42	12.07 ± 0.61	0.623
MN synthesized / kg ADOMR	19.86 ± 2.16	18.59 ± 0.65	0.623
Body weight gain, g / day	180 ± 13	186 ± 11	0.745
Feed efficiency, DMI / gain	15.82 ± 1.61	15.90 ± 1.30	0.957

*Mean of 8 observations; P> 0.05 do not differ significantly

sugarcane tops based feeding system. About 65 per cent (ARC, 1984) of digestible organic matter (1.0 kg) is available in the rumen and it required 33.0 g of nitrogen when considering optimum MN synthesis efficiency of 33 g per kg fermentable organic matter in the rumen (McMeniman *et al*., 1976). However, only 14.81 g nitrogen is available when assuming the same proportion (65 per cent) of digestible nitrogen available in the rumen. Hence, there was 55.12 per cent degradable nitrogen deficit in sugarcane tops based feeding system. For effective rumen synchrony dietary protein and energy sources to the rumen should made available simultaneously in proportions needed by the ruminal micro organisms (Krehbiel *et al*., 2008).

Conclusions

Evolved feeding strategy for sugarcane tops based feeding system did not significantly enhanced the rumen microbial nitrogen synthesis and growth rate in calves. Hence, it was recommended to adopt farmer's feeding strategy without any alterations in the ingredients feeding time in sugarcane tops based feeding system for low milk yielding dairy cows.

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