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The Net Mineral Requirement for Maintenance and Growth of Dorper × Hu Ewe Lambs

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ABSTRACT

A comparative slaughter trial was conducted to estimate the mineral concentrations and distributions in the main body tissues and the net requirements for maintenance and growth of F_1 Dorper × Hu ewe lambs. Thirty-five ewe lambs averaging 33.52±0.56 kg body weight (BW) were used. Seven ewe lambs were randomly chosen and slaughtered at 34.93 ± 0.37 kg BW as the baseline group for measuring initial body composition. Another seven lambs were also randomly chosen and offered a pelleted mixed diet (approximately concentrate : roughage = 60 : 40, DM basis) for ad libitum intake and slaughtered at 41.73 ± 0.53 kg BW. The remaining lambs (n = 21) were allocated randomly on d 0 to 3 treatment intake levels (treatments were ad libitum or restricted to 70 or 40% of the ad libitum intake) within 7 slaughter groups. A slaughter group contained 1 lamb from each treatment, and lambs were slaughtered when the ad libitum treatment lamb reached approximately 50 kg BW. Non-carcass components (head + feet, hide, internal organs + blood) and empty bodies of the lambs were weighed, ground, mixed, and subsampled for chemical analyses. The Ca, P, Na, and Mg were mainly distributed in bone, except for K, which was mainly distributed in the muscle tissues. The net macromineral requirements for maintenance were 20.20 mg Ca, 13.50 mg P, 3.80 mg Na, 7.91 mg K, and 1.10 mg Mg/kg empty body weight (EBW) for ewes. The net requirements for growth ranged from 11.79 to 12.06 g Ca, 6.04 to 6.16 g P, 1.66 to 1.74 g K, 0.40 to 0.42 g Mg, and 0.98 to 0.95 g Na /kg of EBW gain (EBWG) for ewes from 35 to 50 kg BW. These results for the mineral requirements may help to formulate more balanced diets for F_1 Dorper × Hu ewe lambs in the growth phase of 35 to 50 kg BW.

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Authors' Contribution
HZ and HN designed the study, interpreted the data and wrote the article. FW was involved in study design. ZW executed the experimental work.

Key words
Ewe lambs, Macrominerals, Micronutrients, Live weight gain.

INTRODUCTION

The Hu sheep is an indigenous Chinese sheep breed with remarkable precociousness and prolificacy (Yue, 1996). The Dorper sheep is well known for its hardiness, early maturity, and rapid growth (Cloete *et al*., 2000) and has recently been used as a meat sire breed to improve the growth performance and carcass traits of Hu sheep. The Dorper sheep originated in South Africa and is characterized by hardiness, early maturity, and rapid growth (Cloete *et al*., 2000). The Hu sheep is a Chinese indigenous breed that is well adapted to the ecological conditions of high temperature and high humidity areas of China and is noted for its beautiful lamb skin, precociousness and prolificacy (Yue 1996; Zhang *et al*., 2016). The Dorper × Hu crossbred sheep has, therefore, become one of the dominant crossbreeds for lamb meat production in China (Zhang *et al*., 2015, 2017, 2018).

Macromineral requirements have received consideration because any excess or deficiency in one element interferes in the utilization of another, resulting in impaired health, productivity and even survival. Furthermore, accurate prediction of macromineral requirements might minimize their excretion and reduce environmental pollution (Chizzotti *et al*., 2009; Abdelrahman *et al*., 2017).

The breed or genotype of an animal affects its energy and protein requirements (Sahlu *et al*., 2004), and therefore, it is expected that macromineral concentrations, body distribution and requirements are also affected. The estimation of the macromineral requirements of growing sheep necessitates a greater understanding of macromineral deposition in the body.

Comparative slaughter trials (CST) are commonly used to estimate the protein, energy, macromineral, and micromineral requirements of animals (Chizzotti *et al*.,

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2007; Fernandes *et al.*, 2012; Souza *et al.*, 2013). Our objective for this study was to use CST to establish net macromineral requirements for maintenance and growth of F_1 Dorper × Hu ewe lambs by determining macromineral concentrations and distribution in the main body tissues.

MATERIALS AND METHODS

The experiment was conducted at the Haimen Experimental Station of Nantong, Nantong City, Jiangsu Province of China. During the research period, a heated indoor facility was used to keep the temperature within the range of 15.50 ± 1.32 - 26.54 ± 1.61°C. The average relative humidity was 61.25 ± 2.76 %. All trials were conducted in accordance with the Guidelines for the Care and Use of Animals in the College of Animal Science and Technology, Nanjing Agricultural University (SYXK 2011- 0036).

Animals and management

In this study, thirty-five ewe lambs of F_1 crosses of purebred Dorper and Hu sheep were weaned at approximately 90 d of age and offered the diet for ad libitum consumption until the start of the trial when they had an initial BW of 33.52 ± 0.56 kg and 132 ± 4.15 d old. From weaning to experiment, the diet was a pelleted mixture based on cracked corn, soybean meal, and wildrye hay (*Leymus chinensis*), with a concentrate:roughage ratio of 44:56 (DM basis). All of the animals were allocated at the same facility from the same farm and previously raised together. Prior to d 1 of the experiment, all animals were allowed *ad libitum* (AL) access to the experimental diet for a 10 d adaptation period. Thereafter, all lambs were drenched with 0.20 mg ivermectin per kg of BW, and confined in individual stainless steel pens (3.20 by 0.80 m). Each pen was equipped with feeders and automatic water suppliers. The experimental diet was fed as a pelleted mixed diet composed of corn, soybean meal, and soybean straw, with approximately concentrate : roughage ratio of 60 : 40 on a dry matter (DM) basis (Table I). The choice of a pelleted diet was to prevent possible selectivity and waste and to facilitate more accurate measurements of feed intake.

Following the 10 d adaptation period, seven ewe lambs at 34.85 ± 0.37 kg BW and approximately 142 d old were randomly selected for slaughter as the baseline group (BL) for measuring initial body composition. Another seven randomly selected ewe lambs were fed AL and slaughtered as an intermediate slaughter group (IM), which was carried out at approximately 168 d old when they reached 41.73 ± 0.53 kg of BW. On d 0 of the evaluation of treatments, the remaining 21 ewe lambs were randomly allocated to three diet regimens (treatments): AL, 70% of AL, and 40% of AL, which were expected to yield BW gains of approximately 300, 200, and 0 g/d, respectively, according to NRC (2007). The lambs were pair fed in 7 slaughter groups, with each group consisting of 1 lamb from each dietary treatment. All lambs within a slaughter group were slaughtered when the lamb that was fed ad libitum reached approximately 50 kg BW. The entire experiment lasted approximately 60 d.

Animals were fed once daily at 0800 h and had free access to clean water. The amount of feed offered to the AL group was adjusted daily in the morning to ensure a 10% refusal based on the DM intake (DMI) of the previous day. The amount of feed offered to the restricted feed intake groups was also calculated daily, based on the DMI of the AL group from the previous day. Individual samples of the feed offered and orts (approximately 10% of total) were collected daily and frozen (-20°C). Feed offered and orts were sampled to estimate the daily intake of minerals for each animal. These samples were eventually oven-dried at 55°C for 72 h, ground to pass through a 1-mm screen using a Willey mill (Arthur H. Thomas, Philadelphia, PA), and stored until analyses.

Table I.- Ingredient and nutrient composition of the experimental diets on a DM basis.

Item	Value
Ingredients (g kg^{-1})	
Corn	414.4
Soybean meal	193.3
Soybean straw	381.1
Anhydrous calcium phosphate	3.8
Limestone	2.3
Sodium chloride	4
Premix[1]	1.1
Nutrient composition (analyzed)[2]	
CP (g kg^{-1})	171.7
ME (MJ kg^{-1})[3]	9.77
Ether extract (g kg^{-1})	22.6
NDF (g kg^{-1})	456.5
ADF (g kg^{-1})	231.8
Ca (g kg^{-1})	6.8
P (g kg^{-1})	3.2
Na (g kg^{-1})	3.0
K (g kg^{-1})	8.7
Mg (g kg^{-1})	2.2

[1]The premix provided the following nutrients per kg of the diet: 15,000 IU VA, 5,000 IU VD, 50 mg VE, 32 g Na, 92 g K, 23 g Mg, 90 mg Fe, 12.5 mg Cu, 50 mg Mn, 100 mg Zn, 0.3 mg Se, 0.8 mg I, and 0.5 mg Co. [2]Nutrient levels are analyzed values. [3]Energy value estimated according to Deng *et al.* (2012).

Slaughter procedure

The day before slaughter, body weight of lambs in all groups was measured at 1600 h. Lambs were slaughtered by exsanguination after stunning by CO_2 inhalation. Blood was collected and weighed. Mass of the viscera, hide, wool, head, feet, carcass and adipose tissues removed from the internal organs were recorded. The gastrointestinal tract (rumen, reticulum, omasum, abomasum, and small and large intestines) was removed and weighed before and after their contents were removed in order to obtain the empty body weight (EBW), which was determined by subtracting the mass of the content of the gastrointestinal tract (CGIT) and the bladder from the BW. All body components were initially frozen at -6°C, then cut with a stainless steel band saw, ground, and homogenized, and 500 g samples were collected for chemical analysis. These samples were thawed, and 100 g sub-samples were lyophilized for 72 h and then ground in a stainless steel blender. The collection of samples followed the procedures described by Galvani *et al*. (2008) and (2009), with minor modifications. In brief, carcasses and heads were split at dorsal midline. The right-half carcass, the right-half head, and the right anterior and posterior feet were dissected into muscle, bone, and fat. The bone was ground with a bone miller (ModelSGJ-3600, Langfang Huiyong Machinery Plant, Hebei, China) through an 8-mm screen and homogenized by three additional passes through the miller before a 500 g sample was taken for each animal and stored at -20°C. The muscle and fat were cut separately into small pieces, fully ground with an electrical screw grinder (Model-12, Shanghai Xinmai Machinery Plant, Shanghai, China) through a 4-mm screen, and homogenized before a 500 g sample was taken for each animal and stored at -20°C.

Chemical analyses

Feeds, orts and water samples: the DM content of feeds and orts was determined by drying at 135°C for 2 h (AOAC, 1990; method number 930.15). Ash was determined by complete combustion in a muffle furnace at 600°C for 4 h (Myers and Beede, 2009). The ash was dissolved in 3 mol/L HNO_3 suprapur and heated for 10 min in a water bath. Afterwards, the ash solution was filtered into a measuring flask with hot bi-distilled water. The residue on the filter underwent further ashing (450°C) for another 12 h and was filtered again. The final acid concentration in the solution was 0.3 mol/L HNO_3. The concentrations of Ca, P, Na, K and Mg in feeds, orts and water were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES) by Unicam PU701 (now: Thermo Electron GmbH, Dreieich, Germany), power: 1000W, plasma: radial observation, sprayer: Hildebrand-Grid, sprayer pressure: 34–40 p.s.i., argon-cooling gas: 11 L/min, flow of the sample: 1 mL/min, with the emission wavelength specific for each element. For calcium two lines were chosen. As the concentration of potassium was too high in the undiluted samples of muscle tissue they had to be diluted individually (up to 1:100) and measured using an atomic absorption spectroscope [Unicam PU 9400 (now: Thermo Electron GmbH), measuring principle: flames photometric, potassium-hollow-cathode-lamp; burner: 50 mm; flame: air-acetylene; wavelength: 766.5 nm; gap width: 0.5 nm; lamp current: 8 mA] (Bellof *et al*., 2006; Bellof and Pallauf, 2007).

Body components (bone, muscle, hide, viscera, fat, and fleece): a 100 g subsample from each initial sample with the exception of the wool was lyophilized (Galvani *et al*., 2009) for 72 h to determine DM content, and then all the subsamples including the wool were analyzed for macromineral elements (Ca, P, Na, K and Mg) as described above.

Data calculation and analyses

Prediction of the initial body macro mineral content

The body macro mineral content was calculated as the sum of the content of all body components. The initial body macro minerals content of each animal in the intermediate and final slaughter groups was calculated using a regression equation developed for the relationship between the body macro mineral content and EBW of baseline animals (Fernandes *et al*., 2007). The initial EBW of ewe lambs was computed from the BW (Eq. 1):

$$EBW\ (kg) = a + [b \times (BW,\ kg)] \ldots\ldots (1)$$

Macromineral concentrations and distributions

The average macromineral concentration and distribution in carcass tissues were computed from the average values of all treatments with different intake levels and growth stages of 35-50 kg Dorper × Hu lambs (Zhang *et al*., 2015).

Macromineral requirements for maintenance

Maintenance requirements were calculated using the comparative slaughter technique (CST; Lofgreen and Garrett, 1968). Retained macromineral was calculated as the difference between final and initial body mineral content of each animal from intermediate and final slaughter groups, and the total macromineral element losses were calculated as the difference between intake and retained macromineral elements. A linear regression of daily macrominerals retained (mg/kg of EBW) on macrominerals intake (MI) (mg/kg of EBW) was developed to predict their net requirement for maintenance by extrapolating the linear regression until MI = 0. The intercept of this regression represented inevitable macromineral losses equivalent to the net requirement for maintenance (mg/kg EBW·d^{-1}).

Macromineral requirements for growth

Body composition was predicted via a logarithmized allometric equation of the quantity of the macrominerals that were present in the empty body (g) as a function of the EBW (kg), as per ARC (1980).

$$\text{Equation: Log y=a + [b × log x], [2]}$$

where log y is the logarithm of the total amount of macrominerals of the empty body (g), a is the intercept, b is the coefficient of the regression of the macromineral content as a function of the EBW, and log x is logarithm EBW (kg). Eq. [3] was differentiated to compute the estimates of the composition of the gain at various EBWs.

$$\text{Eq. [3]: } y' = b \times 10^{a} \times EBW^{(b-1)}$$

where y' is the nutrient per unit of empty weight gain (in g/kg of gain), EBW is in kilograms, a and b are coefficients determined from a linear regression Eq. [2]. To estimate the net macromineral requirements for BW gain, the values of body composition of gain were divided by the BW to EBW ratio factor. The lambs fed at restricted levels of intake were not included in the prediction equation due to their growth pattern differing from those fed ad libitum.

Statistical analysis

The data were analyzed as a completely randomized design using SAS (SAS Inst. Inc., Cary, NC). The linear regressions analyses were conducted with PROC REG. The analysis of DMI, CP intake (CPI), and ME intake (MEI), body composition, ADG, and EBW were performed using PROC MIXED for the different feeding levels. Residuals plotted against the predicted values were used to check the assumptions of the model for homoscedasticity, independency, and normality of the errors. A data point was deemed to be an outlier and removed from the database if and only if the Studentized residual was outside the ±2.5 range values. The comparison of the means was performed using the Duncan test at $P = 0.05$.

RESULTS

Growth performance and body macromineral composition

As described in Table II, DMI, CPI, MEI, EBW, ADG and EBW gain (EBWG) increased as intake level increased ($P < 0.05$). The Ca, P, Na, K and Mg concentrations decreased as intake level increased ($P < 0.05$) (Table II).

Macromineral content in body tissues and empty body

The Ca, P and Mg concentrations were greater ($P < 0.05$) in bone, Na concentration was higher ($P < 0.05$)

Table II.- Performance and body macromineral concentrations of Dorper×Hu ewe lambs throughout different growing periods and subjected to 3 levels of feed intake.

Item	Treatments					SEM	P-value
	BL	IM	AL	70% AL	40% AL		
n	7	7	7	7	7	–	–
DMI (kg/d)	–	1.69	1.76^{a}	1.24^{b}	0.75^{c}	0.05	0.0146
CPI (kg/d)	–	0.25	0.30^{a}	0.21^{b}	0.13^{c}	0.04	0.03
MEI (MJ/d)	–	15.53	17.20^{a}	12.12^{b}	7.33^{c}	4.25	0.02
BW (kg)	34.85	41.47	48.38^{a}	43.23^{b}	35.67^{c}	0.71	<0.0001
EBW (kg)	29.41	34.56	40.25^{a}	36.18^{b}	31.17^{c}	0.63	<0.0001
ADG (g/d)	–	237.50	220.61^{a}	160.45^{b}	13.44^{c}	21.14	<0.0001
EBWG (g/d)	–	183.94	177.71^{a}	110.99^{b}	28.85^{c}	16.45	<0.0001
Ca (g/kg of EBW)	10.92	11.12	11.23^{c}	11.39^{b}	11.72^{a}	1.13	0.03
P (g/kg of EBW)	5.69	5.81	5.80^{b}	5.89^{a}	5.90^{a}	0.64	0.04
Na (g/kg of EBW)	1.10	1.09	1.06^{c}	1.08^{b}	1.11^{a}	0.31	0.02
K (g/kg of EBW)	1.45	1.50	1.51^{c}	1.53^{b}	1.55^{a}	0.25	0.03
Mg (g/kg of EBW)	0.35	0.36	0.37^{b}	0.38^{b}	0.41^{a}	0.12	0.03

DMI, dry matter intake; CPI, crude protein intake; MEI, metabolic energy intake; BW, body weight; EBW, empty body weight; ADG, average daily gain; EBWG, empty body weight gain; BL, initial slaughter group; IM, middle slaughter group; AL, *ad libitum*. *Ad libitum* (AL) or restricted to 70 or 40% of the ad libitum intake. Animals of each group were slaughtered when the ad libitum lambs reached 50 kg. [a,b,c] Within a row, means without a common superscript letter differ (P < 0.05). Comparisons were made only among feeding levels.

Table III.- Macromineral concentrations in the body tissues (g kg^{-1}) and the distribution (%) in the body of Dorper × Hu ewe lambs on a DM basis.

Item	Bone	Muscle	Hide	Viscera[1]	Fat	Fleece	SEM	P-value
Concentration (g kg^{-1})								
Ca	120.45[a]	0.39[e]	0.59[d]	0.92[c]	0.19[f]	1.18[b]	3.24	<0.001
P	71.78[a]	6.89[b]	2.79[c]	6.65[b]	0.83[d]	0.49[e]	1.89	<0.001
Na	5.98[c]	2.12[d]	6.97[b]	7.74[a]	1.35[e]	2.68[d]	0.71	0.006
K	1.64[e]	11.09[c]	15.89[b]	9.12[d]	1.32[e]	23.89[a]	1.03	<0.001
Mg	2.98[a]	0.86[b]	0.38[d]	0.37[d]	0.08[e]	0.51[c]	0.05	0.008
Distribution (%)								
Ca	98.78[a]	0.45[b]	0.10[d]	0.29[c]	0.04[e]	0.34[bc]	0.89	<0.001
P	82.89[a]	10.78[b]	1.47[d]	3.78[c]	0.76[e]	0.32[f]	0.93	<0.001
Na	43.96[a]	20.10[b]	10.72[c]	17.67[b]	4.08[d]	3.47[d]	1.02	<0.001
K	7.02[d]	37.42[a]	13.28[c]	12.33[c]	3.56[e]	26.39[b]	2.31	<0.001
Mg	65.19[a]	19.54[b]	4.57[c]	3.61[d]	3.30[d]	3.79[d]	1.96	<0.001

[1]Viscera includes blood. [a,b,c,d,e,f] Within a row, means without a common superscript letter differ (P < 0.05).

Table IV.- Macromineral element intake and retention [mg/(kg EBW/d)] of Dorper × Hu ewe lambs fed 3 levels of feed intake using the comparative slaughter technique.

Item	Feed intake level[1]			SEM	P-value
	AL	70% AL	40% AL		
Ca					
Intake	295.32[a]	230.01[b]	163.63[c]	15.15	0.001
Retention	50.11[a]	35.63[b]	18.67[c]	4.24	0.04
P					
Intake	143.31[a]	111.65[b]	79.45[c]	10.23	0.001
Retention	27.71[a]	20.12[b]	9.12[c]	6.32	0.03
Na					
Intake	134.61[a]	106.52[b]	75.92[c]	11.3	0.001
Retention	4.11[a]	3.15[b]	0.62[c]	1.5	0.04
K					
Intake	382.21[a]	302.47[b]	215.49[c]	17.4	0.001
Retention	7.21[a]	4.74[b]	0.92[c]	2.4	0.001
Mg					
Intake	95.51[a]	75.68[ab]	53.96[b]	8.2	0.03
Retention	1.72[a]	1.58[ab]	1.46[b]	0.3	0.03

[1]*Ad libitum* (AL) or restricted to 70 or 40% of the ad libitum intake. Animals of each group were slaughtered when the ad libitum lambs reached 50 kg. [a,b,c] Within a row, means without a common superscript letter differ (P < 0.05).

in viscera (blood included), hide, and bone, and K concentration was higher ($P < 0.05$) in fleece, hide, and muscle than other body tissues (Table III). The main percentages of Ca, P, Na, and Mg are in bone tissue. Bone tissue has more than 98%, 82%, 43% and 65% of the total Ca, P, Na, and Mg, respectively, in the empty body. The main pool of K is muscle tissue, which has more than 37% of the total K in the empty body.

Macromineral intake and retention

The results of macromineral intake and retention of Dorper×Hu crossbred ewe lambs fed different intake levels are given in Table IV. Mineral retention of Ca, P, Na, and K were increased ($P < 0.05$) as intake level increased. The Mg retention was greater ($P < 0.05$) in animals fed ad libitum compared with those fed at 40% feed, but they did not differ from animals fed at 70% intake ($P > 0.05$).

Estimates of macromineral element net requirements for maintenance

The retained macromineral element was highly correlated with macromineral element intake (Table V). Therefore, a linear relationship between retained macromineral element and macromineral element intake was found. Theoretically, a lamb with a macromineral element intake of 0 mg/kg EBW per day is expected to retain macromineral element, which represented the macromineral element requirement for maintenance.

Estimates of macromineral elements body composition and net requirements for growth

Initial EBW of each animal of ewes was computed from initial BW ($r^2 = 0.91$, RMSE = 0.92, n=7, $P < 0.001$): EBW, kg= $1.5123 \pm 0.312 + 0.7984 \pm 0.02 \times$ BW, kg (Table VI).

Table V.- Regression equations to estimate the net maintenance requirements of macrominerals of Dorper × Hu crossbred ewe lambs using the comparative slaughter technique.

Item	Regression equations[1]	R^2	RMSE[2]	P-Value	Net req[3] [mg/(kg EBW·d)]	Net req [mg/(kg BW·d)]
Ca	Ca Ret .= –20.21 ± 3.26 + 0.2394 ± 0.011× Ca Int.	0.90	4.87	<0.001	20.20	17.15
P	P Ret. = –13.50 ± 2.92 + 0.2919 ± 0.023 × P Int.	0.91	3.42	<0.001	13.50	11.46
Na	Na Ret. = –3.80 ± 0.52 + 0.0599 ± 0.011× P Int.	0.92	1.09	<0.001	3.80	3.23
K	K Ret. = –7.91 ± 1.02 + 0.0379 ± 0.0025 × K Int.	0.90	2.17	<0.001	7.91	6.06
Mg	Mg Ret. = -1.10 ± 0.34 + 0.0065 ± 0.0012 × Mg Int.	0.90	0.41	<0.001	1.10	0.93

[1]Ret, retained, mg/kg EBW; Int, intake, mg/kg EBW; [2]RMSE, root mean square error; [3]req, requirement.

Table VI.- Allometric equations to estimate body composition in macrominerals (Ca, P, Na, K and Mg) of Dorper × Hu crossbred ewe lambs.

Item	Regression equation	R^2	RMSE	P-Value	BW (kg)[1]			
					35	40	45	50
EBW (kg)	EBW = 1.5123 ± 0.312 + 0.7984 ± 0.02 × BW	0.91	0.92	<0.001	29.46	33.45	37.44	41.43
Ca (g/kg EBW)	Log Ca (g) = 0.9491 ± 0.074 + 1.0648 ± 0.091 × Log EBW	0.92	0.05	<0.001	11.07	11.17	11.25	11.32
P (g/kg EBW)	Log P (g) = 0.6753 ± 0.032 + 1.0560 ± 0.12 × Log EBW	0.92	0.02	<0.001	5.72	5.76	5.80	5.83
Na (g/kg EBW)	Log Na (g) = 0.2035 ± 0.011 + 0.8903 ± 0.053 × Log EBW	0.91	0.04	<0.001	1.10	1.09	1.07	1.06
K (g/kg EBW)	Log K (g) = -0.0481 ± 0.0025 + 1.1432 ± 0.10 × Log EBW	0.93	0.03	<0.001	1.45	1.48	1.50	1.52
Mg (g/kg EBW)	Log Mg (g) = -0.6975 ± 0.033 + 1.1590 ± 0.11 × Log EBW	0.90	0.06	<0.001	0.34	0.35	0.36	0.36

EBW, empty BW; RMSE, root mean square error. [1]Values were calculated from the equations.

Table VII.- Prediction of the composition of empty body weight gain (g/kg EBWG) of Ca, P, Na, K and Mg at different BW of Dorper × Hu ewe lambs.

	BW (kg)				Equations[1]
	35	40	45	50	
EBW (kg)	29.46	33.45	37.44	41.43	
Ca	11.79	11.89	11.98	12.06	$Ca = 9.4704 \times EBW^{0.0648}$
P	6.04	6.09	6.12	6.16	$P = 4.9999 \times EBW^{0.056}$
Na	0.98	0.97	0.96	0.95	$Na = 1.4224 \times EBW^{-0.1097}$
K	1.66	1.69	1.72	1.74	$K = 1.0233 \times EBW^{0.1432}$
Mg	0.40	0.41	0.41	0.42	$Mg = 0.2326 \times EBW^{0.1590}$

EBW, empty body weight. [1]Component concentration = $b \times 10^a \times EBW^{(b-1)}$, in which a and b are constants determined from the equations in Table VI.

The logarithmic allometric equations used to calculate the relationships between macromineral element quantities and empty body weights were highly significant ($P < 0.001$) and provided a data fit with R^2 values varying between 0.90 and 0.93. By deriving the logarithm regression equations of the body content for Ca, P, K, Na, and Mg according to the logarithm of the EBW, the equations to predict these nutrients per kilogram of EBWG were obtained in Table VII. The relative proportion of Ca, P, K, Na, and Mg at 35, 40, 45, and 50 kg of BW are also presented in Table VI. The proportions of Ca, P, K and Mg increased as BW increased. The proportion of Na decreased as BW increased (Table VI). The macromineral element deposition of ewes in EBWG (Table VII) followed the same concentration pattern as in EBW (Table VI), with increases of approximately 2.29% for Ca, 1.96% for P, 4.82% for K, 5.44% for Mg and decreases of 3.42% for Na as BW increased from 35 to 50 kg.

Table VIII.- Net macromineral requirements for live weight gain (mg/day) of Dorper×Hu crossbred ewe lambs.

BW (kg)	ADG (g/d)	Net requirement (mg/day)				
		Ca	P	Na	K	Mg
35	100	992	508	83	140	34
	200	1984	1016	166	280	68
	300	2976	1524	249	420	102
40	100	994	509	81	141	34
	200	1988	1018	162	282	68
	300	2982	1527	243	423	102
45	100	996	510	80	143	34
	200	1992	1020	160	286	68
	300	2988	1530	240	429	102
50	100	999	510	78	145	35
	200	1998	1020	156	290	70
	300	2997	1530	234	435	105

Net macromineral element requirements for live weight gain

In order to calculate the net macromineral element requirements for live weight gain (Table VIII), the values of composition of empty weight gain were divided by the correction factors that were determined by the BW/EBW ratio, which were calculated as 1.19, 1.20, 1.20, 1.21 for ewes, and corresponded to animals with BWs of 35, 40, 45 and 50 kg, respectively. An increase in the net requirements for live weight gain of ewes was found for Ca, P, K, and Mg with the exception of Na as animal BW increased.

DISCUSSION

Macromineral concentration and distribution in the main body tissues

In the present study, we observed macromineral concentration and distribution in the bone tissue on a DM basis, with Dorper × Hu crossbred ewe lambs at body weight ranging from 35 to 50 kg (Table III). In comparison, ARC (1980) reported concentration ranges of 110 to 200, 50 to 100, 2.0, 4.0, and ≤ 0.05 g/kg bone DM for Ca, P, Mg, Na, and K, respectively, in growing lambs. Bellof *et al*. (2006) reported average concentrations of 97.0, 46.6, 3.4, 1.0, and 2.0 g/kg bone DM for Ca, P, Na, K, and Mg, respectively, in German Merino Land sheep at body weights ranging from 18 to 55 kg. Chizzotti *et al*. (2009) reported that for Nellore and Red Angus crossbred calves, averaging 274 kg BW, bone tissue contained more than 99% of the body Ca, 90% of the body P, 40% of the body Na, 9% of the body K, and 70% of the body Mg. All of these results showed that Ca, P, Na, and Mg were mainly distributed in bone tissue.

Lamb muscle is a valuable source of essential amino acids and B vitamins and for highly bioavailable minerals for human nutrition (Bellof *et al*., 2007). Potassium has long been known to be vitally involved in nerve and muscle excitability and in the water and acid-base balance of the body (Underwood and Suttle, 1999). The K is the third most abundant mineral in the animal body, 2/3 being located in skin and muscles (McDowell, 2003). In the present study, we observed macromineral concentration and distribution in the muscle tissue on a DM basis, with Dorper × Hu crossbred ewe lambs at body weight ranging from 35 to 50 kg (Table III). Similarly, Bellof *et al*. (2006) reported concentrations of 0.3 g Ca, 6.3 g P, 2.1 g Na, 10.9 g K as well as 0.7 g Mg per kg in muscle DM were found in German Merino Land female sheep at 45 kg body weight. In lamb muscle tissue, the major elements K showed the highest concentrations, while Na, Mg, and Ca remained at levels below 100 mg per 100 g meat (wet weight basis) (Bellof *et al*., 2006). Schwarz *et al*. (1995) found for fattening bulls, relative to the total empty body (including blood, skin, and internal organs), the muscle tissue accounted for 0.2% of the total Ca, 13% of the total P, 25% of the total Mg, 18% of the total Na, and 73% of the total K. Bellof *et al*. (2006) reported that the muscle tissue in the carcass of growing German Merino Landsheep (45 kg final body weight) contained 81.0% of the total amount of K. These results all showed that K was mainly distributed in the muscle tissue.

In the present study with F_1 Dorper × Hu crossbred ewe lambs at body weights ranging from 35 to 50 kg, macromineral concentration and distribution were found in the visceral (blood included) tissues on a DM basis (Table III). These results showed that only very small amounts of Ca and Mg were retained in the visceral tissues, and higher concentrations could be found for the elements P, K, and Na in the visceral tissues, which were similar to the results for the visceral tissue of Romneys sheep reported by Grace (1983). Body P is also widely distributed in the fluids and soft tissues of the body where it serves a range of essential functions and plays a vital role in a host of metabolic fuctions; K is the major intracellular ion in tissues. All soft tissues are much richer in K than in Na, making K the third most abundant mineral; Sodium and chloride maintain osmotic pressure, regulate acid-base equilibrium and control water metabolism in the body, with Na^+ being the major cation in the extracellular fluid (Suttle, 2010). These may be the explanation why visceral (blood included) exhibited the differences of concentration among macro-minerals.

The net macro mineral requirements for maintenance

The determination of dietary mineral requirements is important in mineral nutrition. One approach which was adopted in this study is to use the factorial model. The factorial model has two parts that associated with maintenance and that associated with production. This gives a net requirement and if the absorption coefficient and DM intakes are known then a dietary mineral requirement can be calculated (ARC, 1980). The F_1 Dorper × Hu crossbred sheep is specialized in meat production and is characterized by its ability to pass these traits to its descendants (Zhang *et al*., 2015), therefore, mineral retention in fleece and the net macro-mineral requirments for wool production were negligible.

The net Ca requirement of F_1 Dorper × Hu crossbred ewe lambs for maintenance estimated by CST in this study was greater than that value reported by the ARC (1980), which estimated the net Ca requirement for maintenance based on total endogenous loss of 16 mg/kg BW per day mainly through fecal loss (AFRC, 1991). Ji *et al*. (2015) estimated the net Ca requirement of F_1 Dorper × Thin-

Tailed Han crossbred ewe lambs for maintenance was 35.0 mg/kg EBW by using CST, which was greater than that value reported by the present study. This difference may be mainly due to the result of different methodologies, breed, and growth stages.

There is great disparity in the reported net P requirements for the maintenance of small ruminants. The net P requirement of F_1 Dorper × Hu crossbred ewe lambs for maintenance estimated by CST in this study was lower than those values reported by other researchers (NRC, 1985; Ji *et al*., 2013, 2015). Additionally, ARC (1965) estimated the net P requirement for maintenance as 42.5 mg/(kg BW/d) for sheep. This value later was reduced to 14 mg/(kg BW/d) (ARC, 1980) based on the total endogenous loss method. The endogenous loss of P was influenced by amount of feed ingested, quality of the diet, and factors linked to competition for absorbed P via secretion (AFRC, 1991). Salivary P also was shown to play an important role in P metabolism (NRC, 2007). Furthermore, P loss may also occur when dandruff and wool are shed from the body. All of these reasons may explain why the net P requirement for maintenance has been reported differently by many researchers.

The net K and Na requirements of F_1 Dorper × Hu crossbred ewe lambs for maintenance were calculated by CST (Table V). ARC (1980) estimated the net K requirement for maintenance as 38 mg/(kg BW/d) for sheep calculated from endogenous fecal and urinary excretions. Ji *et al*. (2015) estimated the net K requirement of F_1 Dorper × Thin-Tailed Han crossbred ewe lambs for maintenance was 31.4 mg/kg EBW by using CST. NRC (2007) estimated the net Na requirement for maintenance as 10.8 mg/(kg BW/d) for sheep. Ji *et al*. (2015) estimated the net Na requirement of F_1 Dorper × Thin-Tailed Han crossbred ewe lambs for maintenance was 9.3 mg/kg EBW by using CST. These indicated that the net K requirement of Dorper × Hu crossbred ewe lambs for maintenance was lower than the recommendations of the ARC (1980) and Ji *et al*. (2015), and the net Na requirement for maintenance was lower than the recommendations of the NRC (2007) and Ji *et al*. (2015). Except for urine and feces, the losses of K and Na can be also occurred by the skin via perspiration, especially in a hot and humid climate regions (Fernandes *et al*., 2012). These findings also may contribute to the differences in findings between researchers.

The dietary Mg requirements of livestock vary with the species and breed of animal, age, rate of growth or production, and bioavailability in the diet (McDowell and Arthington, 2005). In mature ruminants, the reticulorumen is the principal site of Mg absorption (Thomas and Potter, 1976). Many factors influence the Mg absorption and dietary Mg requirement including amounts of K, Ca, P, Al, Fe, Na, protein, fat, organic acids, carbohydrate type, ionophores, Mg status, and feeding frequency (Fontenot, 1989). The net Mg requirement of F_1 Dorper × Hu crossbred ewe lambs for maintenance estimated by CST in this study was lower than that value reported by NRC (2007) and Ji *et al*. (2015), which estimated the net Mg requirement for maintenance as 3 mg/(kg BW/d) and 2.03 mg/(kg EBW/d) for sheep, respectively. Due to the limited information on the Mg requirement for maintenance of growing lambs, it is difficult to compare our results with the literature, and the reason and implications for different results need to be further study.

The net macro mineral requirements for growth

In this study, we found that for F_1 Dorper × Hu crossbred ewe lambs with BW varying from 35 to 50 kg, Ca, P, K, and Mg concentrations were slightly increased as BW increased. It is well known that the Ca:P ratio is very important for animal growth and health as it affects the absorption and excretion of both minerals (Underwood and Suttle, 1999). According to the AFRC (1991), bone tissue has a Ca:P ratio of 2:1, whereas soft tissues have a ratio of 1.2:1. In this study, we also calculated a body Ca:P ratio of around 2:1.

The macromineral element deposition of ewes in EBWG (Table VII) followed the same concentration pattern as in EBW (Table VI), with increases of approximately 2.29% for Ca, 1.96% for P, 4.82% for K, 5.44% for Mg and decreases of 3.42% for Na, respectively, as BW increased from 35 to 50 kg. The net Ca and P requirement recommendations for growth in sheep were 11 and 6.0 g/kg EBWG, respectively (NRC, 1985; ARC, 1980). The net Na, K and Mg requirement recommendations of the NRC (2007) for growth in sheep were 1.10, 1.80, and 0.41 g/kg live weight gain, respectively. In our study, the results showed that the net Ca and P requirements of F_1 Dorper × Hu crossbred ewe lambs for growth were greater than the recommendations of NRC (1985) and ARC (1980), and the net Na, K, and Mg requirements of F_1 Dorper × Hu crossbred ewe lambs for growth were lower than the recommendations of NRC (2007).

As BW increased, an increase was observed in the net requirements of Ca, P and Mg, which are mostly present in the bones. These results in our study also indicated that these lambs still showed slight bone tissue deposition regardless of the occurrence of more pronounced fat deposition. Furthermore, because of the deposition of fat with the advance of the growth in the body is an energetically expensive process (Resende *et al*., 2008), the elevation in the requirements of Mg for gain as the animal grew is directly linked to the expenditure of energy involved in the formation of adipose tissue deposits

(Garfinkel and Garfinkel, 1985).

As BW increased, a reduction was observed in the net Na requirements for growth of F_1 Dorper × Hu crossbred ewe lambs. The reduction of the Na requirements for growth may be related to the decrease in the amount of water in the body of the animals. There is a decrease in the extracellular volume in the body of living organisms from birth to puberty, which causes a decrease in the concentrations of Na in the body (Ahmed *et al*., 2000). Because of the organism needs to maintain a constant Na:K ratio in order to maintain osmotic equilibrium, decreasing Na levels may accompany the decrease in K concentration (Suttle, 2010). However, the net K requirements for growth was slightly increased as BW increased and Na:K ratio was not constant in our study. This is likely due to the higher concentration of K in the body, which should be associated with the greater deposition of hide, fleece and muscle tissues in our F_1 Dorper × Hu ewe lambs. Because the metabolism of Na and K is interrelated and both are lost through perspiration, variations in their requirements might be dependent on the ability to sweat and on the environmental conditions in which animals are raised (Underwood and Suttle, 1999). Therefore, variability in net Na and K requirements among studies could be a consequence of environmental conditions that favor or oppose water and mineral loss via perspiration in animals.

CONCLUSION

The macromineral elements of F_1 Dorper × Hu crossbred ewe lambs were mainly distributed in bone tissue, except for K, which was mainly distributed in the muscle and fleece tissues. It was noted that the nutritional systems of F_1 Dorper × Hu crossbred ewe lambs for the macromineral elements were different compared to most other nutritional systems. In the present study, these results for the mineral requirements may help to formulate more balanced diets for F_1 Dorper × Hu ewe lambs in the growth phase of 35 to 50 kg BW. It will be critical to perform additional studies using different diets, systems of production, genders, and ages in order to better understand what causes variation in nutritional macromineral element requirements. Further studies to investigate the macromineral element requirements at other stages of growth of F_1 Dorper × Hu crossbred lambs, in particular, are warranted.

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Statement of conflicts of interest

The authors declare no conflicts of interest. The authors are responsible for the content and writing of this article.

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