Trop Anim Health Prod (2011) 43:209–214
DOI 10.1007/s11250-010-9676-1

ORIGINAL RESEARCH

Comparative nutrient utilization, growth, and rumen enzyme profile of mithun (*Bos frontalis*) and Tho-tho cattle (*Bos indicus*) fed on tree-leaves-based ration

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Accepted: 2 August 2010 / Published online: 13 August 2010

Abstract A study was conducted to compare the nutrient utilization, growth, and rumen enzyme profile of mithun (*Bos frontalis*) and Tho-tho cattle (*Bos indicus*) reared in the same feeding and managemental conditions. For the purpose, male mithun (n=8) and male Tho-tho cattle (n=8) of 1.5 years age, selected from the farm of National Research Centre on Mithun, Nagaland, India, were fed on mixed-tree-leaves-based ration as per the requirement of NRC (2001) for cattle for 12 months. Average daily gain (ADG), average dry matter intake (DMI), and feed conversion ratio (FCR) for all animals were recorded. A metabolic trial was conducted at 6 months of the experiment to assess the digestibility coefficient of different nutrients and nutritive value of ration. At 12 months of the experiment, rumen liquor was collected from all animals and analyzed for rumen enzyme profiles, viz., carboxymethylcellulase, xylanase, α-amylase, β-glucosidase, α-glucosidase, urease, and protease. It was found that ADG (507.8 g vs 392.8 g), DM intake (6.59 vs 5.85 kg/day) and DMI/$W^{0.75}$ (98.75 g vs 91.00 g/day), crude protein intake (780 vs 700 g/day), and total digestible nutrient intake (3.65 vs 3.32 kg/day) were higher (p<0.05) in mithun than cattle. The nitrogen balance was higher and FCR was better (p< 0.05) in mithun compared with cattle. The digestibility coefficient of different nutrients was similar (p>0.05) between the species. The microbial enzyme profiles of mithun and cattle were not different (p>0.05). The better growth performance of mithun than cattle as found in the present study clearly indicates that the mithun has higher genetic potential for growth than Tho-tho cattle of north-eastern hilly region of India.

Keywords Mithun · Tho-tho cattle · Growth · Digestibility · Enzyme profile

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Introduction

Mithun (*Bos frontalis*), an important massive ruminant of the north-eastern hilly region (NEHR) of India, is considered to be the domesticated form of wild gaur (*Bos gaurus*). This unique bovine species is believed to be domesticated more than 8,000 years ago and is mainly confined in the north-eastern hilly states of India and is also found, though in lesser number, in Myanmar, China, Bangladesh, and Bhutan. This animal is reared exclusively under free grazing condition. It thrives browsing on the jungle forages, tree fodders, shrubs, herbs, and other natural vegetations in natural condition. Sometimes, lopped branches of fodder trees are also offered to them by the villagers. Generally, farmers do not provide any additional feed except common salt, especially at the time of restraining for different purposes. The owner of the animals sometimes produces a sound out of the musical instrument made of mithun horn, and upon hearing it, mithun comes out from the jungle in search of common salt.

Mithun has lots of similarity with Tho-tho cattle (*Bos indicus*), which is also an inhabitant of the same geo-environmental condition. Recently, the Tho-tho has been recognized as a cattle breed of India (NBAGR, Karnal,

India). Both mithun and cattle thrive on similar types of feed and fodder depending on their availability in this region. Fat and protein content of milk of mithun and Tho-tho cattle are more than other cattle breeds of India (Mondal et al. 2001), and meat of both species is preferred by local tribes.

Many of the tree leaves and shrubs available in NEHR for feeding of mithun and cattle were identified and analyzed for chemical composition. *Lagerstroemia speciosa* is one of the important tree foliage having good nutritive value (8% DCP and 48% total digestible nutrient (TDN)) and is liked by both mithun and cattle under natural condition. Inclusion of *L. speciosa* as green foliage showed higher daily body weight gain, dry matter intake, and feed conversion ratio in growing mithun and cattle compared with straw-based ration. Similarly, *Ficus hirta* is very popular for feeding of cattle and mithun and remains green throughout the year. It has good palatability. *Quercus polystachya* contributes significantly as green fodder during the lean period for feeding of mithun and cattle. Das et al. (2010) used mixed tree leaves in growing male and female mithun and indicated that mithun can be reared in captivity on tree-leaves- and straw-based ration. However, no comparative information in terms of nutrient utilization, growth, and rumen enzyme profile were available after feeding these fodders to mithun as well as local cattle, and the work in this area may generate valuable information on two species of animals. Keeping view of the above fact in mind, a comparative study was conducted to find out the comparison on nutrient utilization, live weight gain, and rumen enzyme profiles of mithun and local cattle fed on tree-leaves-based ration.

Materials and methods

Experimental designs

The experiment was conducted in the mithun farm located at Medziphema, Nagaland. A total of eight male mithun (*B. frontalis*) and eight male Tho-tho cattle (*B. indicus*) aged 1.5 year were individually penned and fed on tree leaves/shrubs (*Q. polystachya*, *L. speciosa*, and *F. hirta*; chaffed and mixed incorporating equal parts on fresh basis) with rice straw (2:1 ratio on fresh basis) along with concentrate mixture as per the requirement of NRC (2001) for cattle with average daily gain of 500 g. The initial mean weights of the two species were 191.0±6.6 and 184.0±8.7 kg in mithun and cattle, respectively. The tree leaves/shrubs were collected daily from the forest, chaffed, and mixed in equal proportion before feeding to the animals. Approximately 50% of DM requirement was met by concentrate and rest by including roughage. The requirement was met by regulating the composition of concentrate mixture, and water was available at all times. The duration of the experiment was 1 year. The composition of concentrate mixture was maize 30 parts, wheat bran 15 parts, rice polish 20 parts, mustard cake 33 parts, mineral mixture one part, and salt one part. Before the start of the experiment, the animals were treated for external and internal parasite with Ivermectin®, and given vitamins A, D_3, and E.

Dry matter intake, nutrient utilization, and growth

The daily offered and residue of roughage was recorded, and DM percentage of each was estimated daily during the entire experimental period to find out the dry matter intake. Live weight of all animals was recorded at every fortnight interval before feeding and watering, and average daily gain was calculated. After 6 months of feeding experiment, a metabolic trial of 6 days' duration was conducted to assess the digestibility of nutrients, nutritive value of ration, level of intake, and balance of nitrogen. During this period, the animals were kept in the metabolic cages, and feces and urine were collected on 24 hourly basis. The representative samples of feces, urine, concentrate mixture, roughage offered, and roughage residue were taken to laboratory for proximate analysis and analyzed as per AOAC (1990). Condensed tannin content of roughage samples were analyzed according to method of Porter et al. (1986). Feed conversion ratio (FCR) of both mithun and cattle was calculated from average DM intake and average daily gain during the experimental period.

Rumen enzyme profile

The rumen liquor samples for enzyme estimation were collected at 3 h post-feeding after 1 year of feeding experiment. The samples in an ice bucket were transported to the laboratory immediately, and the strained rumen liquor was sonicated in ice bath for 5 min with a pulse rate of 30 s. It was followed by centrifugation at 27,000×*g* at 4°C for 30 min. The clear supernatant was used as a source of microbial enzymes estimation (Kamra and Agarwal 2003). For measuring the activities of carboxymethylcellulase and xylanase, the reaction mixture contained 1 ml phosphate buffer (0.1 M, pH 6.8), 0.5 ml enzyme, and 0.5 ml of either carboxymethyl cellulose (1.0%) or xylan (0.25%). The reaction mixture for α-amylase contained 0. 5 ml buffer, 0.25 ml starch (1%), and 0.25 ml enzyme. The reaction mixtures were incubated at 39°C for 60, 15, and 30 min, respectively, and the reducing sugars released were estimated. β-Glucosidase and α-glucosidase activities were estimated using *p*-nitrophenyl-β-D-glucopyranoside and *p*-nitrophenyl-α-D-glucopyranoside as substrate, respectively. Urease activity was measured by incubating the enzyme

sample (0.25 ml) with urea (0.25 ml of 10 mM) for 15 min, and released ammonia nitrogen was estimated. For protease, assay mixture containing l.5 ml buffer, 0.25 ml enzyme, and 0. 25 ml caesin (1%) was incubated for 2 h at 39°C, and the reaction was stopped by adding 20% trichloroacetic acid, and the protein thus hydrolyzed was measured as per the Lowry method.

Statistical analysis

The statistical analyses were done as per Snedecor and Cochran (1980) to determine mean, standard error, and *t* test was used to compare the difference in performance between mithun and cattle.

Results and discussion

Chemical composition of feed and fodder

The chemical composition of feed and fodder during metabolism trial is presented in Table 1. The crude protein content of *F. hirta*, *Q. polystachya*, and *L. speciosa* was 14.6%, 10.90%, and 12.32%, respectively, and crude protein content of mixed tree leaves was 12.6%, which is higher than many conventional fodders. Many works have been conducted on these tree leaves and shrubs, which are available in this region. The tree leaves and shrubs available in this region for feeding of mithun and cattle contain higher quantity of crude protein than cultivated fodder (Das et al. 2006; Prakash et al. 2008, 2009). As per general observations, tree leaves and shrubs are rich in condensed tannin. However, the condensed tannin content of the tree leaves and shrubs used in this experiment were within the acceptable limit of less than 5% for animals, and these data are in conformity with the previous report (Kumar and Vaithiyanathan 1990). The crude protein content of concentrate mixture and rice straw was estimated to be 17.2% and 3.4%, respectively, similar to the values in other regions of India. The crude fiber content of rice straw was found to be higher than that of the tree leaves as also reported by Pal et al. (2001).

Feed intake

The average dry matter intake during the whole experiment, dry matter intake per 100 kg body weight and per kilogram metabolic body weight ($W^{0.75}$) during metabolism trial, and average CP and TDN intake during metabolism trial of both the species of animals are presented in Table 2. There was increased intake of concentrate and roughage by mithun compared to cattle during the whole experiment that resulted in higher crude protein and energy intake ($p<0.05$). The dry matter intake per kilogram metabolic body weight ($DMI/W^{0.75}$) during metabolism trial was also higher than cattle. This showed that mithun had higher intake of nutrients than Tho-tho cattle, and higher intake of nutrient resulted to increased growth rate in this species of animal. When buffaloes were compared with cattle in many experiments, similar results of higher DM intake in buffaloes than cattle were recorded (Kennedy 1990; Ichinohe et al. 2004; Lapitan et al. 2008), and higher DM intake is responsible for higher growth rate of animals (Trach and Thom 2004; Lapitan et al. 2008). Paul et al. (2003) reported that DM intake was significantly lower in buffaloes than cattle. In one of the experiments of comparison between cattle and sheep/goat, higher $DMI/W^{0.75}$ in cattle than sheep or goat was observed (Reid et al. 1990). This showed that increased dry matter intake is one of the important factors for better growth or productive performance in animal, and higher growth rate of mithun than cattle in this present experiment was because of higher DM and nutrient intake. The FCR was better in mithun compared with cattle ($p<0.05$), showing higher efficiency of conversion of nutrients into body tissue in one species than other.

Table 1 Chemical composition of feed and fodder (% DM basis)

Items	Concentrate mixture	*Ficus hirta*	*Quercus polystachya*	*Lagerstroemia speciosa*	Rice straw
DM	89.2	23.6	38.1	36.8	87.3
OM	91.1	93.8	90.2	94.9	85.9
CP	17.2	14.6	10.9	12.3	3.4
EE	8.3	1.8	4.9	2.1	1.1
CF	9.7	18.3	26.9	26.5	33.8
NFE	55.8	59.1	47.5	54.0	47.7
TA	8.9	6.2	9.8	5.0	14.1
CT	-	2.1	3.9	2.9	0.4

DM dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *CF* crude fiber, *NFE* nitrogen-free extract, *TA* total ash, *CT* condensed tannin

Table 2 Feed intake of mithun and Tho-tho cattle

Items	Mithun (n=08)		Tho-tho cattle (n=08)	
	Mean	SE	Mean	SE
Concentrate intake (kg/day)	3.16 a	0.01	2.92 b	0.08
Roughage intake (kg/day)	3.42 a	0.03	2.93 b	0.15
Total DM intake (kg/day)	6.59 a	0.05	5.85 b	0.20
DMI/100 kg b. wt (kg/day)	2.39	0.05	2.26	0.11
DMI/W 0.75 (g/day)	98.75 a	2.06	91.00 b	1.47
FCR	13.14 a	0.82	15.06 b	0.32
CPI (kg/day)	0.78 a	0.01	0.70 b	0. 02
TDNI (kg/day)	3.65 a	0.03	3.32 b	0.09

Similar lowercase letters within each row did not differ (p<0.05)

Digestibility of nutrients

The digestibility of different nutrients of mithun and Tho-tho cattle is presented in Table 3. Digestibility trial conducted after 6 months of feeding experiment showed no statistical difference in the digestibility of different nutrients. This implied that the rumen microbial population in mithun and Tho-tho cattle may be the same as both were given the same type of diet and were from same geo-climatic conditions. Reid et al. (1990) reported that the digestibility of crossbred cattle and crossbred water buffalo were comparable, but the average daily gains were significantly higher in the crossbred buffalo than the crossbred cattle. Literature supports the view that there is increased growth in one species than the other without improving the digestibility (Reid et al. 1990; Lapitan et al. 2008). One of the probable cause is the reduction of digestibility of nutrients as a proportion of intake, when feed intake is increased. In one of the experiments in the author's laboratory, when mithun was compared with cross bred cattle (*B. indicus*×*Bos taurus*), it was found that digestibility of nutrients was more in mithun than crossbred cattle and also the DM intake in mithun was less (unpublished data). Many findings indicated that buffalo rumen microbes have a greater fibrolytic activity than those of cattle when fed a highly fibrous diet (Wanapat et al. 2000; Ichinohe et al. 2004). Paul et al. (2003) reported that the nutrient utilization efficiency of buffaloes was higher than cattle. In some of the experiments, there was reduction in digestibility in buffaloes than cattle fed on different types of diet (Kennedy et al. 1992; Puppo et al. 2002). These results among different studies may be mainly due to difference in species/breed of animals, feed intake level between the various species, and characteristic of the diets.

Table 3 Digestibility of mithun and Tho-tho cattle

Items	Mithun (n=06)		Tho-tho cattle (n=06)	
	Mean	SE	Mean	SE
DM	59.2	1.4	59.9	0.6
CP	60.6	1.2	61.6	1.3
CF	58.9	2.7	59.4	3.3
NFE	55.6	1.6	56.3	1.4
EE	78.4	1.8	80.8	2.1
NDF	52.0	1.6	53.7	1.6
ADF	43.1	3.3	43.2	3.8

Live weight gain and nutritive value of ration

The live weight gain and nutritive value of mithun and cattle is presented in Table 4. After the feeding experiment of 1 year, mithun attained average daily gain (ADG) of 507.8 g, whereas for cattle, it was 392.8 g/day, and the difference was statistically significant. Prakash et al. (2005) and Das et al. (2010) used mixed tree leaves in the ration of mithun and reported essentially similar findings to that of the present study. The growth rate of Tho-tho cattle has not been reported so far, but the animal is comparatively smaller than mithun. The higher growth rate in mithun than

Table 4 Live weight gain and nutritive value of ration in mithun and Tho-tho cattle

Items	Mithun (n=08)		Tho-tho cattle (n=08)	
	Mean	SE	Mean	SE
Initial body weight (kg)	191	6.6	184	8.7
Final body weight (kg)	376 a	13.0	327 b	14.8
Total gain (kg)	185 a	11.9	143 b	7.9
Average daily gain (g)	508 a	32.6	393 b	22.0
CP%	11.7	0.41	11.9	0.68
DCP%	7.1	0.38	7.4	0.64
TDN%	55.4	0.72	56.7	0.74

Different lowercase letters within each row differ significantly (p<0.05)

Table 5 Nitrogen balance of mithun and Tho-tho cattle

Items	Mithun (n=06)		Tho-tho cattle (n=06)	
	Mean	SE	Mean	SE
Nitrogen intake (g/day)	123.0	3.69	113.0	3.29
Fecal nitrogen (g/day)	51.75	2.65	47.25	3.56
Urinary nitrogen (g/day)	34.00	4.26	32.50	3.94
Nitrogen balance (g/day)	37.25 a	1.38	32.75 b	1.11
Nitrogen balance (g/$W^{0.75}$)	0.551 a	0.02	0.502 b	0.01

Different lowercase letters within each row differ (p<0.05)

in cattle in the present study may be due to the increased intake of concentrate and roughage by mithun compared with cattle, which resulted to higher crude protein and energy intake. Similarly, Lapitan et al. (2008) reported higher growth rate in buffaloes compared with cattle, and this higher growth rate was because of increased DM intake. Nutritive values of the ration during the digestibility trial indicated that both the species were meeting the requirement as per the NRC (2001) for cattle. Increased growth rate in mithun without increasing the digestibility of nutrients may explain the better availability of nutrients to the body tissue. It also indicated that the mithun has higher genetic potential for growth than native cattle, Tho-tho.

Nitrogen balance

The nitrogen balance of mithun and cattle during the experiment is presented in Table 5. The results indicated that both the mithun and cattle were in positive nitrogen balance. However, when mithun and cattle were compared, there was higher nitrogen balance (p<0.05) in mithun than cattle, resulting in higher live weight gain. Nitrogen balance is the indicator of growth status of animals, and higher growth rate in mithun than cattle in this experiment was because of higher nitrogen balance. Nha et al. (2008) reported higher nitrogen balance in buffaloes than cattle, and this was also reflected in higher live weight gain.

Rumen enzyme profiles

The rumen enzyme profiles of mithun and cattle are presented in Table 6. There was no significant difference in the rumen enzymatic profiles like carboxymethyl cellulase, xylanase, α-amylase, α-glucosidase, β-glucosidase, β-xylosidase, urease, and protease between mithun and cattle. The probable reason of same enzyme activity in these two species may be due to comparable microbial population in the rumen of both mithun and cattle fed similar type of diet and also reared in the same geographical conditions. This was again supported by the fact that the digestibilities of different nutrients were comparable between two species of animals. Some workers reported higher cellulose digestibility in buffaloes than cattle (Wanapat et al. 2000; Ichinohe et al. 2004), whereas others did not observe any difference (Lapitan et al. 2008). In this experiment of comparison between mithun and cattle, no such difference was noticed in the rumen liquor. As per the observations, it may be concluded that there may be higher metabolism of nutrients or higher bypass protein in mithun than cattle, which may be possible for better growth performance. The result of nitrogen balance study in this experiment also supports the hypothesis.

Conclusion

The present experiment on comparative nutrient utilization, growth, and rumen enzyme profile of mithun (*B. frontalis*)

Table 6 Rumen enzyme profile of mithun and Tho-tho cattle

Items	Mithun (n=06)		Tho-tho cattle (n=06)	
	Mean	SE	Mean	SE
Carboxymethyl cellulase (μmol glucose h^{-1} ml^{-1})	20.30	0.67	21.81	0.96
Xylanase (μmol xylose h^{-1} ml^{-1})	4. 43	0.38	4.86	0.73
Amylase (μmol glucose min^{-1} ml^{-1})	0.64	0.04	0.60	0.03
Alpha glucosidase (mol *p*-nitrophenol min^{-1} ml^{-1})	0.05	0.004	0.04	0.005
β-Glucosidase (mol *p*-nitrophenol min^{-1} ml^{-1})	0.20	0.02	0.15	0.03
Protease (μg hydrolysed protein h^{-1} ml^{-1})	647	47	661	45
Urease (μmol NH_3–N min^{-1} ml^{-1})	0.12	0.01	0.13	0.02

and Tho-tho cattle (*B. indicus*) fed on tree-leaves-based ration indicated that the growth rate and DM intake of mithun was higher than cattle. The FCR was better in mithun than cattle. There was no difference in the digestibility of different nutrients, although nitrogen balance was higher in mithun than cattle. The rumen enzymatic profiles of mithun and native cattle were also comparable.

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