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Original Research Article

A comparison of average daily gain, apparent digestibilities, energy balance, rumen fermentation parameters, and serum metabolites between yaks (*Bos grunniens*) and Qaidam cattle (*Bos taurus*) consuming diets differing in energy level



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ABSTRACT

Yaks (*Bos grunniens*), indigenous to the harsh Qinghai-Tibetan Plateau, are well adapted to the severe conditions, and graze natural pasture without supplements all year round. Qaidam cattle (*Bos taurus*), introduced to the Qinghai-Tibetan Plateau 1,700 years ago, are raised at a lower altitude than yaks, provided with shelter at night and offered supplements in winter. Based on their different backgrounds, we hypothesized that yaks have lower energy requirements for maintenance than cattle. To test this hypothesis, we measured average daily gain (ADG), apparent digestibilities, energy balance, rumen fermentation parameters, and serum metabolites in growing yaks and cattle offered diets differing in metabolizable energy (ME) levels (6.62, 8.02, 9.42 and 10.80 MJ/kg), but with the same crude protein concentration. Six castrated yaks (155 ± 5.8 kg) and 6 castrated Qaidam cattle (154 ± 8.0 kg), all 2.5 years old, were used in 2 concurrent 4 × 4 Latin square designs. Neutral and acid detergent fiber digestibilities were greater ($P < 0.05$) in yaks than in cattle, and decreased linearly ($P < 0.05$) with increasing dietary energy level; whereas, digestibilities of dry matter, organic matter, crude protein and ether extract increased ($P < 0.05$) linearly with increasing energy level. The ADG was greater ($P < 0.001$) in yaks than in cattle, and increased ($P < 0.05$) linearly with increasing energy levels. From the regressions of ADG on ME intake, the estimated ME requirement for maintenance was lower ($P < 0.05$) in yaks than in cattle (0.43 vs. 0.57 MJ/kg BW^{0.75}). The ratios of digestible energy (DE):gross energy and ME:DE were higher ($P < 0.05$) in yaks than in cattle, and increased ($P < 0.05$) linearly with increasing dietary energy level. Ruminal pH decreased ($P < 0.05$), whereas concentrations of total volatile fatty acids (VFAs) and ammonia increased ($P < 0.01$) with increasing dietary energy level, and all were greater ($P < 0.05$) in yaks than in cattle. Concentrations of ruminal acetate and iso-VFAs were greater ($P < 0.05$), whereas propionate was lower ($P < 0.05$) in yaks than in cattle; acetate decreased ($P < 0.001$), whereas butyrate and propionate increased ($P < 0.001$) linearly with increasing dietary energy level. Serum concentrations of β-hydroxybutyrate were lower (interaction, $P < 0.001$) in yaks than in cattle fed diets of 9.42 and

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10.80 MJ/kg, whereas non-esterified fatty acids were greater (interaction, $P < 0.01$) in yaks than in cattle fed diets of 6.62 and 8.02 MJ/kg. Concentrations of serum leptin and growth hormone were greater in yaks than in cattle and serum insulin and growth hormone increased ($P < 0.01$) linearly with increasing dietary energy level. Our hypothesis that yaks have lower energy requirements for maintenance than cattle was supported. This lower requirement confers an advantage to yaks over Qaidam cattle in consuming low energy diets during the long winter on the Qinghai-Tibetan Plateau.

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1. Introduction

The Qinghai-Tibetan Plateau, called “the third pole”, originated about 40 million years ago. The high-altitude plateau is characterized by severe cold, low air oxygen content, high ultraviolet radiation and strong winds (Zhang et al., 2019). The growing season is short, approximately 120 d per year, and the forage availability, especially energy and protein, is insufficient for grazing herbivores during the long cold season (Long et al., 1999; Guo et al., 2021).

The yak (*Bos grunniens*), with a population of 17.6 million, of which 95% are in China (Ma et al., 2013), is indigenous to the Qinghai-Tibetan Plateau and is raised at an altitude between 3,000 and 6,000 m above sea level (a. s. l.). Yaks are well adapted anatomically, physiologically and behaviorally to the harsh Qinghai-Tibetan Plateau. They serve as the symbol of the Tibetan culture and provide pastoralists with meat and milk for food, hair and hides for textiles, leather goods and clothing, dung for fuel and capital (Long et al., 2004). Most yaks are still raised under traditional management; namely, grazing on the alpine rangeland without supplements all year round. Qaidam cattle (*Bos taurus*) were introduced to the Qinghai-Tibetan Plateau from the lowlands by Tibetans about 1,700 years ago (China National Commission of Animal Genetic Resources, 2011; Chen et al., 2018). There are over 10,000 cattle on the Qinghai-Tibetan Plateau that are raised at altitudes between 2,600 and 3,600 m a. s. l., that is, at lower altitudes than yaks. They also graze on natural pasture all year round, but are offered supplements and provided with shelter at night during the cold season (Ren et al., 2011). Genomic studies indicated that yak and cattle separated about 4.38 to 5.32 million years ago (Gu et al., 2007).

It was reported that fasting heat production remained stable in yaks but increased linearly in Qaidam cattle when the altitude ascended from 2,300 to 4,300 m (Hu et al., 1992). Under traditional management, yaks generally lose upwards of 25% body weight during the cold season (Xue et al., 2005). Energy intake is insufficient because of the sparse natural pasture available in this period, which would suggest that energy intake is one of the main limiting factors affecting yak production. However, yaks are able to overcome the long winter without supplements, whereas Qaidam cattle cannot. This led us to hypothesize that yaks have lower energy requirements for maintenance than cattle. To test this hypothesis, we measured average daily gain (ADG), apparent dry matter, nutrient and energy digestibilities, rumen fermentation parameters, and serum metabolites in growing yaks and cattle offered diets differing in energy levels, but with the same crude protein content.

2. Materials and methods

2.1. Study site and animal ethics

The study was conducted at Wushaoling Yak Research Facility of Lanzhou University (37°12.4'N, 102°51.7'E, 3,154 m a. s. l., north-eastern Qinghai-Tibetan Plateau), located in Tianzhu Tibetan

Autonomous County, Wuwei City, Gansu Province, China. The animal use protocol and experimental procedures in this study were approved by the Animal Care and Use Committee of Lanzhou University (No. 201905201).

2.2. Animals and diets

Six yaks (initial body weight, 155 ± 5.8 kg) and 6 Qaidam cattle (initial body weight, 154 ± 8.0 kg), all 2.5 years old and castrated, were selected. The animals were held individually in metabolic cages (1.0 m \times 2.2 m) that allowed for measurements of feed intake and faecal and urine outputs. The animals were offered 2.75 kg DM of 4 diets with different ME contents (6.62, 8.02, 9.42, and 10.80 MJ/kg), but with the same low crude protein content of 74 g/kg DM (Table 1). The dietary CP content was lower than the requirement for ruminants of their body size (National Research Council, 2001), but was similar to the average CP level in the forage of the Qinghai-Tibetan Plateau during the cold season (Xie et al., 1996). The diet was a pelleted total mixed ration (Gansu Runmu Biological Engineering Co., Ltd, Jinchang, China), and was offered in 2 equal portions: half at 08:00 and half at 18:00. The 4 diets, from the lowest to the highest energy yields, provided 0.8, 0.9, 1.0, and 1.1 times maintenance energy requirement (ME_m) of growing cattle (National Research Council, 2001).

The design of the study was a 2 (animal species) \times 4 (energy levels) factorial arrangement and conducted as 2 concurrent 4 \times 4 Latin squares. This study consisted of 4 periods, and each period included 22 d of dietary adaptation (d 1 to 22) and 6 d of measurement (d 23 to 28). In each period, 4 yaks and 4 cattle each received one of the diets, and the 2 other yaks and 2 other cattle received one of the 4 diets, as described by Sarraseca et al. (1988). The 6 animals of each species received all 4 dietary treatments.

2.3. Experimental procedures and collection of samples

Animals were weighed (Weighbridge, Shanghai Jiujin Electronics Apparatus Co. Shanghai, China) before morning feeding on d 1 and 29, and a 100 g sample of each feed was collected on d 23 to 28.

From d 23 to 28, total faeces were collected daily in plastic pans placed under the rear part of the animals, weighed and 50 g/kg were placed in self-sealing bags and stored at -20°C for subsequent analyses. Total urine was collected using a funnel-shaped latex bag (Chinese patent CN201922087846.4; Zhou et al., 2020) into which urine flowed via a polyethylene tube from the bag to a plastic container containing 40 mL of 9 mmol/L sulfuric acid solution to maintain pH < 3.0 . Urine volume was measured daily and 5 mL out of 100 mL were stored in 10-mL centrifuge tubes (Jiangsu Huaxia Ruitai Plastic Industry Co., Ltd, Taizhou, China) at -20°C for later analysis.

On d 28, approximately 150 mL rumen fluid was collected using an oral stomach tube (Anscitech Co. Ltd., Wuhan, China) at 0, 2, 6 h

Table 1
Ingredients and chemical composition of the experimental diets.

Item	Dietary ME levels, MJ/kg			
	6.62	8.02	9.42	10.80
Ingredients, g/kg DM				
Corn straw	850	700	550	400
Corn grain, ground	46.0	117	180	260
Corn husk	11.0	55.0	110	154
Cotton seed meal	27.0	24.3	21.0	18.5
Soybean meal	27.0	24.0	21.0	18.0
Wheat bran	24.0	21.0	16.0	7.0
Corn starch	–	40.0	72.0	104
Rumen bypass palm oil	–	4.00	13.0	20.5
Calcium hydrophosphate	–	–	2.00	3.00
Sodium chloride	10.0	10.0	10.0	10.0
Commercial premix ¹	5.00	5.00	5.00	5.00
Chemical composition, g/kg DM				
ME ² , MJ/kg	6.62	8.02	9.42	10.8
CP ³	74.5	74.4	74.4	74.3
NDF	604	544	486	422
ADF	322	276	232	186
EE	55.7	66.8	80.3	91.6
Calcium	8.70	8.50	7.90	7.00
Phosphorus	2.40	2.30	2.40	2.40
GE, MJ/kg	16.0	16.4	16.9	17.2

ME = metabolizable energy; CP = crude protein; DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract; GE = gross energy.

¹ The premix was provided following as per kilogram: vitamin A 3,000,000 IU, vitamin D 375,000 IU, vitamin E 220 IU, biotin 12 mg, Cu 1 200 mg, Fe 3 000 mg, Mn 2 000 mg, Zn 4 000 mg, I 15 mg, Se 20 mg.

² The ME was calculated according to the Tables of Feed Composition and Nutritive Values in China (Xiong et al., 2018).

³ Calculated as nitrogen \times 6.25.

post morning feeding, of which the first 50 mL was discarded to minimize saliva contamination. The pH was determined immediately using a pH meter (PB-10, Sartorius Co., Göttingen, Germany) and the ruminal fluid was strained through 4 layers of cheesecloth and divided into 3 parts: 10 mL was mixed with 10 mL deproteinizing solution (100 g metaphosphoric acid and 0.6 g croconic acid per liter) for analysis of volatile fatty acids (VFAs); 5 mL was added to 5 mL of 0.5 mmol/L hydrochloric acid solution for measurement of ammonia; and the rest was stored in 50 mL centrifuge tubes and stored at -80°C for determination of free amino acid-N, soluble protein-N and microbial protein-N.

Also, on d 28 before morning feeding, 20 mL jugular blood was collected in evacuated tubes without anticoagulant (Shanghai Kehua Bio-engineering Co., Ltd, Shanghai, China), maintained on ice for 1 h, centrifuged at $3,000 \times g$ for 15 min and the serum was stored at -80°C .

2.4. Laboratory analyses

Feed and faecal samples were dried at 65°C in a forced air oven (DHG-9123 A, Jiecheng Experimental Apparatus, Shanghai, China) for 72 h, air-equilibrated overnight, ground to pass through a 1-mm sieve, and then stored in self-sealing plastic bags. The DM of feed and faecal samples was determined by drying at 105°C for 24 h in a forced air oven (AOAC, 2006; method 925.45) and organic matter (OM) was measured as loss in dry weight upon complete combustion of a sample at 550°C for 6 h in a muffle furnace (AOAC, 2006; method 942.05). Total nitrogen content in feed and faeces was determined by the micro-Kjeldahl method (K1100, Hanon instruments, Jinan, China), and CP was calculated as $\text{N} \times 6.25$. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by an automatic fiber analyzer (Ankom Technology, Fairport, NY, USA) according to Van Soest et al. (1991) and Robertson and Van Soest (1981), respectively. Sodium sulfate (10 g/L of NDF solution)

and heat-stable α -amylase (Termamyl 120 L, Type L, Novozymes A/S) were added to the NDF solution. Ether extract (method 920.29) was measured by using a reflux system (Ankom XT 15, Fairport, NY, USA) with petroleum ether at 90°C for 1 h. Gross energy (GE) of feed, faeces and urine were determined by bomb calorimetry (6400 Calorimeter, Parr Instrument Company, Moline, Illinois, USA). Urine samples were prepared as described by Nijkamp (1969).

Ruminal VFA concentrations were measured by gas chromatography (GC) with a capillary column (AT-FFAP: $30\text{ m} \times 0.32\text{ mm} \times 0.5\text{ }\mu\text{m}$) in a Shimadzu 2010 plus system (Shimadzu Corporation, Kyoto, Japan) following Liu et al. (2021). Ruminal ammonia-N, soluble protein-N and microbial protein-N were analyzed by colorimetry (Spectrophotometer U-2900, Hitachi, Tokyo, Japan) following Hristov et al. (2001), Oosta et al. (1978), and Bradford (1976), respectively.

Serum glucose, β -hydroxybutyrate (BHBA), triglycerides, lactic acid and non-esterified fatty acids (NEFA) were determined by using an automatic biochemistry analyzer (Hitachi 7160, Hitachi High-Technologies Corporation, Tokyo, Japan), following the protocols of commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Insulin, glucagon, leptin, growth hormone, triiodothyronine, thyroxine, norepinephrine and insulin-like growth factor-1 (IGF-1) were measured by enzyme-linked immune sorbent assay (ELISA) kits (Lab systems Multiskan MS Type 352, Helsinki, Finland). The serum metabolites were determined at Shanghai Bangyi Biological Technology Co., Ltd. (Shanghai, China).

2.5. Calculations and statistical analysis

Apparent digestibilities of the nutrients were determined from their contents in the DMI and in the DM faecal output.

Enteric methane energy emission was estimated from gross energy intake (GEI). In cattle it was calculated following Jiao et al. (2014) as:

$$\text{Enteric methane energy (MJ/d)} = 0.057 \times \text{GEI (MJ/d)} + 1.092.$$

This equation was generated from 6 to 22 month old Holstein steers and heifers that were offered a near 55:45 concentrate:forage diet. In yaks, it was calculated following Bai et al. (2021) as:

$$\text{Enteric methane energy (MJ/d)} = 0.040 \times \text{GEI (MJ/d)} + 0.10.$$

This equation was generated from 8 to 16 month old male and female yaks that were offered a 60:40 concentrate: forage diet.

The ME requirement for maintenance (ME_m) was estimated by regressing ADG on daily ME intake (MEI), as ADG is an indirect measure of energy retention, and the MEI at “ADG = 0” was shown to estimate energy requirements accurately (Quigley et al., 2014; Liu et al., 2019). The regression equation took the following form:

$$\text{ADG (g/d)} = a \text{ MEI (MJ/kg BW}^{0.75} \text{ per d)} + b.$$

The data were analyzed according to a 2×4 factorial arrangement in 2 concurrent 4×4 Latin square designs using the mixed model procedure of SAS statistical package (SAS version 9.4, SAS Inst. Inc., Cary, NC). The model was: $Y = \mu + S + E + (S \times E) + P + e$, where Y = dependent variable; μ = treatment mean value; S = effect of animal species; E = effect of dietary energy level; $S \times E$ = interaction between animal species and dietary energy level; P = period; and e = residual error. Dietary energy levels and animal species were fixed effects, and experimental animals and periods were random effects. The parameters in rumen fluid, which were collected serially, were analyzed as repeated measures.

Polynomial contrasts were used to determine whether the effects of dietary energy levels on the measured variables were linear or quadratic. When there was a significant interaction between dietary energy levels and animal species, comparison between animal species at the same dietary energy level was made using a *t*-test. A level of $P < 0.05$ was accepted as significant and $0.05 \leq P < 0.10$ as tended to differ.

3. Results

3.1. Body weight changes and apparent digestibilities

All feed was consumed by both species. The ADG was greater ($P < 0.001$) in yaks than in cattle and increased ($P < 0.001$) linearly with increasing dietary energy level (Fig. 1). For the 8.02 MJ/kg diet, ADG in yaks was positive, whereas in cattle it was negative. From the regression of ADG on ME intake, the estimated ME_m in yaks was 0.43 MJ/kg $BW^{0.75}$ per d, which was lower ($P < 0.05$) than the 0.57 MJ/kg $BW^{0.75}$ per d in cattle (Fig. 2).

The digestibilities of NDF and ADF were greater ($P < 0.05$) in yaks than in cattle, and both decreased ($P < 0.05$) linearly with increasing dietary energy level (Table 2). There was no difference ($P > 0.10$) in apparent digestibilities of DM, OM, CP and EE between species, and digestibilities of DM, OM, CP and EE increased ($P < 0.05$) linearly with increasing dietary energy level.

As designed, GEI increased linearly with increasing dietary energy level, and there was no difference ($P = 0.836$) between species (Table 3). Faecal energy excretion was lower ($P < 0.001$) in yaks than in cattle and decreased ($P < 0.01$) linearly with increasing dietary energy level; whereas urinary energy output was greater ($P < 0.01$) in yaks than in cattle, and also decreased ($P < 0.001$) linearly with increasing dietary energy level. Estimated methane energy emission was lower in yaks than in cattle ($P < 0.001$), and increased ($P < 0.001$) linearly with increasing dietary energy level. The DEI, MEI, and ratios of DE:GE and ME:DE were greater ($P < 0.05$) in yaks than in cattle and all increased ($P < 0.05$) linearly with increasing dietary energy level.

3.2. Rumen fermentation

Ruminal pH and concentration of total VFAs were greater ($P < 0.05$) in yaks than in cattle. As dietary energy increased, ruminal pH decreased ($P < 0.01$), whereas total VFAs increased ($P < 0.001$) linearly (Table 4). As a proportion of total VFAs, acetate was greater ($P < 0.05$) but propionate and iso-VFAs were lower ($P < 0.05$) in yaks than in cattle, whereas butyrate did not differ ($P = 0.979$) between species. The concentration of acetate decreased ($P < 0.001$), whereas propionate, butyrate and iso-VFAs increased ($P < 0.001$) with increasing dietary energy level. The ratio of acetate: propionate was greater ($P = 0.017$) in yaks than in cattle, and decreased ($P < 0.001$) linearly with increasing dietary energy level. Ruminal ammonia-N concentration was greater ($P = 0.021$) in yaks than in cattle, but there was no difference ($P > 0.10$) between species in free amino acid-N, soluble protein-N and microbial protein-N. These ruminal nitrogen sources for both species all increased ($P < 0.001$) linearly with increasing dietary energy level.

3.3. Serum metabolites

Serum glucose concentration did not differ between animal species ($P > 0.10$) and decreased ($P < 0.001$) linearly with increasing dietary energy level (Table 5). Concentration of serum BHBA was lower (interaction; $P < 0.001$) in yaks than in cattle at 9.42 and 10.80 MJ/kg diets, whereas of NEFA was greater (interaction;

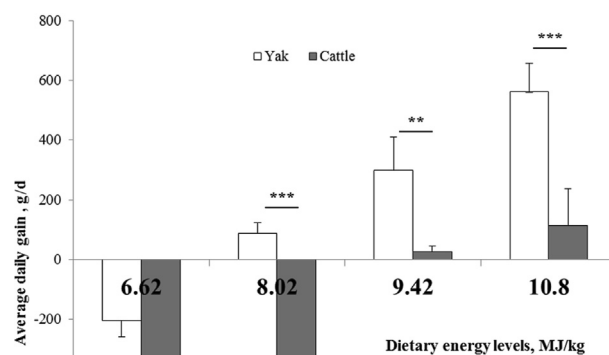


Fig. 1. The average daily gain (ADG) of yaks and Qaidam cattle when offered rations with different energy levels. The *P*-value between animal species, energy level, and their interactions were <0.001 , <0.001 , and <0.01 , respectively. The *P*-values for linear and quadratic effects of dietary energy levels were <0.001 and 0.256, respectively. ** $P < 0.01$, *** $P < 0.001$.

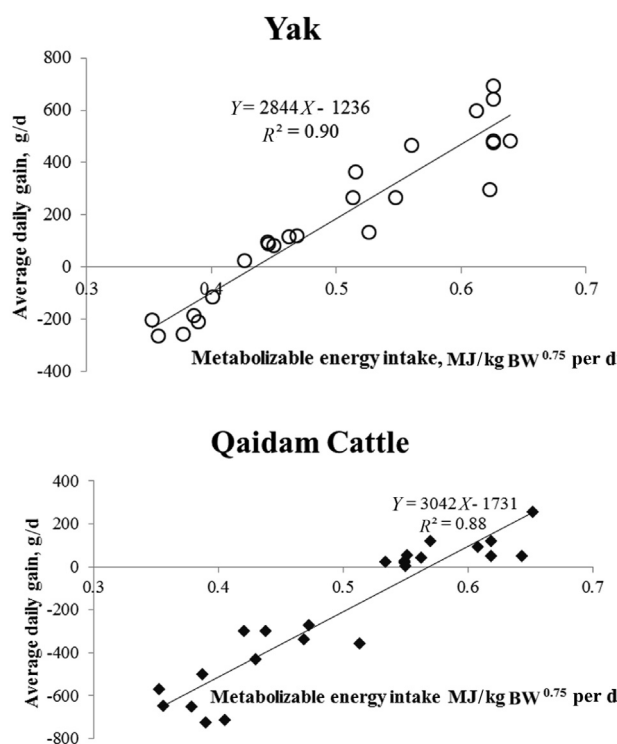


Fig. 2. The linear relationship between metabolizable energy intake (MEI) and average daily gain (ADG) in yaks and Qaidam cattle offered rations with different energy levels. The regression equations were $ADG = 2,844 \text{ MEI} - 1,236$ ($n = 24$, $R^2 = 0.90$) and $ADG = 3,042 \text{ MEI} - 1,731$ ($n = 24$, $R^2 = 0.88$) for yaks and Qaidam cattle, respectively.

$P < 0.01$) in yaks than in cattle at 6.62 and 8.02 MJ/kg diets. As dietary energy level increased, the concentration of serum triglycerides increased ($P < 0.001$) and lactic acid tended to increase ($P = 0.088$) linearly; these 3 variables did not differ ($P > 0.10$) between species.

Concentrations of serum insulin, growth hormone and leptin increased ($P < 0.05$) linearly, whereas glucagon decreased ($P < 0.01$) and triiodothyronine tended to decrease ($P = 0.059$) with

Table 2
Apparent digestibilities of dietary nutrients in yaks and Qaidam cattle offered rations with different energy levels.

Item	Species	Dietary ME levels, MJ/kg				SEM	P-value ¹				
		6.62	8.02	9.42	10.8		S	E	S × E	E-L	E-Q
DM, g/kg	Yak	531	548	588	646	23.4	0.990	<0.01	0.554	<0.001	0.545
	Cattle	539	549	611	603						
OM, g/kg	Yak	581	607	637	684	23.6	0.472	<0.01	0.259	<0.01	0.681
	Cattle	588	596	656	611						
CP, g/kg	Yak	435	461	466	510	11.8	0.383	<0.01	0.994	<0.001	0.709
	Cattle	422	445	463	479						
EE, g/kg	Yak	489	555	581	624	18.3	0.506	<0.01	0.659	<0.001	0.305
	Cattle	499	589	612	610						
NDF, g/kg	Yak	517	478	482	455	11.7	0.047	<0.01	0.897	0.010	0.796
	Cattle	486	479	452	430						
ADF, g/kg	Yak	476	422	415	382	16.8	0.042	<0.01	0.525	<0.01	0.481
	Cattle	426	417	397	350						

ME = metabolizable energy; SEM = standard error of the mean; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber.

¹ S = animal species; E = dietary energy level; E-L = Linear effect of dietary energy levels; E-Q = Quadratic effect of dietary energy levels.

Table 3
Gross, digestible, and metabolizable energy intakes and energy digestibilities in yaks and Qaidam cattle when offered rations with different energy levels.

Item	Species	Dietary ME levels, MJ/kg				SEM	P-value ¹				
		6.62	8.02	9.42	10.8		S	E	S × E	E-L	E-Q
GEI, MJ/d	Yak	44.1	45.2	46.3	47.4	0.14	0.836	<0.001	0.980	<0.001	0.400
	Cattle	44.1	45.2	46.3	47.4						
Faecal energy, MJ/d	Yak	19.9	18.7	17.4	16.3	0.19	<0.001	<0.001	0.631	<0.001	0.642
	Cattle	21.1	20.0	18.9	17.7						
DE intake, MJ/d	Yak	24.2	26.5	29.0	31.0	0.29	<0.001	<0.001	0.945	<0.001	0.999
	Cattle	23.0	25.2	27.4	29.7						
DE/GE, %	Yak	54.9	58.6	62.5	65.5	0.37	<0.001	<0.001	0.755	<0.001	0.825
	Cattle	52.1	56.6	59.2	62.6						
Urinary energy, MJ/d	Yak	1.19	1.08	1.10	1.01	0.03	<0.01	<0.001	0.162	<0.001	0.763
	Cattle	1.04	1.02	0.92	0.86						
Methane Energy, MJ/d	Yak	1.86 ^A	1.91 ^A	1.95 ^A	1.99 ^A	0.01	<0.001	<0.001	0.041	<0.001	0.692
	Cattle	3.60 ^B	3.67 ^B	3.73 ^B	3.79 ^B						
ME intake, MJ/d	Yak	21.2	23.5	25.9	28.0	0.28	<0.001	<0.001	0.689	<0.001	0.912
	Cattle	18.3	20.5	22.8	25.0						
ME/DE, %	Yak	87.2	88.7	89.3	90.3	0.76	<0.001	<0.01	0.658	<0.001	0.712
	Cattle	79.3	81.1	82.6	84.3						

ME = metabolizable energy; SEM = standard error of the mean; GEI = gross energy intake; DE = digestible energy.

^{A,B} Means with different superscript letters in the same column within an item are significantly different from each other ($P < 0.05$).

¹ S = animal species; E = dietary energy level; E-L = Linear effect of dietary energy levels; E-Q = Quadratic effect of dietary energy levels.

increasing dietary energy level for yaks and cattle (Table 6). The concentrations of serum leptin and growth hormone were greater ($P < 0.05$) in yaks than in cattle; whereas serum thyroxine, norepinephrine, and IGF-1 concentrations were not affected ($P > 0.10$) by dietary energy levels and did not differ between animal species.

4. Discussion

4.1. Effect of dietary energy on average daily gain and nutrient digestion

As expected, ADGs of yaks and Qaidam cattle increased with increasing dietary energy level, as was reported in previous ruminant studies (Jing et al., 2019; Tomczak et al., 2019). However, the ADGs were greater in yaks than in cattle across the 4 diets, and was positive in yaks but negative in cattle at the 8.02 MJ/kg diet. Digestion of NDF and ADF and ruminal production of total VFAs were greater, and enteric CH₄ emission was lower in yaks than in cattle; therefore, yaks could allot more energy to growth than cattle, which could explain the difference in ADG between species. From the regression of ADG on MEI, the ME_m for Qaidam cattle was 569 kJ/kg BW^{0.75} per d, which was similar to the 523 kJ/kg

BW^{0.75} per d reported for non-lactating cows (Brosh et al., 2004). The estimated ME_m for yaks in the present study, 435 kJ/kg BW^{0.75} per d was considerably lower than that of Qaidam cattle and was similar to the 458 kJ/kg BW^{0.75} per d in 3-year-old yaks reported by Han et al. (1992). Variations in ME_m are related mainly to basal metabolic rate and the efficiency of utilization of energy for maintenance. Yaks are known for their low metabolic rates (Han et al., 2002), and this could be the main factor in explaining the difference in ME_m between species. Moreover, Ding et al. (2014) reported a ME_m of 545 kJ/kg BW^{0.75} per d for grazing yaks, which was greater than the requirement in the present study. The difference between studies was due to the increased energy expenditure for grazing when compared to confined conditions (Han et al., 1989).

Apparent digestibilities of NDF and ADF in the present study were greater in yaks than in Qaidam cattle, as was observed earlier (Wang, 2009). Studies showed that the relative abundance of ruminal fibrolytic bacteria (Shi et al., 2020) and the activity of fibrolytic enzymes (Zhou et al., 2018) were greater in yaks than in cattle, which could explain the greater fiber digestion. In addition, the size of the rumen relative to omasum was found to be larger in yaks than in lowland cattle, which prolonged the rumen retention time in yaks (Wiener et al., 2003). The apparent digestibilities of

Table 4
Rumen fermentation parameters in yaks and Qaidam cattle offered rations with different energy levels.

Item	Species	Dietary ME levels, MJ/kg				SEM	P-value ¹				
		6.62	8.02	9.42	10.8		S	E	S × E	E-L	E-Q
pH	Yak	7.34	7.21	7.13	7.01	0.13	0.012	<0.01	0.954	<0.01	0.365
	Cattle	7.14	6.97	6.86	6.83						
Total VFA, mmol/L	Yak	65.0	69.2	70.7	75.2	2.09	<0.01	<0.001	0.396	<0.001	0.758
	Cattle	63.1	65.3	68.3	70.1						
VFA, mol/100 mol											
	Acetate	Yak	76.9	74.7	73.4	71.7	0.44	0.042	<0.001	0.973	<0.001
	Cattle	76.1	74.3	72.5	71						
Propionate	Yak	14.1	14.9	15.3	16.0	0.24	0.029	<0.001	0.678	<0.001	0.878
	Cattle	14.5	15.1	16.0	16.8						
Butyrate	Yak	7.65	8.81	9.63	10.6	0.45	0.979	<0.01	0.725	<0.001	0.052
	Cattle	7.91	9.09	9.67	10						
Iso-VFA	Yak	1.40	1.52	1.72	1.71	0.13	0.048	<0.01	0.254	<0.001	0.337
	Cattle	1.49	1.59	1.86	2.24						
Acetate:Propionate	Yak	5.50	5.01	4.84	4.52	0.09	0.017	<0.001	0.620	<0.001	0.318
	Cattle	5.29	4.97	4.57	4.28						
N concentration, mg/100 mL											
	Ammonia-N	Yak	3.46	4.49	6.04	6.87	0.14	0.021	<0.001	0.462	<0.001
	Cattle	3.19	4.08	5.89	6.11						
Free amino acid -N	Yak	9.4	14.5	15.9	18.9	0.81	0.255	<0.001	0.647	<0.001	<0.01
	Cattle	9.2	14.3	15.8	17.3						
Soluble protein-N	Yak	43.5	46.0	48.6	51.7	1.12	0.108	<0.01	0.523	<0.001	0.068
	Cattle	43.1	45.4	47.3	48.4						
Microbial protein -N	Yak	13.0	14.0	15.4	15.6	0.47	0.809	<0.01	0.342	<0.01	0.047
	Cattle	12.9	13.9	15.0	15.1						

ME = metabolizable energy; SEM = standard error of the mean; VFA = volatile fatty acid; N = nitrogen.

¹ S = animal species; E = dietary energy level; E-L = Linear effect of dietary energy levels; E-Q = Quadratic effect of dietary energy levels.

Table 5
Serum metabolite concentrations in yaks and Qaidam cattle offered rations with different energy levels.

Item	Species	Dietary ME levels, MJ/kg				SEM	P-value ¹				
		6.62	8.02	9.42	10.8		S	E	S × E	E-L	E-Q
Glucose, mmol/L	Yak	5.34	5.14	5.04	4.87	0.11	0.140	<0.01	0.591	<0.001	0.427
	Cattle	5.17	5.06	4.89	4.76						
BHBA, μmol/L	Yak	286	264	253 ^A	238 ^A	8.4	<0.01	0.331	0.003	0.163	0.273
	Cattle	270	275	279 ^B	292 ^B						
Triglyceride, mmol/L	Yak	1.80	1.94	2.07	2.11	0.07	0.542	0.005	0.818	<0.001	0.950
	Cattle	1.83	2.01	2.02	2.17						
Lactic acid, mmol/L	Yak	1.17	1.25	1.34	1.44	0.1	0.563	0.097	0.891	0.088	0.983
	Cattle	1.11	1.18	1.23	1.34						
NEFA, mmol/L	Yak	0.74 ^B	0.72 ^B	0.71	0.69	0.02	0.370	0.796	0.037	0.709	0.505
	Cattle	0.67 ^A	0.68 ^A	0.72	0.72						

ME = metabolizable energy; SEM = standard error of the mean; BHBA = β-hydroxybutyrate; NEFA = non-esterified fatty acids.

^{A,B} Means with different superscript letters in the same column within an item are significantly different from each other ($P < 0.05$).

¹ S = animal species; E = dietary energy level; E-L = Linear effect of dietary energy levels; E-Q = Quadratic effect of dietary energy levels.

NDF and ADF decreased linearly in both yaks and cattle as the dietary energy increased, which was consistent with a study in Tibetan sheep (Zhou et al., 2019), but not with crossbred steers (Hales et al., 2014). In the current study, the digestibilities of DM, OM, CP and EE increased linearly with increasing dietary energy level, which was generally in agreement with results in steers (Navarrete et al., 2017), dairy cows (Su et al., 2017), and sheep (Jing et al., 2019). The reason could be attributed to the increase in the proportion of non-fibrous carbohydrates (NFC), which increased with increasing dietary energy level. Dietary NFC is a more fermentable substrate than fibrous matter and, ultimately, improves the digestibilities of nutrients.

4.2. Effect of dietary energy on energy balance

As designed, GEI increased with increasing dietary energy level and there was no difference between bovine species. However, faecal energy output was lower and, consequently, DE intake was greater in yaks than in cattle. As the dietary energy level increased,

faecal energy output decreased, which was due to the reduced dietary NDF content and the increased apparent digestibility, as was reported earlier in cattle (Hales et al., 2014). It was reported that urinary energy excretion was correlated positively with urinary N concentration because urea was the primary energy-generating component in urine (Blaxter et al., 1962; Joseph et al., 1964). However, both urinary N and urea excretion were lower in yaks than in cattle (14.30 g/d vs. 16.65 g/d, unpublished data), whereas urinary energy output was greater. This would suggest that there were other components in urine of yaks, besides urea, that were of greater energy content than in cattle. Urinary energy excretion increased with negative energy balance in the present study, which agreed with a study on heifers (Hemphill et al., 2018). The negative balance resulted in the mobilization of body energy, which increased energy content of the urine.

In the present study, enteric methane energy emission was estimated from prediction equations for cattle (Jiao et al., 2014) and yaks (Bai et al., 2021), which were based on GEI. Methane energy emission was reported to be lower in yaks than in cattle in both *in vivo* (Ding

Table 6
Serum hormone concentrations in yaks and Qaidam cattle offered rations with different energy levels.

Item	Species	Dietary ME levels, MJ/kg				SEM	P-value ¹				
		6.62	8.02	9.42	10.8		S	E	S × E	E-L	E-Q
Insulin, mIU/L	Yak	16.4	17.4	17.9	20.1	1.07	0.827	<0.01	0.512	<0.01	0.322
	Cattle	16.4	17.5	17.1	19.4						
Glucagon, pg/mL	Yak	462	398	376	356	25.9	0.187	<0.01	0.591	<0.01	0.358
	Cattle	419	369	361	342						
Leptin, ng/mL	Yak	17.3	19.7	18.5	17.0	0.99	<0.01	<0.001	0.450	0.997	0.146
	Cattle	14.9	15.5	17.4	14.8						
Growth hormone, ng/mL	Yak	14.2	15.0	15.2	15.6	0.86	<0.01	<0.01	0.159	<0.01	0.396
	Cattle	12.0	12.0	13.1	14.6						
Triiodothyronine, nmol/L	Yak	11.1	10.4	10.0	9.7	0.56	0.848	0.082	0.512	0.059	0.824
	Cattle	10.9	10.6	10.2	9.8						
Thyroxine, nmol/L	Yak	47.6	43.7	48.7	47.6	4.32	0.970	0.298	0.263	0.680	0.950
	Cattle	47.1	50.4	47.8	42.6						
Norepinephrine, ng/mL	Yak	4.70	5.21	4.76	4.48	0.35	0.456	0.359	0.791	0.477	0.344
	Cattle	4.42	4.96	4.61	4.42						
IGF-1, ng/mL	Yak	304	287	352	334	21.0	0.986	0.205	0.384	0.183	0.916
	Cattle	315	320	319	323						

ME = metabolizable energy; SEM = standard error of the mean; IGF-1 = insulin-like growth factor-1.

¹ S = animal species; E = dietary energy level; E-L = Linear effect of dietary energy levels; E-Q = Quadratic effect of dietary energy levels.

et al., 2010; Bai et al., 2021) and *in vitro* studies (Zhang et al., 2016; Mi et al., 2017). In ruminants, methane is produced mainly by methanogenic bacteria in the reduction reaction of carbon dioxide and hydrogen that arises from rumen fermentation (Janssen and Kris, 2008). Yaks harbour a different composition and greater diversity of ruminal methanogen bacteria than Qaidam cattle. For example, the relative abundance of the methylotrophic methanogens in the class Thermoplasmata is greater in yaks than in cattle. This is of particular importance, as this class is characterized by its low methane emission (Huang et al., 2012, 2016; Zhang et al., 2016), which could explain the lower enteric methane emission in yaks than in Qaidam cattle. The ME intake was greater in yaks than cattle, which was attributed mostly to the lower excretion of faecal energy as a consequence of the greater digestion of fibers and production of VFAs in yaks than in cattle. In the present study, the ratio of ME:DE ranged from 0.87 to 0.90 for yaks, which was higher than the generally accepted value of 0.82 (National Research Council, 1976; Garrett, 1980; NASEM, 2016); but was similar to the coefficient reported for Tibetan sheep (Jing et al., 2019). The ratio for Qaidam cattle was lower than for yaks and ranged from 0.79 to 0.84. The ME:DE ratio depends on energy loss in enteric CH₄ and urine energy loss. The energy loss due to CH₄ emission was estimated to be lower in yaks than in cattle, which could explain the higher ME:DE ratios in yaks. The ratio of ME:DE increased as the proportion of dietary concentrate increased, as was also reported in growing beef steers (Fuller et al., 2020; Jennings et al., 2018; Hales et al., 2013, 2014).

4.3. Effect of dietary energy on rumen fermentation parameters

Ruminal pH ranged from 7.01 to 7.34 for yaks and 6.83 to 7.14 for Qaidam cattle. Thus, cattle were within the optimal range of 6.2 to 7.2 for ruminants (Van Soest, 2018), whereas yaks were within or slightly above. Ruminal pH is affected mainly by the concentration of VFAs and ammonia and by saliva secretion. Zhou et al. (2017) reported that the amount of endogenous urea recycled into the gut, which is then hydrolyzed to ammonia, was greater in yaks than cattle. In addition, in the RNA transcriptomes of the ruminal epithelium, yaks upregulated 36 genes associated with VFA transport and absorption compared to cattle (Zhang et al., 2016), which enhanced VFA absorption and reduced VFA accumulation. These differences between species could explain, at least in part, the higher ruminal pH in yaks than in cattle. In the present study, ruminal pH decreased linearly with increasing energy level because the concentration of ruminal VFA increased, and it is likely that

saliva secretion lessened as the ratio of concentrate to roughage increased (Lu et al., 2019; Wang et al., 2019).

More than 70% of the energy supplied to the ruminant is provided by VFAs, which originate mainly from dietary carbohydrates (Zanton and Heinrichs, 2009). When consuming the same diet, yaks produced greater concentrations of ruminal VFAs than cattle *in vivo* (Huang et al., 2012) and also *in vitro* (Zhang et al., 2016) when the same substrate was used with rumen fluid. A previous study, using ultra-deep metagenomic sequencing, demonstrated that the VFA-yielding pathways of rumen microbial genes were greater in yaks than in cattle (Zhang et al., 2016). Moreover, the yak rumen microbiome possesses enriched enzymes for the production of VFAs in the carbon fixation pathways of prokaryotes (Zhang et al., 2016; Jing et al., 2022). These findings could explain, at least in part, the greater ruminal VFA concentration in yaks than in cattle. In this study, ruminal VFA concentration increased with increasing dietary energy due to the increase in NFC, which is in agreement with previous studies in ruminants (Jing et al., 2020; Liu et al., 2021). It was reported that acetate production was associated with fiber digestion, whereas propionate was related to dietary concentrate intake (Dórea et al., 2020). Consequently, the proportion of acetate decreased, whereas propionate and butyrate increased as fiber content decreased with increasing dietary energy level. Furthermore, the greater proportion of acetate in yaks than in cattle could be explained by the higher fiber digestibility in yaks. Zhou et al. (2018) also reported a greater proportion of acetate in yaks than in Qaidam yellow cattle when offered forage-concentrate diets differing in nitrogen concentration. The reason was likely related to a greater abundance of fibrolytic bacteria, such as Ruminococcaceae_NK4A214_group, uncultured_bacterium_f_Muribaculaceae, and Christensenellaceae_R-7_group, harboured in the rumen of yaks than in cattle (Guo et al., 2020; Hu et al., 2021). However, the relative abundance of Succinivibrionaceae_UCG-001, considered predominantly as an amylolytic bacteria, was greater in cattle than in yaks, which resulted in a higher concentration of propionate in cattle than in yaks (Hu et al., 2021; Jing et al., 2022). The iso-VFAs, derived from branched amino acids (Apajalahti et al., 2019), increased with increasing dietary energy level. This suggested that there was more degradable CP in the rumen as dietary energy increased, which was consistent with the CP digestibility. The concentration of ruminal iso-VFAs was lower in yaks than in cattle, which suggested that there were greater amounts of branched amino acids deaminated in cattle than in yaks.

Ruminal ammonia-N is the major N source for the growth of microbial bacteria, especially for *Ruminobacter amylophilus* and *Ruminococcus ablus* (Kim et al., 2014). The optimal concentration of ruminal ammonia-N for microbial growth was reported to range from 5 to 25 mg/100 mL (Preston and Leng, 1987). In the present study, ammonia-N concentrations were below the threshold in both yaks and cattle for the 6.62 and 8.02 MJ/kg diets, which suggested that microbial growth and activities were inhibited. However, ruminal ammonia concentration was greater in yaks than in cattle, which indicated higher microbial protein synthesis in yaks. A previous study demonstrated that an increase in the degree of synchronization in the release of energy and nitrogen into the rumen led to a higher level of microbial protein synthesis, which was accompanied by an increase in *Fibrobacter* and *Ruminobacter* and a decrease in *Klebsiella* and *Succinivibrio* (Zhang et al., 2020). It was reported that yaks harbour a greater relative abundance of *Fibrobacter* and *Ruminobacter* (Mi et al., 2017; Huang et al., 2021) and a lower relative abundance of *Succinivibrio* (Hu et al., 2021), which implies a higher level of microbial protein synthesis in yaks than in cattle. As the dietary energy content increased, ruminal concentrations of free amino acid-N, soluble protein-N and microbial protein-N increased linearly, which may be related to the increased crude protein digestibility and more nitrogen available for rumen bacteria (Zhou et al., 2019).

4.4. Effect of dietary energy on serum metabolite concentrations

Blood metabolite indices reflect the metabolic and health status of livestock. Serum glucose concentrations in the present study were within the normal range in both yaks (3.9 to 5.3 mmol/L; Wang, 2015) and cattle (4.3 to 6.3 mmol/L; Liu et al., 2015). Previous studies showed that a decline in serum glucose concentration occurred due to insufficient energy intake (Jing et al., 2019; Wang et al., 2020). However, serum glucose concentration decreased with increasing dietary energy level in yaks and Qaidam cattle, although ADG increased. This could be explained by the increase in concentration of serum insulin and the decrease in the concentration of glucagon with increasing dietary energy level. Serum NEFA concentration reflects the mobilization of fat, whereas BHBA reflects the completeness of oxidization of fatty acids in the liver (De Koster and Opsomer, 2013). Triglycerides, a vital energy source for cells synthesized by NEFA, and glycerol (Hagenfeldt et al., 1975) increased with increasing dietary energy level in the present study, which was in agreement with findings in dairy cattle (Canfield and Butler, 1990). Mobilization of NEFA from adipose tissue increases the NEFA concentrations in blood when energy intake is below maintenance requirements in ruminants (Bell, 1995). In the present study, serum NEFA concentrations were greater in yaks than in cattle at 6.62 and 8.02 MJ/kg diets, and then decreased in yaks but increased in cattle with an increase in dietary energy level, which was similar to a study in Tibetan sheep and small tailed Han sheep (Jing et al., 2019). It is likely that Qaidam cattle mobilized more fat than yaks initially and then mobilized protein to provide the required energy. Serum BHBA concentration, which increases with the oxidization of fatty acids and the accumulation of ketone bodies in ruminants, has been used to assess the energy status of livestock (Grelet et al., 2019). In the present study, yaks had lower concentrations of serum BHBA than cattle at diets of 9.42 and 10.80 MJ/kg. A concentration of serum BHBA between 0.8 and 1.5 mmol/L is considered as moderately ketotic and at ≥ 1.6 mmol/L as severely ketotic in ewes (Pichler et al., 2014). In present study, serum BHBA ranged from 0.24 to 0.29 mmol/L for yaks and between 0.27 and 0.29 mmol/L for cattle, which were all well below subclinical levels of ketosis, although there was a loss in body weight in the low energy level diets.

Leptin inhibits fat synthesis and stimulates energy expenditure (Myers et al., 2008), and was correlated positively with dietary energy level (Chelikani et al., 2009) and ADG (Nkrumah et al., 2005). In the present study, serum leptin concentration was greater in yaks than in cattle, which was consistent with greater ADG and lower fat synthesis in yaks than in cattle. Growth hormone, a pivotal hormone that regulates growth performance in growing animals, was correlated positively with ADG in yaks (Sarkar et al., 2007). The serum GH concentration was greater in yaks than in cattle and increased linearly with increasing dietary energy level, which was consistent with the changes in ADG in the 2 species.

5. Conclusions

The ADG, NDF and ADF digestibilities, and the ratios of DE:GE and ME:DE were greater in yaks than in cattle. Furthermore, total ruminal VFA concentration, serum leptin and growth hormone concentrations were also greater in yaks than in cattle, but the estimated energy requirements for maintenance were lower in yaks than in Qaidam cattle. We concluded that yaks could utilize low energy diets better than cattle and that this ability confers an advantage to yaks over cattle during the long winter on the Qinghai-Tibetan Plateau when only sparse forage of poor quality is available.

Author contributions

Hu Liu: Conceptualization, Methodology, Investigation, Writing – Original draft preparation. **Jianwei Zhou:** Resources, Writing – Review and Editing, Funding acquisition. **Allan Degen:** Writing – Review and Editing. **Hongshan Liu:** Investigation. **Xuliang Cao:** Investigation. **Lizhuang Hao:** Resources. **Tao Ran:** Writing – Review and Editing. **Zhanhuan Shang:** Project administration. **Ruijun Long:** Project administration, Supervision, Investigation, Resources, Funding acquisition.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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