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Metabolizable Energy Requirements for Growing Afshari Lambs: Insights from the Relative Growth Index Method

Research Article

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ABSTRACT

This study aimed to estimate the energy requirements for maintenance (MEm; kJ/BW$^{0.75}$) and growth (MEg; kJ/g) in growing fat-tailed Afshari lambs using relative growth index (RGI). The RGI was calculated by dividing metabolic body weight (BW$^{0.75}$) by average daily gain (ADG; g). Then, the MEm (kJ/BW$^{0.75}$) and MEg (kJ/g) were extrapolated from the intercept and slope of a linear regression between RGI and metabolizable energy intake (MEI) per unit of gain. Forty-five healthy fat-tailed Afshari male lambs (average weight: 29.5±1.8 kg; age: 140±10 days), individually housed, were assigned to one of three feeding levels: *ad libitum* (ADL, n=15), 80% of ADL (R80, n=15), and 60% of ADL (R60, n=15). Based on the linear relationship between RGI and MEI per g of gain [ME (kJ)=614 RGI (kJ/BW$^{0.75}$) + 17.4 (kJ/g ADG), R^2=0.98, n=45, RMSE=9.65], MEm and MEg were 614 kJ/BW$^{0.75}$, and 17.4 kJ per g of gain. The feed intake level significantly influenced the predicted ADG of fat-tailed Afshari lambs based on the CNCPS-S model with larger discrepancies between observed and predicted ADG in the R60 group. In conclusion, this study indicates that the RGI method is a promising and accessible alternative method for estimating the energy needs of growing lambs. The implications of the results lay the groundwork for more refined, breed-specific feeding strategies for stakeholders that could enhance the productivity and sustainability of Afshari sheep farming.

KEY WORDS energy recommendation, fat-tailed sheep, gain, Iranian Afshari sheep, relative growth index.

INTRODUCTION

The Afshari sheep breed is a fat-tailed breed known for its adaptability to harsh climate and its meat production and high productivity traits; twinning and rapid growth rate (Ebrahimi *et al.* 2020). Afshari sheep are relatively large in size with mature rams and ewes weighing around 88 kg and 70 kg, respectively (Kamalzadeh and Aouladrabiei, 2009). While Afshari sheep plays a crucial role in the economies of rural and nomadic communities, the specific nutrient requirements of these animals are often not well- researched. Only a few studies have attempted to report energy requirement of fat-tailed sheep breeds (Al Jassim *et al.* 1996; Early *et al.* 2001; Kamalzadeh and Shabani, 2007) which may not be sufficient for tabulating nutrient requirements of these breeds. This lack of literature can lead to inefficiencies in sheep managements, impacting productivity of fat- tailed sheep breeds such as Afshari sheep.

Two widely used approaches for estimating livestock energy requirements are comparative slaughter technique (CST) (Early *et al.* 2001; Galvani *et al.* 2008; Deng *et al.* 2012; Costa *et al.* 2018; Martins *et al.* 2019) and indirect calorimetry (Blaxter, 1986; Dawson and Steen, 1998; Kiani *et al.* 2007). The CST is labor-intensive, expensive, and often conflicts with animal welfare standards in many countries. The Indirect calorimetry, considered as standard method, is not available in many countries and requires relatively sophisticated research facilities and financial resources. The limitation of both methods highlights the pressing need for alternative methods to ensure accurate energy requirement estimations across diverse agricultural settings.

A feasible and cost effective method for assessing energy requirements of growing animals is to use gain as an indirect measure of energy retention (Luo *et al*. 2004). This method employs regression equations that plot average daily gain (ADG) against metabolizable energy intake (MEI). Alternatively, relative growth index (RGI; $BW^{0.75}$/g) of an animal can be calculated as the metabolic body weight ($BW^{0.75}$) divided by ADG (g). The RGI serves as an indicator of an animal's efficiency in gaining weight. A high RGI suggests that the animal is gaining weight less efficiently relative to its body weight, while a low RGI indicates more efficient weight gain. To determine energy requirements, the RGI is regressed against MEI per unit of gain, resulting in a linear equation (Y=a+bx) where intercept (a) and slope (b) are the requirement of metabolizable energy for maintenance (MEm; kJ/$BW^{0.75}$) and growth (MEg; kJ/g), respectively. This approach may provide a simpler and more practical alternative to the CST and indirect calorimetry, as it does not require advanced research facilities or substantial financial resources.

This study hypothesizes that the energy requirements of growing Afshari lambs can be effectively estimated using the RGI method. Thus, the aims of the study were to (1) estimate the energy requirements for maintenance and growth of Afshari male lambs from 30 to 40 kg body weight, (2) compare the observed ADG of these lambs with the ADG values predicted by the CNCPS-S system, providing insights into the accuracy of the CNCPS system for estimating nutrient requirements of fat-tailed sheep breeds.

MATERIALS AND METHODS

Site and Ethics Statement

This study was conducted under the supervision of the Department of Animal Science, Faculty of Agriculture, Lorestan University, Iran. The research was carried out from March to April 2021 on a private sheep breeding farm (Reyhan Co., Markazi Province, Iran), (33°38'16.8"N 50°02'31.2"E). All procedures involving animal care and management were approved by the Animal Care and Welfare Committee at Lorestan University, Iran.

Animal management, sampling and experimental design

Forty-five healthy male Afshari lambs (mean body weight: 26.9±2.1 kg, age: 120±10 days) were purchased from a local animal market. Due to their varying nutritional backgrounds, the lambs were acclimated in the research facility for two weeks, during which they were fed a ration consisting of 60% forage and 40% concentrate. During this period, all lambs received an injection of Ivermectin (0.2 mg/kg BW) and oral suspension of Closantel 5% (10 mg/kg BW) to eradicate endoparasites. Lambs were vaccinated against enterotoxaemia (0.2 mg/kg BW, Razi Vaccine and Serum Research Institute, Karaj, Iran). Lambs were individually housed in pens (100 cm×150 cm) with concrete flooring, and were provided with separate buckets for water and feed. The main experiment lasted 75 days, consisting of a 15-day adaptation period followed by 60 days of the experimental phase. At the beginning of the experimental period, the lambs had a mean body weight of 29.5 ± 1.8 kg and an age of 140 ± 10 days. Rations were formulated using the CNCPS-S software (version 1.0.21; Cornell University, Ithaka, NY, USA) and offered as a totally mixed ration at 0800 h and 1630 h. The ingredients and chemical composition of the experimental diet are shown in Table 1. The concentrate:forage ratio in the mixed diet was 70:30. Alfalfa hay, harvested in a single batch, was used throughout the experiment to minimize variability in the forage supplied. The lambs were randomly assigned to three dietary intakes (15 lambs per treatment) in a completely randomized design. Thus, the lambs were either fed *ad libitum* (ADL, n=15) or restricted to 80% (R80, n=15) and 60% (R60, n=15) intake of ADL. The ADL group allowed 10% of feed leftover, and it was collected before feeding the next morning to measure and adjust the amount of DMI-restricted feed groups. The feed of R80 and R60 were adjusted daily according to ADL group intake. Daily feed consumption and refusals were recorded and pooled weekly for each lamb. The lambs always had free access to clean drinking water. The CNCPS-S model was used for predicting ADG of lambs. the ingredient of the ration and their quantity along with the lamb's body weight and age were used as inputs. The predicted daily gain as output of the CNCPS-S model were 252, 179, and 95 g for ADL, 80R, and 60R respectively.

The digestibility trials were conducted using eighteen lambs (six lambs per group) each with a body weight close to the mean of 35 kg. Each trial lasted for seven days, consisting of a 2-day adaptation period followed by 5 days of feces and urine collection. Lambs were housed in metabolic cages (59 cm wide, 160 cm long, and 80 cm high). Daily feces of each lamb were collected in a plastic bucket containing 10 mL formalin 30%. Urine was collected daily using bottles containing 60 mL of 10% sulphuric acid (H_2SO_4) solution; the total daily volume produced by each lamb was measured and 10% of this total volume was collected and then frozen at −18 °C, for further analysis. Lamb body weight was recorded weekly using a digital scale

(Model WIC+, ETEMAD, Tehran, Iran) with measurements taken three hours after the morning feeding.

Table 1 Ingredients and chemical composition of the mixed diet

Ingredients	g per kg DM
Alfalfa hay	300
Barley grain	240
Corn grain	190
Soybean meal	145
Wheat bran	100
Mineral and vitamin premix[1]	15
Salt	7.5
Sodium bicarbonate	2.5
Chemical composition	
Dry matter (DM, g as fed)	873
Organic matter (OM)	964
Crude protein (CP)	18.6
Ether extracts (EE)	52
ASH	40
Neutral detergent fiber (NDF)	313
Acid detergent fiber (ADF)	163
Gross energy (GE, MJ/kg DM)	17.6
Digestible energy (DE, MJ/kg DM)	13.5
Metabolizable energy (ME, MJ/kg DM)	11.0

[1] The premix consisted of (per kg): vitamin A 100000 IU; vitamin D_3: 50000 IU; vitamin E: 1000 IU; Mn: 800 mg; Zn: 800 mg; Cu: 100 mg; Se: 20 mg; Fe: 400 mg; Ca: 146 mg; P: 5 g; Co: 20 mg; Iodine: 20 mg; S: 4 g; Mg: 20 g and Antioxidant: 1000 mg (Mehregan Rooshd Animal Feed Industries Co., Tehran, Iran).

Chemical analyses

The samples of ration fed, orts and feces were dried at 55 ºC for 72 h until their weight was fixed. The DM was determined by drying at 105 °C for at least 8 h by method 930 (AOAC, 2005). Ash by burning at 550 ºC for 2h via method number 942.05 and ether extract EE by method number 920.39 (AOAC, 2005). The NDF and ADF content was calculated as described by Van Soest method (Van Soest *et al.* 1991). The nitrogen content in feed, feces, and urine was determined using the Kjeldahl method using the Tecator-Kjeltec system 1026 (Tecator AB, Höganäs, Sweden) distilling unit by method number 984.13 (AOAC, 2005). Organic matter (OM) was computed as weight loss of samples during burning at 550 °C for 2h. The gross energy (GE) content of feed and feces was measured using an adiabatic bomb calorimeter (System C400, IKA Analysentechnic GmbH, Heitersheim, Germany).

Data calculations

Data from digestibility trial were used to calculate the energy value of the diet. Digestible energy (DE) of the diet was calculated as the difference between GE intake and fecal energy. The energy content of urine was calculated from the equation proposed by Hoffmann and Klein (Hoffmann and Klein, 1980). Methane energy losses were assumed as 5% of GE (CSIRO, 2007). Metabolizable energy was calculated by subtracting urinary and methane energy from DE. Mean body weight (MBW) was calculated from initial body weight (IBW) and final body weight (FBW) of each lamb:

MBW= (IBW+FBW) / 2 (Equation 1)

Relative growth index (RGI) was calculated as gram metabolic body weight ($BW^{0.75}$) divided by average daily gain (ADG). ME per gain was calculated as total daily ME intake divided by ADG:

Total ME requirement= (MEm×$BW^{0.75}$) + (MEg×gain) (Equation 2)

Where:
MEm and Meg: energy requirements for maintenance and growth, respectively.

When the two sides of the above Equation were divided by gain (g), Equation 3 was formed.

Total ME requirement (kJ) / gain (g)= MEm × ($BW^{0.75}$/gain) + Meg (Equation 3)

The slope and intercept of Equation 2 were assumed as metabolizable energy requirement for maintenance (MEm; kJ per $BW^{0.75}$) and ME requirement for growth (MEg; kJ per g ADG). The predicted ADG of lambs were estimated using the CNCPS-S, a mechanistic model that predicts nutrient requirements, biological values of feeds, and sheep performance (Cannas *et al.* 2004). The chemical composition data of the feeds used in this study were input into the feed library of the CNCPS model. Digestion kinetics, protein, and carbohydrate fraction data were sourced from the Tropical Feed Library of the Cornell Net Carbohydrate and Protein System (Tedeschi *et al.* 2010). Observed ADG values were regressed on CNCPS-S model-predicted values. Over- and under- prediction by the CNCPS-S model were indicated by data points below and above of the Y= X line.

Statistical analysis

Data were analyzed using SAS version 9.4 (SAS, 2004). Observations of BW, DMI, apparent digestibility, energy balance, and dietary energy contents from the digestion were analyzed as a randomized design by GLM. Pairwise comparisons of means were performed by Tukey multiple range tests once the significance of the treatment effect was declared at P= 0.05. REG procedure was used to obtain an equation from the linear regression between the ratio of consumed ME to ADG (kJ/g) and ADG to metabolic body weight ($BW^{0.75}$). The slope (b) of the regression line (Y=bX+a) was the energy required for maintenance (kJ per $BW^{0.75}$). The intercept (a) was indicated as energy required for growth (kJ per gram ADG). The standard error of means (SEM) is used as an indicator of variance.

The assumptions of the models, in terms of homoscedasticity, independency, and normality of the errors, were examined by plotting residuals against predicted values.

RESULTS AND DISCUSSION

Dry matter (DM) intake was 22% and 37% lower in the restricted feed intake groups, R80 and R60, respectively, compared to the ADL group (Table 2). The final body weight of R60 lambs was significantly lower ($P<0.05$) than that of both ADL and R80 lambs. Feed intake restriction resulted in a linear decrease ($P<0.05$) in average daily gain. R60 lambs had a significantly higher ($P<0.05$) dry matter and energy intake per g of gain compared to ADL and R80. The difference between observed ADG and predicted ADG using CNCPS-S was negative for ADL and R80 and positive for R60. The calculated RGI for R60 was approximately five and six times higher compared to R80 and ADL, respectively (Table 2).

Energy digestibility increased linearly ($P<0.05$) with feed restriction. Lambs in R60 group exhibited significantly higher ($P<0.05$) energy digestibility compared to ADL lambs (Table 3).

No significant differences ($P>0.05$) in energy digestibility were observed between R80 and ADL groups. The ME intake per metabolic body weight ranged from 670 to 1074 kJ/BW$^{0.75}$. The ME content of DE (ME/DE) was significantly lower ($P<0.05$) in restricted groups compared to ADL lambs. The ME/DE values of R80 and R60 were not significantly different ($P>0.05$). The ME content of GE (q=ME/GE) was significantly higher ($P<0.05$) for ADL lambs compared to restricted lambs. The lambs in R80 and R60 had similar ME/GE values ($P>0.05$).

As expected, the DMI and MEI showed a linear increase ($P<0.01$) as the energy levels in the experimental diets increased (Figure 1, Equation 5).

DMI (g/BW$^{0.75}$)= 4.94 + 0.085 MEI (kJ/BW$^{0.75}$) (R^2 = 0.99) (Equation 5)

Table 2 Effect of level of nutrition on performance and gain efficiency of Afshari male lambs

Item	Level of feed intake			SEM	P-value	Linear	Quadratic
	ADL	R80	R60				
Initial body weight (kg)	29.5	29.7	29.3	0.26	NS	NS	0.51
Final body weight (kg)	39.3^a	38.0^a	32.0^b	0.55	<0.01	<0.01	<0.01
Net gain (kg)	9.92^a	8.25^b	2.67^c	0.52	<0.01	<0.01	<0.01
DM intake (kg)	1.33^a	1.07^b	0.80^c	0.33	<0.01	<0.01	0.77
DM intake(g/BW$^{0.75}$)	94.2^a	76.2^b	61.3^c	2.10	<0.01	<0.01	0.20
OM intake (g/BW$^{0.75}$)	90.7^a	73.5^b	59.0^c	2.01	<0.01	<0.01	0.20
Observed ADG (g)	261^a	217^b	70.3^c	13.6	<0.01	<0.01	<0.01
DMI/ADG (g/g)	5.22^b	5.02^b	19.0^a	1.61	<0.01	<0.01	0.13
ME/ADG (kJ/g)	58.5^b	54.8^b	207^a	17.3	<0.01	<0.01	0.01
DE/ADG(kJ/g)	69.8^b	68.0^b	260^a	22	<0.01	<0.01	0.01
GE/ADG(kJ/g)	91.9^b	88.4^b	335^a	28.3	<0.01	<0.01	0.01
Predicted ADG (g)	232^a	158^b	86.0^c	9.19	<0.01	<0.01	0.74
Predicted-observed (g)	-9.3^b	-38.1^b	+24.4^a	7.32	<0.01	<0.01	<0.01
Predicted-observed (%)	-4.1^b	-21.5^b	+28.4^a	10.7	<0.01	<0.01	<0.01

ADL (*ad libitum*): ration that amount is equal to the limit of appetite; R80: ration that amount is 80% of ADL and R60: ration that amount is 60% of ADL.
DM: dry matter; OM: organic matter; BW: body weight; ADG: average daily gain; DMI: dry matter intake; ME: metabolizable energy; DE: digestible energy and GE: gross energy.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
NS: non significant.

Table 3 Effect of plane of nutrition on energy balance of Afshari growing lambs

Item	Level of feed intake			SEM	P-value	Linear	Quadratic
	ADL	R80	R60				
Energy balance (kJ/BW$^{0.75}$)							
Gross energy	1655^a	1340^b	1078^c	36.7	<0.01	<0.01	0.20
Feces energy	397^a	307^b	239^c	10.3	<0.01	<0.01	0.15
Digestible energy	1258^a	1032^b	840^c	26.7	<0.01	<0.01	0.29
Excreted urinary energy	89.4^b	108^a	94.8^b	2.50	<0.05	0.34	<0.01
Methane energy	116^a	94^b	75^c	2.57	<0.01	<0.01	0.20
Metabolizable energy	1074^a	828^b	670^c	25.9	<0.01	<0.01	0.02
DE/GE (%)	76.0^b	77.0^{ab}	77.8^a	0.17	<0.05	<0.01	0.70
ME/DE (%)	83.7^a	80.4^b	79.7^b	0.32	<0.05	<0.01	<0.01
ME/GE (%)	63.5^a	61.9^b	62.0^b	0.21	<0.05	<0.01	0.03

ADL (*ad libitum*): ration that amount is equal to the limit of appetite; R80: ration that amount is 80% of ADL and R60: ration that amount is 60% of ADL.
DE: digestible energy; GE: gross energy and ME: metabolizable energy.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
NS: non significant.

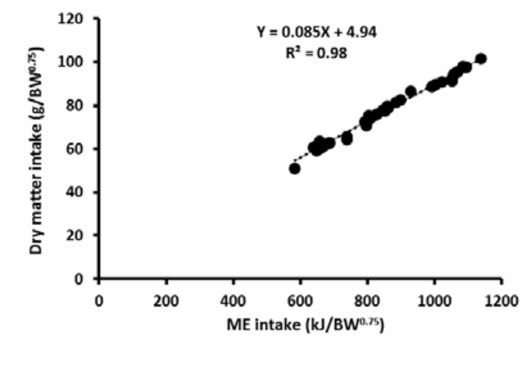

Figure 1 Relationship between dry matter intake (g/BW$^{0.75}$) and metabolizable energy intake (kJ/BW$^{0.75}$) for fat-tailed Afshari sheep. {DM intake, g/BW$^{0.75}$= 4.94 (±1.15) + [0.085 (±0.001) × ME intake, kJ/BW$^{0.75}$]; R2= 0.98; Root mean square error= 1.45; n= 45

The intercept and the slope coefficients in Equation 5 were significantly different from zero (P<0.01). This linear increase in MEI resulted in higher energy availability, leading to an observed increase in daily weight gain.

The ADG increased linearly with higher metabolizable energy concentration in the diet (Equation 6)

ADG (g)= 0.45 (±0.05) ME (kJ/BW$^{0.75}$) + 199 (±44), n=45, R^2= 0.64, RMSE= 55 (Equation 6)

The relationship between metabolizable energy intake (MEI) and average daily gain (ADG) for fat-tailed Afshari sheep is depicted in Figure 2. The intercept and the slope coefficients in Equation 6 were significantly different from zero (P<0.01). The equation 6 showed that the relationship between ADG and ME (Figure 2) has a moderate predictive accuracy (R^2=0.64).

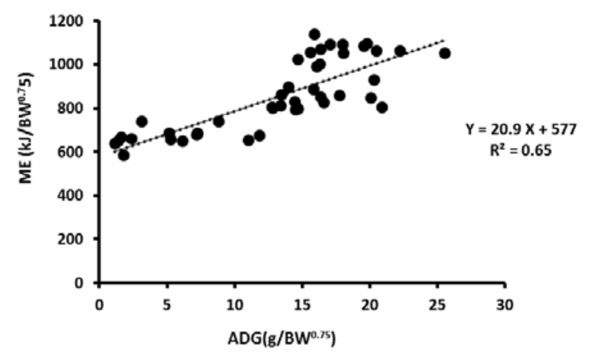

Figure 2 Relationship between metabolisable energy intake (ME; kJ/BW$^{0.75}$) and average daily gain (ADG; g/BW$^{0.75}$) for fat-tailed Afshari sheep. {ME intake, kJ/BW$^{0.75}$= 577 (±39.1) + [20.9 (±2.69) × average daily gain, g/BW$^{0.75}$]; R^2= 0.65; Root mean square error= 100; n= 45}

The extrapolated MEm and MEg were 577 kJ/BW$^{0.75}$ and 20.9 kJ per gram of gain, respectively.

Based on RGI method (Figure 3), the calculated MEm was 614 kJ/BW$^{0.75}$ (Equation 7).

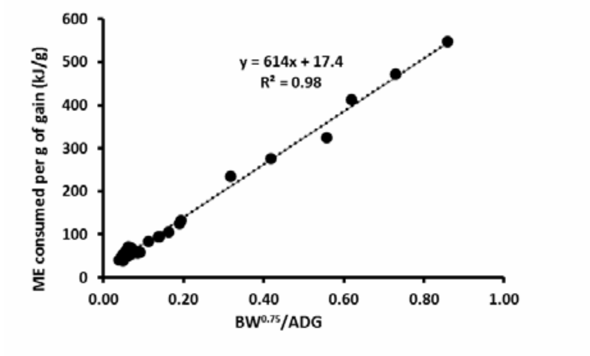

Figure 3 Relationship between relative growth index (BW$^{0.75}$/g) and ME consumed per gain (kJ/g) in fat-tailed Afshari male lambs. {ME intake per g of gain, kJ/g= 17.4 (±1.82) + [614 (±7.1) × relative growth index, BW$^{0.75}$/g]; R^2= 0.98; Root mean square error= 46.4; n= 45}

[ME (kJ/g ADG)]= 614 (±7.1) RGI (BW$^{0.75}$/g) + 17.4 (±1.82) (kJ/g ADG)
R^2= 0.98, n= 45, RMSE= 9.65 (Equation 7)

The total requirement of ME for maintenance and growth of Afshari lambs from 30 to 40 kg body weight with various ADG are presented in Table 4.

In Figure 4, the solid line represents unitary equivalence (Y=X). The intercept and the slope coefficients are both significantly different from zero (P<0.01) and unity (P<0.001).

In this study, the DM intake of the ADL, R80 and R60 groups was 3.9%, 3.2% and 2.6% of body weight, respectively. The DM intake of the ADL group was 34 g per kg body weight and 94.2 g/BW$^{0.75}$ which aligns with the values reported in the literature for fat-tailed sheep (Kamalzadeh and Aouladrabiei, 2009; Arjmand *et al*. 2022; Ben Ettoumia *et al*. 2022a). These values fall within the recommended range of 2.86-3.91% body weight for sheep DMI as suggested by (NRC, 2007). The recommended DM intake according to (NRC, 2007) depends on the ADG of sheep and the energy concentration in the diet. The ME/DE ratio in restricted lambs was slightly lower than that of the ADL lambs. This finding contrasts with the results of (Kamalzadeh and Aouladrabiei, 2009) who observed that feed restriction improved ME/DE in Afshari lambs. The ME/DE found in the ADL group (83.7%) was slightly higher than the generalized value of 82% recommended by (ARC, 1980). The value for restricted lambs (80%) was below the ARC value, which may be attributed to various factors, including dietary composition, animal characteristics and environmental factors (Nikkhah, 2014).

Table 4 Total energy requirement of ME (kJ/d) for the maintenance and growth of Afshari male lambs

BW (kg)	ADG (g/d)								
	0	50	100	150	200	250	300	350	400
30	7867	8733	9599	10464	11330	12196	13062	13928	14794
32.5	8354	9219	10085	10951	11817	12683	13549	14414	15280
35	8831	9697	10563	11429	12294	13160	14026	14892	15758
37.5	9300	10166	11032	11898	12763	13629	14495	15361	16227
40	9761	10627	11493	12359	13225	14090	14956	15822	16688

BW: body weight and ADG: average daily gain.

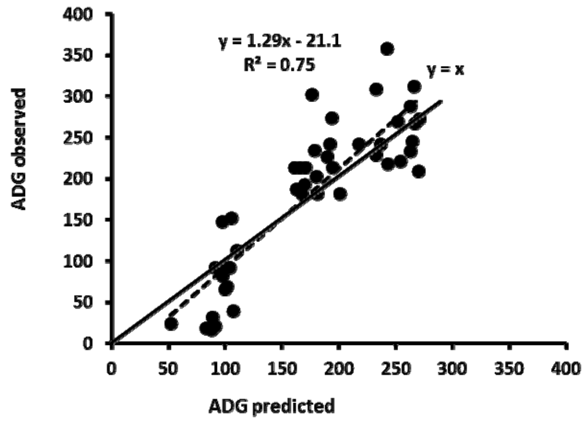

Figure 4 Relationship between observed and predicted ADG by the Cornell Net Carbohydrate and Protein System for Sheep {observed ADG, g/d= 1.29 (±0.11) - [21.1 (±19.3) × predicted ADG, g/d]; R^2= 0.75; Root mean square error= 0.46; n= 45}

The lower ME/DE found in restricted group (R60) compared to ADL lambs might be due to the different composition of the body gain. Feed restriction can affect the composition of the body weight gain during the fattening period (Searle *et al*. 1972; Santos *et al*. 2018).

The digestibility values observed in the present experiment (76.0-77.8%) were similar to those reported for Afshari lambs (Kamalzadeh and Aouladrabiei, 2009). An improvement in digestibility was noted in R60 group compared to ADL. This improvement may be attributed to increased feed utilization efficiency, likely resulting from reduced rumen feed passage (Thomson *et al*. 1982). The metabolizability values obtained in this experiment were between 62 and 63% which was in the range (40 to 64%) proposed in several reports including those of (ARC, 1980; Thomson *et al*. 1982; Kamalzadeh and Aouladrabiei, 2009).

The maintenance energy requirement (MEm) for a growing lamb refers to the energy needed to maintain the body with no changes in body weight or energy reserves. The estimated ME requirement for maintenance of Afshari growing lambs based on ADG was 577 kJ/BW$^{0.75}$ [ME (kJ/BW$^{0.75}$)= 20.9 ADG (g/BW$^{0.75}$) + 577 (kJ/BW$^{0.75}$), R^2= 0.64, n= 45, RMSE= 100]. It worth to mention that this equation shows that the relationship between ADG and ME has a moderate predictive accuracy (R^2=0.64), and show a low fit. It means that the relation between the independent and dependent variables is not strictly linear. One reason for the lack of fit might be due to the small number of animals used in the dataset (n=45).

This MEm value aligns with the recommended value of 560 kJ/BW$^{0.75}$ (Shrunk BW) by NRC (2007) and close to the value of 542 kJ/BW$^{0.75}$ for tropical sheep breeds (Salah *et al*. 2014) and the value of 526 kJ/BW$^{0.75}$ reported for Omani male growing lambs (Early *et al*. 2001). However, the present value (577 kJ/BW$^{0.75}$) was higher than the range of 340 to 500 kJ/BW$^{0.75}$ reported in other studies for fat-tailed sheep (Al Jassim *et al*. 1996; Kamalzadeh and Shabani, 2007). For example, Al-Jassim *et al*. (1996) suggested a range of 342 to 482 kJ/BW$^{0.75}$ for the maintenance energy requirement of fat-tailed Awassi sheep which does not align with the findings of the present study.

The MEm value found in the present study was greater than those reported for European tailed sheep breeds. It was approximately 33% higher than the value (381 kJ/BW$^{0.75}$) obtained for Texel crossbred lambs (Galvani *et al*. 2008), 30% higher than the value (403 kJ/BW$^{0.75}$) reported for Dorper crossbred ram lambs (Deng *et al*. 2012), 27% higher than 417 kJ/BW$^{0.75}$ reported for Texlel lambs (Martins *et al*. 2019), and 19% higher than the value of 460 kJ/BW$^{0.75}$ for English sheep breeds (Dawson and Steen, 1998). The discrepancy in MEm values between the current study and those in the literature can be attributed to differences in breed, body composition, feed ingredients, nutritional quality of diets and experimental conditions. One possible explanation for the variation between fat-tailed and tailed sheep breeds could be linked to their body composition. Fat-tailed sheep deposit more fat compared to tailed breeds (Farid, 1991; Esmailizadeh *et al*. 2012; Ben Ettoumia *et al*. 2022b). The energy cost for the deposition of one gram of fat (68 kJ) is greater than one gram of protein (48 kJ), resulting in a lower net efficiency of ME use for protein deposition compared to fat deposition (Orskov and McDonald, 1970). Therefore, as fatness increases in fat-tailed breeds, the energy required for maintenance also increases. Additionally, the higher MEm value observed in the current study can be partly explained by the experimental condition and method used. In studies using indirect calorimetry, lambs were less active due to being housed in relatively small areas (i.e. metabolic chambers). In contrast, in the current study, lambs were more active because they were kept under more typical farm conditions. This finding aligns with the estimation of ME requirement of goats based on ADG, which resulted in a greater MEm compared to those obtained from indirect calorimetry data (Luo *et al.* 2004). The estimated MEm for a growing male lamb with 35 kg body weight and qm= 0.64 of the diet exceeded the values of 485, 427 kJ/BW$^{0.75}$ in NRC (2007) and AFRC (1993), respectively. This could be attributed to the nutritional status of the lambs used for data generation. In the current study, lambs were in a fed-state, while the data presented in NRC (2007) and AFRC (1993) were derived from fasted lambs. Generally, fed animals have higher energy requirements due to higher metabolic activity of tissues compared to fasted animals (Luo *et al.* 2004).

It has been reported that growing animals typically have higher MEm than mature animals likely due to the influence of body composition on MEm estimation. For growing lambs, energy retention is primarily in the form of protein, whereas in adult ruminants, it is more commonly stored as fat (Searle *et al.* 1972). Furthermore, animals with high growth rates, which are directly or indirectly linked to higher rates of protein synthesis in tissues, tend to exhibit greater basal metabolic rates (Costa *et al.* 2018). These factors contribute to the variation in the maintenance energy requirement and cannot be entirely accounted for (Wang *et al.* 2021).

The metabolizable energy requirement for gain (MEg) in growing Afshari lambs, based on ADG and RGI, was estimated to be 20.9 kJ ME (Figure 2) and 17.4 kJ ME per g of gain (Figure 4) respectively. These estimates fall within the range of values reported in the literature (13.7 to 27.9). Published values for sheep by NRC (NRC, 1985; NRC, 2007), and INRA (INRA, 1989) indicate a value of 20.6 kJ ME per gram of gain. The present values were slightly lower than 24.2 ME for gain reported for sheep in warm environmental conditions (Salah *et al.* 2014). Variations in these estimates can be attributed to differences in methods, genotypes, animal age and body composition (Salah *et al.* 2014; Ma *et al.* 2022).

The total requirement of ME for maintenance and growth of Afshari lambs from 30 to 40 kg body weight with 250 gram ADG range from 12.2 to 14.1 MJ/d (Table 4). For example, for an intact growing Afshari male lamb with 35 kg body weight and qm= 0.64 and 250 g gain, the total ME requirements based on RGI equation would be 14.39 × 614 + 17.3 × 250 = 8835(MEm) + 4325 (MEg) = 13160 (13.2 MJ/d). Similarly, based on ADG equation [ME (kJ/BW$^{0.75}$)= 577 (kJ/BW$^{0.75}$) + 20.9 ADG (g/BW$^{0.75}$), R2= 0.64], the total ME requirements for the same lamb would be 577×14.4 + 20.9 × 250 = 8309 (MEm) + 5225 (MEg) = 13534 (13.5 MJ/d). These two equations predicted the same energy requirement for fat-tailed Afshari breed, however the RGI equation showed better fitness. The value derived from NRC (2007) and the AFRC system (AFRC, 1993) for the same lamb (growing male lamb with 35 kg body weight and qm=0.64 and 250 g gain) are 14.0 and 16.5 MJ/d respectively. It means that the energy required to grow a 35 kg fat-tailed lamb with a daily growth rate of 250 g calculated based on RGI is about 18% and 6% lower than those reported in the English system (AFRC, 1993) and NRC (2007) respectively. These discrepancies may be due to differences in breed, diets, growth pattern and body composition.

The findings of this study suggest that the energy requirements of growing lambs can be effectively estimated using the RGI method. This method offers several advantages over CST and calorimetry methods, as it is non-invasive and does not require sophisticated research facilities. Additionally, the RGI method is more cost-effective as it does not require the measurement of body chemical composition (Sahlu *et al.* 2004). Furthermore, the RGI method aligns better with animal welfare considerations; which can be a limiting factor for the application of CST in many countries. The method has also been successfully applied to goats in previous studies (Luo *et al.* 2004). However, caution should be exercised when comparing results from the RGI with those of other methods. Despite its non-invasive, time-efficient, and cost-effective nature, estimating ME requirements based on ADG has some limitations. It assumes a constant energy concentration in gain and does not account for potential variations in the digestive tract that may affect body weight measurements.

The predicted ADG of lambs were estimated using the CNCPS-S, a mechanistic model that predicts nutrient requirements, biological values of feeds, and sheep performance (Cannas *et al.* 2004). Observed ADG values were regressed on CNCPS-S model-predicted values (Equation 8).

observed ADG= 1.29 (±0.085) × predicted ADG - 21.1 (±0.079); R^2= 0.75) (Equation 8)

Over- and under- prediction by the CNCPS-S model were indicated by data points below and above of the Y = X line. The CNCPS-S model underestimated ADG for the R80 and ADL groups (Figure 4, Equation 8), while it significantly overestimated ADG for lambs in the R60 group. This discrepancy may be attributed to the fact that fat-tailed lambs accumulate more fat in the body compared to tailed sheep breeds. Furthermore, feed restriction can affect the composition of body weight gain during the fattening period (Searle *et al.* 1972; Santos *et al.* 2018). Animals with low growth rates are often associated with higher rates of fat synthesis (Searle *et al.* 1972; Costa *et al.* 2018; Santos *et al.* 2018). Therefore, R60 lambs may accumulate more fat as an adaptation mechanism to unfavourable conditions (Santos *et al*. 2018). Consequently, to gain 1 kg of weight, fat-tailed lambs need to consume more energy because they deposit more fat than protein. This could explain why, in the current study, the discrepancies between CNCPS-S model-predicted and observed ADG tended to decrease as feed intake increased. The findings of this study, along with those of others (Early *et al*. 2001; Kamalzadeh and Aouladrabiei, 2009; Salah *et al*. 2014; Jayanegara *et al.* 2017; Ma *et al*. 2022) clearly show that the nutrient requirements of fat-tailed sheep differ from tailed breeds. Furthermore, this study showed that using feed recommendations from American, and European feeding systems may not be suitable for calculating nutrient requirements in fat-tailed sheep. This discrepancy might be presumably due to the differences in breed, feed composition and nutritional quality, and environmental condition (NRC, 1985; AFRC, 1993; Salah *et al*. 2014; Martins *et al*. 2019). Furthermore, fat-tailed sheep exhibit distinct performance metrics and body composition compared to tailed breeds (Farid, 1991; Esmailizadeh *et al*. 2012; Ben Ettoumia *et al*. 2022b).

CONCLUSION

This study highlights the significant variability in energy requirements between fat-tailed and tailed sheep breeds, emphasizing the importance of breed-specific nutrient guidelines. The use of the RGI method proved to be a promising and accessible alternative method for estimating the energy needs of growing lambs, offering distinct advantages over traditional methods like comparative slaughter and indirect calorimetry. The findings reveal that the maintenance energy requirement (614 kJ/BW$^{0.75}$) and growth energy requirement (17.4 kJ/g ADG) for Afshari lambs are higher than those reported for many tailed sheep breeds, suggesting that fat-tailed sheep have distinct metabolic and growth patterns that should be accounted for in nutritional models. The study also underscores the limitations of applying generalized feeding recommendations across diverse sheep breeds and management systems, calling for more tailored approaches to better meet the unique energy needs of fat-tailed sheep. Ultimately, the implications of the results of the present study lay the groundwork for more refined, breed-specific feeding strategies for stakeholders (farmers, nutritionists) that could enhance the productivity and sustainability of sheep farming in regions where fat-tailed breeds (e.g. Afshari sheep) predominate.

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