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Comparison of Gayal (*Bos frontalis*) and Yunnan Yellow Cattle (*Bos taurus*): Rumen Function, Digestibilities and Nitrogen Balance during Feeding of Pelleted Lucerne (*Medicago sativum*)

Weidong Deng[1,3], Liping Wang[1], Songcheng Ma[1], Bo Jin[1], Tianbao He[2], Zhifang Yang[2] Huaming Mao[1,2] and Metha Wanapat[3,*]

[1]Yunnan Provincial Laboratory of Animal Nutrition and Feed Science, Faculty of Animal Science Yunnan Agricultural University, Kunming 650201, P. R. China

ABSTRACT : Three male Gayal (*Bos frontalis*) and three male Yunnan Yellow cattle (*Bos taurus*) were fed pelleted lucerne and measurements made of digestibility, nitrogen utilisation, rumen fermentation and microbial population and key plasma metabolites. Total actual dry matter intake was similar but when expressed in terms of live weight or metabolic live weight feed intakes were significantly higher ($p<0.05$) for Gayal than cattle. Apparent digestibilities of dry matter, organic matter, fibre and dietary nitrogen were similar for both Gayal and cattle. Rumen ammonia nitrogen and total volatile fatty acids were significantly higher ($p<0.05$) for Gayal than cattle and total numbers of viable rumen bacteria, cellulolytic and amylolytic bacteria, but not proteolytic bacteria nor protozoa, were significantly greater ($p<0.05$) for Gayal than cattle. Although Gayal have a different rumen ecology to cattle, similar digestive parameters were exhibited. Further research is required to establish relationship between rumen ecology and digestive parameters. (**Key Words :** Gayal, Cattle, Nutrient Digestibilities, Rumen Ecology)

INTRODUCTION

The Gayal or Mithun (*Bos frontalis*) is a rare semi-wild bovine species distributed throughout Bangladesh, Bhutan, China, India, Malaysia and Myanmar (Mondal et al., 2004; Rajkhowa et al., 2006). In China, Gayal are found predominantly in the narrow valleys of the Dulong and Nujiang Rivers and adjacent mountainous areas of Yunnan Province where they are described as 'Dulong cattle' (Chi et al., 2005; Mao et al., 2005). Whilst related closely to domesticated cattle (*Bos taurus*) and bison (*Bison bison*), which have a chromosome complement of 2n = 60, and Gaur (*Bos gaurus*), which has a chromosome complement of 2n = 56, Gayal have a chromosome complement of 2n = 58 (Bhambhani and Kuspira, 1969; Gallagher and Womack, 1992; Chi et al., 2005).

Species of ruminants differ in their capacities to digest forages, in particular low quality roughages. For example, it has been found that bison or water buffalo (*Bubalus bubalis*) utilise low quality forages more efficiently than cattle (Hawley et al., 1981; Wanapat et al., 1994). In the case of Gayal which are found in steep mountainous areas where they browse tree leaves and graze grasses including bamboo as well as reeds and other plant species, the animals thrive in adverse environments (Huque et al., 2001a, 2001b) attaining mature live weights which are greater than those of cattle maintained in similar environments (Cheng, 1984; Giasuddin and Islam, 2003; Mao et al., 2005). Gayal also demonstrate good beef traits (Giasuddin et al., 2003) and better meat quality than native yellow cattle (Ge et al., 1996).

To date there is a paucity of information on the digestive physiology of Gayal. The present study was conducted to compare digestion of nutrients and rumen characteristics of Gayal and domestic cattle fed a diet consisting of pelleted lucerne.

MATERIALS AND METHODS

Animals and diet

Three male Gayal (*Bos frontalis*), two years of age with

* Corresponding Author: Metha Wanapat. Tel: +66-43-202368, Fax: +66-43-202368, E-mail: metha@kku.ac.th
[2] Nujiang District Animal Science and Veterinary Bureau, Liuku 673100, P. R. China.
[3] Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand.
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Table 1. Chemical composition of the pelleted diet (% DM basis)

Items	Pelleted lucerne
Dry matter (DM)	90.80
Organic matter (OM)	89.70
Crude protein (CP)	13.67
Crude fiber (CF)	35.47
Ash	10.30
Neutral detergent fiber (NDF)	52.82
Acid detergent fiber (ADF)	44.10
Acid detergent lignin (ADL)	8.83
Total digestible nutrients (TDN)	55.90

mean live weight of 203±26 kg, and three adult male Yunnan Yellow Cattle (*Bos taurus*), with mean live weight of 338±18 kg, were selected for the study. Prior to commencement of the study each animal was given a broad spectrum anthelmintic (Fenbendazole, Shaanxi Hanjiang Pharmaceutical Group Co., Ltd. Hanzhong, China) to control internal parasites. They were confined to metabolism cages within an enclosed area lit by natural light. Pelleted lucerne (*Medicago sativa*) was offered to each animal in two equal portions at 08:00 and 18:00 h daily and orts were recorded daily. The average chemical composition of the diet is shown in Table 1. Water was freely available.

Experimental procedures

In view of the report by Dong et al. (2006) that yak (*Bos grunniens*) took more than two weeks to adapt to a diet high in crude protein (CP), the animals in the present experiment were allowed 20 days to adjust to the diet (days 1-20). Feed intake was measured each day from the commencement of the adjustment period and measurements of digestibility and nitrogen balance were made over five days (days 21-25) after the adjustment period. Samples of blood and rumen fluid were collected on day 25.

Rumen fluid was collected via a stomach tube immediately before and then six hours after fresh feed was offered. Approximately 500 ml of rumen fluid were collected at each sample time from the mid rumen. The first 100 ml collected was discarded and then the pH of the rumen fluid was measured immediately after collection using a pH meter (pHS-3C, Redox, China). The fluid was filtered through four layers of cheese cloth and divided into four portions which were then used to measure ammonia nitrogen (NH_3-N), volatile fatty acids (VFA), numbers of protozoa, and numbers and types of bacteria. Blood samples (5 ml) were collected from the middle auricular vein immediately before and six hours after offering fresh feed. The animals were physically restrained in a crush to facilitate collection of the samples. Immediately after collection blood samples were centrifuged at 3,000 rpm for 10 minutes to prepare plasma which was stored at -20°C pending analyses.

Analyses

Digestibility and nitrogen balance : Between days 21-25 total feed intake and outputs of faeces and urine were measured each day before offering fresh feed. Representative samples of faeces (12.5% by weight) were collected and divided into two equal portions. One portion was acidified by adding 50% sulphuric acid (H_2SO_4) according to the ratio of 5:100 (volume of 50% sulphuric acid: sample weight) then stored at -20°C pending analysis for nitrogen (N) by the Kjeldahl method (AOAC, 1990). The other portion of faeces, together with representative portions of the daily feed offered and orts, were dried at 60°C for 72 h then ground prior to analysing for dry matter (DM), ash and crude protein (CP as N×6.25) according to the methods of the Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) in feed, orts and faeces were measured as described by Goering and Van Soest (1970). NDF was analysed with neutral detergent solution devoid of α-amylase and expressed with residual ash. The ADF also was expressed with residual ash. Urine was collected into plastic trays containing sufficient 6 M HCl to maintain pH below 3.0. An aliquot (5% by volume) of the total daily urine was pooled for each animal and kept at -20°C pending analyses for N by the Kjeldahl method (AOAC, 1990).

Rumen ammonia nitrogen : The aliquot of rumen fluid collected for measurement of NH_3-N was acidified by adding 5 ml 1 M H_2SO_4 to 50 ml of rumen fluid then centrifuging at 16,000 g for 15 minutes. The supernatant was stored at -20°C pending analysis for NH_3-N by the colorimetric method described by Broderick and Kang (1980).

Volatile fatty acids : The portion of rumen fluid (40 ml) obtained for measurement of VFA was deproteinised by addition of 10 ml metaphosphoric acid (20%, v:v) and then centrifuging at 16,000 g for 15 minutes. The supernatant was stored at -20°C pending determination of VFA using a gas liquid chromatograph (Hewlett-Packard, Model 5890; Avondale, PA, USA) fitted with a flame ionisation detector. The injector, column and detector were maintained at 240, 110 and 260°C respectively and the carrier gas (N_2) flow was 50 ml/minute.

Rumen protozoa : Numbers of protozoa in rumen fluid were determined by first mixing 1ml of filtered rumen fluid with 9 ml of formalin (10% v:v in normal saline) as described by Galyean (1989). The protozoa were counted directly using a haemocytometer and a digital light microscope (Model DA1-180M, Xinzhi, China) connected to a computer, following essentially the procedure described by Galyean (1989).

Rumen bacteria : The numbers of total viable bacteria as well as cellulolytic, amylolytic and proteolytic bacteria were measured by the roll tube technique described by

Table 2. Nutrient intakes and, digestibilities and nitrogen balance in experimental animals

Items	Species		SEM	p-value
	Gayal	Cattle		
Intake				
DM (kg/d)	7.4	6.7	0.22	0.135
g/kg liveweight$^{0.75}$	137.8	85.6	13.00	0.004
% live weight	3.66	2.00	0.42	0.003
OM (kg/d)	6.7	6.1	0.18	0.147
NDF (kg/d)	3.5	3.3	0.08	0.149
ADF (kg/d)	2.9	2.6	0.08	0.055
Apparent digestibilities (%)				
DM	61.99	61.33	0.51	0.534
OM	66.57	67.10	0.64	0.687
NDF	51.80	54.12	1.27	0.374
ADF	50.33	52.51	1.05	0.317
Intake of digestible nutrients (kg/d)				
DM	4.6	4.1	0.13	0.052
OM	4.4	4.0	0.09	0.047
NDF	1.8	1.8	0.01	0.228
ADF	1.5	1.4	0.02	0.111
Nitrogen balance				
N intake (g/d)	165	159	5.70	0.663
Faecal N (g/d)	53	43	3.00	0.068
N digestibility (%)	67.47	73.09	2.19	0.256
Urinary N (g/d)	45	53	4.28	0.395
N retention (g/d)	67	62	4.48	0.685
% of N intake	40.53	39.55	1.81	0.832
% of N digested	60.07	54.12	2.53	0.528

SEM: Standard error of the mean.

Hungate (1969).

Plasma metabolites : The concentrations of plasma urea nitrogen and glucose were measured using commercial kits (Zhongsheng Company Ltd, China) and an auto analyser (Technicon RA-500TM Analyser: Bayer Corporation, Tarrytown, NY, USA).

Statistical analyses

The significance of differences between values for the parameters measured for the two groups of animals were assessed using t-tests (Steele and Torrie, 1980) and the statistical package of the Statistical Analysis System Institute (SAS, 1989).

RESULTS

Feed intake and digestibility of nutrients

Data for feed intake and digestibility of nutrients are presented in Table 2. Whilst actual intake of dry matter tended to be higher for Gayal than cattle, when expressed on the basis of live weight or metabolic live weight the intake of dry matter by Gayal was significantly greater ($p<0.01$) than that of cattle. Intake of ADF and digestible DM tended ($p<0.10$) to be higher for Gayal than cattle. Moreover, intake of digestible OM was significantly greater ($p<0.05$) for Gayal than cattle.

No significant differences ($p>0.10$) were found for total organic matter intake, intakes of digestible NDF and ADF nor apparent digestibilities of DM, OM, NDF and ADF.

Nitrogen balance

The data for N balance are also presented in Table 2. The only difference measured was for faecal N which tended ($p<0.10$) to be higher for Gayal than cattle. Intake of N, digestibility of N, urinary N and retention of N were similar for both groups of animals.

Rumen fluid constituents

The concentrations of NH_3-N before and six hours after offering fresh feed and the concentration of total VFA six hours after offering fresh feed were higher ($p<0.05$) for Gayal than cattle. The proportion of iso-valerate in rumen fluid was significantly lower ($p<0.05$) before offering fresh feed, the proportion of propionate in rumen fluid six hours after offering fresh food tended to be higher ($p<0.10$) and the proportion of valerate before feeding tended ($p<0.10$) to be lower before feeding in Gayal than cattle. Differences for rumen pH, other individual VFA and the ratios of acetate: propionate and acetate+butyrate+iso-butyrate: propionate did not differ significantly ($p>0.10$) between Gayal and cattle (Table 3).

Rumen microbes

The number of total viable bacteria ($p<0.05$) as well as cellulolytic ($p<0.001$) and amylolytic ($p<0.001$) bacteria in rumen liquor were significantly higher for Gayal than cattle before and six hours after offering fresh feed. No significant differences ($p>0.10$) between Gayal and cattle were measured for the numbers of proteolytic bacteria or protozoa in rumen liquor either before or six hours after offering fresh feed (Table 4).

Plasma metabolites

Plasma concentrations of urea were significantly higher ($p<0.05$) before (5.36±0.40 mM vs. 3.99±0.01 mM) and six hours after feeding (6.33±0.39 mM vs. 4.35±0.14 mM) for Gayal than cattle. Whereas there was a trend for the concentration of plasma glucose to be higher ($p<0.10$) before feeding for Gayal than cattle (4.65±0.07 mM vs. 4.20±0.01 mM), by six hours after feeding plasma concentrations were not significantly different (5.05±0.35 mM vs. 4.57±0.15 mM; $p>0.10$).

DISCUSSION

Feed intake and digestibility

Intake of DM of the Gayal tended to be higher than for the cattle and when expressed in terms of live weight or

Table 3. Metabolites in rumen fluid of Gayal and cattle

Items	Species		SEM	p-value
	Gayal	Cattle		
Ruminal pH				
0 h (post-feeding)	6.72	6.99	0.14	0.446
6	6.66	6.80	0.03	0.307
NH_3-N (mg/dl)				
0 h (post-feeding)	7.97	6.06	0.53	0.049
6	12.12	10.74	0.36	0.038
TVFA (mmol/L)				
0 h (post-feeding)	65.33	49.80	4.15	0.185
6	86.34	62.53	5.98	0.005
Acetate (C2) (mol/100 mol)				
0 h (post-feeding)	75.65	70.25	1.82	0.164
6	73.66	73.07	1.29	0.858
Propionate (C3, mol/100 mol)				
0 h (post-feeding)	15.83	15.63	0.56	0.887
6	17.80	15.00	0.85	0.094
Butyrate (C4, mol/100 mol)				
0 h (post-feeding)	5.96	7.37	0.47	0.158
6	5.95	8.26	0.91	0.263
Iso-butyrate (C4, mol/100 mol)				
0 h (post-feeding)	1.47	2.16	0.36	0.428
6	1.05	1.28	0.07	0.125
Valerate (C5, mol/100 mol)				
0 h (post-feeding)	0.50	2.02	0.44	0.069
6	1.03	1.28	0.12	0.361
Iso-valerate (C5, mol/100 mol)				
0 h (post-feeding)	0.58	2.57	0.53	0.025
6	0.52	1.11	0.16	0.040
C2:C3 ratio				
0 h (post-feeding)	4.79	4.56	0.21	0.703
6	4.15	4.93	0.30	0.242
(C2+C4):C3 ratio				
0 h (post-feeding)	5.27	5.16	0.65	0.873
6	4.54	5.56	0.33	0.136

SEM: Standard error of the mean.

metabolic live weight intakes of DM were significantly higher for the Gayal compared to cattle. These differences were reflected in the intakes of ADF, digestible DM and OM each of which was increased in Gayal compared to cattle (Table 2).

It might be argued that the differences in intakes of DM and nutrients generally were higher because of the differences in metabolic activity stemming from the differences in maturity of the Gayal and the cattle. The Gayal were younger and could be expected to be at a different stage of growth and thus nutrient use than the cattle. In this connection, it has been reported that in younger humans with relatively leaner bodies the metabolic rate is higher than in older fatter individuals (Piers et al., 1998). Moreover, the energy requirements for maintenance in swine are greater for leaner animals (Campbell and Taverner, 1988). Considering that Gayal studied in the present experiment were younger than the cattle, it might be expected that Gayal would be relatively leaner than the cattle. This is likely to explain, at least in part, the greater intake of feed by Gayal than cattle.

Notwithstanding the above, the present results are in broad agreement with prior observations that the feed intakes of Gayal are higher than for cattle fed similar diets. Intakes of high quality grass by cattle, 100-200 kg live weight have been reported to be 2.9% of live weight (ARC, 1980). By comparison, Huque et al. (2001b) reported that the intake of DM from poor quality roughage by Gayal amounted to 2.4% of live weight. Similarly, Pal et al. (2004) reported intake of DM by Gayal 1.5 years of age, with live weight of 208 kg and consuming jungle grasses (10.24% CP and 27.35% CF), to be 6.86 kg DM amounting to 2.98% of live weight and 115.92 g/kg liveweight 0.75. These observations are similar to the present results obtained when Gayal were fed a medium quality lucerne in pelleted form (Table 2). It is of interest to note that the greater intake of DM of poor/medium quality forage by Gayal compared to cattle, is consistent with the report of Vega et al. (2004)

that intake of DM by Brahman cattle (*Bos indicus*) was lower than that of water buffalo (*Bubalus bubalis*).

In extensive studies with cattle, bison and water buffalo, it has been found that digestibilities of low quality diets in bison or water buffalo exceed those for cattle (Richmond et al., 1977; Liang et al., 1994; Wanapat et al., 1994). In the present study, no differences were measured for digestibilities of DM, OM, NDF, ADF nor N (Table 2) between cattle and Gayal fed a medium quality lucerne diet. Similar results have been reported for bison and cattle fed lucerne hay containing 13.4% CP (Varel and Dehority, 1989). It is of interest to note that Richmond et al. (1977) reported that bison fed poor quality sedge and grass hay containing 7-8% CP demonstrated greater digestibility of the diet than cattle similarly fed, but when lucerne hay containing 19% CP was fed the digestibilities were similar in both cattle and bison. Similar observations have been recorded by others. Thus, Peden et al. (1974) and De Liberto and Urness (1993) reported that bison displayed greater digestibility of DM than cattle only when the diet contained less than 7% CP. Similarly, greater digestibilities of DM, CP, ADF and fat were measured for bison than cattle when animals were fed hay containing 6% CP (Hawley et al., 1981). They concluded that bison are more efficient than cattle in digesting poor quality diets with low CP. In contrast, when a diet containing grass and lucerne supplemented with grain was fed digestibilities of nutrients by bison were lower than those for cattle (Peters, 1958).

From the present results it is not possible to draw firm conclusions about the feed intakes and efficiency of digestion of ingested nutrients of Gayal compared to cattle. There appears to be a capacity for higher intake of DM by Gayal than cattle but differences in the digestibility of nutrients may not have been shown because the diet fed was of reasonable quality in terms of fibre and N contents.

Nitrogen balance

No significant differences were measured between the Gayal and cattle for N balance even though there was a trend for faecal loss of N to be higher for Gayal than cattle. The failure to demonstrate differences between the cattle and Gayal is of interest in view of the higher concentration of NH_3-N in rumen liquor (Table 3) and higher plasma urea of Gayal compared to cattle. As for feed intakes and digestibilities of nutrients, it is possible that the diet fed to the animals masked any actual differences between the two groups of animals. Given this it would be of interest to conduct further studies using diets of poor quality and in particular of low N content.

Rumen fermentation

There were significant differences between Gayal and cattle for the content in rumen liquor of NH_3-N before and after feeding (Gayal>cattle), total VFA after feeding (Gayal>cattle) and the proportion of iso-valerate before feeding (Gayal<cattle). The proportion of valerate tended to be lower ($p<0.10$) for Gayal than cattle before feeding and the proportion of propionate after feeding tended to be higher ($p<0.10$) after feeding for Gayal than cattle. Although differences were not significant ($p>0.10$), there was a consistent trend for the pH of rumen liquor to be lower for Gayal than cattle both before and after feeding.

The latter is of interest in the context of the observation that concentration of total VFA was greater in rumen liquor of Gayal than cattle. Indeed, the optimum ruminal pH is 6.0-6.9 for microbes (Kamra, 2005; Khampa et al., 2006) or 6.5-6.8 for cellulolytic bacteria (Grant and Mertens, 1992a, 1992b) and even though the pH of rumen liquor for both groups of animals fell within 6.66-6.99 before and after feeding, the pH was consistently lower for the Gayal than the cattle.

The above points to a difference in rumen fermentation between Gayal and cattle which might be expected to be greater when low quality diets are fed. Indeed it has been found that compared to cattle Gayal attain higher growth rates and reach greater mature live weight when fed similar diets (Cheng, 1984; Giasuddin et al., 2003; Mao et al., 2005).

It is of interest that the concentration of NH_3-N in rumen liquor was higher for Gayal than cattle. Rumen NH_3-N is absorbed through the rumen wall into the portal blood then transferred to the liver where it is used for the synthesis of urea (Kanjanapruthipong and Thaboot, 2006; Nishida et al., 2006; Wanapat et al., 2006). The concentration of plasma urea was significantly higher before and after feeding for Gayal than cattle. Similar observations have been recorded for bison and swamp buffalo compared to cattle in a number of studies (Norton et al., 1979; Hawley et al., 1982; Kawashima et al., 2006).

There is evidence that the amount of N recycled is related to the plasma concentration of urea (Houpt, 1970) with greater recycling occurring as the concentration of plasma urea increases (Peden et al., 1974). Given this, it is considered that the capacity to re-cycle greater amounts of N, particularly when poor quality/low N diets are consumed, explains the faster growth rates and heavier mature live weight of Gayal compared to cattle.

Rumen microbes

There were significant differences between the microbial populations in the rumen of Gayal and cattle (Table 4). Whereas there was no difference for protozoa, there were significantly more total viable bacteria and cellulolytic as well as amylolytic bacteria in rumen liquor of Gayal than cattle before and after feeding. It is perhaps surprising that the number of proteolytic bacteria was not different given the higher concentrations of NH_3-N in

Table 4. Microorganisms in rumen fluid of Gayal and cattle before (0) and 6 hours after offering fresh lucerne pellets

Items	Species		SEM	p-value
	Gayal	Cattle		
Total viable bacteria ($\times10^9$ CFU/ml)				
0 h (post-feeding)	1.82	0.67	0.13	0.004
6	2.53	0.87	0.15	0.030
Cellulolytic bacteria ($\times10^9$ CFU/ml)				
0 h (post-feeding)	1.50	0.53	0.20	0.001
6	1.85	0.71	0.22	0.001
Proteolytic bacteria ($\times10^8$ CFU/ml)				
0 h (post-feeding)	1.27	1.13	0.21	0.725
6	1.71	1.34	0.22	0.508
Amylolytic bacteria ($\times10^8$ CFU/ml)				
0 h (post-feeding)	2.08	0.47	0.15	0.001
6	3.20	0.75	0.16	0.001
Total protozoa ($\times10^5$ cells/ml)				
0 h (post-feeding)	2.02	2.13	0.10	0.824
6	1.80	2.00	0.11	0.765

SEM: Standard error of the mean.

rumen liquor of Gayal than cattle, but the possible role of rumen protozoa in the production of NH_3 should not be overlooked.

Similar observations to those made in the present studies have been made by others for water buffalo compared to cattle. In these prior studies, water buffalo were found to contain higher numbers of total, cellulolytic and amylolytic bacteria in rumen liquor than cattle when diets high in fibre were fed (Singh et al., 1992; Sommart et al., 1993; Puppo et al., 2002; Wanapat et al., 2003). It is of interest that in the present study, the digestibility of fibre by Gayal was not enhanced in spite of the considerable increase in the number of cellulolytic bacteria. This may have been due to the role of rumen protozoa in digestion of dietary fibre; indeed the number of protozoa was similar for both Gayal and cattle.

Concluding comments

Clearly, both groups of animals were well nourished as shown by plasma concentrations of both glucose and urea. Although the results of the study were not conclusive, there is evidence that Gayal and cattle differ in their digestive physiology. The differences may explain the greater growth rates and heavier mature live weight of Gayal compared to cattle maintained under similar field conditions where the quality of feed is low. In view of the potential for utilising Gayal for meat production, quite apart from any intrinsic interest in Gayal, it would be of interest to conduct further studies to compare Gayal and cattle of similar 'physiological age' and fed poor quality diets similar to those normally consumed under natural conditions.

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