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Determination of maintenance energy requirement and responses of dry ewes to dietary inclusion of lucerne versus concentrate meal

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ABSTRACT

An accurate value for metabolizable energy (**ME**) requirement for maintenance (**ME$_m$**) is essential to enable sheep husbandry practice to reach its potential. The objectives of the study were to use calorimetry chamber data of dry ewes (Hu × thin-tail Han F1 crossbred) to develop updated ME$_m$, examine effects of substituting concentrate feed with lucerne hay on energy partitioning, and explore the relationships between energy utilization and fasting heat production (**FHP**). Data were collected from three experiments. In Exps. 1, 2a and 2b, lucerne hay was used to replace concentrates in three levels (0:40%, 15:25% and 30:10%), with diets containing 60% maize stover (Exp. 1), fresh rye forage (Exp. 2a) or dry rye forage (Exp. 2b). Within each experiment, diets were isoenergetic (digestible energy, **DE**) and isonitrogenous. Exp. 3 aimed at evaluating effects of three BW levels on nutrient utilization of dry ewes offered diets containing 60% maize stover, 15% lucerne hay and 25% concentrates. Energy metabolism data were measured using the respiration calorimeter chamber technique in all three experiments, followed by the measurement of FHP in Exps. 1, 2b and 3. The ME$_m$ derived from the linear regression between energy balance (**EB**) and ME intake was 0.440 MJ/kg BW$^{0.75}$. The average FHP was 0.326 MJ/kg BW$^{0.75}$. The fasting metabolism, net energy requirement for maintenance (**NE$_m$**) and ME$_m$ were estimated to be 0.336, 0.359 and 0.511 MJ/kg BW$^{0.75}$, respectively, through adjustment of FHP using fasting urinary energy output, activity allowance and efficiency of ME use for maintenance. The FHP was negatively correlated to EB/metabolic BW, ME/gross energy (**GE**), ME/DE, EB/GE intake and EB/ME intake, while positively correlated to HP/GE intake, HP/ME intake and CH$_4$-E/GE intake. Compared to zero lucerne hay diet, the 15% lucerne hay intake decreased HP (MJ/d), and had no negative effects on EB (MJ/d) or energy utilization efficiencies. The results indicate that nutrient requirement standards currently used across the world are likely to underestimate ME$_m$ for dry ewes, and the selection of low FHP ewes for breeding has the potential to improve sheep production efficiency.

Implications

The modern dry ewes require more energy than that currently recommended to maintain their basal metabolic activities. The selection of breeding ewes with low fasting heat production has the potential to improve production efficiencies and reduce the environmental footprint of sheep production. Diet manipulation using lucerne hay to replace a certain amount of concentrates could sustain similar energy intake and energy retention which provides an alternative way to reduce concentrate input.

Introduction

Energy use for maintenance is the largest portion of feed requirement for sheep production. Evidence reported by Yang et al. (2019) indicated that some sheep breeds (Belclare, Highlander, Lleyn, Meatlinc, Suffolk and Texel) with higher productivity and lean mass proportion required more energy for maintenance than that recommended by AFRC (1993). The energy feeding standard of AFRC (1993) was developed using the data obtained in the

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1970s, which may be incompatible to the requirements of current sheep productivity and feed management. A range of diet and animal factors can influence metabolizable energy (**ME**) requirement for maintenance (**ME$_m$**) of sheep, including diet composition, plane of nutrition, sheep breed/genotype, production level and grazing management (Ferrell and Jenkins, 1985; Koong et al., 1985; Burrin et al., 1992; AFRC, 1993). These effects are particularly relevant to tropical and/or arid-semiarid areas (e.g., Northwest China), where the extremely vast range of climatic and environmental zones greatly influence the forage resource and supply, and sheep production activities. However, there is little information available in the literature on the quantification of basal metabolic rates of sheep in these arid-semiarid areas.

There is an increasing demand for livestock products across the world, which encourages livestock farmers to increase concentrate input and rear high genetic merit livestock (Baldwin et al., 1983; Kennedy et al., 2002). However, increasing concentrate input has placed a great pressure on the global food security and environmental sustainability (Rojas-Downing et al., 2017; Godfray et al., 2018). Therefore, there is an urgent need to explore alternative strategies to reduce concentrate input, but maintain animal health and productivity, especially in low-quality forage feeding systems. Part replacement of concentrates with lucerne hay can sustain similar daily milk yield of dairy cows (Baldwin et al., 1983), and feed intake of calves (Kobayashi et al., 2017). However, there is a research gap in the effects of feeding lucerne hay to replace equivalent concentrate input on energy utilization of nonlactating and nonpregnant ewes (dry ewes) using respiration calorimeter measurements.

Therefore, the present study was to use respiration calorimeter chamber data of dry ewes to develop ME$_m$, to evaluate responses in energy partitioning to levels of replacing concentrates by lucerne hay, and to detect the associations between fasting heat production (**FHP**) and energy utilization.

Material and methods

Metabolic experiments design

The data used in the current study were obtained from three metabolic experiments with 81 dry ewes of Hu × thin-tail Han F1 crossbred (BW = 23.8–40.7 kg and the age of 3 years old). These experiments were undertaken in Linze Grassland Ecological Experiment Station of Arid Area, Lanzhou University in China, situated at 39.24°N, 100.06°E (1 370 m a.s.l.) with a mean precipitation of 121.5 mm.

Exp.1 was a 3 (diets) × 3 (periods) Latin Square design study of 12 dry ewes (32.2 ± 0.83 kg BW), which evaluated the effects of replacing concentrate meal with lucerne hay on nutrient utilization. All the three diets contained (DM basis) 60% of maize stover and the ratios of lucerne hay to concentrates were 0%:40% (LH0), 15%:25% (LH15) and 10%:30% (LH30), respectively. In Exp. 2a, 12 dry ewes (32.0 ± 0.74 kg BW) were randomly allocated to three diets, and each diet contained (DM basis) 60% fresh rye forage and 40% mixtures of lucerne hay and concentrates (ratios of lucerne hay to concentrates were same as in Exp. 1). Exp. 2b used the same animals, and repeated the experimental design and arrangement as used in Exp. 2a, except that dry rye forage rather than fresh rye was used. There was a 24-d interval gap between Exp. 2a and 2b. Within each experiment, diets were isoenergetic (digestible energy, DE) and isonitrogenous. In Exp. 3, 21 dry ewes (33.5 ± 3.24 kg BW) were divided into three groups to evaluate the effects of three BW levels on nutrient utilization for ewes offered diet containing (DM basis) 60% maize stover, 15% lucerne hay and 25% concentrates.

The rye (*Secale cereale* L. – local Chinese variety) forage used in Exp. 2a and 2b was harvested daily from a single paddock. The paddock was divided into 12 plots, and each plot was designed to provide sufficient forage for 2 d. Each plot was thoroughly trimmed at a residual height of 5 cm and then allowed 22 d regrowth before cut. The trimming of these 12 plots was in a sequential order with 2 d interval. The grass was cut daily at 1 700 during the 1st regrowth and then divided into two equal portions. One portion was stored at 4 °C and then offered to ewes as fresh grass in the following day in Exp. 2a, and the other portion was spread out for drying in the field. The dry forages were stored until offered to ewes as hay in Exp. 2b. Maize stover (Exps. 1 and 3) was harvested in October and purchased from local farmers. Lucerne (*Medicago sativa* L. – Golden Empress cv.) hay used in all experiments was sown at the Experiment Station in 2010 and harvested at the beginning of flowering at first growth. Concentrates used in all experiments contained wheat bran, maize and soybean meal, with mineral and vitamin premix. The ingredients of diets for each experiment are presented in Table 1.

In all experiments, forages were chopped into 3–5 cm lengths and offered as mixtures in two equal portions at 1 000 and 1 700, respectively. Concentrate pellets were offered at 1 330 daily. All diets were formulated based on the Feeding Standards for Sheep and Goats Production in China (MOA, 2004). Daily amounts

Table 1
Diet ingredient composition for dry ewes (% DM basis).

Items	Exp. 1			Exp. 2a and 2b			Exp. 3
	LH0	LH15	LH30	LH0	LH15	LH30	
Maize stover	60	60	60				60
Rye (fresh or hay)[1]				60	60	60	
Lucerne hay	0	15	30	0	15	30	15
Wheat bran	28.4	13.5	0.01	38.5	1.50	0.50	5.5
Soybean meal	10.9	10.5	9.48	0.50	5.00	3.00	12.0
Maize	2.00	5.00	0.01	0.50	18.0	6.00	7.00
Mineral premix[2]	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin premix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10

Abbreviation: Exp. = Experiment.

[1] Fresh rye was used in Exp. 2a, and rye hay was used in Exp. 2b.

[2] Mineral premix (Zhenjiang Tianhe Biotechnology Co., Ltd., Yangzhong, Jiangsu Province, China) contains 2 g Cu, 20 g Fe, 12 g Zn, 15 g Mn, 0.15 g I, 0.1 g Se, and 300 g Ca per kg DM.

[3] Vitamin premix (Shandong Shenlong Animal Health Products Co., Ltd., Weifang, Shandong Province, China) contains 1102.3 × 104 IU vitamin A, 165.3 IU VD3, 5 512 IU vitamin E, 4 409 mg vitamin K, 551 mg vitamin B1, 1 102 mg vitamin B2, 7 560 mg vitamin C, 8 112 mg folic acid, 7 560 mg L-pantothenic acid, 10 g K+, and 7.5 g Na + per kg DM.

of concentrates offered were adjusted according to the experimental design and forage DM intake which was offered *ad libitum* to ensure refusal of at least 10%. The adjustment was undertaken based on averaged concentrate and forage DM intakes in the previous three days.

On average, total DM intake was 814 g/d, including (g/d) 140 lucerne, 455 other forage and 299 concentrates. The differences between maximum and minimum values of CP, NDF, ADF, **NFC** (nonfibre carbohydrate) and **EE** (ether extract) concentrations were 82, 246, 179, 286 and 23.3 g/kg DM, respectively (Table 2).

Metabolism measurements

Following an acclimation period of 14 d, during which sheep were individually penned and fed their experimental diet, ewes were then transferred to an individual crate for 7 d in which feed intake was recorded, and faeces and urine output were collected over the final 6 d. Immediately on the completion of the digestibility measurements, ewes were moved to an indirect open-circuit respiration calorimetric chamber (one sheep/chamber) for further 3 d in which gas exchanges (O_2, CO_2 and CH_4) were measured over the final 2 d.

All equipment, procedures, analytical methods and calibration of respiration chambers are as described by Wang et al. (2020) in the Supplementary Materials.

After chamber measurements, ewes ($n = 45$) in Exps. 1, 2b and 3 were fasted continuously for 5 d with free access to water, the first 3 d in the individual crates and the final 2 d in individual respiration chambers with measurement of urine output, O_2 consumption, and CO_2 and CH_4 production measured during the final 24 h (96–120 h).

Sample collection for laboratory analysis

BW was measured immediately before and after ewes entered and exited both crates and chambers. Fasted ewes were weighed at the beginning and end of the gaseous exchange measurement in respiration chambers. Offered feed and all residues were weighted and sampled daily. Samples of offered forages were bulked to be a representative of fed forage either each week (Exps. 1 and 3), or in each 3 d period (Exp. 2), and a representative sample of fed forage was then taken for further analysis. Concentrate samples were taken weekly. Residuals of each ewe were pooled for one sample during the measurement period. All samples were dried at 65 °C for 72 h for measuring DM concentration, and then milled through a 1 mm mesh, for determining **GE** (gross energy), **OM** (organic matter), CP, NDF, ADF, NFC, and EE concentrations.

Table 2
Feed intake and feed quality data of dry ewes in the experiments used in the present study ($n = 81$).

Items	Exp. 1	Exp. 2a	Exp. 2b	Exp. 3
Feed intake, g/d				
Total DM	837	709	704	896
Lucerne DM	140	139	138	139
Other forage DM	468	359	356	506
Concentrate DM	228	211	211	251
Nutrient concentrations of total diets, kg/kg DM or MJ/kg DM				
DM	0.930	0.593	0.952	0.933
OM	0.900	0.918	0.917	0.905
GE	17.9	18.2	18.2	17.8
CP	0.140	0.122	0.122	0.145
NDF	0.564	0.528	0.481	0.515
ADF	0.340	0.305	0.280	0.313
NFC	0.196	0.262	0.317	0.252
EE	0.0204	0.0281	0.0247	0.0169

Abbreviations: Exp. = Experiment; OM = Organic matter; GE = Gross energy; NFC = nonfibre carbohydrate; EE = Ether extract

In all experiments, daily faecal output for each ewe was recorded and all stored in plastic bag. Daily urine output was collected in containers with 20 ml of 30% sulfuric acid added before the start of the collection. The volume of urine for each ewe was measured and then a sample of 20% of this volume was taken. Faeces and urine samples were stored at 4 °C during the first 5 d. After the last day of collection, faeces and urine outputs were thoroughly admixed, respectively, and then representative samples were taken. Fasting urine samples were also taken during the last 24 h of fasting measurements. Each fresh faecal sample was divided into two subsamples. One portion was used for analysing N content on a fresh basis, and the other portion for measuring DM content at 65 °C for 96 h and then forced through a 1.0 mm sieve for determining GE, OM, CP, NDF, ADF, NFC and EE concentrations. Laboratory analyses of feed, residuals and excreta followed the methods described by Wang et al. (2020).

Calculation of maintenance energy requirement

The ME intake was calculated as GE intake subtracted a sum of faecal, urinary and CH_4 energy outputs. Energy balance (**EB**) was calculated as ME intake less heat production (**HP**), with HP calculated according to the formula proposed by Brouwer (1965).

The ME_m in the present study was developed using the linear regression of EB (MJ/kg$^{0.75}$) against ME intake (MJ/kg$^{0.75}$). The ME_m was also estimated using FHP data, with which fasting metabolism (**FM**, MJ/kg BW$^{0.75}$), net energy requirement for maintenance (**NE_m**, MJ/kg BW$^{0.75}$) and ME_m values were estimated.

The FM was calculated as a sum of fasting urinary energy output and the coefficient of the linear regression between FHP and metabolic BW (MBW, kg BW$^{0.75}$). The NE_m was calculated as FM plus activity allowance (MJ/kg BW$^{0.75}$), and ME_m was derived as NE_m divided by k_m (the efficiency of ME use for maintenance). The activity allowance for housed ewes and k_m were estimated using the recommendation of AFRC (UK, 1993) and MOA (China, 2004), and q_m was 0.567 in the present study.

Statistical analysis

Linear Mixed Model procedure (GenStat, 19th edition, VSN International Ltd., UK) was performed to analyse all data. The linear relationships between EB and ME intake, FHP and MBW, and energy utilization parameters and FHP were undertaken with ewes as the random factor. The data of energy partitioning across in Exps. 1 and 2 were pooled to evaluate the responses to lucerne hay replacement rates for concentrates, in which differences between mean data were performed using the least significant difference (LSD) test. Because forage types (maize stover in Exp.1, fresh rye forage in Exp. 2a and dry rye forage in Exp. 2b) influenced energy partitioning, this factor was fitted as covariable effect. Ewes were fitted as the random effect. The significance level was declared at 0.05 using the Wald test. A pseudo R^2 was used to describe the goodness of fit for predicted regressions, which were calculated by the actual and fitted response variables in each case.

Outliers in the dataset were identified through the standardized residual procedure, which were outside the range of -2.5 and 2.5. The outliers were then removed from the dataset. Standardized residuals were calculated according to the actual and predicted values for the regression.

Table 3
Energy intake, energy output and utilization efficiency data of dry ewes in the experiments used in the present study ($n = 81$).

Items	Exp.1	Exp.2a	Exp.2b	Exp.3
Energy intake and output, MJ/d				
GE intake	15.0	12.9	12.8	15.9
Faecal energy output	5.61	3.96	4.28	5.59
Urinary energy output	0.336	0.193	0.212	0.370
CH_4-E output	0.895	0.735	0.778	0.989
DE intake	9.38	8.95	8.48	10.33
ME intake	8.15	8.02	7.49	8.98
HP	6.46	5.85	6.00	6.56
EB	1.69	2.16	1.49	2.42
Energy utilization efficiencies, MJ/MJ				
DE/GE	0.626	0.695	0.664	0.649
ME/GE	0.544	0.622	0.586	0.564
ME/DE	0.868	0.896	0.882	0.868
HP/ME intake	0.802	0.731	0.809	0.733
EB/ME intake	0.198	0.269	0.191	0.267

Abbreviations: Exp. = Experiment; GE = Gross energy; CH_4-E = Methane energy; DE = Digestible energy; ME = Metabolizable energy; HP = Heat production; EB = Energy balance.

Results

Summary statistics for energy utilization

The average ME intake and EB were respectively 8.25 (from 4.93 to 10.7) and 1.92 (from −0.709 to 4.34) MJ/d. On average, DE/GE, ME/GE, ME/DE, HP/ME intake and EB/ME intake were 0.648, 0.567, 0.874, 0.775 and 0.225 MJ/MJ (Table 3).

Effects of lucerne replacement level for concentrate on energy metabolism data

Increasing lucerne replacement levels for concentrates decreased DE and ME intake, CH_4-E output, HP, DE/GE, ME/GE and CH_4-E/GE intake, but increased faecal energy output, especially between 0 and 30% lucerne input. The EB and EB/ME intake for 15% lucerne diet were significantly greater than those for 30% lucerne diet, and numerically greater than those for zero lucerne diet. Lucerne replacement levels had no effects on GE intake, urinary energy output and ME/DE (Table 4).

Development of metabolizable energy requirement for maintenance

The linear regressions of observed EB (MJ/kg $BW^{0.75}$) against ME intake (MJ/kg $BW^{0.75}$) and FHP (MJ/d) against metabolic BW (kg $BW^{0.75}$) are shown in Eqs. (1) and (2) (Fig. 1). Both linear relationships were significant ($P < 0.001$), and R^2 values were 0.542 and 0.635, respectively.

$$EB = -0.376_{(0.0511)} + 0.853_{(0.0830)}\ MEI\ (R^2 = 0.542,\ P < 0.001,\ n = 80) \quad (1)$$

$$FHP = 0.326_{(0.0047)}\ MBW\ (R^2 = 0.635,\ P < 0.001,\ n = 45) \quad (2)$$

The ME_m derived from Eq. (1) was 0.440 MJ/kg $BW^{0.75}$. The FM, NE_m and ME_m derived from Eq. (2) were 0.336, 0.359 and 0.511 MJ/kg $BW^{0.75}$, respectively.

Linear relationships between energy utilization and fasting heat production

All linear relationships were significant ($P < 0.05$), and R^2 of linear regression ranged from 0.079 to 0.482 (Table 5 and Fig. 2). The FHP was negatively related to EB/MBW, ME/GE, ME/DE, EB/GE intake and EB/ME intake, while positively related to CH_4-E/GE intake and HP expressed as a proportion of GE and ME intake.

Discussion

Determination of energy requirement for maintenance

The ME_m developed from linear regression of EB against ME intake and FHP data in the present study were 0.440 and 0.511 MJ/kg $BW^{0.75}$, respectively. These values are higher than 0.342 MJ/kg $BW^{0.75}$ recommended by AFRC (1993), 0.392 MJ/kg $BW^{0.75}$ for Thin-tail Han dry ewes (Yang and Feng, 2004), and 0.424 MJ/kg $BW^{0.75}$ for ewe lambs suggested by MOA (2004). The ME_m of AFRC (1993) was derived from FHP data of sheep offered restricted feed (near to maintenance level) before fasting (ARC, 1980), which might contribute to the low ME_m. Nutritional restriction, even for short intervals, could directly decrease microbial activities, and consequently reduce VFAs and NH_3-N production in the rumen (Kim et al., 2019) and rates of blood flow (Ferrell and Jenkins, 1985), and increases the action of Cori cycle for main-

Table 4
Effects of lucerne hay replacement rates for concentrates on energy intake, output and utilization efficiency of dry ewes ($n = 60$).

Items[1]	LH0	LH15	LH30	SED	P-value
Energy intake and output, MJ/kg $BW^{0.75}$					
GE intake	1.046	1.023	1.035	0.0327	0.790
Faecal energy output	0.343^{b}	0.350^{b}	0.396^{a}	0.0193	0.019
Urinary energy output	0.0203	0.0212	0.0193	0.00141	0.403
CH_4-E output	0.064^{a}	0.062^{ab}	0.058^{b}	0.0025	0.070
DE intake	0.703^{a}	0.676^{ab}	0.639^{b}	0.0194	0.007
ME intake	0.620^{a}	0.594^{ab}	0.561^{b}	0.0178	0.004
HP	0.483^{a}	0.446^{b}	0.452^{ab}	0.0140	0.017
EB	0.136^{ab}	0.147^{a}	0.105^{b}	0.0214	0.106
Energetic utilization efficiencies, MJ/MJ					
DE/GE	0.677^{a}	0.659^{a}	0.622^{b}	0.0102	<0.001
ME/GE	0.596^{a}	0.579^{a}	0.546^{b}	0.0096	<0.001
ME/DE	0.879	0.878	0.878	0.0040	0.901
CH_4-E/GE intake	0.062^{a}	0.060^{ab}	0.056^{b}	0.0021	0.028
HP/ME intake	0.786^{ab}	0.759^{b}	0.822^{a}	0.0338	0.101
EB/ME intake	0.214^{ab}	0.241^{a}	0.178^{b}	0.0338	0.101

Abbreviations: GE = Gross energy; CH_4-E = Methane energy; DE = Digestible energy; ME = Metabolizable energy; HP = Heat production; EB = Energy balance.
$^{a–b}$Values within a row with different superscripts differ significantly at $P < 0.05$.
[1] The three diets contained (DM basis) 60% rye (fresh or hay) or maize stover, and, respectively, 40, 25 and 10% concentrates and 0, 15 and 30% lucerne hay.

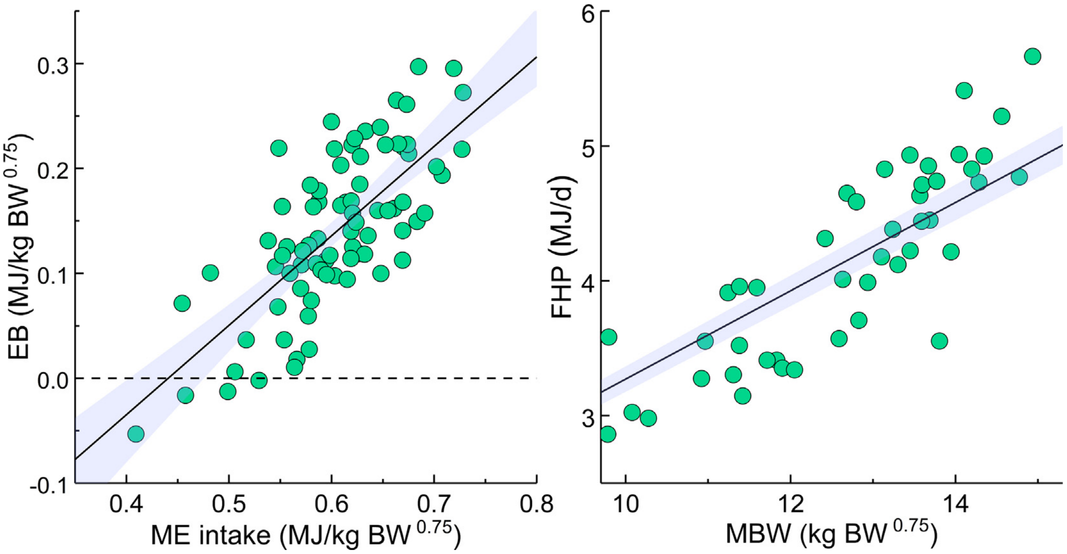

Fig. 1. Linear regressions of EB with ME intake and FHP with MBW of dry ewes, Abbreviations: EB = Energy balance; ME = Metabolizable energy; FHP = Fasting heat production; MBW = Metabolic BW. The blue shade lines represent 95% confidence limits of the regression.

Table 5
Linear regression equations between energy utilization and FHP of dry ewes.

Equations	R^2	*P*-value	*n*
EB/MBW = $0.559_{(0.0829)} - 1.258_{(0.2540)}$ FHP	0.348	<0.001	45
CH_4-E/GE intake = $0.012_{(0.0094)} + 0.148_{(0.0289)}$ FHP	0.372	<0.001	44
ME/GE = $0.671_{(0.0494)} - 0.325_{(0.1518)}$ FHP	0.079	0.038	43
ME/DE = $0.947_{(0.0225)} - 0.226_{(0.0691)}$ FHP	0.181	0.002	45
HP/GE intake = $0.153_{(0.0545)} + 0.832_{(0.1680)}$ FHP	0.365	<0.001	42
HP/ME intake = $0.062_{(0.1091)} + 2.155_{(0.3345)}$ FHP	0.479	<0.001	45
EB/GE intake = $0.556_{(0.0651)} - 1.292_{(0.1997)}$ FHP	0.482	<0.001	45
EB/ME intake = $0.938_{(0.1091)} - 2.155_{(0.3345)}$ FHP	0.479	<0.001	45

Abbreviations: FHP = fasting heat production; EB = Energy balance; MBW = Metabolic BW; CH_4-E = Methane energy; GE = Gross energy; ME = Metabolizable energy; DE = Digestible energy; HP = Heat production.
Units: MJ/kg BW$^{0.75}$ for EB/MBW and FHP; MJ/MJ for CH_4-E/GE intake, ME/GE, ME/DE, HP/GE intake, HP/ME intake, EB/GE intake and EB/ME intake.

tenance of blood glucose levels (Bowden, 1973). Hence, the restricted feeding for a long-term period would force animals to reduce their basal metabolic rates to adapt to the lower nutrient intake (Chowdhury and Ørskov, 1994). As reported, animals with the same BW that offered high feeding level before fasting had 25–53% greater FHP values than those offered low feeding level (Koong et al., 1985). Therefore, when applying fasting technique to derive ME$_m$, it is more appropriate to feed animals *ad libitum* prior to fasting (Yan et al., 1997b). In the present study, the high ME$_m$ derived from FHP data was probably contributed by the underestimated k$_m$, because ME/GE used to calculate k$_m$ in the present study was derived from *ad libitum* feeding experiments. The ME/GE used to calculate k$_m$ of AFRC (1993) is recommended to be obtained with sheep offered diets near to maintenance feeding levels. Hence, 0.440 MJ/kg BW$^{0.75}$ was recommended as the ME$_m$ for dry ewes of Hu × thin-tail Han F1 crossbred.

Recommendations for ewe lambs by MOA (2004) were based on comparative slaughter data (Hao et al., 1991). The ME$_m$ varies with the measurement technique and physiological stage. For growing ruminants, the energy retention is mainly deposited as protein, as against fat retention for adult ruminants (Tiwari et al., 2000). The cost of protein deposition is 68.0 kJ/g, which is higher than 47.9 kJ/g for fat deposition (Ørskov and McDonald, 1970). Hence, the energy required for maintenance is thought to decrease with increasing stage of maturity. Under comparative slaughter experiments, changes in body energy content are measured with different groups of animals at various ME intake levels in a longer-term period. The effects of these factors on the derived maintenance energy requirement cannot be removed completely. As reported, HP for ewes fed at high feeding level (415 kJ/kg BW$^{0.75}$) was 30% greater than that at low feeding level (318 kJ/kg BW$^{0.75}$) (Yang and Feng, 2004), and ME$_m$ derived from metabolism data was 13.9% lower than that from comparative slaughter data (Hao et al., 1991). It is suggested, compared to comparative slaughter techniques, maintenance energy requirement derived from data measured by calorimetric chambers is more reliable and repeatable.

Estimated ME$_m$ for dry ewes obtained from ME intake and EB data in the present study is 28.7%, 10.6%, 12.8% and 20.9% greater than that recommended by AFRC (1993, 342 kJ/kg BW$^{0.75}$), INRA (1989, 398 kJ/kg BW$^{0.75}$), NRC (2007, 390 kJ/kg BW$^{0.75}$) and CSIRO (2007, 364 kJ/kg BW$^{0.75}$), respectively. Although ME$_m$ for dry ewes varies according to variations of animal breed and diets factors (Burrin et al.,1992), ME$_m$ obtained in the present study is similar to that of 449 kJ/kg BW$^{0.75}$ averaged from the recent publications estimated by indirect calorimetry data (Zhao, 2013; Yang et al., 2019), but is higher than that of 372 kJ/kg BW$^{0.75}$ obtained using the C-N balance technique (Lou et al., 2015). In general, variation in ME$_m$ appears to be directly associated with genetic potential for productivity (e.g., growth rate or milk production, Ferrell and Jenkins 1985). As a consequence of genetic improvement, current breeds have higher lean or protein mass proportion with high growth rate, and relatively greater feed intake with higher mass of internal organs (Koong et al., 1985; Ferrell, 1988). Ruminants having high fat free mass contribute to the increased ME$_m$, because the net efficiency of ME use for protein deposition is much lower than that for fat deposition (Ørskov and McDonald, 1970; CSIRO, 2007) and lean tissue is metabolically more active (Tiwari et al., 2000). Genetically lean rats or pigs have higher FHP or maintenance requirement per kg MBW (Ferrell and Jenkins, 1985). Meanwhile, animals with high growth rates, being directly or indirectly associated with the high rates of protein synthesis in these tissues (Ferrell and Jenkins, 1985), tend to have greater basal metabolism rate (Costa et al., 2013). Hence, it is necessary to update ME$_m$ to reflect the genetic merit for productivity for current sheep flocks.

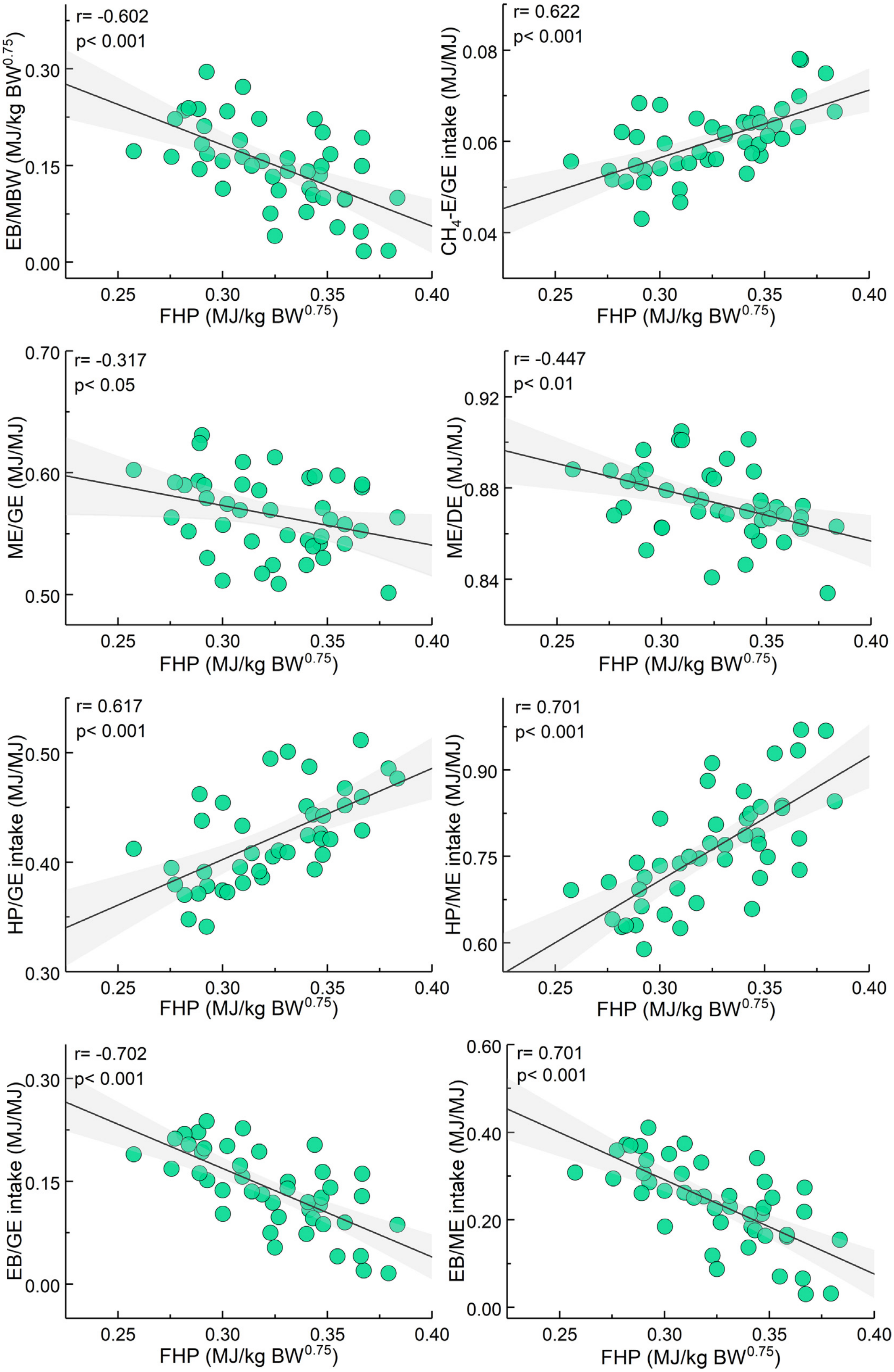

Fig. 2. Linear relationships between energy utilization and FHP of dry ewes, FHP = Fasting heat production; EB = Energy balance; MBW = Metabolic BW; CH_4-E = Methane energy; GE = Gross energy; ME = Metabolizable energy; DE = Digestible energy; HP = Heat production. *r* represents Pearson's correlation coefficient, and *P* is the significant level, which were tested by two-tailed test.

Relationships between energy utilization and fasting heat production

In the present study, FHP was negatively related to EB/MBW, ME/GE, ME/DE and EB as a proportion of GE and ME intake, and positively related to CH_4 emissions. The results indicated the energy utilization was also affected by the variation in FHP, besides plane of nutrition (Lou et al., 2015) and forage proportion (Yan et al., 1997a; Dong et al., 2015). Hence, future genetic selection could focus on selecting the genotypes of low FHP, and explore the transmitted direct and maternal genetic effects of low FHP to evaluate if the genetic character is stable and heritable. Some previous studies indicated that the FHP was highly associated with the productivity levels and differences in body protein and fat contents (Ferrell and Jenkins, 1985), and weights of liver and gut organs (Koong et al., 1985). In this study, the BW was not correlated with FHP, hence, sheep with low FHP was probably attributed to small sizes of internal organs of total body masses, and/or less energy (GE and ME) intake used for HP. Meanwhile, based on EB and retained N data, neither energy retained as protein nor the proportion of protein relative to fat retained in the ewes was related to FHP, although energy retained as fat was negatively related to FHP ($P < 0.05$). The effects of FHP on productivity and lean mass proportion need to be evaluated in a range of further studies using data obtained from fasting experiments and comparative slaughter experiments.

Effects of lucerne input level on energy partitioning

The present study found the negative effects of feeding high lucerne diet on DE/GE and ME/GE, which is in agreement with the result of Dong et al. (2015). Meanwhile, this result also showed a reduced CH_4 energy output with sheep fed lucerne diet, which was probably attributed to decreased DE intake. It has been well documented that HP was positively related to ME intake (Koong et al., 1985; Ferrell, 1988; Lou et al., 2015) and forage proportion (Yan et al., 1997a; Dong et al., 2015). Increasing dietary lucerne levels in the present study would increase indigestible fibre content and feed particle sizes, and consequently increase energy expenditure on rumination, chewing and digestion (Dong et al., 2015). Furthermore, high fibre content could lead to higher gut fill with consequently an increase in gut mass and the size of internal organs (Koong et al., 1985; Ferrell, 1988; Reynolds, 1996), and acetic acid in rumen contributing higher energy loss as CH_4. However, compared to the zerolucerne diet, the present study found that using lucerne hay to replace concentrates at 15% could sustain similar energy intake, EB and energy utilization efficiency, and significantly lower HP. At this replacement level, the difference in lucerne hay intakes might not be enough to influence the sheep to adjust their internal body metabolism. A decrease in urinary N output for lucerne diets being attributed to lower N intakes could reduce energy expenditure for protein degradation in rumen (Yang and Feng, 2004) and energy cost of excreting urea (Cannas et al., 2004).

Conclusion

The 0.440 MJ/kg $BW^{0.75}$ calculated by the linear regression of EB against ME intake is recommended as the ME_m for dry ewes of Hu × thin-tail Han F1 crossbred, rather than 0.511 MJ/kg $BW^{0.75}$ derived from FHP data, which is higher than that currently recommended across the world. This indicates that the present feeding standards need to be updated to reflect the production potential of the current sheep flocks. The sheep with low FHP could have higher EB/GE intake and EB/ME intake, and lower CH_4-E/GE intake. The present study also found that compared to zero lucerne input, lucerne hay could be used to replace 15% concentrates without negative effects on feed intake and energy utilization efficiency.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100200.

Ethics approval

All experimental procedures were approved by the Animal Ethics Committee of Lanzhou University, and conducted under the rules and regulations of experimental field management protocols (file No. 2010-1 and 2010-2).

Data and model availability statement

None of the data were deposited in an official repository.

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F.J. Hou, C.M. Wang and T. Yan designed the study. **F.J. Hou** supervised the study. **C.M. Wang, K. Xie, S.H. Chang and C. Zhang** conducted the data curation. All authors wrote the draft. **F.J. Hou, T. Yan and C.M. Wang** revised the draft. All authors approved the submitted version.

Declaration of interest

None.

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