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RESEARCH ARTICLE

Effect of Altering Feeding Time of the Ingredients in Napier - Bajra Hybrid Grass - Based Feeding System on Ruminal Microbial Protein Synthesis in Calves

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ABSTRACT

The study was designed to develop a possible ruminal delivery of synchronized energy and nitrogen to enhance the synthesis of ruminal microbial biomass in cattle by evolving an appropriate time of feeding without altering the feed ingredients (Evolved feeding strategy-EFS) in Napier- bajra hybrid grass-based feeding system. Napier-bajra hybrid grass, groundnut cake and de-oiled rice bran were fed at 65.8, 8.8 and 25.4 per cent level, respectively to the low milk yielding dairy cows by the farmers twice daily. The respective half time ($T_{1/2}$) of organic matter and nitrogen determined by an *in vitro* technique (TANUVAS RUSITEC) for Napier-bajra hybrid grass were 16±1 h and 14±2 h, respectively. The respective $T_{1/2}$ for organic matter and nitrogen of the commonly fed supplemental feeds *viz.*, groundnut cake and de-oiled rice bran were 7 ± 1 and 9 ± 1, 8 ± 0 and 7 ± 1 h, respectively. The influence of farmer feeding strategy (FFS) and EFS on their efficiency of synthesizing the rumen microbial protein were tested by simulated feeding in semi- continuous culture system (RUSITEC). The results indicated that at 48 h of incubation, the dry matter and organic matter degradability was significantly ($P<0.05$) higher in EFS when compared to FFS. The *invitro* rumen microbial protein production per day and the microbial nitrogen synthesis per kg metabolic body weight of cattle was comparable between EFS and FFS. Based on our findings we conclude that EFS for Napier-bajra hybrid grass- based feeding system did not significantly enhance the rumen microbial nitrogen synthesis.

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INTRODUCTION

Farmers usually adopt traditional feeding practices to feed their cattle using locally available feed resources. Farmers do not give due importance to the time of feeding or the sequence of feeding or even the quantity of the various ingredients that make up the ration. However, it has been scientifically proven that rumen microbial protein synthesis could be maximized by synchronization of the delivery of nutrients (Dewhurst *et al.* 2000; Krehbiel *et al*. 2008).

The synchronization can be achieved either by altering the relative time at which the ingredients in the feed are fed or by dosing specific forms of energy and nitrogen into the rumen at specified time intervals. The frequency and the exact time to feed specific protein and energy concentrate feedstuffs in relation to other feedstuff have become highly relevant to practical dairy operations, this synchronization is still not practiced in small scale livestock holdings. Thus, this study was designed to bring out a possible ruminal delivery of synchronized energy and nitrogen to enhance the synthesis of ruminal microbial biomass in cattle by developing an appropriate time of feeding without altering the feed ingredients in Napier- bajra hybrid grass- based feeding system.

MATERIALS AND METHODS

A survey on feeding practices by farmer's was carried out in five agro-climatic zones of Tamil Nadu where five

farmers in each zone were selected to assess the type, quantity and time of feeding of different feed ingredients to low milk yielding cattle (5-8 kg / day) that are fed with Napier- bajra hybrid grass (NB hybrid grass) as a staple roughage. The degradability characteristics of the commonly fed supplemental feeds and NB hybrid grass were assessed by semi- continuous culture system (RUSITEC) as described by Czerkawski and Brackenridge (1977) to determine the half life ($T_{1/2}$) of organic matter and nitrogen. The half time ($T_{1/2}$) required for the respective roughage or supplemental feeds to deliver half of the organic matter or nitrogen were calculated as, ln 2/c = 0.693/c, where, c is the degradation rate of organic matter or nitrogen (Orskov *et al*. 1980).

The feeding time practiced by the farmer's or the order by which the feeding ingredients are fed to cows by the farmers (Farmer's feeding strategy -FFS) were modified without altering the type and quantity of the ingredients in the feed to explore the possibility of increasing the production of the ruminal microbial biomass. Thus, 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 cows by the farmers (Evolved feeding strategy- EFS). The difference between $T_{1/2}$ of the sources of the organic matter and $T_{1/2}$ of the sources of nitrogen were calculated and the difference was used to determine the feeding time of ingredients. These two treatment groups (FFS and EFS) were evaluated in the RUSITEC for their efficacy on the microbial protein synthesis. Feed samples were incubated up to 48 h. The proportions of various feed ingredients fed to cows by the farmers per day (ration) were calculated for 20 g to simulate in the RUSITEC.

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 as per the method of Weatherburn (1967). Microbial protein, in terms of total bacterial protein was estimated as described by Makkar *et al*. (1982). In order to estimate the flow of microbial protein at post rumen in the animal, the total effluent volume was multiplied with the concentration of microbial protein (mg / dl) to estimate the microbial protein synthesis per day. The efficiency of microbial protein synthesis (EMPS) was measured by calculating the 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 strategy on rumen microbial protein synthesis were validated in eight male calves of crossbred cattle. Metabolic trial was conducted for 7 day during last week of each period. The calves were harnessed with specially made urinary collection devices for collecting the urine. The total quantity of urine excreted in 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 pH of the urine. The urine voided was weighed and measured every day. Purine derivatives were estimated using diluted and filtered urine (IAEA, 1997) along with the nitrogen (AOAC, 2006) from the preserved urinary samples. 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 nitrogen supply per kg apparently digestible organic matter intake (ADOMI). The data obtained regarding various parameters of the study were subjected to statistical analysis by one-way ANOVA as per the procedure of Snedecor and Cochran (1980) and SPSS (2001). P value less than 0.05 is considered statistically significant.

RESULTS

Farmer's feeding strategy

The results of our survey revealed that a large number of farmers practiced feeding of NB hybrid grass in combination with groundnut cake and de-oiled rice bran twice a day (Table 1). Our survey revealed that the NB hybrid grass, groundnut cake and de-oiled rice bran were fed in the proportions of 65.8, 8.8 and 25.4 per cent, respectively.

Rate of nutrient degradability

The organic matter and nitrogen content in the NB hybrid grass were degraded up to 63.35 and 73.70 %, respectively at 72 h of incubation. The time required to deliver half ($T_{1/2}$) of the organic matter and nitrogen in the rumen were 16 and 14 h, respectively. The organic matter and nitrogen content in the groundnut cake were degraded up to 83.15 and 86.56 %, respectively at 24 h of incubation and the $T_{1/2}$ of the organic matter and nitrogen were 8 and 9 h, respectively. The organic matter and nitrogen content in de-oiled rice bran were degraded up to 59.18 and 74.03 %, respectively at 24 h of incubation. The $T_{1/2}$ of the organic matter and nitrogen were 8 and 7 h, respectively.

The $T_{1/2}$ of organic matter from the NB hybrid grass was 16 h and the $T_{1/2}$ of nitrogen from the groundnut cake was 9 h. Thus, there was a 7 h difference between the energy released from the grass and the nitrogen released from the groundnut oil cake. The nitrogen from groundnut

Table 1: Feeding time of the ingredients, quantity and proportion of feed ingredients used in the *in vivo* and *in vitro* studies

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 (%)
09:00	NB hybrid grass	7.4	37.0	07:00	NB hybrid grass	7.4	37.0
11:00	Groundnut cake	0.9	4.5	14:00	Groundnut cake	0.9	4.5
	De-oiled rice bran	2.7	13.7		De-oiled rice bran	2.7	13.7
16:00	NB hybrid grass	5.8	28.8		NB hybrid grass	5.8	28.8
17:30	Groundnut cake	0.9	4.3	21:00	Groundnut cake	0.9	4.3
	De-oiled rice bran	2.3	11.7		De-oiled rice bran	2.3	11.7
Total		20	100	Total		20	100

oil cake was rapidly degradable compared to the energy released from NB hybrid grass as the structural carbohydrate present in this forage (NB hybrid grass) has taken a longer time for degradation (9 h in groundnut oil cake vs. 16 h in NB hybrid grass). The silages, green fodder contain medium degradable protein and carbohydrates (Srinivas and Malik, 2009). Hence, taking $T_{1/2}$ of organic matter from NB hybrid grass into account, groundnut cake was fed 7 h after feeding of the NB hybrid grass in our EFS without any altering the quantity of the feed ingredients. In the FFS, NB hybrid grass was introduced into RUSITEC reaction vessel twice at 9:00 and 16:00 h and groundnut cake and de-oiled rice bran at 11:00 and 17:30 h (Table 1). In the EFS, NB hybrid grass was introduced in to the RUSITEC reaction vessel twice at 07:00 and 14:00 h and groundnut cake along with de-oiled rice bran was introduced twice at 14:00 and 21:00 h (Table 1).

Effect of feeding strategies on *in vitro* rumen microbial nitrogen synthesis

The influence of feeding strategy in NB hybrid grass-based feeding system on the ration degradability and rumen parameters is presented in Table 2. The % apparent dry matter (DM) and organic matter (OM) degradability of the rations at 24 and 48 h of incubations were significantly higher ($P<0.05$) in EFS when compared to FFS. The pH and NH_3-N of ruminal fluid in the reaction vessels measured at nitrogen $T_{1/2}$ of oil cake between two feeding strategies did not vary significantly (Table 2). However, quantitative reductions in pH and NH_3-N were observed in the EFS compared to FFS. A lower NH_3-N concentration in synchronized diets was also reported in a previous study (Kolver *et al*. 1998). The comparative reduction in the values of pH and NH_3-N concentration in EFS supports better utilization of the released nitrogen for microbial protein synthesis. Microbial protein (MP) concentration at nitrogen $T_{1/2}$ of oil cake and microbial protein synthesized per day in the EFS were comparatively higher when compared to FFS. However, there were no statistically significant differences between the values of MP and the microbial protein synthesized between FFS and EFS groups. The efficiency of microbial protein synthesized (EMPS) per kg apparently degraded organic matter (ADOM) observed in the EFS did not vary significantly but showed slightly higher numerical values, when compared to FFS.

Feeding strategies pertaining to *in vivo* rumen microbial nitrogen synthesis

The influence of feeding strategy on the nutrient intake, nutrient digestibility and performance of the calves maintained on NB hybrid grass- based feeding system is presented in Table 3. The intakes of digestible OM and digestible nitrogen between FFS and EFS were comparable. Thus this study showed that changing the feeding time did not influence the digestibility of nutrients, a finding that which concurs with the reports of Kolver *et al.* (1998). The average daily body weight gain values of calves between FFS and EFS did not vary significantly and our values were in agreement with the report of Richardson *et al*. (2003). The influence of feeding strategy on nitrogen balance, urinary purine derivatives excretion and rumen microbial nitrogen yield in male calves maintained on NB hybrid grass- based feeding system indicated that the quantity of nitrogen

Table 2: Influence of the feeding strategies in Napier-bajra hybrid grass- based feeding system on the ration degradability and rumen parameters in RUSITEC (Mean* ± SE)

Parameters	FFS	EFS	P value
% Apparent DM degradability			
at 24 h	39.18 ± 0.50	41.76 ± 0.42	0.002
at 48 h	49.55 ± 0.43	51.11 ± 0.28	0.035
% Apparent OM degradability			
at 24 h	38.03 ± 0.45	40.34 ± 0.38	0.001
at 48 h	48.20 ± 0.49	49.59 ± 0.33	0.054
Ruminal parameters at N $t_{1/2}$ of oil cake			
pH	6.94 ± 0.01	6.91 ± 0.02	0.262
NH_3-N, mg %	10.19 ± 0.45	9.42 ± 0.64	0.188
MP concentration, mg %	52.55 ± 2.60	60.84 ± 2.41	0.056
MP synthesized, mg / day	343.54 ± 9.88	376.99 ± 8.22	0.071
EMPS, g MP / kg ADOM	52.01 ± 1.95	53.72 ± 1.33	0.530

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

Table 3: Influence of the feeding strategies on the digestible nutrients intake, rumen microbial nitrogen synthesis and the performance of crossbred cattle male calves*

Parameters	FFS	EFS	P value
Organic matter intake, g / day	2106 ± 213	2098 ± 168	0.977
Nitrogen intake, g / day	51.46 ± 5.23	51.40 ± 4.34	0.993
Nitrogen retained, g / day	15.26 ± 1.69	16.22 ± 1.39	0.629
Apparently digestible organic matter intake, g / day	1425 ± 131	1436 ± 98	0.946
Apparently digestible nitrogen intake, g / day	35.04 ± 3.18	36.12 ± 3.13	0.821
Purine derivatives excretion / kg $BW^{0.75}$, mmol / day	1.153 ± 0.057	1.192 ± 0.029	0.557
Rumen microbial nitrogen synthesized, g/ kg ADOMI)	12.92 ± 1.18	13.27 ± 0.64	0.802
Body weight gain, g / day	222 ± 11	239 ± 12	0.347
Feed efficiency, DMI / gain	10.85 ± 0.92	10.15 ± 0.85	0.602

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

retained in FFS and EFS did not vary significantly and this observation was in agreement with the report of Richardson *et al*. (2003).

The urinary total purine derivatives excretions and the total purine derivatives absorption did not vary significantly between the two feeding strategies. Therefore, the microbial nitrogen synthesized in the two feeding strategies showed no significant difference a finding that is in contrast with the observations of Chumpawadee *et al.* (2006). The efficiency of rumen microbial nitrogen (MN) production (Table 3) was within the range reported by ARC (1984) and Hoover and Stokes (1991). In contrast, to our findings, Clark *et al*. (1992), Dewhurst *et al.* (2000) and Ayyappan *et al.* (2007) had opined that synchronization of organic matter and nitrogen release in the rumen leads to increased microbial protein synthesis.

DISCUSSION

The feeding patterns recorded in this study concurred with the findings of Meena *et al.* (2008), who reported that stall feeding during mornings and evenings for all type of animals was practiced by the farmers and that only few farmers provided feed and fodder more than twice per day. The $T_{1/2}$ values of grass and concentrate ingredients estimated in this study were also similar to findings of Zewdu Tessema *et al*. (2002) who reported $T_{1/2}$ values for dry matter and nitrogen for Napier grass and organic matter (Ayyappan *et al*., 2007) and nitrogen (Lohan and Gupta, 1988) of groundnut cake. However, from the degradability studies of Singh and Kundu (2008), the calculated $T_{1/2}$ value for DM and nitrogen of de-oiled rice bran were lower when compared to the values observed in this study.

The significantly higher degradability of the rations at 24 and 48 h of incubations in EFS compared to FFS concurred with the observations of Chumpawadee *et al.* (2006) and Ayyappan *et al.* (2007). The lower NH_3-N concentration in synchronized diet was also reported by Kolver *et al.* (1998) as observed in this study. The comparative reduction in pH and NH_3-N concentration in EFS, although not statistically significant, supports the idea of a better utilization of the released nitrogen for microbial protein synthesis. The comparatively higher values (although not statistically significant) of microbial protein (MP) concentration, microbial protein synthesized per day and efficiency of microbial protein synthesized (EMPS) per kg apparently degraded organic matter (ADOM) observed in the EFS, when compared to FFS were in line with the findings of Ayyappan *et al*. (2007). The moderate rate of organic matter degradability of NB hybrid grass probably supplied the required energy for sustenance of the rumen microbes and therefore, it is possible that NB hybrid grass can be fed along with moderate rate of nitrogen degradable oil cakes for a better nutrient utilization. The similar medium rate of nitrogen degradability in NB hybrid grass to that of organic matter indicates synchronized release of both the nutrients. Hence, the released nitrogen from the grass might have been available for microbial protein synthesis in the rumen along with utilizing the released energy from the same grass.

Conclusion

Based on our findings, we conclude that EFS for NB hybrid grass- based feeding system did not significantly enhance the rumen microbial nitrogen synthesis and hence we recommend FFS for farmers.

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