Influence of Isobutyric Acid Supplementation on Nutrient Intake, Its Utilization, Blood Metabolites and Growth Performance of Crossbred Calves Fed Wheat Straw Based Low Protein Diets

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ABSTRACT : The effects of dietary supplementation with the sodium salt of isobutyric acid in a low protein (10% CP) wheat straw based diet on nutrient utilization, blood metabolites and growth performance were studied with male crossbred calves. The calves were divided into two equal groups of 6 each. The animals of the control group were fed a basal diet consisting of wheat straw, concentrate mixture and green oat fodder in 40:40:20 proportion whereas BCFA supplemented group received the basal diet+isobutyric acid at 0.75 percent of basal diet. The duration of study was 120 days. The feed intake between experimental groups did not differ significantly and the average total DMI (% BW) was 1.99 and 1.95 kg day^{-1} in control and BCFA supplemented diets. The dietary supplementation of BCFA improved ($p<0.01$) the DM, OM, CP ($p<0.05$), NDF and cellulose digestibilities by 8.50, 9.01, 5.39, 17.78 and 18.44 per cent over those fed control diet. The total N retention on BCFA supplementation was improved ($p<0.01$) due to the decreased ($p<0.05$) faecal N excretion. The BCFA supplementation did not alter the blood circulatory levels of glucose, total protein, albumin, urea N and amino acids. However after 120 days of experimental feeding a significant ($p<0.05$) increase in the concentrations of non-esterified fatty acid was observed in control group. The DCP intake and the DCP content of experimental diets was similar in both groups. However, the TDN content of BCFA supplemented diet was significantly ($p<0.01$) higher (64.35%) than that of control (59.60%). The total live weight gain in BCFA supplemented diet increased by 15.94% over control. The average daily gain and efficiency of feed conversion were also improved in BCFA fed calves by 13.38 and 26.71% respectively, compared to control. It is concluded that dietary supplementation with isobutyric acid improved the digestibility of nutrients and growth performance of calves. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 2 : 200-205)*

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

INTRODUCTION

The branched chain volatile fatty acids (BCFA) isobutyric (IB), 2-methyl butyric (2-MB) and isovaleric (IV) are considered as essential nutrients for many predominant rumen cellulolytic bacteria (Bryant, 1973) and any of these can be used to meet their requirements (Gorosito et al., 1985). Inclusion of a mixture of BCFA in the diet resulted in improved microbial growth *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; Peirce-Sandner et al., 1985) 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 or urea supplementation (Umunna et al., 1975). In India, 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 (Judkins et al., 1987; 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 (WS) based diet on nutrient utilization and growth performance in crossbred calves.

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MATERIALS AND METHODS

Animals and diets

Twelve healthy crossbred male calves (age 7 to 13 months) were randomly divided 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 *ad libitum* a basal diet consisting of WS, concentrate mixture (maize grain, 65; ground nut cake, 26; wheat bran, 6; mineral mixture, 2; common salt, 1) and green oat fodder in a 40:40:20 (DM basis) proportion (control) and basal diet+IB at 0.75 percent of basal diet (BCFA). The WS and concentrate mixture (CM) were offered at 09:00, while the oat fodder was offered at 14:00. 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.

Experimental procedure

Metabolism trial

After a 60 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. Separate sets of samples of feces and urine from the daily collections were preserved in dilute sulfuric (40% v/v) acid for nitrogen (N) estimation. The BW of animals was recorded on two consecutive days at 15 d intervals as well as before and after the metabolism trial.

Chemical analysis of feeds, feces and urine

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 Van Soest (1970). The NDF in CM was estimated by the method of Robertson and Van Soest (1977) using amylase enzyme (Sigma chemicals, USA).

Blood metabolites

Blood samples were collected by jugular vein puncture in the morning hours before the meal, at initiation and at the end of the 120 days feeding period. Glucose was determined in whole blood (Somogyi, 1945), while urea-nitrogen (Varley et al., 1980), total protein, albumin (Oser, 1971) and non-esterified fatty acids (Shipe et al., 1980) were determined in blood plasma. Blood plasma samples were also analyzed for their amino acid content on an HPLC system (Waters, Model 510) according to the Waters PICO-TAG amino acid analysis method (Waters Chromatography division, Millipore corporation, Milford, USA).

Statistical analysis

Means for intake and digestibility were compared by using 't' test as described by Snedecor and Cochran (1989). Blood biochemical 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 software products, Marketing department, SPSS Inc., Chicago, IL 60606-6307, USA):

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

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

RESULTS

Chemical composition of dietary components

The chemical composition of the dietary components used is presented in table 1. The chemical

Table 1. Chemical composition (g kg^{-1} DM) of wheat straw (WS), oat fodder and concentrate mixtures (CM) used in metabolic trial

Particulars	WS	Oat	CM-I	CM-II
DM	89.48	14.87	91.93	91.66
OM	88.40	89.05	89.70	89.30
CP	3.940	7.66	18.10	18.06
NDF	79.00	61.00	23.00	23.00
ADF	48.00	32.00	7.00	6.50
Hemicellulose[1]	31.00	29.00	16.00	16.50
Cellulose[2]	39.00	29.00	6.00	5.50
Acid detergent lignin	9.00	3.00	1.00	1.00

[1] NDF-ADF; [2] ADF-ADL.

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

Attributes	Control	BCFA
DMI		
Total	3.02±0.20	2.96±0.32
Body weight (%)	1.99±0.16	1.95±0.18
g kg^{-1} $W^{0.75}$	70.23±5.25	68.35±6.38
Proportion of dietary components (%)		
Wheat straw	44.57±2.10	43.34±0.90
Concentrate mixture	40.53±0.95	41.40±0.85
Oat	14.90±1.12	15.26±0.75
DCP intake (g day^{-1})	217.90±6.55	226.94±2.49
DCP of ration (%)	7.19±0.13	7.51±0.30
TDN intake (kg day^{-1})	1.80±0.11	1.90±0.20
TDN of ration (%)**	59.60±0.49	64.35±0.45
Nutrient digestibility (%)		
Dry matter**	60.25±0.41	65.43±0.46
Organic matter**	62.99±0.55	68.71±0.49
Crude protein*	70.63±1.30	74.44±0.70
Neutral detergent fiber**	44.15±1.45	52.00±0.68
Acid detergent fiber**	39.44±1.16	48.78±0.58
Hemicellulose**	49.20±2.16	56.26±1.26
Cellulose**	55.41±0.89	65.63±0.94

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

composition of WS and oat fodder was within the range of normal values. Both the CMs were iso-nitrogenous at 18.1% CP.

Dry matter intake and digestibility of nutrients

There were no differences in total DM intake (% BW and g kg^{-1} $W^{0.75}$) between the two groups (table 2). The proportion of WS, oat fodder and CM in total diet was statistically similar in both the groups.

The mean digestibility coefficients of DM, OM and fiber fractions are presented in table 2. The digestibility of DM, OM, CP and fiber fractions (NDF, ADF, cellulose and hemicellulose) were higher ($p<0.01$) in the BCFA supplemented group compared to control. However the digestible crude protein (DCP) intake (g^{-1} day) and DCP content of rations (%) were similar in both the groups. The intake of TDN in control and BCFA supplemented groups was 1.80 and 1.90 kg day^{-1} respectively, and these differences are non-significant. However the TDN content of the BCFA supplemented diet was significantly ($p<0.01$) higher than that of control.

Nitrogen balance

The N intake, and N excretion in feces and urine are presented in table 3. There was no significant difference between groups in N intake and N excreted through urine. However, losses of faecal N were greater ($p<0.01$) and per cent of absorbed nitrogen retained was lower ($p<0.01$) for calves fed the control diet than those fed BCFA supplemented diet. The N retained was significantly ($p<0.05$) higher in the BCFA supplemented group than control (18.27 vs 15.11 g day^{-1}).

Blood metabolites

Concentrations of blood metabolites are presented in table 4. The concentrations of blood glucose, total protein (TP), albumin (AB) and urea N were similar in both the control and BCFA supplemented group. The concentration of NEFA was higher ($p<0.01$) in control than in BCFA supplemented group. Concentrations of the total amino acids (TAA), essential (EAA), non-essential (NEAA) and branched chain (BCAA) amino acids in blood plasma were not

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

Particulars	Control	BCFA
N intake	49.67 ± 2.40	48.70 ± 3.78
N excreted		
Feces**	14.80 ± 1.06	12.39 ± 1.63
Urine	19.75 ± 1.49	18.05 ± 1.42
Total*	34.56 ± 1.23	30.44 ± 1.85
N retained*	15.11 ± 1.26	18.27 ± 1.18
Faecal N as % of N intake*	29.80 ± 1.30	25.44 ± 0.71
Urinary N as % of N intake	39.76 ± 1.22	37.06 ± 2.01
% retention of N intake*	30.42 ± 0.83	37.50 ± 2.68
N absorbed as % of N intake*	70.63 ± 1.30	74.44 ± 0.71
N retained as % of N absorbed*	43.34 ± 1.07	50.32 ± 1.10

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

Table 4. Concentrations of blood metabolites for calves fed control and BCFA supplemented diets

Blood metabolites	Diets	0 day	after 120 days	Factorial analysis		
				T	P	T×P
Glucose (mg dl^{-1})	Control	69.11 ±4.15	68.33 ±3.04	NS	NS	NS
	BCFA	70.82 ±3.30	72.70 ±2.20			
Total protein (g dl^{-1})	Control	7.75 ±0.76	9.33 ±0.46	NS	NS	NS
	BCFA	6.46 ±0.57	9.48 ±0.59			
Albumin (g dl^{-1})	Control	3.43 ±0.15	3.27 ±0.13	NS	NS	NS
	BCFA	4.00 ±0.24	3.25 ±0.11			
Urea-N (mg dl^{-1})	Control	15.04 ±1.14	18.48 ±1.70	NS	NS	NS
	BCFA	16.35 ±0.60	19.82 ±1.04			
Non-esterified fatty acids (mML^{-1})	Control	0.117 ± 0.009	0.148 ± 0.013	*	NS	NS
	BCFA	0.107 ± 0.0006	0.114 ± 0.008			

T=Treatment; P=Period; * $p<0.05$; NS=Non-significant.

Table 5. Influence of supplemental BCFA on amino acid composition (g 100^{-1} g amino acid) of plasma[#]

Particulars	Control	BCFA
Essential amino acids	2.11±0.03	2.29±0.03
Non-essential amino acids	2.16±0.02	2.25±0.02
Branched chain amino acids (BCAA)	0.78±0.03	0.84±0.06
Total amino acids (g 100^{-1} g protein)	4.26±0.25	4.54±0.21
BCAA:TAA ratio	5.46±0.08	5.43±0.03
Amino acid composition		
Arginine	0.25±0.02	0.26±0.01
Histidine	0.11±0.01	0.13±0.02
Isoleucin	0.15±0.01	0.17±0.02
Leucin	0.35±0.02	0.37±0.02
Lysine	0.33±0.02	0.34±0.01
Methionine	0.16±0.01	0.18±0.01
Phenylalanine	0.18±0.01	0.20±0.03
Threonine	0.31±0.02	0.34±0.03
Tyrosine	0.18±0.01	0.21±0.01
Valine	0.29±0.01	0.30±0.02
Alanine	0.25±0.02	0.25±0.01
Cystine	0.16±0.01	0.16±0.01
Aspartic acid	0.37±0.02	0.37±0.02
Glutamic acid	0.50±0.02	0.50±0.01
Glycine	0.23±0.01	0.24±0.02
Proline	0.12±0.02	0.14±0.01
Serine	0.35±0.01	0.39±0.02

[#] Treatment comparison did not differ ($p<0.05$).

Table 6. Growth performance of calves fed BCFA supplementated diet (n=6; days=120)

Parameters	Control	BCFA
Initial body weight (kg)	116.83± 7.65	118.00± 7.18
Final body weight**	160.67± 3.48	168.83± 3.05
Total gain (kg)**	43.84± 1.14	50.83± 2.87
Average daily gain (g)**	373.75±28.46	423.75±27.09
Feed efficiency (%)**	12.50± 0.08	10.94± 0.50

** $p<0.01$.

altered by BCFA, nor was the ratio of BCAA to TAA altered (table 5).

Growth performance

Growth performance data of calves are presented in table 6. The total live weight gain and average daily growth rate (ADG) and feed conversion efficiency were significantly ($p<0.05$) higher in the BCFA supplemented group than in control.

DISCUSSION

Nutrient intake and utilization

The level of IB (0.75% of total diet) to be incorporated in whole diet was derived from earlier *in vitro* studies (Misra, 1998). The level of IB amounts to 0.78% (on DM basis) in the BCFA fed group. A similarity in DM intake between the two groups indicated that dietary supplementation with BCFA had no effect on DM intake (Felix et al., 1980b; Peirce-Sandner et al., 1985; Klusmeyer et al., 1987). The deviation in proportion of WS, CM and oat fodder in both the experimental diets, i.e. control and BCFA (45:41:14 and 44:41:15), from the stipulated proportion (40:40:20), occurred primarily due to variable intake of WS.

Dietary supplementation of with BCFA improved 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 the diets. The improvement in cell wall digestibility on BCFA supplementation observed in the present experiment could be due to increased numbers and activity of cellulolytic microbes (Van Gylswyk, 1970). Robinson and Sniffen (1983) and Hefner et al. (1985) also reported improvement in ruminal digestibility of NDF, ADF, hemicellulose and cellulose with BCFA supplementation.

There was a significant difference between control and the BCFA fed group in fecal N excretion (14.80 vs 12.39 g day^{-1}) and N retention ($p<0.01$) (15.11 vs 18.27 g day^{-1}). Van Gylswyk (1970) and Oltjen et al. (1971) reported similar findings of decreased N excretion with subsequent improvement in N balance. Some researchers have observed decreased rumen ammonia concentration and improvement in efficiency of N utilization (Felix et al., 1980a, b). The reduction in fecal N excretion with subsequent improvement in body weight gains in control vs BCFA fed calves in the present experiment (43.84 vs 50.83 kg during 120 days) suggests improved utilization of N and, consequently, more microbial synthesis of protein.

Blood metabolites

The mean concentrations of blood glucose and TP, AB and urea N in plasma samples obtained at initiation and after 120 days of feeding of BCFA supplemented diet were not different between treatments. The values of these blood metabolites were the in normal range of variation (Oser, 1971). Similarly the treatment by time interactions during the feeding trial sampling period also were not significant. The initial concentrations of NEFA were statistically similar between the two groups, but after 120 days of experimental feeding the calves fed the control diet a exhibited a significantly ($p<0.05$) higher NEFA concentration (0.148 mML^{-1}) than the BCFA supplemented group (0.114 mML^{-1}). This indicated that the animals receiving BCFA were on a good plane of nutrition because such animals, which are not under

negative energy balance or not poor utilizers of dietary ME, do not exhibit any change in plasma NEFA levels (Verman and Schultz, 1968). Several studies have shown that feed restriction or poor utilization of feeds results in an increased concentration of NEFA in an plasma of ruminants (Jackson et al., 1968; Bassett et al., 1971; Bauman et al., 1979), probably because of the increased mobilisation of fat from adipose tissues. However, Bartle et al. (1983) reported that energy levels in the diet did not effect the plasma NEFA levels of ruminants and concluded that NEFA have limited usefulness as indicators of energy status.

The concentrations of TAA, EAA, NEAA and BCAA were statistically not different between BCFA supplemented and control group. It appeared that supplementation with BCFA did not alter the concentration of their precursor amino acids (BCAA) in plasma. The variation in dietary supply had relatively little effect on plasma amino acid concentration because of microbial degradation of protein and resynthesis. The microbial protein is almost static in amino acid composition irrespective of dietary variations and unless the major proportion of dietary protein is bypassed to small intestine, when it can be absorbed without alteration, the greater alterations in plasma amino acid profile are unlikely to occur (Orskov, 1982).

Growth performance

The total gain in live weight in the BCFA supplemented group was increased by 15.9% over the control. Similarly, the average daily gain in BCFA supplemented calves was 13.4% higher ($p<0.01$) than in control (table 6). The positive influence of BCFA supplementation in present growth trial, is substantiated by several other investigations (Felix et al., 1980a; Deetz et al., 1985). The average DM consumption per kg live weight gain was lower ($p<0.01$) in the BCFA group than control. The efficiency of feed conversion was improved by 12.5% on BCFA supplementation over that of control. The BCFA fed calves gained 16% more live weight with 12.5% less feed DM consumption for each kg gain as compared to control. The improvement in feed conversion efficiency in calves fed BCFA supplemented diets may be due to improved digestibility of nutrients and nitrogen metabolism (Deetz et al., 1985).

CONCLUSION

The sodium salt of isobutyric acid when incorporated at 0.75% of total diet DM did not affect the feed intake of crossbred calves. The digestibility of major nutrients was increased significantly with BCFA supplementation. The nitrogen retention was significantly ($p<0.01$) higher, whereas nitrogen excretion in feces was significantly ($p<0.05$) lower in BCFA fed animals than in control. It was observed that BCFA addition at the 0.75% level in concentrate mixture alters the nutrient utilization beneficially, resulting in better growth. Therefore it may be concluded that BCFA can be used as one of the feed additives in low protein straw based diets to improve nutrient utilization and growth performance of crossbred calves.

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