The net iron, manganese, copper, and zinc requirements for maintenance and growth of Dorper × Hu ewe lambs

Hao Zhang\textsuperscript{a,b}, Haitao Nie\textsuperscript{c}, Ziyu Wang\textsuperscript{c} and Feng Wang\textsuperscript{c}

\textsuperscript{a}Laboratory of Metabolic Manipulation of Herbivorous Animal Nutrition, College of Animal Science and Technology, Yangzhou University, Yangzhou, China; \textsuperscript{b}Joint International Research Laboratory of Agriculture & Agri-Product Safety, Yangzhou University, Yangzhou, China; \textsuperscript{c}Jiangsu Engineering Technology Research Center of Mutton Sheep & Goat Industry, Nanjing Agricultural University, Nanjing, China

ABSTRACT
A comparative slaughter trial was conducted to estimate the net trace element requirements of Dorper × Hu ewe lambs. Thirty-five ewe lambs with an initial body weight of 33.52 ± 0.56 kg, were used. Seven ewe lambs were randomly chosen and slaughtered at 34.85 ± 0.37 kg body weight as the baseline group for measuring initial body composition. Another seven lambs were also randomly chosen and offered a pelleted mixed diet for ad libitum intake and slaughtered at 41.47 ± 0.53 kg body weight. The remaining ewe lambs (n = 21) were allocated randomly on d 0–3 treatment intake levels within seven 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 body weight. The daily net trace element requirements for maintenance were 310.4 mg Fe, 4.4 mg Mn, 35.7 mg Cu, and 110.3 mg Zn/kg empty body weight. The net trace element requirements for growth decreased from 41.02 to 37.05 mg Fe, 0.52–0.49 mg Mn, and 3.39–3.10 mg Cu and increased from 34.21 to 35.80 mg Zn/kg empty body weight gain (EBWG) from 35 to 50 kg body weight. The net Fe, Mn, Cu and Zn requirements of Dorper × Hu ewe lambs for maintenance were greater than the recommendations of the NRC, and the net Fe and Mn requirements of Dorper × Hu ewe lambs for growth were lower than the recommendations of the NRC, except that the net Cu and Zn growth requirements were greater than the recommendations of the NRC.

Introduction
The Dorper sheep breed was imported into China as a meat sire breed to improve growth performance and carcase traits of the Hu sheep. It originated in South Africa and is characterised 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 crossbreds for lamb meat production in China (Zhang et al. 2015, 2017). The breed or genotype of an animal affects its energy and protein requirements (Sahlu et al. 2004), and therefore, it is expected that trace element requirements are also affected.

Trace element requirements have received a great deal of consideration, as any excess or deficiency in one element interferes in the utilisation of another (Gerseev et al. 2000), impairing health, productivity, and even survival. Excess of Zn is known to induce a Cu deficiency, and Cu is essential for numerous enzymes involved in Fe transport and metabolism (Mateos et al. 2004). Furthermore, the majority of trace elements ingested by sheep are excreted in faeces and urine; feeding high levels of Zn and Cu increases the excretion of these elements and becomes a potential environmental threat. Therefore, accurate prediction of trace element requirements might minimise trace element excretion and environmental pollution (Chizzotti et al. 2009).

The estimation of the trace element requirements demands exact knowledge of trace element deposition in the body (Bellof and Pallauf 2007). Thus, an accurate
technique with direct measurement, as comparative slaughter trials (CST), has recently been used in trials to estimate the trace element requirements for sheep (Ji et al. 2014; Zhang et al. 2015). Therefore, the present study was performed to use CST to establish net trace element requirements for maintenance and growth of Dorper × Hu ewe lambs.

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 to 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, 35 ewe lambs of F₁ 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 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 1). 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 lambs were fed ad libitum and slaughtered as an intermediate slaughter group (IM), which was carried out at approximately 170 d old when they reached 41.47 ± 0.53 kg of BW. On d 0 of the evaluation of treatments, the remaining 21 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 seven slaughter groups, with each group consisting of one 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 trace element 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, USA), and stored until analyses.

Slaughter procedure

The day before slaughter, body weight was measured at 1600 h. Shrunk BW (SBW) was measured as BW after

### Table 1. Ingredient and nutrient composition of the experimental diets on a DM basis.

| Item                        | Value  |
|-----------------------------|--------|
| **Ingredients (%)**         |        |
| Corn                        | 41.44  |
| Soybean meal                | 19.33  |
| Soybean straw               | 38.11  |
| Anhydrous calcium phosphate | 0.38   |
| Limestone                   | 0.23   |
| Sodium chloride             | 0.40   |
| Premix²                    | 0.11   |
| **Nutrient composition (analysed)b** |         |
| CP, %                       | 17.17  |
| ME, MJ/kg                   | 9.77   |
| Ether extract, %            | 2.26   |
| NDF, %                      | 45.65  |
| ADF, %                      | 23.18  |
| Fe, mg/kg                   | 415.96 |
| Mn, mg/kg                   | 101.69 |
| Cu, mg/kg                   | 5.41   |
| Zn, mg/kg                   | 135.82 |

*The premix provided the following nutrients per kg of the diet: 15,000 U VA, 5,000 U VD, 50 mg VE, 32 g Na, 92 g K, 23 g Mg, 90 mg Fe, 2.5 mg Cu, 50 mg Mn, 100 mg Zn, 0.3 mg Se, 0.8 mg I, and 0.5 mg Co.

*Nutrient levels are analysed values.
a 16-h fast of feed and water. Lambs were slaughtered by exsanguination after stunning by CO₂ inhalation. Blood was collected and weighed. Mass of the viscera, hide, wool, head, feet, carcase 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 SBW. All body components were initially frozen at −6°C, then cut with a stainless steel band saw, ground, and homogenised, and 500 g samples were collected for chemical analysis. These samples were thawed, and 100 g sub-samples were lyophilised for 72 h and then ground in a stainless steel blender. The collection of samples followed the procedures described by Galvani et al. (2008, 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 homogenised 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 homogenised 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 HNO₃ 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 HNO₃. The concentrations of Fe, Mn, Cu and Zn 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: 10000W, 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. The measurement of the Cu contents in the fat tissues led to results below the detection limit of 0.05 mg/L measure solvent. In the meat, bone and fat samples, the measurement of Mn led to results below the detection limit of 0.05 mg/L. Therefore, a re-measurement of Mn was carried out for the bone samples using an atomic absorption spectrometer (graphite furnace AAS by PerkinElmer [PE 5100 Z]; details of the instrument: wave length: 279.5 nm; gap width: 0.2 nm; lamp current: 20 mA; base correction: Zeeman; cuvette: not coated; injection capacity: 20 μL; modifier: none; integration: area; drying: two-stage [90°C and 120 °C]; ashing: two-stage [500°C and 900°C]; atomisation: 2200°C). The calibration took place at a concentration of 1–10 μg/L. Cu in bones was analysed by flame AAS (Unicam PU 9400 [now: Thermo Electron GmbH, Dreieich, Germany], measuring principle: flame: air-acetylene; wavelength: 324.8 nm; gap width: 0.5 nm). For quality control of the trace element analysis, certified bovine liver (NIST bovine liver 1577 b) was used. The recovery rate measured was 107% for Fe, 102% for Cu, 103% for Mn and 98% for Zn (Bellof et al. 2006; Bellof and Pallaf 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 lyophilised (Galvani et al. 2009) for 72 h to determine DM content, and then all the subsamples including the wool were analysed for trace elements (Fe, Mn, Cu and Zn) as described above.

**Data calculation and analyses**

**Prediction of the initial body trace element content**

The body trace element content was calculated as the sum of the content of all body components. The initial body trace element content of each animal in the intermediate and final slaughter groups was calculated using a regression equation developed for the relationship between the body trace element content and EBW of baseline animals (Fernandes et al. 2007). The initial EBW of intermediate and final slaughter groups was calculated according the regression equation between EBW and BW [Eq. (1)]:

\[
\text{EBW}, \text{ kg} = a + \left[ b \times (\text{BW}, \text{ kg}) \right] \tag{1}
\]

**Trace element requirements for maintenance**

Maintenance requirements were calculated using the comparative slaughter technique (CST; Lofgreen and
Garrett 1968). Retained trace elements was calculated as the difference between final and initial body trace elements content of each animal from intermediate and final slaughter groups, and the total trace elements losses were calculated as the difference between intake and retained trace elements. A linear regression of daily trace elements retained (mg/kg of EBW) on trace elements 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 trace elements losses equivalent to the net requirement for maintenance (mg/kg EBW/C1)

Trace elements requirements for growth

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

\[
\log y = a + [b \times \log x]
\]

where \(\log y\) is the logarithm of the total amount of trace elements of the empty body (g), \(a\) is the intercept, \(b\) is the coefficient of the regression of the trace elements content as a function of the EBW, and \(\log x = \log \text{EBW (kg)}\). Equation (3) was differentiated to compute the estimates of the composition of the gain at various EBWs.

\[
y' = b \times 10^a \times \text{EBW}^{(b-1)}
\]

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 trace elements 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 analysed as a completely randomised design using SAS (SAS Inst. Inc., Cary, NC, USA). 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 \(p = .05\).

Results

Growth performance and body trace element composition

As described in Table 2, DMI, CPI, MEI, EBW, ADG, and EBW gain (EBWG) increased as intake level increased \((p < .05)\). For Dorper × Hu ewe lambs, Fe, Mn, Cu, and Zn concentrations slightly decreased as intake level increased \((p < .05\); Table 2).

Table 2. Performance and body trace element content of Dorper × Hu ewe lambs throughout different growing periods and subjected to three levels of feed intake.

| Item               | BL            | IM           | AL            | 70%           | 40%           | SEM        | p Value |
|--------------------|---------------|--------------|---------------|---------------|---------------|------------|---------|
| DMI, kg/d          | –             | 1.69         | 1.76          | 1.24          | 0.75          | 0.05       | .0146   |
| CPI, kg/d          | –             | 0.25         | 0.30          | 0.21          | 0.13          | 0.04       | .03     |
| MEI, MJ/d          | –             | 15.53        | 17.20         | 12.12         | 7.33          | 4.25       | .02     |
| BW, kg             | 34.85         | 41.47        | 48.38         | 43.23         | 35.67         | 0.71       | <.0001 |
| EBW, kg            | 29.41         | 34.56        | 40.25         | 36.18         | 31.17         | 0.63       | <.0001 |
| ADG, g/d           | –             | 237.50       | 220.61        | 160.45        | 13.44         | 21.14      | <.0001 |
| EBWG, g/d          | –             | 183.94       | 177.71        | 110.99        | 28.85         | 16.45      | <.0001 |
| Fe, mg/100 g of EBW| 6.06          | 5.72         | 5.43          | 6.42          | 7.48          | 1.04       | .03     |
| Mn, mg/100 g of EBW| 0.064         | 0.062        | 0.060         | 0.071         | 0.074         | 0.034      | .04     |
| Cu, mg/100 g of EBW| 0.47          | 0.45         | 0.43          | 0.58          | 0.67          | 0.31       | .04     |
| Zn, mg/100 g of EBW| 2.99          | 3.07         | 3.14          | 3.93          | 4.55          | 0.26       | .02     |

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. Within a row, means without a common superscript letter differ \((p < .05)\). Comparisons were made only among feeding levels.
Trace element intake and metabolism

The results of trace element intake and metabolism of Dorper × Hu crossbred ewe lambs fed different intake levels are given in Table 3. Trace element retention of Fe, Mn, Cu and Zn was markedly increased (\( p < .05 \)) as the intake level increased.

Estimates of trace element net requirements for maintenance

The retained trace element was highly correlated with trace element intake (Table 4). Therefore, a linear relationship between retained trace element and trace element intake was found. Theoretically, a lamb with a trace element intake of 0 mg/kg EBW per day is expected to retain trace element, which represented the trace element requirement for maintenance.

Estimates of trace elements body composition and net requirements for growth

Initial EBW of each animal of ewe lambs was computed from initial BW (\( R^2 = 0.96, \text{root mean square error (RMSE)} = 0.96, n = 7, \ p < .001 \)): EBW, kg = 1.5123 + 0.7984 × BW, kg (Table 5). The logarithmic allometric equations used to calculate the relationships between trace element quantities and EBWs were highly significant (\( p < .001 \)) and provided a data fit with \( R^2 \) values varying between 0.89 and 0.96. By deriving the logarithm regression equations of the body content for Fe, Mn, Cu, and Zn according to the logarithm of the EBW, the equations to predict these nutrients per kilogram of EBWG were obtained in Table 6.

The relative proportions of Fe, Mn, Cu, and Zn at 35, 40, 45, and 50 kg of BW are also presented in Table 5. The proportions of Fe, Mn and Cu slightly decreased as BW increased. The proportion of Zn slightly increased as BW increased (Table 5). The trace element deposition of ewe lambs in EBWG followed the same concentration pattern as in EBW (Table 6).

Net trace element requirements for live weight gain

In order to calculate the net trace element requirements for live weight gain (Table 7), 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, and 1.21 for ewe lambs, and corresponded to animals with BWs of 35, 40, 45, and 50 kg, respectively. An increase in the net requirement for live weight gain of ewe lambs was found for Zn as animal BW increased. A decrease in the net requirement for live weight gain was found for Fe, Mn and Cu as animal BW increased.

Discussion

EBW, ADG, and EBW gain (EBWG) increased as intake level increased, which was according to the report by Zhang et al. (2015). Similar results were also reported by Silva et al. (2013), who showed that the EBW and EBWG of Canindé goats with BW varying from 15 to 25 kg increased as feed intake level increased. In our study, for Dorper × Hu ewe lambs, Fe, Mn, Cu, and Zn

Table 3. Trace element intake and metabolism [mg/(kg empty body weight/d)] of Dorper × Hu ewe lambs feeding three levels of feed intake using the comparative slaughter technique.

| Item | Feed intake level | AL | 70% | 40% | SEM | \( p \) Value |
|------|-------------------|----|-----|-----|-----|-------------|
| Fe   | Intake            | 17.67<sup>a</sup> | 15.34<sup>b</sup> | 12.45<sup>c</sup> | 3.60 | <.001        |
|      | Retention         | 0.40<sup>a</sup>  | 0.31<sup>b</sup>  | 0.19<sup>c</sup>  | 0.14 | <.001        |
| Mn   | Intake            | 4.32<sup>a</sup>  | 3.77<sup>b</sup>  | 3.08<sup>c</sup>  | 1.80 | .001         |
|      | Retention         | 0.003<sup>a</sup> | 0.0012<sup>b</sup> | 0.0009<sup>c</sup> | 0.0003 | <.001     |
| Cu   | Intake            | 0.23<sup>a</sup>  | 0.19<sup>b</sup>  | 0.16<sup>c</sup>  | 0.03 | .03          |
|      | Retention         | 0.025<sup>a</sup> | 0.017<sup>b</sup> | 0.006<sup>c</sup> | 0.0003 | <.001     |
| Zn   | Intake            | 5.77<sup>a</sup>  | 4.69<sup>b</sup>  | 4.03<sup>c</sup>  | 1.00 | .001         |
|      | Retention         | 0.22<sup>a</sup>  | 0.16<sup>b</sup>  | 0.12<sup>c</sup>  | 0.03 | .001         |
<sup>a,b,c</sup>Within a row, means without a common superscript letter differ (\( p < .05 \)).

Table 4. Regression equations to estimate the net maintenance requirements of trace element of Dorper × Hu ewe lambs using the comparative slaughter technique.

| Item | Regression equations<sup>1</sup> | \( R^2 \) | \( p \) Value | Net req<sup>2</sup> mg/(kg EBW-d) | Net req mg/(kg BW-d) |
|------|----------------------------------|--------|-------------|-------------------------------|------------------|
| Fe   | \( \text{Fe Ret} = -0.3104 + 0.0403 \times \text{Fe Int} \) | 0.90   | <.001       | 0.3104                        | 0.2587           |
| Mn   | \( \text{Mn Ret} = -0.0044 + 0.0016 \times \text{Mn Int} \) | 0.88   | <.001       | 0.0044                        | 0.0037           |
| Cu   | \( \text{Cu Ret} = -0.0357 + 0.2676 \times \text{Cu Int} \) | 0.92   | <.001       | 0.0357                        | 0.0298           |
| Zn   | \( \text{Zn Ret} = -0.1100 + 0.0573 \times \text{Zn Int} \) | 0.83   | <.001       | 0.1103                        | 0.0917           |

<sup>1</sup>Ret: retained, mg/kg EBW; Int: intake, mg/kg EBW.

<sup>2</sup>req = requirement.
Hu crossbred lambs ranged from 20-35 kg, Fe, Mn, Cu, and Zn concentrations slightly decreased as BW increased, except for Zn concentration of males. In this study, we also found that for Dorper × Hu crossbred ewe lambs, in general, trace element retention of Fe, Mn, Cu, and Zn was markedly increased as the intake level increased, similar to the results reported by Zhang et al. (2015). Different concentration and retention patterns were reported in different kinds of lamb, which may be due to the different diets, systems of production, genotypes, body composition, genders, and ages.

Iron supplies are rarely inadequate for older farm livestock, in which the major problem is presented by excess dietary Fe (Suttle 2010). Usually Fe supply is not a problem, also because for Fe there is an active system of absorption. Net Fe requirement of Dorper × Hu crossbred ewe lambs for maintenance estimated with the CST in this study was 9.05–12.94 mg/d, which was much greater than that value reported by NRC (2007), which estimated the net Fe requirement for maintenance as 0.49–0.70 mg/d from 35 to 50 kg BW. Net Fe requirement of Dorper × Hu crossbred ewe lambs for maintenance estimated with the CST in this study was also much greater than those values reported by Zhang et al. (2015), who estimated the net Fe requirements for maintenance as 6.14–10.75 and 2.63–4.60 mg/d for males and females of Dorper × Hu crossbred lambs, respectively, from 20 to 35 kg BW. The results of this study could be different from other study because the intake is quite high. In our study, we found that the trace element retention of Fe, Mn, Cu and Zn was markedly increased as the intake level increased. Further studies are warranted to evaluate the effect of intake on retention could be interesting.

Manganese is an essential dietary element for ruminants, required for skeletal development and reproductive efficiency (Araújo et al. 2017). Deficiency of Mn results in more services per conception and in irregular or absence of oestrus in females. In males, Mn deficiency is associated with seminal tubular degeneration (Zhang et al. 2015). In ruminants, a minimum

| Table 5. Allometric equations to estimate body composition in trace elements (Fe, Mn, Cu and Zn) of Dorper × Hu ewe lambs. |
| --- |
| **Item** | **Regression equations** | \( R^2 \) | **RMSEA** | \( p \) Value | **35** | **40** | **45** | **50** |
| **EBW**, kg | \( \text{EBW} = 1.5123 + 0.7984 \times \text{BW} \) | 0.96 | 0.96 | <0.001 | 29.46 | 33.45 | 37.44 | 41.43 |
| **Fe**, mg/kg EBW | Log Fe, mg = 2.2586 + 0.6761 \times \log \text{EBW} | 0.96 | 0.07 | <0.001 | 60.63 | 58.19 | 56.10 | 54.29 |
| **Mn**, mg/kg EBW | Log Mn, mg = 0.0754 + 0.8147 \times \log \text{EBW} | 0.93 | 0.06 | <0.001 | 0.64 | 0.62 | 0.61 | 0.60 |
| **Cu**, mg/kg EBW | Log Cu, mg = 1.0636 + 0.7304 \times \log \text{EBW} | 0.89 | 0.04 | <0.001 | 4.65 | 4.50 | 4.40 | 4.24 |
| **Zn**, mg/kg EBW | Log Zn, mg = 1.2631 + 1.1446 \times \log \text{EBW} | 0.91 | 0.07 | <0.001 | 29.89 | 30.45 | 30.95 | 31.40 |

\( *\text{RMSEA: root mean square error.} \)

\( ^b\text{Values were calculated from the equations.} \)

\( ^c\text{EBW: empty body weight.} \)

| Table 6. Prediction of the composition of gain in empty body weight (EBWG, mg/kg) of Fe, Mn, Cu and Zn at different BW of Dorper × Hu ewe lambs. |
| --- |
| **BW, kg** | 35 | 40 | 45 | 50 | **Equations** |
| **EBW, kg** | 29.46 | 33.45 | 37.44 | 41.43 | \( \text{Fe} = 122.63 \times \text{EBW}^{-0.3239} \)
| **Fe** | 41.02 | 39.58 | 37.98 | 37.05 | \( \text{Fe} = 0.97 \times \text{EBW}^{-0.1663} \)
| **Mn** | 0.52 | 0.51 | 0.50 | 0.49 | \( \text{Mn} = 8.45 \times \text{EBW}^{-0.2967} \)
| **Cu** | 3.39 | 3.29 | 3.18 | 3.10 | \( \text{Cu} = 20.98 \times \text{EBW}^{-0.1446} \)
| **Zn** | 34.21 | 34.68 | 35.16 | 35.80 | \( \text{Zn} = 0.79 \times \text{EBW}^{-0.7984} + 2.2586 \)

\( ^d\text{Component concentration} = b \times 10^a \times \text{EBW}^{0.96}, \text{in which } a \text{ and } b \text{ are constants determined from the equations in Table 5.} \)

| Table 7. Net trace element requirements for live weight gain (mg/day) of Dorper × Hu crossbred ewe lambs. |
| --- |
| **BW, kg** | ADG, g/d | **Net requirement, mg/day** |
| **Fe** | **Mn** | **Cu** | **Zn** |
| **35** | 100 | 3.42 | 0.043 | 0.29 | 2.85 |
| | 200 | 6.84 | 0.086 | 0.58 | 5.70 |
| | 300 | 10.26 | 0.129 | 0.87 | 8.55 |
| **40** | 100 | 3.30 | 0.043 | 0.27 | 2.89 |
| | 200 | 6.60 | 0.086 | 0.54 | 5.78 |
| | 300 | 9.90 | 0.129 | 0.81 | 8.67 |
| **45** | 100 | 3.17 | 0.042 | 0.26 | 2.93 |
| | 200 | 6.34 | 0.084 | 0.52 | 5.86 |
| | 300 | 9.51 | 0.126 | 0.78 | 8.79 |
| **50** | 100 | 3.09 | 0.041 | 0.25 | 2.98 |
| | 200 | 6.18 | 0.082 | 0.50 | 5.96 |
| | 300 | 9.27 | 0.123 | 0.75 | 8.94 |
requirement for Mn is difficult to define because Mn feed availability varies depending on the composition of the ration, particularly Ca and P content (Underwood and Suttle 1999; NRC 2007). Assessment of the optimal Mn requirement of sheep by measurement of the pyruvate carboxylase activity in the liver could be useful (Hidiroglou 1979). Net Mn requirement for maintenance of Dorper × Hu crossbred ewe lambs estimated with the CST in this study was 3.7 μg/(kg BW/d), which was larger than that value reported by the NRC (2007), which estimated the net Mn requirement for maintenance as 2 μg/(kg BW/d). Net Mn requirement for maintenance in our study was much greater than that value reported by Zhang et al. (2015), who estimated the net Mn requirement for maintenance as 2.7 μg/(kg BW/d) for Dorper × Hu crossbred ewe lambs from 20 to 35 kg BW.

Copper is an essential trace element that plays an important role in the biochemical reactions of the body; however, its requirement and interaction with other minerals is not clearly understood (Solaiman et al. 2006). Antagonists, such as Mo, S, and Fe, at high concentrations can increase dietary requirements for Cu (McDowell 2003). The maintenance component of copper consists mainly of faecal endogenous losses (Hopkins and Solaiman 2002); because the faeces is a major route for excreting excess of absorbed copper, not all losses need to be replaced (Suttle 2010). Net Cu requirement of Dorper × Hu crossbred ewe lambs for maintenance in this study was 29.8 μg/(kg BW/d), which was much greater than those values reported by the NRC (2007), Suttle (2010) and Grace and Clark (1991), which have reported the net Cu requirement for maintenance as 4 μg/(kg BW/d). Net Cu requirement for maintenance in our study was also greater than that value reported by Zhang et al. (2015), who estimated the net Cu requirement for maintenance as 23.8 μg/(kg BW/d) for Dorper × Hu crossbred ewe lambs from 20 to 35 kg BW.

Zinc is a nutritionally essential trace element that plays a major role in the growth and health of all animals and interacts with numerous metalloenzymes (Underwood and Suttle 1999). Its functions in gene expression (Dreosti 2001) and appetite (Suttle 2010) are perhaps even more important. However, information on the maintenance components of net Zn requirement in factorial models has been lacking (Suttle 2010; Ji et al. 2014). In the present study, the net Zn requirement of Dorper × Hu crossbred ewe lambs for maintenance estimated with the CST was 91.7 μg/kg BW daily, which was greater than that value reported by the NRC (2007), which estimated the net Zn requirement of sheep with the factorial method for maintenance as 76 μg/(kg BW/d). The net Zn requirement for maintenance in our study was also greater than those reported by Zhang et al. (2015), who estimated the net Zn requirement for maintenance as 72 and 81.6 μg/(kg BW/d) for males and females of Dorper × Hu crossbred lambs, respectively, from 20 to 35 kg BW. However, net Zn requirement for maintenance in our study was much lower than those reported by Ji et al. (2014), who estimated the net Zn requirements for maintenance as 97 and 165 μg/(kg BW/d) for males and females of Dorper × thin-tailed Han crossbred lambs, respectively, from 20 to 35 kg BW.

The net requirement recommendation of the NRC (2007) for growth in sheep was 55 mg Fe per kilogram of live weight gain by using factorial method. Zhang et al. (2015), working with Dorper × Hu crossbred ewe lambs with BW varying from 20 to 35 kg, observed the net Fe requirement for growth decreased from 24.70 to 17.60 mg per kilogram live weight gain. Bellof and Pallauf (2007), working with German Merino Land sheep, observed net requirement for growth was 26.1 mg Fe per kilogram of EBWG for the animals with BW varying from 18 to 55 kg. However, in our study, the net requirement for growth decreased from 34.20 to 30.90 mg Fe per kilogram live weight gain (Table 7) and from 41.02 to 37.05 mg Fe per kilogram of EBWG from 35 to 50 kg BW (Table 6). These indicated that the net Fe requirement of Dorper × Hu crossbred ewe lambs for growth in this study was greater than the recommendations of Zhang et al. (2015) and Bellof and Pallauf (2007), except that the net Fe growth requirement for Dorper × Hu crossbred ewe lambs was lower than the recommendation of the NRC (2007).

The net Mn requirement recommendation of the NRC (2007) for growth in sheep was 0.47 mg per kilogram of live weight gain by using factorial method. Zhang et al. (2015), working with Dorper × Hu crossbred ewe lambs with BW varying from 20 to 35 kg, observed the net Mn requirement for growth decreased from 0.35 to 0.25 mg per kilogram live weight gain. Bellof and Pallauf (2007), working with German Merino Land sheep, observed net requirement for growth was 1.04 mg Mn per kilogram of EBWG from 35 to 50 kg BW (Table 6). These indicated that the net Mn requirement of Dorper × Hu crossbred ewe lambs for growth in this study was greater than the recommendations of Zhang et al. (2015) and Bellof and Pallauf (2007), except that
the net Mn growth requirement for Dorper \times Hu crossbred ewe lambs was greater than the recommendation of Zhang et al. (2015).

The net Cu requirement recommendation of the NRC (2007) for growth in sheep was 1.06 \mu g per kilogram of live weight gain by using factorial method. Zhang et al. (2015), working with Dorper \times Hu crossbred ewe lambs with BW varying from 20 to 35 kg, observed the net Cu requirement for growth decreased from 2.30 to 1.70 mg per kilogram live weight gain. Bellof and Pallauf (2007), working with German Merino Land sheep, observed net requirement for growth was 1.41 mg Cu per kilogram of EBWG for the animals with BW varying from 18 to 55 kg. However, in our study, the net Cu requirement for growth decreased from 2.90 to 2.50 mg per kilogram live weight gain (Table 7) and from 3.39 to 3.10 mg per kilogram of EBWG from 35 to 50 kg BW (Table 6). These indicated that the net Cu requirement of Dorper \times Hu crossbred ewe lambs for growth in this study was greater than the recommendations of the NRC (2007), Bellof and Pallauf (2007), and Zhang et al. (2015).

The net Zn requirement recommendation of the NRC (2007) for growth in sheep was 24 mg per kilogram of live weight gain by using factorial method. Zhang et al. (2015), working with Dorper \times Hu crossbred ewe lambs with BW varying from 20 to 35 kg, observed the net Zn requirement for growth decreased from 22.30 to 20.50 mg per kilogram live weight gain. Ji et al. (2014) observed the net Zn requirement of Dorper \times thin-tailed Han crossbred ewe lambs for growth decreased from 23.8 to 22.1 mg/kg BW gain as their BW increased from 20 to 35 kg. Bellof and Pallauf (2007), working with German Merino Land sheep, observed net requirement for growth was 30.0 mg Zn per kilogram of EBWG for the animals with BW varying from 18 to 55 kg. However, in our study, the net Zn requirement for growth increased from 28.50 to 29.80 mg per kilogram live weight gain (Table 7) and from 34.21 to 35.80 mg per kilogram of EBWG from 35 to 50 kg BW (Table 6). These indicated that the net Zn requirement of Dorper \times Hu crossbred ewe lambs for growth in this study was greater than the recommendations of the NRC (2007), Bellof and Pallauf (2007), Ji et al. (2014) and Zhang et al. (2015).

In general, these differences might be related in part to breed, feed, management practices, and physiological and environmental conditions. In particular, the level of intake influence the absorption: higher is the availability higher is the absorption. The physiological condition and in particular the kind of growth with different proportion of bone, muscle, adipose tissue deposition, and the retention of mineral result differently. The determination of dietary trace element requirements is important in trace element 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 net mineral requirements underestimate gross dietary needs for minerals because ingested minerals are incompletely absorbed, the degree of underestimate being inversely related to the efficiency with which a given mineral is absorbed from a given diet (Suttle 2010). However, the main advantage of the factorial approach is that requirements can be predicted for a wide range of production circumstances, provided that reliable data are available for each model component (ARC 1980; White 1996). Therefore, these extrapolated nutrient recommendations can be used as a starting point for the minimum suggested nutrient requirements and concentrations in formulating diets (Robert and Van 2006).

**Conclusions**

In conclusion, the net Fe, Mn, Cu and Zn requirements of Dorper \times Hu ewe lambs for maintenance were greater than the recommendations of the NRC (2007), and the net Fe and Mn requirements of Dorper \times Hu ewe lambs for growth were lower than the recommendations of the NRC (2007), except that the net Cu and Zn growth requirements were greater than the recommendations of the NRC (2007). It will be critical to perform studies using different diets, systems of production, genders, and ages to better understand what causes variation in nutritional trace element requirements. Further studies are warranted to investigate the trace elements requirements at other stages of growth of Dorper \times Hu crossbred lambs. However, additional research is also necessary to determine the absorption coefficient to complete the factorial model for trace elements nutritional systems.

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