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Effect of carbon-4 and carbon-5 volatile fatty acids inclusion in the substrate varying in protein levels and sources on organic matter and cell wall degradability *in vitro*

A K MISRA[1] and S S THAKUR[2]

National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

Effects of Na-salts of branched chain volatile fatty acids (IB, IV and 2–MB (@ 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 sources (with and without urea) on dry matter, organic matter and cell wall digestion *(in vitro*) were investigated. Inclusion of BCFA @ 0.75 and 1.50% in the substrate, increased DM, OM and cell wall digestibility compared to control. Higher dose of BCFA (1.50%) did not show any additional advantage. The inclusion of BCFA was most effective in the substrates containing 10 and 12% protein level as the higher *in vitro* digestion coefficients of DM, OM and cell wall were associated with these protein levels. Among 3 BCFA, the isobutyric acid was most effective in improving the *in vitro* digestion of DM, OM and cell wall at 24 h, whereas at 48 h, all the 3 BCFA were equally effective. Substrate combinations containing urea showed higher response. Effect of BCFA addition was more pronounced during first 24 h of fermentation compared 48 h. Higher TVFA and acetate concentrations were found associated with the substrate combinations incubated with BCFA. It is evidenced in the study that sodium salt of BCFA has potential to be used as feed supplement with the diets largely based on straw and other crop residues.

Key words: Branched chain volatile fatty acid, BCFA, Dose, Fatty acid, Fiber digestibility, Protein level, Sodium salt

The branched chain volatile fatty (BCFA) acids (C-4 and C-5) i.e. isobutyric, isovaleric and 2-methyl butyric acid, are mainly produced in rumen by the microbial degradation of branched chain amino acids of the dietary protein. Under normal feeding conditions, particularly the 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 India, the animal production system is solely based on crop residues and meagre amounts of energy and protein supplements. Studies have shown that feeding of such feedstuffs leads to ruminal branched chain fatty acids deficiency and their concentration reaches even below the detectable limits (Krysal *et al*. 1989). Hence, in present investigation the attempts were made to select the most effective branched chain fatty acid and its dose and optimum dietary protein level to optimise *in vitro* DM, OM and NDF degradation.

Present address: [1] Principal Scientist (asimkmisra@gmail.com), Plant Animal Relationship Division, Indian Grassland and Fodder Research Institute, Jhansi 284003 India.[2] Principal Scientist, Dairy Cattle Nutrition Division.

MATERIALS AND METHODS

Preparation of sodium salts of BCFA and basal substrate: BCFA were added in the substrate in the aqueous form of sodium salts. Sodium salts of isobutyric, isovaleric and 2 methyl butyric acid were prepared by neutralizing these acids with sodium hydroxide solution on molar basis. At the end of process, the pH of solution was recorded to ensure the complete neutralization and slightly alkaline pH (7.5 8.0) value was preferred as index for complete neutralization. Substrates used in this study were consisting of wheat straw, 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 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 protein/ nitrogen source, the urea was used to replace 1/3rd of total

Table 1. Feed ingredients and their proportion in concentrate mixtures

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

*Urea is 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!
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; !NDE-ADF.

nitrogen of the substrate at each protein level. Thus, total of 54 treatment combinations of protein levels (3), branched chain volatile fatty acids (3), doses (3) and protein sources (2) were assayed.

In vitro *digestion assay and chemical analysis:* The *in vitro* digestibility assay was carried out for 24 and 48 h as per the method prescribed by Tilley and Terry (1963). The ruminal fluid inoculum was collected 4 hours post feeding and watering from two fistulated cattle fed on the same diet, which was to be tested as substrate. The DM, OM (AOAC 1995) and NDF (Van Soest *et al.* 1991) contents of the substrate were determined. Total volatile fatty acids were determined (TVFA) as per the method prescribed by Barnett and Reid (1957) whereas individual fraction of VFA was estimated (Ervin *et al.* 1961) by gas liquid chromatography. Data were analyzed in 3×3×3×2 factorial arrangements of treatments using SPSS Base 10 (SPSS software) 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, wheat straw and oven dried green fodder (maize) indicated that chemical constituents in most of the feed ingredients were within the acceptable levels. However, the CP content of groundnut cake was lower (37.0%) and the reason might be the high ash (11.0%) and fibre content (22.2%).

Dry matter, Organic matter and Cell wall digestibility

Addition of Na-salt of BCFA increased (P<0.01) DM, OM and NDF digestion at 24 and 48 h post *in vitro* incubations (Table 3). Both the doses (075 and 1.50) of sodium slats of BCFA were similar in their effect on DM, OM and NDF digestion. The BCFA addition in the substrate with 10 and 12% protein level showed maximum (P<0.01) effect on DM, OM and NDF digestion (Table 3) at 24 h post incubation. Among the Na-salts of 3 BCFA, IB showed maximum (P<0.05) response on DM, OM and NDF digestion at 24h post incubation. Addition of urea in the substrate increased the effect of Na-salt of BCFA addition on digestion of DM at 24 and 48h, OM and NDF at 48h (Table 3). The significant improvement in DM, OM and NDF at 24 and 48 h post incubation on BCFA supplementation as compared to control (zero dose), indicated that on providing these microbial growth factors, *in vitro* fermentation got stimulated resulting in enhancement of DM and OM digestibility (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 digestibility of these constituents 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 the digestibility. While substrate having 12% CP level, which is considered optimum for microbial fermentation, also responded positively on BCFA inclusion. The effect of inclusion of all the three BCFA in the substrate, on IVDMD and IVOMD at 24 and 48 h, varied significantly and was highest on inclusion of isobutyrate followed by 2 methyl butyrate and isovalerate. However, the differences between isobutyrate and 2 methyl butyrate, and 2 methyl butyrate and isovalerate were statistically non significant. The variability in responses of each BCFA might be due to the difference in inter convertibility of one BCFA to other, and thereby, replacing the requirements of each other (Gorosito *et al*., 1985). The nitrogen sources (U_0 and U_1) were also found to have significant effect on IVDMD and the substrate having urea (U_1), showed highest IVDMD (54.18 and 70.51%) compared to substrate incubated without urea (52.20 and 69.11%) at 24 and 48 h of incubation. However, the effect of of both the protein sources (U_0 and U_1) on OM and cell wall digestion at 24 h of incubation was similar. This was primarily due to reason that at 8% CP level, the high quantity of starch was used as compared to other substrates, which

Table 3. Effect of addition of sodium salts of BCFA addition in the substrate varying in protein levels and sources on *in vitro* DM, OM and NDF digestion at 24 and 48 h

Particulars	Hours	*In vitro* digestibility of DM, OM and NDF (%)											P and SEM			
		Dose (%)			Protein Levels (%)			BCFA (Na-Salt)			Protein source		Dose	Protein levels	BCFA	Protein source
		0	0.75	1.50	8	10	12	IB	IV	2-MB	U0	U1				
DM	24	50.06 ^{a}	55.47 ^{b}	55.71 ^{b}	47.41 ^{a}	56.75 ^{b}	57.67 ^{b}	54.58^{ab}	52.86 ^{b}	53.60^{ab}	52.20 ^{a}	54.18 ^{b}	0.31**	0.31**	0.31*	0.28**
	48	68.08 ^{a}	70.46 ^{b}	70.89 ^{b}	69.63	69.99	69.82	70.30	69.33	69.83	69.11 ^{a}	70.51 ^{b}	0.25**	0.25^{NS}	0.25^{NS}	0.23**
OM	24	48.61 ^{a}	54.17 ^{b}	54.26 ^{b}	45.93 ^{a}	54.82 ^{b}	56.33 ^{b}	52.85	51.98	52.23	51.74	51.44	0.45**	0.45**	0.45 ^{NS}	0.36 ^{NS}
	48	69.64 ^{a}	69.75 ^{b}	69.75 ^{b}	68.61	68.97	69.10	68.96	68.25	67.90	67.87 ^{a}	69.87 ^{b}	0.34*	0.34^{NS}	0.34 ^{NS}	0.27 *
NDF	24	38.26 ^{a}	45.54 ^{b}	46.10 ^{b}	34.61 ^{a}	47.66 ^{b}	48.08 ^{b}	43.84 ^{a}	42.51 ^{b}	43.55 ^{ac}	43.18	43.42	0.40**	0.40**	0.40*	0.33 ^{NS}
	48	56.36 ^{a}	59.64 ^{b}	59.97 ^{b}	54.38 ^{a}	61.69 ^{b}	59.90 ^{c}	58.49	59.02	58.46	56.09 ^{a}	60.62 ^{b}	0.40**	0.40**	0.40^{NS}	0.32 **

DM, Dry matter; OM, organic matter; NDF, neutral detergent fibre; IB, iosbutyric; IV, isovaleric; 2-MB: @-methyl butyric; U1, with urea; U0, without urea. P: Statistical significance; SEM, standard error of means; **, P<0.01; * , P<0.05; NS, nonsignificant.

Table 4. Effect of addition of sodium salt of BCFA addition in substrate varying in protein levels and sources on individual and total VFA concentration (mM/l) at 24 and 48 h in *in vitro*

Particulars	Hours	*In vitro* concentration of individual and TVFA (mM/l)											P and SEM			
		Dose (%)			Protein Levels (%)			BCFA (Na-Salt)			Protein source		Dose	Protein levels	BCFA	Protein source
		0	0.75	1.50	8	10	12	IB	IV	2-MB	U0	U1				
Acetic	24	42.56 ^{a}	45.63 ^{b}	46.36 ^{b}	34.24 ^{a}	46.26 ^{b}	54.08 ^{c}	47.16 ^{a}	42.76 ^{b}	44.63 ^{b}	38.44 ^{a}	51.27 ^{b}	0.78**	0.78**	0.78*	0.78**
	48	56.14	57.83	57.16	49.26 ^{a}	57.50 ^{b}	63.22 ^{c}	61.54 ^{a}	54.65 ^{b}	54.63 ^{b}	56.39	57.17	1.08^{NS}	1.08**	1.08*	1.08**
Propionic	24	19.34	19.57	19.57	20.13	19.92	20.02	19.95	19.33	19.19	20.86	18.12	0.78^{NS}	0.78 ^{NS}	0.78 ^{NS}	0.78NS
	48	20.94	22.60	22.93	19.94 ^{a}	22.13 ^{b}	24.02 ^{b}	20.84	19.36	19.83	20.98 ^{a}	22.46 ^{b}	0.73^{NS}	0.73**	0.73^{NS}	0.73**
Butyric	24	4.27	4.62	4.69	5.10 ^{a}	3.99 ^{b}	4.52 ^{b}	4.28	4.43	4.87	4.55	4.52	0.35^{NS}	0.35**	0.35^{NS}	0.35*
	48	5.60	6.55	6.58	6.26	6.10	6.53	6.56	6.43	6.46	6.35	6.24	0.49^{NS}	0.49 ^{NS}	0.49 ^{NS}	049NS
TVFA	24	66.12 ^{a}	70.86 ^{b}	72.86 ^{b}	60.80 ^{a}	68.55 ^{b}	79.60 ^{c}	72.12 ^{a}	67.16 ^{b}	69.73 ^{b}	64.65 ^{a}	74.70 ^{b}	0.77*	0.77**	0.77**	0.77**
	48	83.02 ^{a}	88.37 ^{b}	88.82 ^{b}	76.49 ^{a}	87.38 ^{b}	96.06 ^{c}	96.18 ^{a}	81.68 ^{b}	82.36 ^{b}	84.61 ^{a}	88.02 ^{b}	1.11**	1.11**	1.11**	1.11**

DM, Dry matter; OM, organic matter; NDF, neutral detergent fibre; IB, iosbutyric; IV, isovaleric; 2-MB: @-methyl butyric; U1, with urea; U0, without urea. P: Statistical significance; SEM, standard error of means; **, P<0.01; * , P<0.05; NS, nonsignificant.

might have resulted in lower IVNDFD. The inclusion of starch in high quantity or starch rich concentrate, in mixed diet reduce cell wall degradation. Gylswyk and Schwartz (1984) also reported that the addition of starch increases the lag time in the degradation of grass fibre and those strains of *B. succinogenes*, which can use both starch and cellulose, preferentially digested the starch. As the fermentation progressed and the availability of readily soluble/fermentable components of substrate (mainly starch) was exhausted, the more complex nutrients and structural components, i.e., cell wall etc. was fermented during 24 to 48 h of incubation and as a result higher cell wall digestibility at 48 h on starch + urea substrate was observed. The overall improvement in cell wall digestibility on inclusion of BCFA exhibited the pattern similar to that reported elsewhere (Gorosito *et al*. 1985) with various types of substrates.

Volatile fatty acid concentration: Concentration of acetic acid was increased (P<0.01) due to addition of Na-salts of BCFA @ 0.75 and 1.50% in the *in vitro* medium (Table 3) at 24 h post incubation, whereas no effect was observed on butyric and propionic acid concentration. The TVFA concentration was also increased on BCFA addition at 24 (P<0.05) and 48 h (P<0.01) post incubation. Efficacy of two doses of BCFA was similar. Acetic and TVFA concentrations in *in vitro* medium at 24 and 48 h post incubation were increased (P<0.01) with increasing protein levels of the substrate (Table 4). Increased concentration of acetic acid (P<0.05) and TVFA (P<0.01) at 24 and 48 h post incubation were associated with the substrate incubated with Na-salts of IB (Table 4) compared to the other two BCFA (IV and 2-MB). Substrate containing urea showed greater (P<0.01) concentrations of acetic acid and TVFA in the in vitro medium at 24 and 48 h post incubation. The increased acetate and TVFA concentrations on BCFA inclusion substantiated the fact that addition of these growth factors improved the microbial fermentation and digestibility. The amount and profile of VFA is governed by the type of feed and microbial metabolism. Higher celluloytic activity as a consequence of greater NDF digestibility elevated the levels of acetate in the *in vitro* medium. Addition of urea in the substrate exhibited stimulatory effect on fermentation characteristics.

Inclusion of BCFA in the substrate @ 0.75 and 1.50%, increased the DM, OM and cell wall digestibility *in vitro*. 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% protein level. Among the 3 BCFA, the isobutyric acid was found to be the most effective in improving the *in vitro* digestion at 24 h, whereas at later stage of fermentation i.e., 48 h, all the 3 BCFA were equally effective. Addition of urea stimulated 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.

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