Effect of Dietary Supplementation of Sodium Salt of Isobutyric Acid on Ruminal Fermentation and Nutrient Utilization in a Wheat Straw Based Low Protein Diet Fed to Crossbred Cattle

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ABSTRACT : The effect of dietary supplementation of sodium salt of isobutyric acid in low protein (10% CP) wheat straw based diet on nutrient utilization and rumen fermentation was studied in ruminally fistulated male crossbred cattle. The study included a 7 day metabolism and a 3 day rumen fermentation trials. The cattle were distributed into two equal groups of 4 each. The animals of control group were fed a basal diet consisting of wheat straw, concentrate mixture and green maize fodder in 40:40:20 proportion whereas branched chain volatile fatty acid (BCFA) supplemented group received a basal diet + isobutyric acid at 0.75 percent of basal diet. The duration of study was 36 days. The feed intake between experimental groups did not differ significantly and the average total DMI (% BW) was 2.01 and 2.28 kg day^{-1} in control and BCFA supplemented diets. The dietary supplementation of BCFA improved ($p<0.05$) the DM, OM, NDF and cellulose digestibility by 4.46, 6.63, 10.57 and 11.31 per cent over those fed control diet. The total N retention on BCFA supplementation was improved ($p<0.01$) due to decreased ($p<0.05$) urinary N excretion. The concentrations of ruminal total N was 37.07 and 34.77 mg 100 ml^{-1} in control and BCFA fed groups, respectively. Dietary supplementation BCFA significantly ($p<0.01$) reduced the ruminal ammonia N concentration as compared to control and the mean values (mg 100 ml^{-1}) were 13.18 and 9.42 in control and BCFA fed groups. The total VFA concentration was higher ($p<0.01$) in BCFA supplemented group (101.14 mM) than the control (93.05 mM). Among the VFAs, the molar proportion of acetate was higher ($p<0.01$) in BCFA supplemented group (71.07 mM) as compared to control (64.98 mM). However, the concentration of propionate and butyrate remained unchanged. Amino acids composition of bacterial hydrolysates was similar in both the groups. Ruminal outflow rate of liquid digesta was higher ($p<0.01$) in BCFA fed group (67.56 l day^{-1}) than control (52.73 l day^{-1}). It is concluded that the dietary supplementation of Na-salt of isobutyric acid in low protein diet improved the nutrient utilization and ruminal fermentation characteristics. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 4 : 479-484*)

Key Words : Branched Chain Volatile Fatty Acid, Sodium Salt, Isobutyric Acid, Nutrient Utilization, Rumen Fermentation, Cattle

INTRODUCTION

The branched chain volatile fatty acids (BCFA) such as isobutyric (IB), 2-methyl butyric (2-MB) and isovaleric (IV) are considered as essential nutrients for many predominant rumen cellulolytic bacteria (Bryant, 1973) and either can be used to meet their requirements (Gorosito et al., 1985). Inclusion of mixture of BCFA in the diet resulted in improved microbial growth in in vitro (Russel and Sniffen, 1984), cellulose digestion (Gorosito et al., 1985), nitrogen utilization (Oltjen et al., 1971; Umunna et al., 1975), milk production (Papas et al., 1984) and feedlot performance of steers (Deetz et al., 1985). Under normal feeding conditions, particularly if the diet is sufficient in protein, the deficiency of BCFA is unlikely to occur and de novo synthesis of BCFA would meet microbial requirements. However, in spite of de novo synthesis, a BCFA deficiency is possible in ruminants fed low protein diets and such a deficiency would not be corrected by non-protein nitrogen (NPN) or urea supplementation (Umunna et al., 1975). In India, where animal production systems are solely based on crop residues with meager amounts of energy and protein supplements. Studies have shown that such feeding regimens lead to ruminal BCFA deficiency and their concentrations may reach even below the detectable limits (Krysl et al., 1989). The present experiment was conducted to determine the effect of dietary supplementation of the sodium salt of isobutyric acid in a low protein wheat straw based diet on ruminal fermentation and nutrient utilization in crossbred cattle.

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MATERIALS AND METHODS

Animals and diets

Eight healthy crossbred male growing cattle (age 2 to 2.5 years) fitted with permanent rumen cannula, were randomly distributed into two groups on the basis of body weight (BW) and housed in individual pens. The animals were vaccinated against common contagious diseases and routinely treated for ecto- and endo-parasitic infestations. Animals were fed a basal diet consisting of wheat straw (WS), a concentrate

mixture (maize grain, 65; ground nut cake, 26; wheat bran, 6; mineral mixture, 2; common salt, 1) and chopped green maize fodder (GMF) in a 40:40:20 (DM basis) proportion (control) and basal diet+IB at 0.75 percent of basal diet. The WS and concentrate mixture (CM) were offered at 900 h, while the GMF was offered at 1400 h. The sodium salt of IB (1.88 kg pure isobutyric acid, w/w 100 kg^{-1} DM of CM-II) was added at 0.75 per cent of total diet (on DM basis) in the form of aqueous solution in CM-II. All the diets were iso-nitrogenous at 10% CP. Animals were offered clean drinking water twice a day at 10:00 and 14:00 h.

Experimental procedure

Metabolism trial: After a 25 day preliminary period during which the assigned diets were fed, a 7 day metabolism trial was carried out in individual metabolism stalls with facility for quantitative collection of feces and urine. Samples of feed offered, residue left and feces and urine voided were collected daily and representative samples were collected for further analysis. Pooled samples were dried at 60℃ and ground for chemical analysis. A separate set of samples of feces and urine from the daily collection were preserved in dilute sulfuric acid for nitrogen (N) estimation. The BW of animals was recorded on two consecutive days at 14 d intervals as well as before and after the metabolism trial.

Rumen metabolism study

Feeding schedule and sampling of rumen liquor: A rumen metabolism study was carried out immediately after completion of the metabolism trial. All feed stuffs (WS, CM and GMF) and clean drinking water were offered two hours before sampling. Rumen liquor samples (50-60 ml) were collected for three consecutive days, from day 33 at 0, 2, 4, 6, 8, 10 and 12 h post-feeding through specially made perforated stainless steel probes covered with nylon cloth from four different sites in the rumen. For determination of rumen volume and outflow rate of liquid digesta polyethylene glycol (PEG-4000) solution was infused into the rumen of each animal on 36th day of feeding (Smith, 1959). The samples drawn from the rumen were acidified with 0.2 ml of 10 N H_2SO_4 and stored at -5℃ for later analysis.

Chemical analysis

The DM, N, and ash contents were determined according to AOAC (1984), while the neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the methods described by Goering and VanSoest (1970). The NDF in CM was estimated by the method of Robertson and Van Soest (1977) by using amylase enzyme (Sigma chemicals, USA). Rumen liquor samples were analyzed for total-N (AOAC, 1984), NH_3-N (Conway, 1962) and total volatile fatty acids (Bernett and Reid, 1957). Individual VFA was estimated as described by Ervin et al. (1961) using Gas Liquid Chromatography (Nucon Series-5500) fitted with flame ionization detector and a pair of stainless steel column (200 cm long and 0.20 cm diameter) packed with chromosorb 101 as stationary phase. The bacterial hydrolysates were prepared by centrifuging whole ruminal fluid to remove feed ingesta and protozoa (Cummins and Papas, 1985). The harvested bacterial pellets were analyzed for the various amino acids on HPLC system (Waters, Model 510) according to the Waters PICO-TAG amino acid analysis method (Millipore corporation, Milford, USA). Rumen fluid volume (Smith, 1959) and out flow rate of liquid digesta (Hyden, 1961) were determined by PEG-4000. Ruminal fluid passage rate was calculated by regressing the natural logarithm of PEG concentration against time after marker administration. Ruminal fluid volume was calculated by deviding PEG dose by ruminal PEG concentration extrapolation to 0 h.

Statistical analysis

Intake and digestibility data were analyzed by using student 't' test as described by Snedecor and Cochran (1980). Rumen fermentation variables were analyzed for treatment and sampling time as main effects and treatment by sampling time interaction using the following mathematical model in a two way analysis of variance procedure of SPSS Base 10.0 (SPSS Inc., Chicago, USA). These variables were also analyzed for linear, quadratic and cubic effects.

$$Y_{ijk}=(\mu+T_i+P_j+(TP)_{ij}+e_{ijk}$$

Where, μ=General mean, T_i=Effect of ith treatment, P_j=Effect of jth period, $(TP)_{ij}$=Interaction effect of ith treatment with j th period, e_{ijk}=Random error.

RESULTS

The chemical composition of different dietary components used in metabolism and rumen fermentation study is presented in table 1. The DM content of CM-II was comparatively lower than the CM-I. Both the concentrate mixtures were iso-nitrogenous. There were no differences in total DM intake (% BW and $g/kg^{0.75}$) between the two groups (Table 2). However, the DM intake through wheat straw was significantly ($p<0.05$) higher in BCFA supplemented group (2.84 kg day^{-1}) than control (2.21 kg day^{-1}). The DM intake through other two dietary components viz. concentrate mixture and green maize

was statistically similar.

The mean digestibility coefficients of DM, OM and fiber fractions are presented in table 2. The digestibility of DM, OM and fiber fractions (NDF, ADF, cellulose and hemicellulose) were higher ($p<0.05$) in BCFA supplemented group compared to control. The digestibility of CP and also the DCP intake were similar in both the groups. The intake of TDN in control and BCFA supplemented group was 2.78 and 3.42 kg day^{-1} respectively, and these differences are significant ($p<0.05$).

The N intake, N excretion in feces and urine are presented in table 3. There is no statistically significant difference between groups in N intake and N excreted through feces. However, losses of urinary N was greater ($p<0.05$) and per cent of absorbed nitrogen retained was lower ($p<0.01$) for cattle fed the control diet than those fed BCFA supplemented diet. The N retained g day^{-1} was significantly ($p<0.05$) higher in BCFA supplemented group (6.69) compared to control (1.17).

The values averaged across the sampling times of 0, 2, 4, 6, 8, and 12 h post-feeding for pH, total-N, NH_3-N and total VFA are presented in table 4. The mean values of ruminal pH and total-N were statistically similar in both experimental groups. However, the cattle supplemented with BCFA have lower ($p<0.01$) ruminal NH_3-N concentrations compared with control. Total VFA concentrations (mM) was significantly ($p<0.01$) higher in BCFA supplemented group (101.14) compared to control (93.05). The molar proportions of acetate and branched chain fatty acids (IB, 2-MB and IV) were higher ($p<0.05$) in BCFA supplemented cattle as compared with those fed control diet. No differences ($p<0.05$) were detected in molar proportions of propionate and butyrate. The ruminal fluid volume (l day^{-1} and % of BW) was statistically similar however, the passage rate of ruminal fluid was higher ($p<0.05$) in BCFA supplemented group compared to control.

Concentrations of total amino acids (TAA), essential (EAA), non-essential (NEAA) and branched chain (BCAA) amino acids in bacterial hydrolysates were not altered by BCFA, nor were the ratio of BCAA to TAA altered (table 5).

Table 1. Chemical composition (g kg^{-1} DM) of wheat straw, green maize fodder (GMF) and concentrate mixtures used in metabolic and rumen fermentation trial

g kg^{-1} DM	Wheat straw	GMF	Concentrate (Control)	Concentrate (BCFA)
Dry matter	911.0	209.8	924.5	918.0
Organic matter	874.3	860.4	910.0	898.9
Crude protein	35.0	87.5	179.4	175.0
NDF	830.0	680.0	220.0	215.0
ADF	505.0	360.0	65.0	60.0
Hemicellulose[1]	325.0	320.0	155.0	155.0
Cellulose	400.0	305.0	55.0	50.0
Acid detergent lignin	105.0	55.0	10.0	10.0

[1] NDF-ADF.

DISCUSSION

Nutrient intake and utilization

The lower DM content of CM-II may be attributed to addition of aqueous solution of Na- Salt of IB. The level of IB (0.75% of total diet) to be incorporated in whole diet was arrived at based on in vitro Studies (Misra, 1998). The level of IB amounts to 0.74 per cent (on DM basis) in BCFA fed group. A similarity in DM intake between both the groups indicated that dietary supplementation of BCFA had no effect on DM intake (Felix et al., 1980). The deviation in proportion of WS, CM and GMF in both the experimental diets i.e. control and BCFA (44:43:12 and 49:39:12), from stipulated proportion (40:40:20), occurred primarily due to variable intake of WS.

Dietary supplementation improved ($p<0.05$) the digestibility of DM (DMD) and OM (OMD). Increased DMD and OMD reflects the increased ($p<0.05$) utilization of fiber fractions (NDF, ADF, cellulose) of diets. The improvement in cell wall digestibility on BCFA supplementation observed in present experiment could be due to increased numbers and activity of cellulolytic microbes (Van Gylswyk, 1970). The higher

Table 2. Intake and digestibility of experimental diets used in metabolic and rumen fermentation trial

Attriutes	Control	BCFA	SEM[1]
Initial BW (kg)	251.25	250.88	15.54
Final BW (kg)	246.50	255.75	8.01
DM intake (kg day^{-1})			
Wheat straw*	2.21	2.84	0.33
Concentrate mixture	2.17	2.27	0.11
Green maize fodder	0.64	0.68	0.05
Total	5.01	5.79	0.68
DCP intake (g day^{-1})	271.40	284.56	23.23
TDN intake (kg day^{-1})**	2.78	3.42	0.27
Nutrient digestibility (%)			
Dry matter*	56.73	59.26	1.05
Organic matter**	54.79	60.55	1.23
Crude protein	54.91	54.04	1.34
Neutral detergent fiber*	45.85	50.70	1.34
Acid detergent fiber*	41.56	47.44	0.88
Hemicellulose*	50.26	57.75	2.17
Cellulose*	55.61	61.90	1.64

* $p<0.05$; ** $p<0.01$.

[1] Standard error of mean.

($p<0.01$) concentration of total VFA coupled with higher ($p<0.01$) proportion of acetate observed in this experiment further supports this view. Robinson and Sniffen (1983) also reported improvement in ruminal digestibility of NDF, ADF, hemicellulose and cellulose with BCFA supplementation.

There was a significant difference ($p<0.05$) between control and the BCFA fed group in N excretion in urine (42.25 vs 38.84) and N retention ($p<0.01$) (1.17 vs 38.84). Van Gylswyk (1970) and Oltjen et al. (1971) reported similar findings of decreased N excretion in urine with subsequent improvement in N balance. Some researchers have observed decreased rumen ammonia concentration and improvement in efficiency of N utilization (Felix et al., 1980). The reduction in urinary N excretion with subsequent improvement in body weight gains in control vs BCFA fed cattle in present experiment (-4.8 kg vs +4.9 kg during 37 days) suggest improved utilization of N and, consequently, more microbial synthesis of protein.

Rumen fermentation and diurnal variations

No significant effect on pH in spite of higher ($p<0.05$) total VFA concentrations due to BCFA supplementation was observed. This agrees with several reports on BCFA supplementation (Oltjen et al., 1971). The reason might be the inclusion of IB as Na-salt in CM-II. On hydrolysis of sodium isobutyrate, the release of Na molecule and subsequent attachment of H^+ ions at the place of Na molecule on the fatty acids moiety, in the medium might have resulted in reducing the preponderance of H^+ ions, responsible for pH depression. The average pH values recorded in this

Table 4. Influence of supplemental BCFA on ruminal metabolites

Particulars	Control	BCFA	SEM[1]
pH	6.74	6.73	0.08
N fractions (mg dl^{-1})			
Total-N	37.07	34.77	2.80
NH_3-N**	13.18	9.42	1.12
NH_3-N as % of total-N*	35.55	27.09	2.26
VFAs (mM)**	93.05	101.14	2.31
Acetate*	64.98	71.07	1.87
Propionate	18.98	19.18	1.59
Isobutyrate**	0.21	0.98	0.02
Butyrate	8.60	9.19	1.95
2-metyl butyrate*	0.10	0.42	0.02
Isovalerate*	0.18	0.30	0.02
Rumen fluid volume (l)	26.90	33.30	3.24
Rumen fluid volume as % of BW	10.23	12.92	3.54
Flow rate of liquid digesta (l day^{-1})*	52.73	67.56	5.14
Water intake (l day^{-1})*	14.17	22.67	2.88

* $p<0.05$; ** $p<0.01$.
[1] Standard error of mean.

Table 3. Nitrogen balance (g day^{-1}) on experimental diets

Particulars	Control	BCFA	SEM[1]
N intake	79.29	84.69	7.39
N excreted			
Feces	35.87	39.16	3.99
Urine*	42.25	38.84	1.38
Total	78.12	78.00	6.62
N retained**	1.17	6.69	1.34
Fecal N as % of N intake	45.24	46.34	3.73
Urinary N as % of N intake*	53.29	45.94	2.16
% retention of N intake	1.48	7.85	2.86
N absorbed as % of N intake	54.91	53.79	3.12
N retained as % of N absorbed*	2.69	14.62	4.69

* $p<0.05$; ** $p<0.01$.
[1] Standard error of mean.

Table 5. Influence of supplemental BCFA on amino acid composition (g 100 g amino $acid^{-1}$) of bacterial hydrolysates*

Particulars	Control	BCFA	SEM[1]
Essential amino acids	48.49	48.92	0.95
Non-essential amino acids	51.51	51.08	1.62
Branched chain amino acids (BCAA)	18.47	19.14	1.59
Total amino acids (TAA g 100 g $protein^{-1}$)	49.80	50.80	3.50
BCAA:TAA ratio	0.18	0.19	0.09
Amino acid composition (g 100 g $protein^{-1}$)			
Arginine	5.27	52.00	0.68
Histidine	1.91	2.54	0.25
Isoleucin	5.51	5.72	0.45
Leucin	8.10	8.57	2.01
Lysine	9.29	8.81	1.03
Methionine	2.03	2.11	0.68
Phenylalanine	4.32	4.36	0.49
Threonine	7.20	6.76	0.42
Tyrosine	4.36	4.22	0.51
Valine	4.86	4.85	0.72
Alanine	7.42	7.23	0.95
Cystine	10.91	10.80	0.94
Aspartic acid	1.80	1.81	0.39
Glutamic acid	13.41	13.37	0.69
Glycine	7.04	6.84	0.42
Proline	2.79	2.86	0.58
Serine	3.78	3.98	0.52

* Treatment comparison did not differ.
[1] Standard error of mean.

experiment were in the optimal range of fiber digestion (Mertens, 1979) and cellulolytic bacterial growth (Ørskov, 1982). The higher ($p<0.05$) total VFA concentrations on BCFA diet occurred mainly due to the differences observed in DM and fiber digestibility. Increased total VFA concentrations with BCFA supplementation were observed in several studies reviewed by Cook (1985). Sampling time and treatment interactions ($p<0.05$) were noted for molar proportion of acetic, isobutyric, 2-methyl butyric and iso-valeric acids. Concentrations of these acids increased with BCFA supplementation. The concentration of IB, 2-MB and IV at 0 h were not different ($p<0.05$) between both the treatments. Isobutyrate concentration increased linearly ($p<0.05$) with BCFA addition at 2, 4 and 6 h after feeding. However 2-MB and IV responded in a linear ($p<0.05$) fashion across treatment only at 2 and 4 h. In general effects of BCFA were maintained for less than 12 h with no apparent carry over effects. Consumption of wheat straw based diet (control) in this study, provided IB, 2-MB and IV levels (0.11, 0.10 and 0.13 mM) at the lower end of the optimal concentration suggested by Dehority et al. (1967) for cellulolytic bacteria.

The peak values for ruminal NH_3-N across the treatments, were noticed at 4 h post-feeding and thereafter, declined gradually to reach minimum. At no time did ruminal NH_3-N concentrations decline below 5 mg 100 ml^{-1} strained rumen liquor, the minimum concentration suggested by Satter and Roffler (1975). Both the treatment and sampling time had significant ($p<0.01$) effects on ruminal NH_3-N concentrations, however the interactions between treatment and interval were not significant ($p<0.05$). The cattle supplemented with BCFA, have a lower ($p<0.01$) ruminal NH_3-N concentrations compared to control. This agrees with the reports that BCFA addition increases N assimilation and reduces ruminal NH_3-N concentrations (Cline et al., 1966; Oltjen et al., 1971). The lower ruminal NH_3-N concentration and reduction in urinary N excretion with subsequent improvement in body weight gains in BCFA fed cattle in present study may be attributed to increased microbial protein synthesis in rumen with subsequent improvement in efficiency of N utilization. The amino acid composition of bacterial protein presented to host for post ruminal digestion was not altered by BCFA supplementation. This agrees with the findings of Cline et al. (1966) that the iso-VFA and valeric acid to the urea-starch purified diet did not change the amino acid pattern of the bacterial hydrolysates.

CONCLUSIONS

Sodium salt of isobutyric acid when incorporated at 0.75% of total diet in concentrate mixture for crossbred cattle did not affect the feed intake. The digestibility of major nutrients, except CP, was significantly increased by BCFA supplementation. The nitrogen retention was significantly ($p<0.01$) higher, whereas nitrogen excretion in urine was significantly ($p<0.05$) lower in BCFA fed animals compared to control. It is observed that BCFA addition at the 0.75% level in concentrate mixture alters the rumen fermentation beneficially, resulting in better nutrient utilization. Therefore it may be concluded that BCFA can be used as one of the feed additives in low protein straw based diets to manipulate rumen fermentation and nutrient utilization.

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