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Effect of paddy straw based complete rations with different levels of neutral detergent fibre on microbial protein synthesis in dairy cows during early and mid lactation

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ABSTRACT

A study was conducted in 18 lactating dairy cows (mean milk yield 10 kg/day) for 6 months by feeding paddy straw based complete rations containing different levels of NDF, in two phases, viz. early and mid lactation, to assess the microbial protein synthesis using purine derivatives (PD) excreted in urine. Isonitrogenous and isocaloric complete rations; T1, T2 and T3 with 25, 30 and 35 per cent NDF, respectively were formulated as per ICAR (1998). Paddy straw was the sole source of roughage NDF and the rest of NDF met by non-forage sources, in 3 diets. PD excreted through urine were comparable between the dietary treatments, in phases 1 and 2. The microbial protein (MBP) synthesis in T1, T2 and T3 were 997.01, 825.91, 842.06 g, respectively, in phase 1 and 1127.93, 1106.74 and 1157.24 g, respectively, in phase 2. The TDN intake calculated from the values of digestibility coefficients of nutrients in T1, T2 and T3 were 5.18, 5.86, 6.10 kg in phase 1 and 8.63, 8.81, 9.00 kg, in phase 2, respectively. The efficiency of MBP production/kg of TDN was 192.47, 140.94, and 138.04 g in phase 1 and 130.70, 125.62 and 128.58 g in phase 2, respectively in T1, T2 and T3. Results suggest that complete diets with 25 per cent are the most ideal NDF level in early lactation while all three NDF levels, viz. 25, 30 and 35% are ideal in mid lactation.

Key words: Complete feeds, Cows, NDF, Paddy straw, Purine derivatives, Urine, Microbial protein synthesis

Energy requirements of lactating dairy cows should be balanced in neutral detergent fibre (NDF), with optimum proportion of fibrous and non-fibrous carbohydrate (NFC) to meet energy needs of rumen microbial biomass and host animal. The National Research Council (NRC) of USA has recommended that a milking cow should be fed with a ration containing at least 25 to 33% of fibre in the form of NDF (NRC 2001). In India, crop residues are staple feeds and provides major portion of NDF in the diet while NFC is often limited which is necessary for microbial growth in the rumen (Singh and Srinivas 2016).

However, providing even this minimum quantity of fibre, from forage sources alone, is very difficult because currently in India, green fodder is deficit to the tune of 56.73% (Datta 2013). This scarcity of green fodder could only be met in a sustained manner through the efficient use of crop residues that do not compete with human food.

The need of the time was to blend crop residues such as straws along with locally available agro-industrial by products like brans, which are good sources of NDF, to which other concentrate ingredients were added to form a complete feed or complete diet (Lailer *et al.* 2005).

Microbial protein synthesis estimated form purine derivatives in urine, is a very good tool to measure the efficiency of protein degradation in the rumen (Sannes *et al*. 2002). Pina *et al*. (2006) compiled the results of microbial protein synthesis estimated from urinary purine derivatives, obtained from several studies carried out in milking cows, subjected to different feeding conditions and recommended a microbial protein synthesis of 120 g/kg of TDN intake as a reference standard for tropical conditions.

Hence, this study was conducted to assess the microbial protein synthesis of cows fed on paddy straw plus non-forage fibre sources based complete rations having different levels of NDF, by estimating urinary purine derivatives, in both early and mid lactation.

MATERIALS AND METHODS

Eighteen crossbred dairy cows in the early stage of lactation (within 2 weeks of calving) were selected from the University Livestock Farm and Fodder Research and Development Scheme, Mannuthy and randomly divided

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Table 1. Ingredient and chemical composition of experimental complete feeds offered to cows in phases 1 and 2

Phase	1			2		
Treatment	T1	T2	T3	T1	T2	T3
Ingredient composition (kg)						
Maize	37	27	16	38	30	21
Coconut cake (de-oiled)	11	12	17	5	8	12
Rape seed meal	11	12	11	10	10	10
De-oiled rice bran	20	19	16.50	26	23	21
Paddy straw	14	21	29	14	22	29
Molasses	5	5	5	5	5	5
Calcite	1.50	1.50	1.50	1.5	1.5	1.5
Salt	0.50	0.50	0.50	0.5	0.5	0.5
Vegetable fat	-	2.00	3.50	-	-	-
Total	100*	100*	100*	100*	100*	100*
Chemical composition (% DM basis)						
Crude protein	12.23	12.94	12.18	12.40	12.14	12.08
Crude fibre	10.73	12.68	15.01	10.53	12.70	14.85
Ether extract	4.00	3.60	3.80	4.50	3.50	3.60
Total ash	14.00	12.90	12.90	14.10	13.30	12.98
Nitrogen free extract	59.04	57.88	56.11	58.47	58.36	56.49
Acid insoluble ash	5.30	5.60	6.00	4.80	5.40	6.20
Neutral detergent fibre (NDF)	25.88	30.03	35.59	25.94	30.86	35.38
Acid detergent fibre (ADF)	21.60	23.10	24.80	21.80	23.00	25.01
Calcium	0.83	0.85	0.87	0.81	0.84	0.86
Phosphorus	0.54	0.52	0.48	0.59	0.55	0.52
Metabolisable energy** (MJ/kg DM)	9.00	8.87	8.41	9.04	8.54	8.24

*0.10g of the vitamin premix (vitamin AD_3E supplement) provided per 1 kg of diet contained 10,000 IU of vitamin A, 2,000 IU of vitamin D_3 and 1,000 IU of vitamin E; 0.50g of trace mineral mixture (KERAMIN FORTE) and 0.50g of toxin binder (CURATOX) were added per 1 kg of complete diets. **Calculated value.

into 3 equal groups, viz. T1, T2 and T3, based on age, parity, milk yield and body weight.

The cows were housed individually in a well ventilated shed with cemented floor and fed isonitrogenous (12 to 12.7% CP) and isocaloric (63 to 65% TDN) complete rations containing NDF%, 25 (T1), 30 (T2) and 35 (T3), in two consecutive phases consisted 90 early lactation followed by 90 mid lactation. The ingredient and chemical composition of diets are depicted in Table 1.

Complete diets were fed *ad lib.* and feed left was manually weighed, twice a day, at 9 AM and 2 PM, respectively. *Ad lib.* drinking water was provided to the cows using automatic watering system. Proximate principles of feed were determined as per standard procedure (AOAC 2012). The acid detergent fibre and neutral detergent fibre (NDF) were estimated by the method of Van Soest *et al.* (1991). The calcium and phosphorus contents of the feed were analysed as per the standard procedure described in AOAC (2012).

A metabolism trial of 5 days duration was conducted during the last week in phase 1 and phase 2. Total collection method was adopted to collect the dung and urine samples from all the animals, on all the five days of the metabolism trial. Daily feed intake, dung and urine output were recorded individually. Representative samples of the feed were taken every day during the trial for proximate analysis. Dry matter content of the feed as well as the waste was determined daily.

Dung and urine voided by each animal was collected manually, by total collection method, as and when they were voided and stored in individual containers, on a continuous 24 h basis. All possible precautions were taken to ensure the quantitative collection of dung and urine, uncontaminated with each other as well as feed residue or dirt. The entire quantity of dung voided by each animal during the previous 24 h were weighed separately at 8 AM. every day and representative samples at the rate of 1% of the total weight were taken after mixing thoroughly and stored in double lined polythene bags. The samples collected each day were kept in a deep freezer. The process of collection, weighing and preservation of dung was continued till the end of the trial. At the end of the metabolism trial in each phase, aliquots collected from each animal for five days were pooled, mixed together and composite samples were taken after thorough mixing for chemical analysis. The moisture was determined immediately and the crude protein was estimated using the fresh samples. The rest of the analysis was done using the dried dung samples. The proximate principles of feed and dung were determined as per standard procedure (AOAC 2012).

Urine was collected manually from each cow in plastic buckets of 25 litre capacity, in which 100 ml of 25% sulphuric acid was added as preservative. The urine

Table 2. Purine derivatives* in urine and microbial protein* synthesis of animals maintained on the three experimental rations in phases 1 and 2

Phase	Treatment	Allantoin (mmol/day)	Uric acid (mmol/day)	Total purine derivatives (mmol/day)	Intestinal flow of microbial N (g/day)	Microbial protein synthesis (g/day)
1	T1	176.21±18.41	43.22±9.37^{a}	219.42±22.05	159.52±16.03	997.01±100.17
	T2	162.12±24.76	19.65±3.77^{b}	181.77±27.55	132.15±20.03	825.91±125.18
	T3	163.28±27.98	22.05±3.27^{b}	185.32±27.54	134.73±20.02	842.06±125.14
2	T1	178.35±17.12	69.89±13.28^{a}	248.24±28.53	180.47±20.74	1127.93±129.64
	T2	203.78±36.66	39.79±5.98$^{b\#}$	243.57±41.71	177.08±30.33	1106.74±189.53
	T3	213.91±49.27	40.78±4.90$^{b\#}$	254.69±51.36	185.16±37.34	1157.24±233.35

*Average of 6 values; ^{a,b}means with different superscripts in the same column, for each phase differ significantly (P<0.05); #significantly different from corresponding treatment value in phase 1 (P<0.05).

collected from each cow for 24 h was measured at 8 AM and 1% was taken as sub-sample. Sub-samples collected during the trial were used for subsequent analysis.

Urine samples were analysed for the purine derivatives (PD) such as allantoin and uric acid and calculated microbial protein (MBP) production (IAEA-TECDOC-945 1997). Urinary allantoin was determined by colorimetric method and uric acid was determined by uricase method using commercial kits (M/s. Agappe Diagnostics Kochi, India). Urinary purine derivative excretion is the sum of urinary allantoin and uric acid excretion in mmol/d. MBP production was calculated according to Chen and Gomes (1992) using the formula:

$$\text{Microbial N (g N/d)} = 70\,X/(0.116 \times 0.83 \times 1000) = 0.727\,X$$

where X, urine purine derivatives (mmol/d).

$$\text{Microbial protein (g/d)} = \text{Microbial N (g/d)} \times 6.25$$

where, N content of PD was 70 mg/mmol, digestibility of microbial purines was 0.83 and the ratio of purine N to total N in mixed microbial population in rumen was 11.6:100.

Data were analysed statistically as per Snedecor and Cochran (1994) by analysis of variance (ANOVA) technique, by statistical product and service solutions (SPSS), version 21.0.

RESULTS AND DISCUSSION

The data on urinary purine derivative, viz. allantoin and uric acid excretion, total purine derivative excretion and microbial protein synthesis from the experimental animals in phases 1 and 2 of the experiment are given in Table 2.

The urinary allantoin in all the 3 diets, uric acid in T2 and T3, total PD and MBP synthesis of cows fed on all three treatments in phase 1 were comparable to observations of Ronquillo *et al*. (2004) who reported values of 224.80 to 256.00 mmol/d, 18.50 to 34.70 mmol/d, 243.30 to 290.60 mmol/d and 710.63 to 1186.88 g/d for allantoin, uric acid, total PD and MBP, respectively, in early lactation HF cows fed on a mixed diet which consisted forage and concentrate at varying levels. Nevertheless, the uric acid values in cows fed on T1 in phase 1 were higher than those of Ronquillo *et al*. (2004). However, the present values of allantoin in all three diets, uric acid in T2 and T3, total PD and MBP in cows fed on all 3 treatments in phase 1 were lesser than those of Pina *et al*. (2006) who reported values of 326.25 to 377.45 mmol/d, 37.16 to 43.34 mmol/d, 368.88 to 417.26

Table 3. Efficiency of microbial protein synthesis of animals fed on the three experimental rations in phases 1 and 2

Nutrient	Phase 1			Phase 2		
	T1	T2	T3	T1	T2	T3
Crude protein*	47.72±1.28	48.37±1.56	48.61±1.35	52.25±0.56	54.86±2.81	55.29±2.65
Crude fibre*	46.01±2.01	48.53±1.58	49.18±2.08	45.54±1.36	47.03±1.57	47.71±2.11
Ether extract*	86.38±1.88	82.41±2.80	82.21±4.24	85.34±2.78	84.84±1.66	82.94±3.04
Nitrogen free extract*	70.12±1.47	70.23±1.49	68.11±2.21	66.78±1.64	68.15±1.38	66.60±1.22
TDN$^{\gamma}$	43.09±1.98	45.44±2.01	46.27±1.68	55.49±2.64	56.38±2.16	56.61±2.34
Average DMI$^{\Omega}$	12.03±0.43	12.89±0.58	13.19±0.38	15.56±0.33	15.62±0.61	15.89±0.31
Average TDN intake$^{\Omega}$	5.18±0.28	5.86±0.24	6.10±0.21	8.63±0.13	8.81±0.44	9.00±0.19
Microbial protein synthesis$^{\alpha}$	997.01±100.17	825.91±125.18	842.06±125.14	1127.93±129.64	1106.74±189.53	1157.24±233.35
Efficiency of microbial protein synthesis$^{\beta}$	192.47±26.27	140.94±24.13	138.04±23.66	130.70±28.19	125.62±37.06	128.58±40.98

*Average of six values expressed in %, on DM basis; $^{\gamma}$average of 6 values expressed in %, on DM basis calculated from digestibility coefficients of nutrients; $^{\Omega}$average of 6 values expressed in kg/animal/day; $^{\alpha}$average of 6 values expressed in g/animal/day; $^{\beta}$average of 6 values expressed in g/kg TDN intake/animal/day.

mmol/d and 1492.86 to 1718.29 g/d, for allantoin, uric acid, total PD and MBP, respectively, in lactating Holstein cows fed on complete diets which included corn silage as basal roughage supplemented with 4 different protein sources. On the other hand, the uric acid values in cows fed on T1 in phase 1 were comparable to those observed by Pina *et al*. (2006).

Allantoin, uric acid, total PD excreted and MBP synthesised in 3 dietary treatments in phase 2 were higher than the values of 137.20 and 150.20 mmol/d; 4.80 and 5.20 mmol/d; 142.50 and 155.40 mmol/d; 583.75 and 650.00 g/d for allantoin, uric acid, total PD and MBP, respectively, reported by Dey *et al*. (2013) in crossbred cows fed on complete rations, in the fourth month of lactation. In phase 2, the allantoin, total PD and MBP of cows in all 3 dietary treatments was lesser while the uric acid excreted in T2 and T3 was comparable and that in T1 was higher than those of Yang and Beauchemin (2006a) who reported allantoin, uric acid, total PD and MBP values ranging from 320.40 to 367.30 mmol/d, 38.50 to 41.90 mmol/d, 362.30 to 409.00 mmol/d and 1750.00 to 1983.13 g/d, respectively, in dairy cows in mid lactation, fed on corn silage based complete rations of varying particle length.

The efficiency of microbial protein synthesis (g/kg TDN intake), calculated from the digestibility coefficients of nutrients in percentage and the average daily dry matter intake in kg per animal per day in phases 1 and 2 of the experiment are given in Table 3.

Pina *et al*. (2006) compiled the results of MBP synthesis estimated from urinary PD, obtained from several studies carried out in milking cows, subjected to different feeding conditions and recommended a MBP synthesis of 120 g/kg of TDN intake. The TDN intake of cows fed on the respective experimental rations, viz. T1, T2 and T3 was 5.18, 5.86 and 6.10 kg/d in early lactation and 8.63, 8.81 and 9.00 kg/d in mid lactation. In the present study, the efficiency of MBP/kg of TDN in cows fed on the respective experimental rations, viz. T1, T2 and T3 was 192.47, 140.94 and 138.04 g in early lactation and 130.70, 125.62 and 128.58 g in mid lactation.

The observations of Valadares *et al*. (1999) who found that the urinary excretion of allantoin, uric acid and total PD as well as MBP synthesis of lactating dairy cows, increased with increase in concentrate/NFC content of the diet; Pina *et al*. (2006) who opined that the highest MBP production was consistent with the highest consumption of soluble carbohydrates which provides greater amount of fermentable substrates and thereby greater amount of energy to the rumen microbes for MBP production; Yang and Beauchemin (2006b) who reported that the ruminal MBP synthesis of lactating dairy cows fed on barley silage based complete rations with 32 to 33% NDF and varying peNDF, decreased numerically with increase in peNDF in the ration, even though the decrease in values were not statistically significant and Boguhn *et al.* (2010) who reported that the intensity of fermentation as well as MBP synthesis was lower in high NDF beet pulp silage based TMRs when compared to low NDF maize based TMRs in dairy cows, were in total agreement with the findings of the present study, with respect to phase 1, where the numerical value of allantoin, uric acid, total PD and MBP synthesis was the highest in cows of T1 fed on the ration with the highest amount of soluble carbohydrate, while those of T2 and T3 fed on rations with lesser amount of soluble carbohydrate than T1 had lower numerical value.

On the other hand, in phase 2, cows fed on diet T3 with 35% NDF had numerically higher MBP synthesis than those fed on T2 with 30% NDF and T1 with 25% NDF. Nevertheless, there was little difference in the efficiency of MBP synthesis, with T1 having the highest efficiency, followed by T3 and T2 in descending order.

The probable reason could be that, in cows fed on rations T2 and T3 with lesser soluble carbohydrate content than T1, a simultaneous adaptation mechanism of the rumen ecosystem must have come into play, wherein, rumen microorganisms with shorter generation time and which use less amount of substrates for their own maintenance were selected, predation by protozoa was reduced and nutrient recycling occurred, with the net result that MBP synthesis increased in T2 and T3 in phase 2 as compared to phase 1 (Pina *et al.* 2006).

A thorough evaluation of the results obtained in the present study in phases 1 and 2, indicated that animals in all the three dietary treatments performed well, having adequate microbial protein synthesis, with respect to the TDN intakes. These results suggest that complete rations with 25 to 35% NDF, containing paddy straw as the sole source of roughage NDF, with the rest of NDF being met from non-forage sources, can be recommended for use in lactating dairy cows, with 25% being the most ideal NDF level in early lactation and all three NDF levels, viz. 25, 30 and 35% being ideal in mid lactation.

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