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Comparison of Nitrogen Metabolism in Yak (*Bos grunniens*) and Indigenous Cattle (*Bos taurus*) on the Qinghai-Tibetan Plateau*

Hucheng Wang[1,2], Ruijun Long[1,2,], Juan Boo Liang[3], Xusheng Guo[2], Luming Ding[2] and Zhanhuan Shang[1,2]**

[1] Institute of Rangeland and Yak Research, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, China

ABSTRACT : The objective of the present study was to examine whether yaks possess any adaptive mechanisms of nitrogen (N) metabolism to survive in the harsh foraging environment of the Qinghai-Tibetan Plateau. A grazing experiment on native alpine meadows was conducted to determine availability of herbage biomass and body weight (BW) change of yaks over the year, followed by two indoor feeding trials to investigate adaptation mechanisms of N metabolism in yaks fed at similar intake level to grazing conditions. Three castrated males of each of three genotypes; yak (*Bos grunniens*), indigenous cattle (*Bos taurus*) and their crossbred - cattleyak (*Bos taurus*♂×*Bos grunniens*♀), were used in the housed trials. Results showed that: i) Monthly herbage biomass production and daily grazing intakes by yaks over the year ranged from 220 to 4,664 kg DM per ha, and 1.90 to 8.50 kg DM, respectively. For about seven months each year, yaks suffer from malnutrition as a result of inadequate pasture conditions; ii) Urinary N excretion and N retention by yaks were significantly affected by feeding level, and yaks had a lower ($p<0.05$) average daily urinary N excretion (0.39 g/kg $BW^{0.75}$) and a greater ($p<0.05$) N retention (-0.09 g/kg $BW^{0.75}$) than indigenous cattle (0.47 and -0.16 g/kg $BW^{0.75}$, respectively). Fasting daily urinary N excretion was greater ($p<0.05$) for indigenous cattle than yaks (353 vs. 248 mg/kg $BW^{0.75}$). Purine derivative N excretion and purine derivative N index (PNI) increased with increasing feeding level, while the value of PNI was greater ($p<0.05$) for yaks and cattleyak (0.11 and 0.12, respectively) than for indigenous cattle (0.09) during the feeding trials. These results suggest that yaks could rely, in part, on the recycling of N to adapt to the harsh forage environment on the Qinghai-Tibetan Plateau. (**Key Words :** Yak, Grazing Intake, Nitrogen Balance, Plasma Urea Nitrogen, Purine Derivative)

INTRODUCTION

With an area of 1.29×10^8 ha, the Qinghai-Tibetan plateau accounts for 32.5% of the total grassland area of China (Long and Ma, 1996). There are 14 million yaks (*Bos grunniens*), and fewer indigenous cattle (*Bos taurus*) and their crossbred - cattleyak (*Bos taurus*♂×*Bos grunniens*♀), that are largely dependent on the native grasslands for survival. The growth period of native herbage, from mid-May to mid-September, is short compared with its almost 8-month senescent season. Therefore, the surplus of fodder in the warm season and inadequate supply in the cold season from the native rangelands leads to a large shift in the quantity and quality of forages across the year on the plateau. Given this unique pattern of above-ground forage mass distribution, productive performance of yaks is significantly affected by seasonal changes (Long et al., 1999a). Although yaks suffer from inadequate feeding and malnutrition in the long cold season (November to next June), they provide milk and meat which are important sources of food and income for Tibetan herders (Wiener et al., 2003; Long et al., 2005). Long et al. (1999b) reported that yaks are able to use dietary nitrogen (N) more efficiently than cattle kept at low altitudes. Wang et al. (2009) found that yaks had lower glomerular filtration rate (GFR, 3.9 L/kg $BW^{0.75}$) and the ratio of net secretion in renal tubular load to total excretion of purine derivatives ranged from 26.2% to 66.3%, which reflected that yaks may have developed special regulatory mechanisms in the kidney as adaptive mechanisms to malnutrition in the harsh

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** Corresponding Author : Ruijun Long. Tel: +86-931-8915650, Fax: +86-931-8915650, E-mail: Longrj@lzu.edu.cn
[2] International Centre for Tibetan Plateau Ecosystem Management Lanzhou University, Lanzhou, China.
[3] Laboratory of Industrial Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia.
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environment of the plateau.

The current study consisted of two experiments: the first was a grazing trial to estimate the average monthly intake of yaks to identify the gap between fodders supplied from native pastures and maintenance requirement of yaks across a year. Based on results of the above trial, different feeding levels for the subsequent indoor trails were designed to determine the characteristics of nitrogen metabolism in yaks compared with indigenous cattle and cattleyak. The overall aim of the present study was to examine whether yaks possess any adaptive mechanisms of N metabolism to survive in the harsh foraging environment of the Qinghai-Tibetan Plateau.

MATERIALS AND METHODS

Experimental site for grazing trial

A grazing experiment was conducted at the Wushaoling Farm, situated in alpine meadow grassland of the Tibetan Autonomous County, Gansu province, at lat 37°12′N, long 102°52′ E. The altitude of the grassland ranges from 2,700 to 3,300 m. The average annual rainfall is approximately 400 mm and there are no frost-free periods during the year. The average annual temperature is 0.2°C, with the lowest mean monthly temperature of -20.6°C occurring in January, and the highest 25.8°C in July. Annual solar radiation ranges from 5,700 to 6,000 MJ/m^2. Native plants only grow for 120 to 140 days of the year and herbage biomass production is frequently reduced by drought. In addition, during late winter and early spring periodic snowfalls further prohibit grazing and cause death to some animals. The native forages consist mainly of sedges such as *Kobresia humilis* and *Carex atrofusca*, grasses such as *Elymus nutans* and *Rorgnevia kamoji*, forbs such as *Polygonum viviparum* and *Potentilla reptans*, and shrubs such as *Dasiphora fruticosa* and *Salix* species. Sedge forages are the dominant indigenous species, and shrub communities are often mixed with grass, sedge and forb species in high mountain pasture where they are grazed only in the summer. Sedge and sedge-grass meadows are of particular importance in the winter and spring seasons (Long et al., 1999a; Gu et al., 2005; Zhou et al., 2006).

Experimental animals and diets

Throughout the period of the grazing experiment, six 7-year-old female yaks with a mean initial body weight (BW) of 220±21 kg were used to measure grazing intake and average monthly BW change on the summer natural pastures at the Wushaoling Farm. Intake at pasture was estimated from the difference in forage mass immediately pre- and post-grazing according to the procedure described by Long et al. (2003). Pre- and post-grazing herbage mass was measured immediately before and after grazing for 3 consecutive days on the selected uniform plot (100 m×60 m) by cutting 6 random quadrates of 50 cm×50 cm to ground level every other week during the warm season (from June to September) and every four weeks in the cold season (from October to next May). All of the samples were oven dried at 65°C for 48 h. Body weights of yaks were determined on day 20 of each month using an electronic scale, and DMI for maintenance requirement was recommended as 2.5% BW (Han et al., 1990; Long, 1995). Referring to the local yak feeding system and availability of forage in winter (November to January) and spring (February to May) the animals were supplemented with 0.5 to 1.2 kg and 1.0 to 1.5 kg of oat hay daily, respectively, as biomass yield from native rangeland in spring was more inadequate than in winter. These additional feeds were equivalent to 15 to 51% of the metabolizable energy maintenance requirements (0.46 MJ/kg BW$^{0.75}$ per day) recommended by Xue et al. (1994).

Two indoor trials were conducted at the Wushaoling farm site from September to December in 2008. Three 3-year-old castrated males of each genotype, yak (160±5 kg BW), indigenous cattle (110±5 kg BW) and cattleyak (170 ±2 kg BW), were selected from the same pasture for use in the housed experiments. The experimental diets offered during the housed experiments were 100% oat hay containing 8.3 MJ ME/kg DM (*in vitro* determination) and 87.9 g CP/kg DM, cut into 10 cm lengths with a hay cutter and mixed during each period. In the first trial, animals were kept in pens and fed individually oat hay *ad libitum* three times daily at 08:00 h, 12:00 h, and 17:00 h for 15 days to measure their daily voluntary intake (g/kg BW$^{0.75}$). On the last day, body condition scores (BCS) of the nine animals were recorded according to the procedure of Ferguson et al. (1994). The lowest voluntary intake (VI), measured among the animals, was used to calculate the three feeding levels in the next intake trial (i.e. 0.3×VI, 0.6 ×VI, and 0.9×VI) for all the animals in order to avoid any refusals at the highest feeding level. The feeding trial was conducted using three 3×3 Latin squares, one for each genotype over three periods of 22 days each (15 days of adaptation and 7 days of measurement). The three Latin squares were conducted concurrently. The animals were housed individually in metabolism crates to facilitate feces and urine collections, and fresh water was freely accessible at all times. On day 1, 3, 5 and 7 of measurement in each period, a blood sample (07:00 h) was taken from a jugular vein of each animal. Two 10 ml heparinized tubes containing 150 IU heparin sodium were used to collect jugular blood by venipuncture, then centrifuged at 2,500 g for 10 min at 4°C and heparinzed plasma was stored - 80°C for later analysis of plasma urea N (PUN). Total daily feces

output was collected into a plastic box attached to the crates, weighed and two samples were taken. One fecal sample from each animal was bulked over the 7-day collection period and oven dried at 65°C for 48 h. The second sample was placed in a plastic bottle, containing 10% (v/v) sulphuric acid to stop further microbial activity, for subsequent determination of N content. Urine was collected using a latex funnel attached round the penis of the animal and through a flexible latex pipe into a plastic tray beneath the metabolism crate. Excreted urine was collected daily into buckets containing 10% (v/v) sulphuric acid to keep the final pH at 2 to 3. The urine was weighed, thoroughly mixed, and sampled. Samples were stored at -20°C.

Following the feeding trial, a fasting trial as described by IAEA (1997) was carried out according to a single factor (genotype) with three genotypes (yak, indigenous cattle and cattleyak) design to determine endogenous N excretion profile. The daily DM intake by animals was reduced to 1.5% of their BW for two weeks, and then reduced stepwise to 1%, 0.5% and 0% over the next three days. The fasting period lasted for 6 days, followed by a 2-day period of re-alimentation, during which the feed was increased stepwise to 0.5% and 1% DM of their respective BW. The animals were housed as in the previous feeding trial and feces and urine were collected as described above. Animals were cared for throughout all experimental periods according to the Guide for the Care and Use of Laboratory Animals (Gansu Province Animal Care Committee).

Calculation model, chemical and statistical analysis

Purine derivatives nitrogen index (PNI) was considered as purine derivative nitrogen (PDN) vs. total N in urine (Chen et al., 1998). N contents of oat hay, feces and urine were determined as described by (AOAC, 1984). Plasma urea N was determined using the Sigma Diagnostics-Urea N Procedure NO. 640 (Sigma-Aldrich, St. Louis, MO). Purine derivative concentrations in urine were measured using HPLC following the procedure described by Balcells et al. (1992).

Data from the feeding experiment was analyzed as a Latin Square design following the model:

$$y_{ijk} = \mu + L_i + A_j + P_k + \varepsilon_{ijk}$$

where y_{ijk} is the dependent variable, μ is the overall mean, L_i is the fixed effect of feeding level treatment i, A_j is the random effect of animal j, P_k is the random effect of period k, and ε_{ijk} is the random residual error. When the treatment effect was significant ($p<0.05$), polynomial contrasts for linear and quadratic effects of different feeding levels were also tested.

To determine the effects of genotype, the data were regrouped and analyzed in a factorial design using the model:

$$y_{ijkl} = \mu + G_i + L_j + GL_{ij} + A(G)_k + P_l + \varepsilon_{ijkl}$$

where G_i, L_j, $A(G)_k$, P_l, GL_{ij} represent genotype, feeding level, animal within breed, period and the genotype×intake level interaction, respectively, contrasted against the residual error term (ε). When the genotype effect was significant ($p<0.05$), multiple comparisons were tested according to the Tukey method.

Data obtained from the fasting trial were analyzed using a one-way ANOVA procedure. All analyses were performed using the computing SPSS software (SPSS Inc., Chicago, IL) according to the procedure described by Landau and Everitt (2004).

RESULTS

Grazing experiment

The above ground herbage mass varied dramatically across the year. During the warm season (June to October), herbage in the native rangeland was surplus to that required by the yaks, whereas in the cold season (November to May) yaks suffered malnutrition because intakes were far below their requirements for maintenance of growth. The highest and the lowest monthly biomass production were 220 to 4, 664 kg DM/ha, respectively, and the daily intakes were estimated to range from 1.90 to 8.50 kg DM, over the year (Figure 1). The average monthly BW change was 19.4 kg from May to October, and -6.05 kg from November to the following April. At the same time, BCS ranged from 3.6 to 4.0 from August to November, 2.6 to 3.0 from February to July, and 3.1 to 3.4 for the other months. Using the value of 11 g N/kg DM for Tibetan forages from sedge-grass meadow (Long, 1995), daily N intakes for seven months were estimated to range from 0.41 to 0.94 g N/kg $BW^{0.75}$ except for the period from June to October (1.09 to 1.69 g N/kg $BW^{0.75}$), which was far below the maintenance requirements of digestible N (0.97 g/kg $BW^{0.75}$ per day) recommended by Xue et al. (1994).

Indoor feeding experiment

Daily voluntary intake by the animals ranged from 61.5 to 90.3 g DM/kg $BW^{0.75}$ (Table 1), with the lowest intake (61.5 g DM/kg $BW^{0.75}$) by a cattleyak. Based on the lowest intake value, the three daily intake levels (0.3×VI, 0.6×VI, 0.9×VI) calculated for the feeding trial were 18.5, 36.9, 55.4 g DM/kg $BW^{0.75}$, respectively, with ME intakes of 0.15, 0.31, 0.46 MJ/kg $BW^{0.75}$, respectively. No feed residues were found for any animal throughout the feeding trial. Furthermore, the changes of BW and BCS ranged from 0.9

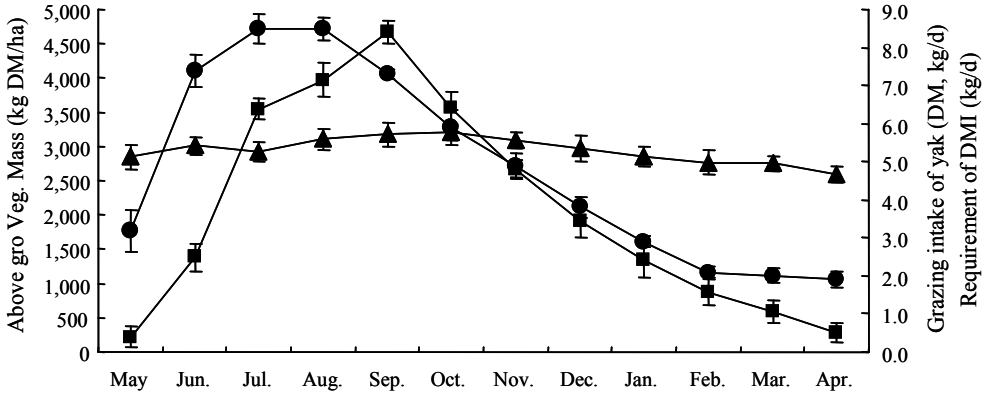

Figure 1. Biomass supply of above-ground vegetation and grazing intake (■ above ground vegetation, ● grazing intake of yak, ▲ maintenance requirement of DMI) by yak during the year on the Qinghai-Tibetan plateau (Exp. 1).

Table 1. Daily voluntary intake (VI, g/kg $BW^{0.75}$) by animals fed oat hay *ad libitum* for 15 days (Exp. 2)

Animal	Yak	Indigenous cattle	Cattleyak
No.1	69.2±13.37	90.3±17.93	66.3±12.64
No.2	69.9±11.06	89.9±13.97	64.8±10.68
No.3	65.7±11.27	87.6±10.68	61.5±10.12

to 1.2 kg and 0.1 to 0.15 at pre- and post- trial, respectively, and were not significantly different compared with their initial values.

Nitrogen metabolism

N intake and fecal N excretion for the three genotypes increased linearly ($p<0.05$) as feeding level increased from 0.3×VI to 0.9×VI; Urinary N excretion and N retention in yaks and indigenous cattle were affected linearly ($p<0.05$) by feeding level, but not for cattleyak (Table 2). Daily urinary PDN excretion and PNI increased linearly with increasing feeding level for the three genotypes (Table 3). PUN concentration decreased linearly with increasing feeding level ($p<0.05$) for yak and cattleyak, but an opposite tendency was evident for indigenous cattle (Table 4). N intake and fecal N excretion did not differ among the three genotypes, but yaks had a lower ($p<0.001$) daily average urinary N excretion (0.39 g/kg $BW^{0.75}$) and greater

Table 2. Nitrogen balance (g/kg $BW^{0.75}$ daily) in yak, indigenous cattle and cattleyak fed restricted levels of oat hay (Exp. 2)

	Level of voluntary intake[1]			SEM[2]	p[3]	
	0.3	0.6	0.9		L	Q
Yak						
Intake N	0.26^{c}	0.57^{b}	0.73^{a}	0.007	0.001	0.02
Fecal N	0.13^{b}	0.19^{b}	0.33^{a}	0.011	0.01	0.13
Urinary N	0.38^{b}	0.37^{b}	0.43^{a}	0.006	0.03	0.04
N balance	-0.25^{c}	0.01^{ab}	-0.03^{a}	0.013	0.01	0.02
Indigenous cattle						
Intake N	0.25^{c}	0.56^{b}	0.74^{a}	0.015	<0.001	0.01
Fecal N	0.12^{b}	0.18^{ab}	0.33^{a}	0.014	0.01	0.12
Urinary N	0.44^{b}	0.47^{ab}	0.49^{a}	0.026	0.03	0.81
N balance	-0.31^{c}	-0.09^{ab}	-0.08^{a}	0.031	0.03	0.07
Cattleyak						
Intake N	0.27^{c}	0.58^{b}	0.75^{a}	0.011	0.001	0.03
Fecal N	0.14^{b}	0.18^{b}	0.36^{a}	0.023	0.02	0.13
Urinary N	0.41	0.42	0.44	0.042	0.92	0.69
N balance	-0.28	-0.02	-0.05	0.048	0.07	0.18

[1] The lowest voluntary intake determined in the pre-feeding trial. [2] Standard error of the means.
[3] p-values for linear (L) and quadratic (Q) effects of feeding level.
[a,b,c] Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 3. Daily urinary purine derivative nitrogen excretion (PDN, mg/kg $BW^{0.75}$) and PDN index (PNI) by yak, indigenous cattle and cattleyak fed restricted levels of oat hay (Exp. 2)

	Level of voluntary intake[1]			SEM[2]	p[3]	
	0.3	0.6	0.9		L	Q
Yak						
Urinary PDN	25.4[b]	35.2[b]	64.5[a]	1.99	0.01	0.06
PNI	0.07[b]	0.10[ab]	0.15[a]	0.006	0.01	0.23
Indigenous cattle						
Urinary PDN	25.8[c]	40.2[b]	51.7[a]	1.32	0.01	0.45
PNI	0.06[c]	0.09[ab]	0.11[a]	0.002	0.01	0.45
Cattleyak						
Urinary PDN	25.7[c]	48.9[b]	75.5[a]	1.99	0.003	0.55
PNI	0.06[c]	0.12[b]	0.17[a]	0.013	0.02	0.58

[1] The lowest voluntary intake determined in the pre-feeding trial. [2] Standard error of the means.
[3] p-values for linear (L) and quadratic (Q) effects of feeding level.
[a,b,c] Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 4. Plasma urea N concentration (PUN, mg/dl) in yak, indigenous cattle and cattleyak fed restricted levels of oat hay (Exp. 2)

	Level of voluntary intake[1]			SEM[2]	p[3]	
	0.3	0.6	0.9		L	Q
Yak	17.1[a]	16.3[ab]	10.3[b]	0.78	0.03	0.11
Indigenous cattle	11.4	13.9	13.1	0.42	0.11	0.08
Cattleyak	16.7[a]	9.8[b]	5.8[c]	0.14	<0.001	0.01

[1] The lowest voluntary intake determined in the pre-feeding trial. [2] Standard error of the means.
[3] p-values for linear (L) and quadratic (Q) effects of feeding level.
[a,b,c] Means in the same row with different superscripts differ significantly ($p<0.05$).

($p<0.05$) N retention (-0.09 g/kg $BW^{0.75}$) than indigenous cattle (0.47 and -0.16 g/kg $BW^{0.75}$, respectively); The values of PNI for yaks and cattleyak (0.11 and 0.12, respectively) were greater ($p<0.05$) than for indigenous cattle (0.09); cattleyak had a greater ($p<0.05$) daily average PDN excretion (50.0 mg/kg $BW^{0.75}$) than yaks (41.7 mg/kg $BW^{0.75}$) and indigenous cattle (39.2 mg/kg $BW^{0.75}$). PUN concentration for yak was significantly greater ($p<0.05$) than the other two genotypes (Table 5). Fasting daily urinary N excretion was greater ($p<0.05$) for indigenous cattle (353 mg/kg $BW^{0.75}$) than for yaks (248 mg/kg $BW^{0.75}$); fasting PDN excretion and PNI were not different among the three genotypes ($p>0.05$) (Table 6).

DISCUSSION

Han et al. (1990) reported that the DMI of 2- to 4-year-old yaks varied from 2.4 percent of BW for an oat hay diet to 1.8 percent for a wheat straw diet. Long (1995) reported that the ratio of DMI to BW was 2.56% and then BW increased by 1.2 kg in October, but when the ratio decreased to 2.43% in November grazing yaks started to lose weight. Considering the results obtained from indoor feeding and outdoor grazing trials, therefore, the ratio of DMI to BW at the stage of maintenance requirement is recommended as 2.5% from the present grazing trial. Using this parameter as the basis of calculation, the maintenance

Table 5. Nitrogen balance, daily urinary purine derivative nitrogen excretion (PDN), PDN index (PNI) and plasma urea nitrogen (PUN) in yak, indigenous cattle and cattleyak fed restricted levels of oat hay (Exp. 2)

	Yak	Indigenous cattle	Cattleyak	SEM
N balance (g/kg $BW^{0.75}$)				
Intake N	0.52	0.52	0.53	0.005
Fecal N	0.22	0.21	0.23	0.009
Urinary N	0.39[b]	0.47[a]	0.42[ab]	0.011
N balance	-0.09[a]	-0.16[b]	-0.12[ab]	0.015
Urinary PDN (mg/kg $BW^{0.75}$)	41.7[b]	39.2[b]	50.0[a]	2.36
PNI	0.11[a]	0.09[b]	0.12[a]	0.006
PUN (mg/dl)	14.6[a]	12.8[b]	10.8[b]	0.75

[a,b] Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 6. Daily urinary N and purine derivative nitrogen (PDN) excretion (mg/kg $BW^{0.75}$) and PDN index (PNI) of yak, indigenous cattle and cattleyak during the fasting trial (Exp. 2)

	Yak	Indigenous cattle	Cattleyak
Urinary PDN	12.8±4.16	16.6±2.18	13.3±1.18
Urinary N	248^{b}±19.3	353^{a}±41.1	321^{ab}±27.5
PNI	0.05±0.005	0.05±0.009	0.04±0.007

[a, b] Means in the same row with different superscripts differ significantly ($p<0.05$).

requirement for yaks in the grazing trial was estimated to be from 4.65 to 5.75 kg DM/d across a year. Based on the annual pattern of vegetation production on the Qinghai-Tibetan plateau (Figure 1), the available forages were able to meet or exceed requirements for maintenance for yaks during only 5 months of the year (June to October). During the rest of the year, forages decline sharply in quantity which leads to a large reduction in BW, or even death during deep winter and spring, if yaks are without sufficient supplementation. However, yaks are able to recover this lost BW quickly from June to August and achieve peak BW by September or October (Long et al., 1999a; 2005). Domestication of yaks occurred 8, 000 years ago (Guo et al., 2006), and over millions of years of adaptation to their harsh foraging environment yaks must have evolved some mechanisms to maximize utilization of the limited feed resources to survive on the plateau.

The grazing trial (Exp. 1) showed huge variation in forage production between warm and cold seasons, resulting in a malnutrition status for yaks for almost seven months a year. In order to test the hypothesis that yaks may have developed an adaptive mechanism to survive under malnutrition conditions, the indoor feeding trials were designed to simulate a real foraging situation for grazing yaks, i.e. their intakes in terms of digestible crude protein (DCP) and ME were below maintenance levels in the cold season. Therefore, the animals (all three genotypes) were fed an oat hay diet of low N content (87.9 g CP/kg DM) at different intake levels below maintenance requirements except for the 0.9×VI treatment, which was close to maintenance level.

Voluntary intake is governed by many factors, including quality of feed, rate of chewing and rate of passage, which influence feed digestibility and ultimately voluntary intake. The higher voluntary feed intake on a metabolic weight basis for cattle compared to yaks and cattleyak (Table 1) is rather unexpected and difficult to explain. However, higher rate of passage of feed particles in cattle could be one reason. Liu et al. (1991) reported that rumen fluid volumes ranged from 32.3 to 35.8 L for yaks with a mean BW of 150 kg, while similar values for cattle weighing 530 kg were from 40 to 50 L. Based on the above data, the estimated rumen fluid volume relative to BW for yaks (0.23) was 2.4 fold higher than that for cattle (0.09). Larger rumen volume relative to BW is normally associated with slower passage rate of feed, which allows feed to be retained longer in the rumen for better digestion, especially under restricted intake level. This is an adaptive feature of ruminants, including water buffaloes, to poor quality feed (Liang and Samiyah, 1988).

Long et al. (1999b) reported that fasting urinary N excretion of dry female yaks was lower than that of low-altitude cattle but similar to water buffaloes, suggesting that yaks may have evolved a mechanism to recycle more N to the rumen than cattle. Therefore, in the present study, yaks and indigenous cattle had similar initial body condition scores and were treated identically. Daily urinary N excretion, on a metabolic weight basis, in the indigenous cattle was 18%, 27% and 13% greater than in yaks in the 0.3×VI, 0.6×VI and 0.9×VI treatment groups, respectively. Daily endogenous N excretion in the indigenous cattle was 40% greater than in the yaks. Meanwhile, the recommended maintenance requirement for DCP for growing yaks was 6.09 g/kg $BW^{0.75}$ (Xue et al., 1994) and 0.46 MJ/kg $BW^{0.75}$ for ME (Han et al., 1993). In the present study, daily DCP intakes for the three feeding levels ranged from 0.65 to 2.57 g/kg $BW^{0.75}$ and ME intakes ranged from 0.15 to 0.46 MJ/kg $BW^{0.75}$. The above values were far below the recommended requirements except for MEI in 0.9×VI; therefore, it is not surprising that negative N balances were recorded in this study (Table 2). However, the N retention for yaks was significantly greater than that for the cattle treatment group, even though they were under malnutrition conditions. This implies that yaks have a greater N utilization capacity to adapt to a harsh environment than cattle.

Previous research has also indicated that PNI provides a simple and rapid means of assessing the efficiency of conversion of dietary N to microbial protein, and, in conjunction with protein degradability and dietary N intake, can be useful in calculating ruminant feeding efficiency at the rumen level (Chen et al., 1998). In the present study, with increasing feeding level values for the three genotypes increased linearly, which reflected the positive efficiency of conversion of dietary N to microbial protein (Table 3). Meanwhile, the PNI value for yaks was significantly greater than that for indigenous cattle fed under the restricted intake level. This suggested yaks and cattleyak have a greater

efficiency of conversion of endogenous and exogenous N to microbial protein than cattle.

The observed decrease for yak and cattleyak in PUN concentrations in relation to increased feeding level did not agree with other observations in the literature (Preston et al., 1965; Vasconcelos et al., 2006), but was similar to observations in grazing yaks by Long et al. (1999c), and is probably a result of an insufficient supply of dietary N. Blood urea sources are dietary protein and deamination of tissue protein. A proportion of blood urea is excreted via the kidney and some is recycled via the salivary glands and the rumen wall. In the present study, PUN concentration for yaks and cattleyak showed greater sensitivity to malnutrition conditions than cattle, and the greater PUN concentration and lower urinary N excretion rate suggested that, in time of need, yaks may catabolise tissue protein for glucose precursors. However, this assumption needs further investigation. Lower urinary N excretion, greater PNI features and PUN metabolism were also found in the cattleyak, indicating that the crossbred may have inherited some of the adaptive characteristics of its parents.

These data indicate that yaks can recycle more N than the indigenous cattle, particularly at lower feeding levels. The ability to recycle more N and thus reduce urinary N excretion in yaks compared to cattle appears to be a physiological adaptation to the inadequate and poor feed supply during the cold season. The lower excretion of urinary N in yaks is probably due to the more efficient utilization of the N recycled between gastrointestinal tract and liver and the efficient recovery of urea N in the kidney as compared to the other two genotypes. To date, the quantification of N recycling and urea flux in dairy cattle (Marini and Amburgh, 2003), beef cattle (Archibeque et al., 2001; Huntington et al., 2009), sheep (Sarraseca et al., 1998) and goats (Brun, 1996) has been extensively studied, but not in yaks.

It is essential to highlight that, due to limited resources and local constraints; the number of animals used for this study was rather small. One should, therefore, interpret the results with caution. In conclusion, results in this study suggest that yaks rely on better nutrient utilization together with an enhanced efficiency of microbial protein synthesis in the rumen to adapt to the inadequate and poor forage environment in the Qinghai-Tibetan plateau. However, quantification of N recycling in yaks requires further investigation.

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