

Relationship between chemical composition of native forage and nutrient digestibility by Tibetan sheep on the Qinghai–Tibetan Plateau

Chuntao Yang,* Peng Gao,* Fujiang Hou,*¹ Tianhai Yan,[†] Shenghua Chang,*
Xianjiang Chen,* and Zhaofeng Wang*

*State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China; and [†]Sustainable Agri-Food Sciences Division, Agriculture Branch, Agri-Food and Biosciences Institute, Hillsborough, County Down, BT26 6DR, United Kingdom

ABSTRACT: To better utilize native pasture at the high altitude region, three-consecutive-year feeding experiments and a total of seven metabolism trials were conducted to evaluate the impact of three forage stages of maturity on the chemical composition, nutrient digestibility, and energy metabolism of native forage in Tibetan sheep on the Qinghai–Tibetan Plateau (QTP). Forages were harvested from June to July, August to October, and November to December of 2011 to 2013, corresponding to the vegetative, bloom, and senescent stages of the annual forages. Twenty male Tibetan sheep were selected for each study and fed native forage ad libitum. The digestibility of DM, OM, CP, NDF, ADF, DE, DE/GE, and ME/GE were greatest ($P < 0.01$) from the vegetative stage, intermediate ($P < 0.01$) from the bloom stage, and least ($P < 0.01$) from the senescent stage. Nutrient digestibility and energy parameters correlated positively (linear, 0.422 to 0.778; quadratic, 0.568 to 0.815; $P < 0.01$) with the CP content of forage but correlated negatively with the content of NDF (linear, 0.343 to 0.689; quadratic, 0.444 to 0.777; $P \leq 0.02$), ADF (linear, 0.563 to 0.766; quadratic, 0.582 to 0.770;

$P < 0.01$), and ether extract (EE, linear, 0.283 to 0.574; quadratic, 0.366 to 0.718; $P \leq 0.04$) of forage. For each predicted variable, the prediction of DMI expressed as grams per kilogram of BW (g/kg BW·d) yielded a greater R^2 value (0.677 to 0.761 vs. 0.616 to 0.711) compared with the equations of DMI expressed as g/kg metabolic BW by step-wise regression. The results suggest that parameters of forage CP, NDF, and ADF content were most closely related to nutrient digestibility. Contrary to previous studies, in this study, ADF content had a greater linear relationship (0.766 vs. 0.563 to 0.732) with OM digestibility than the other parameters of nutrient digestibility. The quadratic relationship between forage CP content and CP digestibility indicates that when forage CP content exceeds the peak point (9.7% DM in the present study), increasing forage CP content could decrease CP digestibility when Tibetan sheep were offered native forage alone on the QTP. Additionally, using the forage CP, EE, NDF, and ADF content to predict DMI (g/kg BW·d) yielded the best fit equation for Tibetan sheep living in the northeast portion of the QTP.

Key words: chemical composition, correlation analysis, digestibility, native forage, sheep

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¹Corresponding author: cyhoufj@lzu.edu.cn

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INTRODUCTION

Grasslands represent one of Earth's main biological communities and cover approximately 20% of the total land surface (Wang et al., 2009a), and contribute substantially to global agricultural production (Campos et al., 2016). Qinghai–Tibetan Plateau (QTP) is the largest grassland ecosystem in

Eurasia and spans an area of approximately 2.5 million km² (Cai et al., 2014) and are the source of the headwaters of major rivers of East Asia, Southeast Asia, and South Asia, including the Yangtze, Yellow, Ganges, Indus, and Mekong rivers. The growth dynamics and productivity of native forage in QTP are strongly influenced and restricted by the region's climate (Wang et al., 2009a), which affects livestock productivity. There is little information concerning seasonal effects on the chemical composition, nutrient digestibility, and energy metabolism of the native forage at high altitudes in this region. Moreover, the prediction of DMI for livestock on the QTP has not been reported. Tibetan sheep (*Ovis aries*) are one of the most important livestock species on the QTP, with over 50 million animals providing meat, milk, and income for most inhabitants of the plateau (Xin et al., 2011). However, there is a relative paucity of data on forage nutrient utilization of Tibetan sheep compared with Hu sheep (Lin et al., 2013), Mongolian sheep (Wang et al., 2009b), and Dorper sheep (De Waal and Combrinck, 2000), among others. Hence, this research was designed to investigate the impact of chemical composition of native forage on growth performance, nutrient digestibility, and energy metabolism of Tibetan sheep during the three major periods of forage growth and developed equations to predict native forage DMI of this sheep on the QTP.

MATERIALS AND METHODS

All trial procedure strictly followed the rules and regulations of experimental field management protocols (file No: 2010–1 and 2010–2), which were approved by Lanzhou University.

Study Site and Vegetation

The research site is located in Maqu County, Gansu Province, China (33°06′ to 34°33′N, 100°46′ to 102°29′E; elevation 3,500 m), which is in the

northeast portion of the QTP and has a climate typical of Asian plateau meadows. A mean annual temperature of 2.6 °C and rainfall of 678 mm were recorded at the local Agro-meteorological Information Station during the experimental period (Figure 1). The climate is the continental cold/humid type, with the rainy season in summer and dry season in winter.

In the general area, the vegetation consists of typical alpine meadows. The primary foliage in the field is perennial herbaceous and comprised of *Kobresia* spp. (e.g., *K. graminifolia*, *K. capillifolia*, *K. humilis*, *K. Tibetica*), *Elymus* sp. (e.g., *E. nutans*), *Potentilla* L. sp. (e.g., *P. anserina*), *Stipa* spp. (e.g., *S. aliena*), *Festuca* spp. (e.g., *F. ovina*), and various other species.

Forage, Animal, and Experimental Design

Native forage was harvested daily from June to July of 2012 and 2013 for the vegetative stage (VS), August to October of 2011, 2012, and 2013 for the bloom stage (BS), and November to December of 2011 and 2012 for the senescent stage (SS). In the trial period, forage was harvested at 1700 h daily by cutting 5.0 cm above ground level using a forage harvester with a sickle bar mower, and the forage was chopped into approximately 10-cm lengths and stored at 4 °C before to feeding to the experimental animals. The forage was rotationally harvested from three plots within the experimental fields. Each plot was harvested and fed to the sheep for a week. Forage was sampled daily, at the last day of each week the seven samples were mixed completely and a sample was taken for chemical analysis.

Every year, 20 male Tibetan sheep (25.9 ± 3.4 kg BW) with average age of 1 yr were used and placed individually in stainless steel pens (3.2 by 0.8 m²) on a slatted floor to conduct the evaluations of the different maturity stages of forage. All sheep were fed indoors with natural light. Temperature was similar

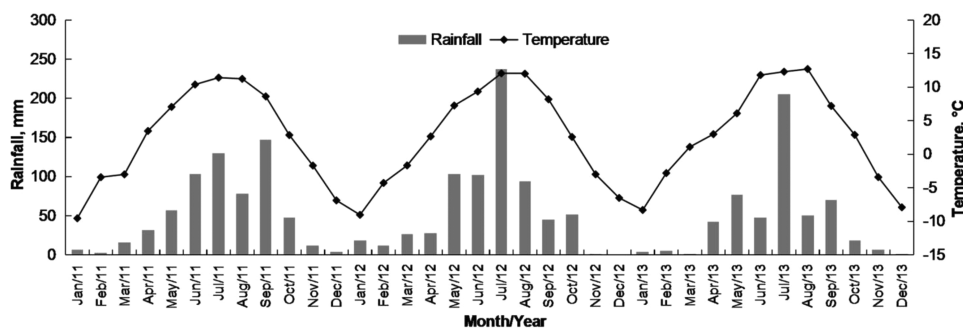


Figure 1. Data of rainfall and temperature for the research site located in Maqu County from 2011 to 2013. Temperature, average 2.6 °C, high 12.7 °C, low -9.5 °C; Rainfall, average 52.4 mm, high 237.1 mm, low 0.1 mm.

between the pens. Native forage was provided ad libitum with 5% refusal as a basal diet throughout the trial. Animals had free access to fresh water and block mineral lick (guaranteed value (per kg): 8.07 g Zn, 0.60 g Cu, 5.53 g Fe, 0.36 g Mn, 0.45 g Co, 0.70 g K, 1.83 g Se, 5.00 g Mg, 284.66 g Na, and 38.23 g Ca; Cangzhou Leysin Biotechnology Co. Ltd). During each period of forage, the initial and final BW of each sheep were recorded to calculate ADG. The weight of native forage that was supplied to each animal, as well as any orts, were recorded to calculate DMI of forage. Gain-to-feed ratio (**G:F**) was calculated as the proportion of ADG to DMI.

Each year, six sheep were selected from the flock of 20 sheep to obtain accurate measures of forage nutrient digestibility. A total of seven metabolism trials, one for each forage maturity period, were conducted from 2011 to 2013. During the experimental period, sheep were offered the experimental forage in individual pens and then placed individually in specialized metabolism cages for 10 d with total feed intake and feces and urine output collected during the final 7 d. The metabolism cages were located near by the pens indoors. Native forage and fresh water were provided ad libitum throughout the trial. Total feces from each sheep were collected, weighed, mixed, and sampled twice daily (0900 and 1700 h). Representative samples (100 g each) were mixed with 10 mL of 10% (wt/wt) hydrochloric acid and stored in a sterile plastic bag. Urine was collected twice daily (0900 and 1700 h) in a plastic bucket placed under the metabolism cage. A sample (1% of total urine) was filtered through two layers of gauze into a plastic bottle, and the pH of the filtered urine was adjusted to <3 with 10% (wt/wt) hydrochloric acid. The weight of forage that was supplied to each animal, as well as any orts, were recorded to calculate forage intake, and the samples were collected daily before the morning feeding during the metabolism trial. Forage, orts, feces, and urine samples were pooled for each sheep after the collection period and stored at -20 °C until analysis.

Chemical Analysis

Samples of forage and feces were dried at 60 °C for 24 h in a forced-air drying oven and ground to pass through a 1-mm screen. The ground samples were dried in a forced-air oven at 135 °C for 2 h to calculate DM of forage and feces (method 930.15; AOAC 1990). Organic matter was measured as the difference between DM and ash content (method

938.08; AOAC 1990). Gross energy was measured by bomb calorimetry (6400, PARR Inc., Moline, IL, USA). Total N content was determined by the Kjeldahl method using selenium as a catalyst and CP was calculated as $6.25 \times N$ (Ma et al., 2015). Ether extract (EE) was measured by weight loss of the DM on extraction with diethyl ether in an extractor (XT-15, ANKOM, Macedon, NY, USA). Neutral detergent fiber and ADF were determined according to Van Soest et al. (1991) and Goering and Van Soest (1970), respectively. Urinary total N and urinary GE were determined as described above. Before GE measurements, a 10-mL sample of urine was absorbed by a piece of quantitative filter paper (Grade 3, Whatman International Ltd., Kent, UK) and the paper was then oven-dried at 55 °C for 30 min. The urine GE content was calculated as the difference in GE contents between filter papers with and without urine samples (Deng et al., 2012).

Digestible energy, ME, and digestibility of each of DM, OM, CP, EE, NDF, and ADF were calculated based on the metabolism trials for each of the three maturity stages. Methane energy was calculated as 7.5% of GE based on the results of Deng et al. (2014).

Statistical Analysis

Data for chemical composition, growth performance, nutrient digestibility, and energy parameters of forage were analyzed using the GLM procedures of SAS (version 9.2, SAS Institute Inc., Cary, NC, USA) based on the statistical model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$$

where Y_{ij} = dependent variable of the ij th forage or sheep, μ = overall mean, α_i = fixed effect of the i th year ($i = 2011, 2012, 2013$), β_j = fixed effect of the j th stage of forage maturity ($j = VS, BS, SS$), and e_{ij} = residual error. Significance was declared at $P < 0.05$, and the comparison of least square means was carried out using the PDIF option.

Correlations between nutrient digestibility and energy parameters of forage and forage chemical composition were implemented with linear and quadratic regression analyses, using the REG procedure of SAS. The residual diagnostics were assessed using normality plots. To evaluate the effect of chemical composition of forage on DMI of sheep, step-wise multiple linear regression analysis with backward elimination was carried out to yield prediction equations as

well as the equations for linear regression (equation I) and quadratic regression (equation II), as shown below:

$$Y = a + b_1x_1 + b_2x_2 + b_3x_3 + \dots + b_nx_n \quad (\text{I})$$

$$Y = a + b_1x_1 + c_1x_1^2 + b_2x_2 + c_2x_2^2 + b_3x_3 + c_3x_3^2 + \dots + b_nx_n + c_nx_n^2. \quad (\text{II})$$

Significant differences of the effect of the linear, squared term, and each individual predictor were accepted at $P < 0.05$.

RESULTS

Chemical Composition of Forage and Growth Performance of Sheep

Except for GE and EE content, which did not differ ($P \geq 0.18$) across the stages of maturity, chemical composition (i.e., DM, OM, CP, NDF, ADF) of forage was affected by stage of maturity ($P \leq 0.02$; Table 1). The SS forage had a mean DM content of 88.7%, which is approximately 2.3-fold of the lowest value measured from the BS ($P < 0.01$). Forage CP content decreased over the stages of maturity ($P < 0.01$), whereas both NDF and ADF increased ($P < 0.01$). Forage OM of SS was greatest, intermediate from the BS, and lowest

from the VS, with differences observed between VS and SS ($P = 0.02$).

The ADG of Tibetan sheep ranged from 103 to 112 g/d during the warm season (from VS and BS; Table 2), but the values were negative (-62.7 g/d) when the SS forages were offered. The DMI (g/d) and DMI (g/kgW^{0.75}·d) decreased with increasing maturity of forage ($P < 0.01$). Similar to the ADG, G:F of Tibetan sheep was greater from the VS and BS compared with the SS ($P < 0.01$).

Nutrient Digestibility and Energy Metabolism

In line with the observed large variation in chemical composition of forage, nutrient digestibility ($P \leq 0.02$; Table 3) and energy parameters ($P < 0.01$; Table 4) also varied greatly. The digestibility of DM, OM, CP, NDF, and ADF were greatest from the VS, intermediate from the BS, and lowest from the SS ($P \leq 0.02$). The EE digestibility decreased with increasing forage maturity, with differences observed among VS, BS, and SS ($P < 0.01$).

Similar findings were observed for forage GE intake, DE, ME, apparent digestibility of energy (DE/GE), and ME/GE, with greatest from the VS, intermediate from the BS, and least from the SS ($P < 0.01$). Urine energy (UE) and ME/DE were greater ($P < 0.01$) from VS and BS compared with SS. However, fecal energy (FE) of BS was greater than VS and SS ($P \leq 0.01$).

Table 1. Effects of stage of maturity on chemical composition of native forage

Item	Stage of maturity, % of DM			SEM	P value
	VS	BS	SS		
OM	93.8 ^b	94.2 ^{ab}	94.5 ^a	0.31	0.016
CP	9.58 ^a	6.14 ^b	3.02 ^c	0.414	<0.001
GE, MJ/kg	18.0	17.8	18.1	1.59	0.175
EE	2.91	3.16	3.05	0.316	0.291
NDF	61.2 ^b	61.5 ^b	75.5 ^a	1.46	<0.001
ADF	28.9 ^c	30.9 ^b	36.3 ^a	0.65	<0.001

^{a-c}Mean within a row with different superscripts differ ($P < 0.05$).

Table 2. Effects of stage of maturity on growth performance of Tibetan sheep ($n = 20$)

Item	Stage of maturity			SEM	P value
	VS	BS	SS		
DMI, g/d	994 ^a	880 ^b	602 ^c	31.3	<0.001
DMI, g/kgW ^{0.75} ·d	98.8 ^a	81.2 ^b	43.6 ^c	3.42	<0.001
ADG, g/d	112 ^a	103 ^a	-62.7 ^b	14.81	<0.001
G:F*, g/kg	119 ^a	116 ^a	-78.6 ^b	18.23	<0.001

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

*G:F = gain-to-feed intake ratio (ADG/DMI).

Table 3. Effects of stage of maturity on nutrient digestibility of forage in Tibetan sheep ($n = 6$)

Item	Stage of maturity, % of DM			SEM	P value
	VS	BS	SS		
DM	76.1 ^a	65.7 ^b	52.2 ^c	1.49	<0.001
OM	77.2 ^a	68.8 ^b	57.2 ^c	1.22	<0.001
CP	75.9 ^a	61.6 ^b	21.0 ^c	2.99	<0.001
EE	42.0 ^a	40.0 ^a	31.4 ^b	1.13	<0.001
NDF	71.5 ^a	60.0 ^b	51.1 ^c	1.33	0.018
ADF	67.3 ^a	61.8 ^b	48.4 ^c	1.45	<0.001

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

Table 4. Effects of stage of maturity on energy metabolism (MJ/kg·W^{0.75}) of forage in Tibetan sheep ($n = 6$)

Item	Stage of maturity			SEM	P value
	VS	BS	SS		
GE intake	1.81 ^a	1.42 ^b	0.78 ^c	0.065	<0.001
FE	0.41 ^b	0.47 ^a	0.40 ^b	0.010	0.005
UE	0.03 ^a	0.02 ^a	0.01 ^b	0.001	<0.001
DE	1.40 ^a	0.95 ^b	0.37 ^c	0.064	<0.001
ME	1.23 ^a	0.81 ^b	0.30 ^c	0.058	<0.001
DE/GE, %	76 ^a	67 ^b	53 ^c	1.6	<0.001
ME/GE, %	67 ^a	57 ^b	43 ^c	1.8	0.001
ME/DE, %	88 ^a	86 ^a	81 ^b	1.9	0.002

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

Relationships Between Forage Chemical Composition and Nutrient Digestibility and Energy Parameters

Table 5 presents the correlation coefficients for linear and quadratic relationships between forage chemical composition and nutrient digestibility or energy parameters. Figure 2 shows representative examples of linear and quadratic relationships between forage chemical composition and digestibility or energy parameters.

There were no linear relationships between EE content and digestibility of CP, EE, and ADF and ME/DE ($P \geq 0.073$), but the remaining linear and all quadratic relationships between EE content and digestibility of different nutrients and energy parameters were negative (r , 0.283 to 0.574; $P \leq 0.043$). Furthermore, concentrations of NDF (r_{linear} , 0.343 to 0.689; $r_{\text{quadratic}}$, 0.444 to 0.777; $P \leq 0.016$) and ADF (r_{linear} , 0.563 to 0.766; $r_{\text{quadratic}}$, 0.582 to 0.770; $P < 0.001$) were correlated negatively (both linear and quadratic) with nutrient digestibilities and energy parameters with the exception of ME/DE, which was positively correlated (linear, $r_{\text{NDF}} = 0.557$ and $r_{\text{ADF}} = 0.629$; quadratic, $r_{\text{NDF}} = 0.626$ and $r_{\text{ADF}} = 0.644$; $P < 0.01$). Concentrations of CP were correlated positively (both linear and quadratic) with nutrient digestibilities and energy parameters

($P \leq 0.003$). Comparing the degree of the above relationships revealed greater correlation coefficients were for contents of CP (linear, 0.422 to 0.778; quadratic, 0.568 to 0.815), NDF (linear, 0.343 to 0.689; quadratic, 0.444 to 0.777), and ADF (linear, 0.563 to 0.766; quadratic, 0.582 to 0.770) when compared with EE content (linear, 0.283 to 0.574; quadratic, 0.366 to 0.718).

Equations for Predicting DMI

The addition of NDF as a predictor for the linear relationship or replacing CP with NDF for the quadratic relationship further improved the R^2 of equations for DMI expressed as gram per kilogram of metabolic weight (g/kgW^{0.75}·d), but the lowest R^2 of the linear prediction was still markedly greater than the greatest R^2 of the quadratic prediction (0.696 vs. 0.618; Table 6). In comparison with the prediction of DMI (g/kgW^{0.75}·d), however, the prediction of DMI expressed as grams per kilogram of BW (g/kg BW·d) using forage chemical composition yielded a greater R^2 (0.677 to 0.761 vs. 0.616 to 0.711). The best fit in the regression of DMI (g/kg BW·d) was achieved when forage CP, NDF, and ADF content were used as explanatory variables in each linear relation. Moreover, a relatively greater R^2 for the quadratic regressions was obtained for

Table 5. Correlation coefficients of linear (Lin) and quadratic (Quad) relationships between nutrient digestibility or energy parameters and forage chemical composition

Item*	Forage chemical composition							
	CP		EE		NDF		ADF	
	Lin	Quad	Lin	Quad	Lin	Quad	Lin	Quad
Nutrient digestibility								
DM	0.674	0.722	-0.425	-0.597	-0.572	-0.638	-0.659	-0.659
OM	0.676	0.716	-0.355	-0.598	-0.482	-0.525	-0.766	-0.768
CP	0.641	0.815	—	-0.535	-0.689	-0.777	-0.635	-0.637
EE	0.453	0.656	—	-0.366	-0.444	-0.444	-0.642	-0.712
NDF	0.436	0.568	-0.574	-0.718	-0.636	-0.636	-0.563	-0.582
ADF	0.422	0.632	—	-0.614	-0.615	-0.617	-0.732	-0.770
Energy parameter								
GE intake	0.745	0.754	-0.283	-0.421	-0.347	-0.466	-0.630	-0.632
DE intake	0.647	0.672	-0.387	-0.569	-0.471	-0.556	-0.613	-0.630
DE	0.776	0.780	-0.351	-0.503	-0.345	-0.481	-0.633	-0.634
ME	0.778	0.782	-0.358	-0.512	-0.343	-0.482	-0.630	-0.632
DE/GE	0.656	0.708	-0.343	-0.602	-0.518	-0.597	-0.672	-0.692
ME/GE	0.658	0.711	-0.345	-0.603	-0.515	-0.597	-0.668	-0.687
ME/DE	0.573	0.681	—	-0.549	0.557	0.626	0.629	0.644

*Empty cells represent nonsignificant effect in the relation. The *P* values of linear relationship between EE content and digestibility of CP, EE, and ADF and ME/DE were 0.075, 0.457, 0.073, and 0.175, respectively. The *P* values for the remaining values were <0.05.

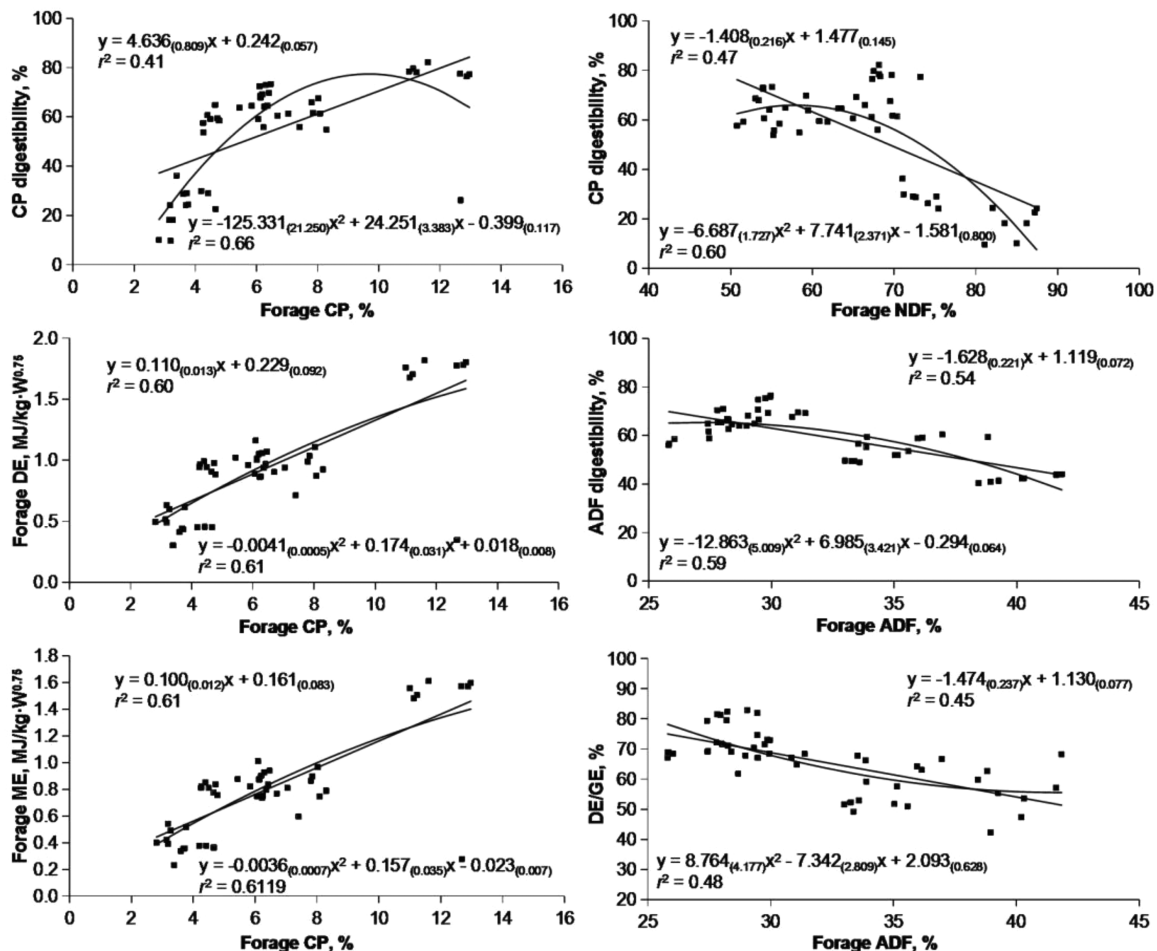


Figure 2. Relationship between nutrient digestibility parameters and chemical composition of forage fed to Tibetan sheep living on the Qinghai–Tibetan Plateau.

Table 6. Linear and quadratic prediction of dry matter intake of Tibetan sheep using forage chemical composition parameters

Item	Equation*	R ²
DMI, g/kg BW·d	= $-1,920.199_{(739.086)} + 1,106.754_{(481.876)} EE - 184.863_{(78.452)} EE^2 + 7.280_{(1.114)} NDF - 0.054_{(0.008)} NDF^2 + 5.557_{(3.175)} ADF - 0.112_{(0.047)} ADF^2$	0.761
	= $60.071_{(7.918)} + 2.152_{(0.316)} CP - 0.294_{(0.092)} NDF - 0.622_{(0.227)} ADF$	0.727
	= $45.707_{(6.336)} + 2.517_{(0.307)} CP - 0.413_{(0.087)} NDF$	0.682
	= $-2,109.230_{(332.901)} + 207.427_{(34.498)} GE - 5.311_{(0.904)} GE^2 + 4.750_{(1.124)} NDF - 0.040_{(0.008)} NDF^2$	0.677
DMI, g/kg W ^{0.75} ·d	= $122.758_{(16.785)} + 4.393_{(0.671)} CP - 0.294_{(0.095)} NDF - 1.686_{(0.482)} ADF$	0.711
	= $114.994_{(16.187)} + 4.270_{(0.675)} CP - 2.028_{(0.430)} ADF$	0.696
	= $-1,650.116_{(760.302)} + 13.016_{(3.876)} CP - 0.509_{(0.024)} CP^2 + 175.474_{(80.103)} GE - 4.589_{(0.210)} GE^2$	0.618
	= $-4,560.568_{(746.802)} + 449.827_{(77.389)} GE - 11.524_{(2.027)} GE^2 + 9.760_{(2.522)} NDF - 0.081_{(0.019)} NDF^2$	0.616

*Units: % of DM for forage chemical composition of OM, CP, EE, NDF, and ADF; MJ/kg of DM for forage GE. Values in subscripted parentheses represent SEM.

the prediction of DMI (g/kg BW·d) using EE, NDF, and ADF compared with linear regressions.

DISCUSSION

Forage Nutrient Content and Sheep Growth Performance

Similar to the results of Wang et al. (2009b) and Campos et al. (2016), the ability of Tibetan sheep to utilize the nutrients in QTP forage was a direct consequence of their physiological state at the time (i.e., from different forage stages of maturity), which, of course, depended on local weather conditions. In the study, the DM content was maximal (88.7%) in the SS. This is because the low proportion of forage leaves to stems during the cold season decreased palatability of the pasture forage (Kandel et al., 2013) and decreased forage DMI of the Tibetan sheep as with previous research (Sun et al., 2015). Moreover, the combined effects of low CP content and high NDF and ADF content of forage from the SS led to decreased growth performance of sheep (low ADG and G:F). Forage CP content is the most limiting nutrient parameter (Ephrem et al., 2015), and CP content of native pasture hay falls below the minimum threshold level (7.0%) that would suppress rumen microbial activity and reduce the forage intake (Van Soest, 1994; McDonald et al., 2002). However, the CP content of forage in the study varied from 6.1% to 9.6% from the VS and BS, which improved the ADG of Tibetan sheep (from 103 to 112 g/d), and implies that the CP of these native forage can meet the basic requirements of rumen microbial activity during the warm season on the QTP. The NDF and ADF content of forage also are major factors affecting feeding intake and feed conversion efficiency of ruminants, which in turn

are believed to be important factors that influence animal performance (McDonald et al., 2002; Ephrem et al., 2015). In the present study, the CP, NDF, and ADF content of forage varied over the three stages of maturity. A possible explanation is that the observed lower temperature and rainfall amount during the cold season compared with the warm season may have reduced the yield and quality of pasture on the QTP (Xue et al., 2009). Hence, the nutrient composition of native forage is crucial for the assessment of the livestock production and productivity. Therefore, it is vital, based on chemical composition of forage, to select the appropriate time to market livestock to maximize both the economic return for smallholder farmers on the QTP and the export value for the country.

Effect of Forage Chemical Composition on Nutrient Digestibility

Various interrelated factors influence the digestibility of forage (Hughes et al., 2014). This research found that the digestibility of forage on the QTP decreased as the forage matured. Forages are most nutritious and digestible from the VS when tissues are succulent and growing rapidly (Blair et al., 1977; Beever et al., 1986). Therefore, forage nutrient content influences the nutrient digestibility in Tibetan sheep on the QTP.

Hoekstra et al. (2007) and Stergiadis et al. (2015) suggested that increasing N content improved N digestibility when total forage N content was less than about 3.5% (equivalent to 21.9% CP); over this content, any increase in forage N content will decrease N digestibility. In the present study, the increase in CP content of forage improved CP digestibility, but the relationship between forage CP content and digestibility was best described using

a quadratic relationship. Comparing their reported CP content of 21.9%, when forage CP content was over the peak point (9.7% DM in the present study) in the quadratic relationship between forage CP content and CP digestibility, further increasing forage CP content would decrease CP digestibility when Tibetan sheep were offered native forage alone, indicating that the local climate of the high-elevation region of the QTP had the effect of decreasing the threshold of CP digestibility in the rumen of livestock. Additionally, the linear regression analysis revealed that an increase in forage NDF or ADF content by 10 percentage unit would proportionately decrease CP digestibility or ADF digestibility by 14 and 16 percentage units, respectively. This may be due to that increasing NDF content is likely to reduce the availability of nonstructural carbohydrates and thus restrict the supply of fermentable energy for microorganisms to degrade feed protein in the rumen for microbial growth, as a result, increasing fecal nitrogen output. However, the quadratic relationship for these predictions explained more of the variation. In the present study, NDF and ADF content correlated negatively with all nutrient digestibility parameters, owing in part to the fact that these fiber components are partially indigestible in the rumen (Hopkins and Wilkins, 2006). Morgan and Stakelum (1987) suggested that forage ADF content correlates poorly with OM digestibility when cows are fed a fresh-grass diet. In the present study, oppositely, ADF content had a greater relationship with OM digestibility than the other parameters of nutrient digestibility. However, forage NDF content had a lower correlation with OM and EE digestibility than with digestibility of the other components measured in the present study. This difference in the impact of forage NDF and ADF content on nutrient digestibility indicates that hemicellulose may strongly influence forage nutrient value for ruminants living in the northeast portion of the QTP.

Effect of Forage Nutrient Content on Energy Parameters

Similar to the results of Givens et al. (1990) and Stergiadis et al. (2015), in the current experiment, forage CP content was positively related to DE and ME contents. However, the positive relationships between the CP content and DE/GE, ME/GE, and ME/DE, which differed from the relationships reported previously (Givens et al., 1990; Stergiadis et al., 2015), could be explained by the proportionately greater increase in energy utilization on the increase in CP

intake in the present study. Metabolizable energy increased at a greater rate than GE or DE on the CP content increasing resulted in greater ME/GE and ME/DE, indicating that increased CP content of forage provide a greater contribution to energy requirements of Tibetan sheep. Published results are in line with present research showing that a greater content of NDF and ADF in fresh forage reduces DE and ME of forage and enhances ME/DE (Givens et al., 1990; Stergiadis et al., 2015), and this may be due to a relatively greater effect on FE output compare with UE, thereby decreasing DE at a greater rate than ME. Wilson (1976) suggested that, under conditions that promote rapid forage growth, the proportion of leaves to stems decreases and the available assimilates are drained off by the highly active meristematic regions. This results in a greater fiber content and least carbohydrate availability, which increases FE.

Prediction of DMI

The use of the chemical composition of forage to predict DMI (g/kg BW·d) or DMI (g/kgW^{0.75}·d) was previously proposed by Andueza et al. (2011), and in the current work, the parameters such as CP, GE, EE, NDF, and ADF appeared among the equations that yielded the best fit for predicting the DMI (g/kg BW·d) or DMI (g/kgW^{0.75}·d) for Tibetan sheep living in the northeast portion of the QTP. Similar to the results of Mertens (1994) and Van Soest (1994), forage fiber (NDF, ADF) is an important predictor of DMI through its effects of a low digestion rate and high rumen fill for ruminants. However, it was found that factors such as forage CP, GE, and EE content maximized the variance in DMI (g/kg BW·d) or DMI (g/kgW^{0.75}·d), indicating an additional important contribution of other nutrients in predicting DMI. In the current study, the *R*² values of equations for predicting DMI (g/kg BW·d) were greater than those of DMI (g/kgW^{0.75}·d), which differed from the results of Donefer (1966), who obtained best fit when the DMI were expressed as gram per kilogram of metabolic weight (g/kgW^{0.75}·d). The differences in the equations of DMI may be attributable to the different experiment conditions. In the current study, the Tibetan sheep were investigated with similar BW and the forage were provided ad libitum. Andueza et al. (2011) suggested that the effect of difference of BW of the animals should be taken into account when DMI is expressed in gram per kilogram of metabolic weight (g/kgW^{0.75}·d). In addition, the environmental conditions e.g.,

temperature (Chai et al., 1985), day length (Hicks et al., 1990), and breed of animal (Andueza et al., 2011) may also influence the amount of forage consumed by sheep and hence the results generated by the equations.

CONCLUSION

Results from this study show that the parameters of forage CP, NDF, and ADF content were most closely related to nutrient digestibility. Contrary to previous studies, in this study, ADF content had a greater relationship with OM digestibility than the other parameters of nutrient digestibility. The high-elevation climate in this region had the effect of decreasing the threshold of CP digestibility. When forage CP content was over the peak point (9.7% DM in the present study) in the quadratic relationship between forage CP content and CP digestibility, further increasing forage CP content would decrease CP digestibility when Tibetan sheep were offered native forage alone on the QTP. The CP content and available energy of forage harvested from the northeast portion of the QTP could not meet nutrient requirements of Tibetan sheep. Additionally, comparing with predicting DMI ($\text{g/kgW}^{0.75}\cdot\text{d}$), using the forage CP, EE, NDF, and ADF content to predict DMI ($\text{g/kg BW}\cdot\text{d}$) yielded the best fit equation for Tibetan sheep living in the northeast portion of the QTP.

LITERATURE CITED

- Andueza, D., F. Picard, P. Pradel, D. Egal, P. Hassoun, J.R. Peccatte, and R. Baumont. 2011. Reproducibility and repeatability of forage *in vivo* digestibility and voluntary intake of permanent grassland forages in sheep. *Livest. Sci.* 140:42–48. doi:10.1016/j.livsci.2011.02.005
- AOAC. 1990. *Official Methods of Analysis*. 15th ed. Arlington (VA): Assoc. Off. Anal. Chem.
- Beever, D.E., H.R. Losada, S.B. Cammell, R.T. Evans, and M.J. Haines. 1986. Effect of forage species and season on nutrient digestion and supply in grazing cattle. *Br. J. Nutr.* 56:209–225. doi:10.1079/BJN19860101
- Blair, R.M., H.L. Short, and E.A. Epps. 1977. Seasonal nutrient yield and digestibility of deer forage from a young pine plantation. *J. Wildl. Manage.* 41:667–676.
- Cai, Y.J., X.D. Wang, L.L. Tian, H. Zhao, X.Y. Lu, and Y. Yan. 2014. The impact of excretal returns from yak and Tibetan sheep dung on nitrous oxide emissions in an alpine steppe on the Qinghai-Tibetan Plateau. *Soil Biol. Biochem.* 76:90–99. doi:10.1016/j.soilbio.2014.05.008
- Campos, F.P., D.R.O. Nicácio, P. Sarmiento, M.C.P. Cruz, T.M. Santos, A.F.G. Faria, M.E. Ferreira, M.R.G. Conceição, and C.G. Lima. 2016. Chemical composition and *in vitro* ruminal digestibility of hand-plucked samples of Xaraes palisade grass fertilized with incremental levels of nitrogen. *Anim. Feed Sci. Technol.* 215:1–12. doi:10.1016/j.anifeedsci.2015.12.013
- Chai, K., P.M. Kennedy, L.P. Milligan, and G.W. Mathison. 1985. Effects of cold exposure and plant species on forage intake, chewing behaviour and digesta particle size in sheep. *Can. J. Anim. Sci.* 65:69–76. doi:10.4141/cjas85-007
- De Waal, H.O., and W.J. Combrinck. 2000. The development of the Dorper, its nutrition and a perspective of the grazing ruminant on veld. *Small Rumin. Res.* 36:103–117. doi:10.1016/S0921-4488(99)00155-8
- Deng, K.D., Q.Y. Diao, C.G. Jiang, Y. Tu, N.F. Zhang, J. Liu, T. Ma, Y.G. Zhao, G.S. Xu. 2012. Energy requirements for maintenance and growth of Dorper crossbred ram lambs. *Livest. Sci.* 150:102–110. doi:10.1016/j.livsci.2012.08.006
- Deng, K.D., C.G. Jiang, Y. Tu, N.F. Zhang, J. Liu, T. Ma, Y.G. Zhao, G.S. Xu, and Q.Y. Diao. 2014. Energy requirements of Dorper crossbred ewe lambs. *J. Anim. Sci.* 92:2161–2169. doi:10.2527/jas.2013–7314
- Donefer, E. 1966. Collaborative *in vivo* studies on alfalfa hay. *J. Anim. Sci.* 25:1227–1231. doi:10.2527/jas1966.2541227x
- Ephrem, N., F. Tegegne, Y. Mekuriaw, and L. Yeheyis. 2015. Nutrient intake, digestibility and growth performance of Washera lambs supplemented with graded levels of sweet blue lupin (*Lupinus angustifolius* L.) seed. *Small Rumin. Res.* 130:101–107. doi:10.1016/j.smallrumres.2015.07.019
- Givens, D.I., J.M. Everington, and A.H. Adamson. 1990. The nutritive value of spring-grown herbage produced on farms throughout England and Wales over four years. III. The prediction of energy values from various laboratory measurements. *Anim. Feed Sci. Technol.* 27:185–196. doi:10.1016/0377-8401(90)90081-I
- Goering, K.H., and P.J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some application). *Agric. Handbook*. No. 379. Washington (DC): ARS-USDA.
- Hoekstra, N.J., R.P.O. Schulte, P.C. Struik, and E.A. Lantinga. 2007. Pathways to improving the N efficiency of grazing bovines. *Eur. J. Agron.* 26:363–374. doi:10.1016/j.eja.2006.12.002
- Hicks, R.B., F.N. Owens, D.R. Gill, J.W. Oitjen, and R.P. Lake. 1990. Dry matter intake by feedlot beef steers: influence of initial weight, time on feed and season of year received in yard. *J. Anim. Sci.* 68:254–265.
- Hopkins, A., and R.J. Wilkins. 2006. Temperate grassland: key developments in the last century and future perspectives. *J. Agric. Sci.* 144:503–523. doi:10.1017/S0021859606006496
- Hughes, M., V. Mlambo, P.G.A. Jennings, and C.H.O. Lallo. 2014. The accuracy of predicting *in vitro* ruminal organic matter digestibility from chemical components of tropical pastures varies with season and harvesting method. *Trop. Agric.* 91:131–146. doi:10.5829/idosi.mejsr.2014.19.12.11432
- Kandel, T.P., S. Sutaryo, H.B. Møller, U. Jørgensen, and P.E. Lærke. 2013. Chemical composition and methane yield of reed canary grass as influenced by harvesting time and harvest frequency. *Bioresour. Technol.* 130:659–666. doi:10.1016/j.biortech.2012.11.138
- Lin, B., Y. Lu, A.Z.M. Salem, J.H. Wang, Q. Liang, and J.X. Liu. 2013. Effects of essential oil combinations on sheep ruminal fermentation and digestibility of a diet with fumarate included. *Anim. Feed Sci. Tech.* 184:24–32. doi:10.1016/j.anifeedsci.2013.05.011

- Ma, T., D.D. Chen, Y. Tu, N.F. Zhang, B.W. Si, K.D. Deng, and Q.Y. Diao. 2015. Effect of dietary supplementation with resveratrol on nutrient digestibility, methanogenesis and ruminal microbial flora in sheep. *J. Anim. Physiol. Anim. Nutr* 99:676–683. doi:10.1111/jpn.12264
- McDonald, P., R.A. Edwards, J.F.D. Greenhalgh, and C.A. Morgan. 2002. *Animal Nutrition*. Harlow (UK): Pearson ed. Ltd.
- Mertens, D.R. 1994. Regulation of forage intake. In: Fahey G.C., M. Collins, D.R. Mertens, and L.E. Moser, editors, *Forage quality, evaluation and utilization*. Madison: American Society of Agronomy. p. 450–532.
- Morgan, D.J., and G. Stakelum. 1987. The prediction of the digestibility of herbage for dairy cows. *Ir. J. Agric. Res.* 26:23–34.
- Stergiadis, S., M. Allen, X.J. Chen, D. Wills, and T. Yan. 2015. Prediction of nutrient digestibility and energy concentrations in fresh grass using nutrient composition. *J. Dairy Sci.* 98:3257–3273. doi:10.3168/jds.2014-8587
- Sun, Y., J.P. Angerer, and F.J. Hou. 2015. Effects of grazing systems on herbage mass and liveweight gain of Tibetan sheep in Eastern Qinghai-Tibetan Plateau, China. *Rangeland J.* 37:181–190. doi:10.1071/RJ14062
- Van Soest, P.J. 1994. *Nutritional ecology of the ruminant*, 2th ed. Ithaca (NY): Cornell Univ Press. p. 476.
- Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2
- Wang, H., X.B. Li, H.L. Long, and W.Q. Zhu. 2009a. A study of the seasonal dynamics of grassland growth rates in Inner Mongolia based on AVHRR data and a light-use efficiency model. *Int. J. Remote Sens.* 30:3799–3815. doi:10.1080/01431160802552702
- Wang, C.J., S.P. Wang, and H. Zhou. 2009b. Influences of flavomycin, ropadiar, and saponin on nutrient digestibility, rumen fermentation, and methane emission from sheep. *Anim. Feed Sci. Tech.* 148:157–166. doi:10.1016/j.anifeedsci.2008.03.008
- Wilson, J.R. 1976. Variation of leaf characteristics with level of insertion on a grass tiller. II. Anatomy. *Aust. J. Agric. Res.* 27:355–364. doi:10.1071/AR9760355
- Xin, G.S., R.J. Long, X.S. Guo, J. Irvine, L.M. Ding, L.L. Ding, and Z.H. Shang. 2011. Blood mineral status of grazing Tibetan sheep in the Northeast of the Qinghai-Tibetan Plateau. *Livest. Sci.* 136:102–107. doi:10.1016/j.livsci.2010.08.007
- Xue, X., J. Guo, B.S. Han, Q.W. Sun, and L.C. Liu. 2009. The effect of climate warming and permafrost thaw on desertification in the Qinghai-Tibetan Plateau. *Geomorphology* 108:182–190. doi:10.1016/j.geomorph.2009.01.004.