Indian Journal of Animal Sciences **82** (4): 400–404, April 2012

Effect of sodium salts of branched chain volatile fatty acids on extra cellular ammonia utilization and protein production *in vitro*

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Received: 10 December 2010; Accepted: 28 October 2011

ABSTRACT

Effects of Na salts of branched chain volatile fatty acids (isobutyric, isovaleratic and 2–methyl butyric) addition (@ 0.75 and 1.50%) in the substrates containing wheat straw, green fodder and concentrate mixture (40:40:20) and varying in protein content (8, 10 and 12%) and nitrogen sources (with and without urea) on dry matter digestion, utilization of extra cellular ammonia and protein production in the *in vitro* medium were investigated. Inclusion of BCFA in the substrate @ 0.75 and 1.50%, increased (P<0.01) the DM digestibility, ammonia utilization and protein content compared to control (0 dose). No added advantage was observed when the dose of BCFA was increased from 0.75 to 1.50%. The BCFA addition was found to be the most effective in the substrates containing 10 and 12% protein compared to 8% protein level as the higher (P<0.01) DM digestion, ammonia utilization and protein production were observed with these protein levels. All the 3 BCFA were equally effective. Substrate combinations containing urea showed higher (P<0.01) response of BCFA *in vitro* digestion, ammonia utilization and protein concentration. The responses of BCFA addition on *in vitro* degradation were more pronounced during first 24 h of fermentation compared to the later part i.e., 48 h. It may be concluded that the inclusion of sodium salts of BCFA @ 0.75 and 1.50% of the substrate, improved the DM digestion, ammonia utilization and protein production at *in vitro* medium. For optimum efficacy of the BCFA, the protein content of the substrate or diet should be in the range of 10 to 12% and part (up to33%) of this can be replaced by the urea.

Key words: Ammonia, Branched chain volatile fatty acids, Digestibility, Protein level, Rumen fermentation, Sodium salt

The branched chain volatile fatty acids (BCFA) i.e. isobutyric, isovaleric and 2–methyl butyric acid, are essential growth factors for a number of strains of important ruminal bacteria (Allison *et al.* 1958, Wegner and Foster 1960). These BCFA are mainly produced in rumen by the microbial degradation of branched chain amino acids of the dietary protein. Under normal feeding conditions, diet is sufficient in protein content, the deficiency of BCFA is unlikely to occur and *de novo* synthesis of BCFA would suffice the microbial requirements. However, in spite of *de novo* synthesis, the deficiency of BCFA is possible in ruminants fed low protein diet and such deficiency would not be corrected by non protein nitrogen or urea supplementation (Umunna *et al.* 1975). In Asia, the animal production system is solely based on crop residues and meagre amounts of energy and protein supplements. Studies have shown that such feeding regimens lead to ruminal BCFA deficiency and their concentration reaches even below the detectable limits (Judkins *et al.* 1987, Krysal *et al.* 1989). Available information indicates that inclusion of BCFA in *in vitro* and *in vivo* studies, resulted in improved microbial growth, beneficial in promoting the growth rate and feed conversion efficiency (Deetz *et al.* 1985, 1990, Misra and Thakur 2001a, b), improved milk production and persistency of lactation (Papas *et al.* 1984, Peirce Sander *et al.* 1985). The responses of BCFA were higher when supplemented to high fibre and low protein but otherwise adequate in nitrogen diet. Objective of our study was to see the response of BCFA addition in the substrate varying in protein level and nitrogen source on DM digestion, utilization of extra cellular ammonia and protein production in the *in vitro* medium. Efficacy and dose responses of different BCFA on these parameters were also studied.

MATERIALS AND METHODS

Preparation of sodium salts of BCFA

BCFA were added in the substrate in the aqueous form of sodium salts. Sodium salts of isobutyric, isovaleric and 2-

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methyl butyric acid were prepared by neutralizing these acids with sodium hydroxide solution on molar basis.

Basal substrate

Substrates used in this study were consisting of wheat straw (WS), concentrate mixture (maize grain, wheat bran, groundnut cake, maize, starch, urea) and oven dried green maize fodder, in 40:40:20 proportions on dry matter basis. Total 6 concentrate mixtures (CM) were prepared using different feed ingredients (Table 1). The proportion of each feed ingredient in different concentrate mixtures was adjusted in such a way that the incorporation of that particular concentrate mixture at 40% level with another 40 and 20 parts of wheat straw and maize fodder could provide the desired CP content i.e. 8, 10 and 12% of the finished substrate. To vary the nitrogen source, the urea was used to replace 1/3rd of total nitrogen (N) of the substrate in each protein level.

Treatment variables

The treatment variables were, protein levels of substrate (8, 10 and 12%), sodium salts of the 3 BCFA (isobutyric, isovaleric and 2 methyl butyric acids), doses of BCFA (0, 0.75 and 1.50% of the substrate), nitrogen sources {without and with urea (to replace 1/3 of total nitrogen in substrate)}. The 54 treatment combinations of protein levels (3), BCFA (3), doses (3) and N sources (2) were assayed.

In vitro *digestion assay*

In vitro DM digestibilities were (Tilley and Terry 1963) obtained in triplicate following 24 h and 48 h incubation. The ruminal fluid inoculum was obtained 4 h post feeding from a fistulated crossbred male cattle (240 kg BW; 28 months old) consuming a diet of WS and CM (roughage to concentrate ratio 65:35) containing 11.6% CP and 3.15 Mcal/kg digestible energy. Incubations were initiated 25 min after collection of rumen fluid.

Chemical analysis

The DM content was determined by drying the materials in hot air oven at 60°C until a constant weight was achieved. The OM and CP were determined as per AOAC (1990). The NDF and ADF contents were estimated following the method of Van Soest *et al.* (1991). Ammonia nitrogen was determined by the method prescribed by Conway (1957). Estimation of protein content (TCA-precipitable nitrogen) under *in vitro* medium was carried out by the method of Tagari *et al.* (1964).

Statistical analysis

Data were analyzed in 3×3×3×2 factorial arrangements of treatments using SPSS Base 10 (SPSS software products, USA) package. The group differences were compared by using Duncan's Multiple Range Test (Duncan 1955). The factors were the different BCFA (3), doses of BCFA(3), protein levels (3) and protein sources (2).

RESULTS AND DISCUSSION

The chemical analysis (Table 2) of feed ingredients, WS and oven dried green fodder (maize) indicated that chemical constituents in most of the feed ingredients were within the normal range of variations. However, the CP content of

Table 1. Feed ingredients and their proportion in concentrate mixtures (CM)

Concentrate mixtures	CP (%)	Ingredients of concentrate mixture (on DM basis)					Total
		Maize grain	Groundnut cake	Wheat bran	Starch	Urea*	
CM1	8	79.00	5.00	16.00	–	–	100
CM2	8	52.50	–	–	45.10	2.40	100
CM3	10	68.75	25.00	6.25	–	–	100
CM4	10	81.25	–	7.50	8.23	3.02	100
CM5	12	49.00	43.00	8.00	–	–	100
CM6	12	77.75	7.25	11.36	–	3.64	100

*Urea was used to replace 1/3rd of total nitrogen of the finished substrate.

Table 2. Chemical composition (% on DM basis) of wheat straw, maize fodder and ingredients of concentrate mixtures used in substrate

Ingredients	DM	OM	CP (N*6.25)	NDF	ADF	Hemicellulose[1]
Wheat straw	89.8	88.0	4.2	77.6	53.8	23.8
Maize fodder	14.0	90.2	8.0	67.7	44.0	23.7
Maize (grain)	91.6	98.1	9.8	13.8	5.6	8.2
Groundnut cake	91.4	88.9	37.0	22.2	11.0	11.2
Wheat bran	87.0	93.8	13.8	40.6	12.8	27.8
Maize starch	98.9	99.9	-	-	-	-
Urea	98.8	100.0	44.1*	-	-	-

* Nitrogen; [1]NDF-ADF.

groundnut cake was lower (37.0%) and the reason might be the high ash (11.0%) and fibre content (22.2%).

Dry matter digestibility

The significant improvement in the *in vitro* dry matter digestibility (IVDMD) at 24 and 48 h post-incubation on BCFA addition (Table 3) as compared to control (zero dose), indicated that on providing these microbial growth factors, *in vitro* fermentation got stimulated resulting in enhancement of DM digestibility (Soofi *et al.* 1982, Cummins and Papas 1985, Quipse *et al.* 1991). Further increase in dose of BCFA from 0.75 to 1.50% did not show any added advantage. The greater response of BCFA inclusion on IVDMD at 10% protein content indicated the deficiency of precursor amino acids required for the synthesis of BCFA. In case of 8% CP content, the substitution of microbial BCFA requirements through extraneous source though stimulated the microbial fermentation, but at the same time, inadequacy in nitrogen supply probably restricted the true enhancement in IVDMD. While substrate having 12% CP level, which is considered optimum for microbial fermentation, also responded positively on BCFA inclusion. The efficacy of all the 3 BCFA in the substrate, on IVDMD at 24 and 48 h was similar indicating the inter convertibility of one BCFA to other, and thereby, replacing the requirements of each other (Gorosito *et al.* 1985). The N sources were also found to have significant effect on IVDMD and the substrate having urea, showed highest IVDMD (54.18 and 70.51%) compared to substrate incubated without urea (52.20 and 69.11%) at 24 and 48 hours of incubation.

Utilization of extra cellular ammonia

The addition of sodium salts of BCFA in the substrate significantly reduced the ammonia nitrogen in the *in vitro* medium (Table 3), indicating the enhanced utilization of ammonia by the microbes. In present experiment, the reduction in ammonia nitrogen concentration in the *in vitro* medium due to addition of BCFA in the substrate was 13.62 and 12.36% at over the control (0 BCFA). About 8 to 9% reduction in ammonia N concentration has been reported by Russel and Sniffen (1984) and Gorosito *et al.* (1985) in the *in vitro* medium containing WS cell wall and defined medium, respectively. The effect of two doses (0.75 and 1.50%) of BCFA was similar. Surprisingly, at 48 h the substrate incubated without BCFA (0 dose), showed lower (P<0.01) ammonia N concentration (Table 3) compared to two other BCFA doses and the exact reason for this pattern is not known. In respect of protein levels, the ammonia N varied greatly (P<0.01) and increased with the protein content of the substrate was increased (Table 4) at 24 and 48 h of post-incubations *in vitro*. The effect of sodium salts of 3 different BCFA was similar on utilization of ammonia N in

Table 3. Effect of doses of sodium salts of BCFA addition on DM digestion (%), ammonia nitrogen and TCA precipitable nitrogen (mg 100^{-ml}) at 24 and 48 h of post incubations *in vitro*

Doses (% of substrate)	DM digestibility		Ammonia nitrogen		TCA precipitable -N	
	24 h	48 h	24 h	48 h	24 h	48 h
0	50.06[a]	68.08 [a]	6.31 [a]	9.82 [a]	7.09 [a]	9.07 [a]
0.75	55.47 [b]	70.46 [b]	5.45 [b]	10.16 [b]	9.03 [b]	11.16 [b]
1.50	55.71[b]	70.89 [b]	5.53 [b]	10.00 [b]	9.10 [b]	11.25 [b]
P-value	**	**	**	**	**	**
SEM	0.31	0.25	0.03	0.03	0.02	0.03

SEM, standard error of means; **,P<0.01, *,P<0.05; means in a column bearing similar superscripts do not differ significantly (P<0.01).

Table 4. Effect of addition of sodium salts of BCFA in the substrate varying in protein levels on DM digestion (%), ammonia nitrogen and TCA precipitable nitrogen (mg 100^{-ml}) at 24 and 48 h of post-incubations *in vitro*.

Doses (% of substrate)	DM		Ammonia nitrogen		TCA precipitable -N	
	24 h	48 h	24 h	48 h	24 h	48 h
0						
8	47.41[a]	69.63	4.19 [a]	5.90 [a]	5.07 [a]	6.88 [a]
10	56.75 [b]	69.99	4.95 [b]	8.16 [b]	7.68 [b]	12.28 [b]
12	57.67 [b]	69.82	8.17 [c]	15.92b[c]	12.54 [c]	12.37 [b]
P-value	**	NS	**	**	**	**
SEM	0.31	0.25	0.03	0.03	0.02	0.03

SEM, standard error of means; **,P<0.01; *,P<0.05; means in a column bearing similar superscripts do not differ significantly (P<0.01).

Table 5. Effect of addition of sodium salts of BCFA on DM digestion (%), ammonia nitrogen and TCA precipitable nitrogen (mg/100 ml) at 24 and 48 h of post incubations *in vitro*

Sodium salts	DM		Ammonia nitrogen		TCA precipitable -N	
of BCFA	24 h	48 h	24 h	48 h	24 h	48 h
Isobutyric	54.58	70.30	5.67	9.97 [a]	8.66	10.33
Isovaleric	53.86	69.33	5.73	10.20 [b]	8.54	10.28
2-methyl butyric	53.60	69.83	5.70	9.79 [c]	8.50	10.42
P-value	NS	NS	NS	**	NS	NS
SEM	0.31	0.25	0.03	0.03	0.02	0.03

SEM, standard error of means; **,P<0.01, *,P<0.05; Means in a column bearing similar superscripts do not differ significantly (P<0.01).

Table 6. Effect of addition of sodium salts in the substrate varying in nitrogen source on DM digestion (%), ammonia nitrogen and TCA precipitable nitrogen (mg/100 ml) at 24 and 48 h of post incubations *in vitro*

Nitrogen source	DM		Ammonia nitrogen		TCA precipitable -N	
	24 h	48 h	24 h	48 h	24 h	48 h
No urea	52.20 [a]	69.11 [a]	4.77 [a]	6.50 [a]	7.65 [a]	9.01
Urea	54.18 [b]	70.51 [b]	6.76 [b]	13.49 [b]	10.16 [b]	10.02
P	**	**	**	**	**	NS
SEM	0.28	0.23	0.03	0.03	0.02	0.03

SEM, standard error of means; **,P<0.01, *,P<0.05; Means in a column bearing similar superscripts do not differ significantly (P<0.01).

the *in vitro* medium (Table 5). The addition of urea in the substrate elevated (P<0.01) the ammonia N levels in the *in vitro* medium (Table 6).

Microbial protein production

Inclusion of BCFA in the substrate significantly (P<0.01) increased the concentration of TCA-precipitable nitrogen at 24 and 48 h of post-incubation (Table 3) compared to control. Further increase in dose of BCFA from 0.75 to 1.50 (% of the substrate) did not show any additional advantage in protein content in the *in vitro* medium. The increased utilization of extra cellular ammonia on BCFA addition in the substrate might have converted into the protein by the microbial population (Cummins and Papas 1985) and thus enhancing the protein production reflected as higher TCA-precipitable nitrogen under the *in vitro* system. An increase of 18.7% in microbial protein synthesis was observed by Russel and Sniffen (1984) on inclusion of blends of 3 BCFA in the *in vitro* medium. Maximum (P<0.01) TCA-precipitable nitrogen was observed with the substrate having 12% protein (Table 4). All the 3 BCFA salts showed similar impact on the protein concentration in the *in vitro* medium. However, the variations due to nitrogen sources in TCA-precipitable nitrogen were significant (P<0.01) and higher concentration was found to be associated with the substrate having urea as compared to the substrate incubated without urea at 24 and 48 h of post incubations. Further, with in the substrates having urea, the increase in TCA-precipitable nitrogen was significantly (P<0.01) greater wherein the urea and starch were added together in the substrate. The readily availability of keto acids and ammonia arising from degradation of the starch and urea, respectively ensured the synchronization in release of energy and nitrogen, thereby stimulating the microbial growth and increasing the protein synthesis (Cummins and Papas 1985).

SUMMARY

Inclusion of BCFA in the substrate @ 0.75 and 1.50%, increased (P<0.01) the DM digestibility, ammonia utilization and protein synthesis in the *in vitro* medium. No added advantage was observed when the dose of BCFA was increased from 0.75 to 1.50%. The BCFA supplementation was found to be the most effective in the substrates containing 10 to 12% protein. All the 3 BCFA were equally effective in improving the DM digestion, ammonia utilization and protein production. Addition of urea in the substrate stimulated the efficacy of BCFA in improving the *in vitro* digestion. The responses of BCFA addition on *in vitro* digestion were more pronounced during first 24 h of fermentation compared to the later part i.e., 48 h.

ACKNOWLEDGEMENT

Authors wish to thank the Director, National Dairy Research Institute, for providing the facilities to carry out this experiment.

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