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Bacterial and Total Volatile Fatty Acids Production Rates in Cross-bred Calves Fed on Various Hay Diets

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ABSTRACT

TVFA production and bacterial production rates were determined in four experiments with male cross-bred calves. In experiment I and II, the animals were fed *ad lib* on cowpea hay and berseem hay diets, respectively either as such or treated with formaldehyde (2 per cent of CP) or supplemented with synthetic saliva (after feeding). In experiment III and IV, animals were fed oat hay and maize hay diets, respectively either as such or mixed with berseem/cowpea hay (1 : 1) or sprinkled with urea to provide N equivalent to berseem/cowpea hay.

On leguminous hay diets, TVFA production rate and ammonia-N levels were significantly low and bacterial production rate was significantly higher with synthetic saliva in comparison to untreated or formaldehyde treated hay. In case of non leguminous hays, ammonia-N levels and bacterial production rates were significantly higher on mixing non-legume and legume hay in equal parts or sprinkling urea in comparison to feeding hay as such. However, TVFA concentration and production rate were significantly lower in these treatments as compared to non-legume hay fed as such.

Key words : Bacterial productian rate, TVFA concentration, TVFA production, ATP production and NH_3-N concentration

Availability of substrates for optimum microbial cell synthesis in the rumen is of prime importance for efficient animal production. A continuous supply of fermentable carbohydrates and ammonia-N as precursors for cell growth is paramount to efficient use of ATP. At low ammonia levels in rumen fluid, reactions that fix ammonia into amino acids require ATP whereas, when ammonia levels are above a certain optimum, the ammonia is incorporated into amino acids without using ATP[1]. Under optimum conditions of nitrogen, minerals and energy availability in the rumen, microbial protein synthesis can take care of maintenance needs of protein requirement of ruminant animal. Thus, the bacterial protein synthesis was determined in crossbred calves fed legume and non legume hay diets in relation to ammonia-N and TVFA levels in the rumen and the results are incorporated in this communication.

Mateirals and Methods

Four experiments were conducted on male cross bred fistulated animals using legume and non legume hay diets. In all the experiments, six rumen fistulated calves were divided into three groups of two each In experiment I while the animals in Gr. A were fed on cowpea hay *ad lib*, those in Gr. B were fed on formaldehyde treated (2 per cent of CP) cowpea hay *ad lib*. Animals in Gr. C were fed on cowpea hay *ad lib* but in addition were offered with synthetic saliva[2] after feeding (6 litres each in the morning and evening). Experiment II was conducted on berseem hay with dietary treatments (Gr. D, E and F) similar to experiment I. In experiment III animals in Gr. G were fed on oat hay *ad lib*, in Gr. H on oat and berseem hay (1 : 1) *ad lib* and in Gr. I on oat hay *ad lib* sprinkled with urea to provide N equivalent to Gr. H. In experiment IV, animals were fed on maize hay (Gr. J, K and L) with dietary treatments similar to experiments III except in Gr. K, where maize and cowpea

hay (1 : 1) were fed *ad lib.* In each experiment, 20 g mineral mixture was given to each animal daily. The animals were fed twice a day but after a preliminary feeding of three weeks, the total ration was divided into 12 equal parts and fed at 2 hr intervals to ahieve a near steady state in the rumen.

Bacterial production rate was determined by single injection isotope dilution technique using ^{35}S sodium sulphate[3]. Samples of rumen liquor were collected with the help of specially designed stainless steel probes wrapped in nylon cloth. The bacterial pellets were dissolved in 'soluene' TM 100 and counted for radioactivity in Packard Tricarb Scintillation Counter model BPLD (PRIAS) using the scintillation fluid consisting of 500 ml dioxane, 500 ml toluene, 300 ml ethanol and 6.5g PPO (2,5-diphenyl oxazole). The TVFA production rates using 1,2 ^{14}C-sodium acetate were determined[4]. Ammonia-N (mg/100 ml) in the strained rumen liquor (SRL) was also determined[5].

Data were analysed statistically and linear and multiple regression equations developed[6].

Results and Discussion

Proximate composition of different hay diets fed under different experiments has been shown in Table 1.

Table 1. % chemical composition of different hays (D. M. basis)

Particulars	Organic matter	Crude protein	Ether extract	Crude fibre	Total ash	N-free extract
Cowpea hay	89.88	17.22	3.20	25.20	10.12	44.26
Berseem hay	88.16	15.20	3.50	26.40	11.84	43.06
Oat hay	88.30	10.60	3.39	29.80	11.70	44.51
Maize hay	86.70	10.20	3.20	32.20	13.30	42.10

Ammonia-N (mg/100 ml SRL) was significantly lower in Gr. C as compared to Gr. A and B in experiment I and in Gr. F as compared to Gr. D and E in experiment II (Table 2). This may be due to lesser microbial degradation of dietary protein on providing synthetic saliva which increases the flow rate of digesta from the rumen[7]. In experiment III and IV, ammonia-N in SRL (mg/100 ml) was significantly higher in Gr. H and I and K and L as compared to Gr. G as well as Gr. J, respectively. The higher values for ammonia-N in Gr. H and K may be attributed to higher crude protein contents[8,9] in the diet due to mixing of leguminous hay in equal parts and in Gr. I and L due to sprinkling of urea on hay[10,11].

Bacterial protein synthesis (g/day) was significantly higher in Gr. C as compared to Gr. A and B in experiment I and in Gr. F as compared to Gr. D and E in experiment II. However, bacterial production rate (g/kg DOMI) in experiment I and II was not found significantly different amongst verious groups due to higher OM intake in Gr. C and F, respectively. It is reported that infusion of artificial saliva into the rumen of sheep increases the dilution rate and efficiency of bacterial protein synthesis[7,12]. It was shown[13] that the Y-ATP for a continuous culture of ruminant bacteria could be altered from 8 to about 17 by increasing the

Table 2. Bacteria and TVFA production rate, TVFA concentration and ammonia-N concentration in the rumen fed on various hay diets

Group No.	NH_3-N (mg 100 ml SRL)	Bacterial production rate (g/day)	Bacterial production rate (g/kg DOMI)	TVFA conc. (mM/100 ml SRL)	TVFA production (moles/day)	ATP production (moles/day)	Bacterial production mg/mole ATP (Y-ATP)
			Experiment-I (Cowpea hay)				
A	$22.95 \pm 1.14^{a**}$	$178.15 \pm 0.45^{a**}$	89.97 ± 1.95	8.25 ± 0.05^{a}	$8.45 \pm 0.04^{a*}$	$18.59 \pm 0.08^{a*}$	$9.57 \pm 0.35^{a*}$
B	21.45 ± 1.15^{a}	174.02 ± 3.62^{a}	92.07 ± 3.16	8.55 ± 0.05^{a}	8.87 ± 0.12^{a}	19.51 ± 0.26^{a}	8.91 ± 0.44^{a}
C	17.36 ± 1.39^{b}	191.66 ± 0.41^{b}	87.12 ± 4.12	7.70 ± 0.10^{b}	7.91 ± 0.13^{b}	17.40 ± 0.29^{b}	11.03 ± 0.58^{b}
			Experiment-II (Berseem hay)				
D	$21.37 \pm 0.94^{a**}$	$176.81 \pm 0.41^{a**}$	82.23 ± 1.98	8.20 ± 0.10^{a}	$8.45 \pm 0.20^{a*}$	$18.59 \pm 0.44^{a*}$	$9.52 \pm 0.54^{a*}$
E	22.12 ± 1.14^{a}	169.66 ± 0.44^{a}	81.96 ± 1.43	8.05 ± 0.15^{a}	9.07 ± 0.07^{a}	19.95 ± 0.15^{a}	8.51 ± 0.39^{a}
F	17.86 ± 1.31^{b}	196.15 ± 1.29^{b}	81.73 ± 2.12	7.30 ± 0.10^{b}	7.63 ± 0.03^{b}	16.76 ± 0.07^{b}	11.69 ± 0.34^{b}
			Experiment-III (Oat hay)				
G	$18.92 \pm 0.37^{a**}$	$130.16 \pm 3.64^{a*}$	$69.97 \pm 1.55^{a*}$	$10.05 \pm 0.05^{a*}$	$10.37 \pm 0.11^{a*}$	$22.81 \pm 0.24^{a*}$	$5.71 \pm 0.11^{a*}$
H	21.56 ± 0.52^{b}	150.34 ± 1.26^{b}	79.54 ± 2.12^{b}	9.25 ± 0.06^{b}	9.57 ± 0.03^{b}	21.05 ± 0.07^{b}	7.14 ± 0.17^{b}
I	23.17 ± 0.47^{c}	151.32 ± 3.99^{b}	78.40 ± 1.92^{b}	9.00 ± 0.10^{b}	9.26 ± 0.14^{b}	20.37 ± 0.31^{b}	7.38 ± 0.08^{b}
			Experiment-IV (Maize hay)				
J	$17.92 \pm 0.64^{a**}$	$140.90 \pm 3.86^{a**}$	$71.16 \pm 2.31^{a*}$	$9.25 \pm 0.05^{a*}$	$9.56 \pm 0.30^{a*}$	$21.03 \pm 0.66^{a*}$	$6.71 \pm 0.33^{a*}$
K	20.72 ± 0.73^{b}	156.69 ± 0.27^{b}	78.06 ± 2.42^{b}	8.30 ± 0.05^{b}	9.20 ± 0.05^{b}	20.24 ± 0.11^{b}	7.74 ± 0.12^{b}
L	24.11 ± 0.66^{c}	157.62 ± 0.52^{b}	79.78 ± 1.96^{b}	8.30 ± 0.10^{b}	9.18 ± 0.17^{b}	20.20 ± 0.37^{b}	7.81 ± 0.27^{b}

^{a,b,c}Figures bearing different superscripts in a column differ significantly; * $P < 0.05$; ** $P < 0.01$.

*Significant at 5% level.

**Significant at 1% level-

dilution rate of the medium. This indicates that a large pool of micro-organisms growing at a slow rate uses ATP less efficiently than a smaller pool of microbes growing rapidly. These observations suggest that how an increased turnover rate of rumen digesta should increase microbial cell yield and Y-ATP and the results of the experiments in which fluild turnover of the rumen was increased by feeding or infusing salts supports this view. Bacterial production rate was also higher in Gr. H and I and K and L as compared to Gr. G in experiment III aud Gr. J in experiment IV, respectively. Microbial N is derived from ammonia-N and (or) preformed amino acids, with the latter highly dependent on the dietary N source. The percentage of microbial N derived from ammonia-N has been reported to range from 40 to 100 per cent under various conditions[14–18]. Thus, higher bacterial protein production in Gr. H, I and K, L in experiment III and IV may be due to higher ammonia-N level which has been reported upto 29 mg/100 ml SRL[19]. Similarly, bacterial production rate (g/kg DOMI) in these groups was significantly higher (Table 2).

TVFA concentration (mM/100 ml SRL) and production rate (moles/day) were significantly lower in Gr. C and F as compared to Gr. A and B as well as D and E, respectively in experiment I and II. Carbohydrates on fermentation enter into either microbial constituents or TVFA plus methane and carbon dioxide[1]. If microbial cell components are synthesised from the glucose, then growth is highly efficient. If, however, the end products of fermentation (*i. e.*, VFAs) are used, the synthesis of microbial cell is much lower per unit of organic matter fermented[1]. Due to these reasons, it is reported that microbial protein synthesis and TVFA production are inversely related[20]. Thus, the low TVFA concentration and production rate in Gr. C and F in experiment I and II is explainable. Similar reason may be attributed to low TVFA production rate in Gr. H and I in experiment III and Gr. K and L in experiment IV.

Considering that TVFA produced in the rumen may serve as source of energy for bacterial protein syntnesis[21,22] and 2.2 moles of ATP are generated per mole of VFA produced[23], an attempt was made to study the relationship between b acterial production rate and TVFA concentration or total ATP production. Since ba cterial protein synthesis is also reported to be highly dependent on ammonia-N concentration in the rumen[14], a relationship between bacterial protein synthesis and ammonia-N concentration was developed separately for leguminous and non leguminous hay diets as under:

$$(1)\quad Y_{BP} = 383.95 - 25.27\ X_C \quad (r = 0.95;\ n = 24;\ p < 0.01)$$

$$(2)\quad Y_{BP} = 382.02 - 11.0417\ X_P \quad (r = 0.95;\ n = 24;\ p < 0.01)$$

$$(3)\quad Y_{BP} = 262.26 - 3.96\ X_{AN} \quad (r = 0.83;\ n = 12;\ p < 0.01)$$

Where,

Y_{BP} = Bacterial production rates (g/day)

X_C = TVFA concentration (mM/100 ml SRL)

X_P = ATP production (moles/day)

X_{AN} = Ammonia-N concentration (mg/100 ml SRL)

TVFA concentration, ATP production and ammonia-N (leguminous hay diets only) were significantly and negatively correlated with bacterial production rate. A signi-

ficant relationship between bacterial protein synthesis (g/day) and TVFA production rate (moles/day) has also been reported earlier[24–26]. It is, thus, obvious that bacterial production rate in the rumen is highly dependent upon TVFA concentration or ATP production as well as ammania-N concentration. Therefore, combined multiple regression equations were attempted and developed between these parameters as under:

$$(4)\quad Y_{BP} = 383.27 + 0.047\ X_{AN} - 25.31\ X_O \quad (R^2 = 0.91,\ n = 24;\ p < 0.01)$$

$$(5)\quad Y_{BP} = 382.45 - 0.029\ X_{AN} - 11.03\ X_P \quad (R^2 = 0.90;\ n = 24;\ p < 0.01)$$

These studies, thus, indicate that micrabial protein synthesis, in the rumen is highly and significantly correlated with ammonia-N, ATP production, TVFA, concentration and TVFA production rate[27].

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