Determination of the trace minerals requirements for maintenance and growth of 35–50 kg Dorper × Hu crossbred ram lambs

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ABSTRACT

This study aimed at estimating the trace minerals net requirements for maintenance and growth of Dorper × Hu ram lambs using the comparative slaughter techniques in 35 lambs of 35–50 kg body weight (BW). Seven lambs were slaughtered at the initial BW (34.93 ± 0.37 kg) to determine the basal whole-body composition. Another seven lambs were fed ad libitum (AL) and then slaughtered when it reached 41.73 ± 0.53 kg BW. The remained 21 lambs were assigned into three treatment groups, seven animals each, and fed 100, 70 or 40% of AL intake, respectively, and were slaughtered when they reached a BW of 49.93 ± 1.03 kg. The net requirements of maintenance were 5.1, 429.3, 94.0 and 48.8 mg/kg empty BW (EBW) for Mn, Fe, Zn and Cu, respectively. The net requirements of growth at 35 kg BW were 0.86, 70.41, 33.46 and 4.31 mg/kg EBW for Mn, Fe, Zn and Cu, respectively. At a BW of 50 kg, the net growth requirements were 0.93, 68.40, 35.20 and 4.15 mg/kg EBW for Mn, Fe, Zn and Cu, respectively. Our data indicated that the Mn and Zn requirements increase, whereas those of Fe and Cu decrease when BW increases. In addition, the net Cu, Mn and Fe requirements for maintenance and Zn, Cu, Mn and Fe requirements for growth of Dorper × Hu hybrid ram lambs were higher than those reported in the NRC but Zn requirements for maintenance matched that of the NRC.

HIGHLIGHTS

- Trace minerals requirements for maintenance and growth vary with sheep genotype or breed.
- The comparative slaughter method could be a reliable tool for estimating the net requirements of trace minerals in Dorper × Hu ram lambs.
- Our estimated values for maintenance (Cu, Mn and Fe) and growth (Zn, Cu, Mn and Fe) requirements were higher than those reported in the NRC (2007).

Introduction

Having a high meat production potential and a good adaptation to various environmental conditions, Dorper sheep were brought to China from South Africa with the aim to improve the growth performance and carcase traits of the native Hu sheep (Cloete et al. 2000; Kovács et al. 2008; Li et al. 2015). The Chinese native breed, Hu sheep, are well-adapted to various environmental conditions in certain areas of China and are famous for their superior genotype and phenotype traits (Yue 1996; Xu et al. 2018). Therefore, Dorper × Hu crossbred sheep are the dominant breed for meat production in China. Because the nutrient requirements of livestock can be affected by the genetic and environmental factors, great attention has been paid to precisely determine the nutrient requirements of Dorper × Hu crossbred sheep to optimise their production in China. However, most of the work...
had been done on energy and protein requirements (Deng et al. 2012, 2014; Nie et al. 2015) with a little attention being paid to the mineral requirements (Zhang et al. 2015, 2018a,b) particularly the trace mineral in ram lambs; the future meat producing sheep in China.

The fundamental role of trace minerals in maintaining the health and performance of livestock is well-known (Underwood and Suttle 1999; Suttle 2010). Although required in minute amounts in diets, trace mineral nutrition should be precisely monitored because nutritional imbalances and interactions of trace mineral result in various metabolic and health problems (Froslie 1990; Grace and Clark 1991). For example, it is known that excessive zinc (Zn) intake induces a deficiency of copper (Cu), which is important for various enzymes involved in iron (Fe) metabolism (Minervino et al. 2018; Hill and Shannon 2019). Moreover, most trace minerals ingested by sheep are largely excreted in urine and faeces (Underwood and Suttle 1999). In addition, feeding a high level of Cu and Zn exaggerates the excretion of these minerals, which is a potential threat to the environment (Underwood and Suttle 1999). Hence, a precise determination of trace mineral requirements together with their balanced feeding could minimise their excretion and consequently contribute to environment protection (Chizzotti et al. 2009; Costa e Silva et al. 2015; Castro et al. 2019; Pereira et al. 2019).

It has been indicated that estimating the deposition of the trace minerals in the animals’ body is a prerequisite for determining their requirements (Bellof and Pallauf 2007). The direct measurement of trace minerals deposition using the comparative slaughter technique (CST) has been recently used to determine trace minerals requirements in sheep and goat (Ji et al. 2014; Araujo et al. 2017). Previously, our team conducted a series of studies on the Dorper × Hu crossbred lambs and estimated the trace minerals requirements of ewe lambs with a body weight (BW) of 35–50 kg (Zhang et al. 2018a) and of both ewe and ram lambs with a BW of 20–35 kg (Zhang et al. 2015). As an extension to that series, this study aimed to determine the trace minerals (Zn, Cu, Mn, Fe) requirements for maintenance and growth of 35–50 kg Dorper × Hu crossbred ram lambs using the CST.

### Materials and methods

All experimental procedures were performed according to the Guide for Animal Care and Use, Animal Science & Technology School, Nanjing Agricultural University (SYXK 2011-0036).

#### Animals and management

The experiments were conducted at the Nantong Haimen Experimental Station (Nantong, Jiangsu, China). The Dorper × Hu crossbred ram lambs (no, 35; BW, 33.45 ± 0.42 kg; age, 132 ± 4.15 days old) were housed in one indoor farm facility with the ambient temperature ranging from 15.50 ± 1.32 to 26.54 ± 1.61 °C and the mean relative humidity was 61.25 ± 2.76%. Each animal was restricted within its own stainless steel pen (dimension, 3.20 × 0.80 m) and each pen had an automatic waterer and feeder. All lambs were drenched with ivermectin (0.20 mg/kg BW) for deworming. All animals were fed a pelleted diet of forage and concentrate mix (Table 1) ad libitum (AL) for 10 days as an adaptation period. The pelleted diet was designed to enable a more precise measurement of feed intake and to minimise the potential of feed sorting.

Following the 10-day adaptation period, animals were randomly assigned to five treatment groups, seven animals each. The first group was nominated as a baseline group where animals were slaughtered to measure the initial whole-body composition at the BW.

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

| Items                             | Value     |
|-----------------------------------|-----------|
| Ingredients, %                    |           |
| Corn                              | 42.83     |
| Soybean meal                      | 16.04     |
| Soybean straw                     | 40.02     |
| Anhydrous calcium phosphate       | 0.40      |
| Limestone                         | 0.20      |
| Sodium chloride                   | 0.40      |
| Premix*                           | 0.11      |
| Nutrient composition (analysed)b  |           |
| DM, % air dry basis               | 90.13     |
| CP, %                             | 17.89     |
| ME, MJ/kg                         | 10.02     |
| Ether extract, %                  | 2.66      |
| NDF, %                            | 49.17     |
| ADF, %                            | 20.89     |
| Fe, mg/kg                         | 426.29    |
| Mn, mg/kg                         | 112.87    |
| Cu, mg/kg                         | 5.40      |
| Zn, mg/kg                         | 135.12    |

The premix provided the following nutrients per kg of the diet: 15,000 U VA, 8000 U VD, 50 mg VE, 32 g Na (sodium chloride source), 92 g K (potassium iodate source), 23 g Mg (magnesium sulphate source), 90 mg Fe (iron sulphate source), 2.5 mg Cu (copper sulphate source), 50 mg Mn (manganese sulphate source), 100 mg Zn (zinc sulphate source), 0.3 mg Se (sodium selenite source), 0.8 mg I (potassium iodate source) and 0.5 mg Co (cobalt sulphate source).

Nutrient levels are analysed values.

DM: dry matter; CP: crude protein; ME: metabolisable energy; NDF: neutral detergent fiber; ADF: acid detergent fiber.
of 34.93 ± 0.37 kg. The second group was designated as an intermediate slaughter group where animals in this group were fed 100% AL until reaching a BW of 41.73 ± 0.53 kg, and then were slaughtered (at an age of 168 days old).

The animals in the other three treatment groups were fed at 100, 70 or 40% of AL intake, respectively. These respective feeding regimens were expected to produce 300, 200 and 0 g/day of BW gain, respectively, based on the NRC (2007) recommendations. The feed was offered once a day at 08:00 am and the daily feed amount was adjusted based on the dry matter intake (DMI) of the previous day considering the 10% feed refusal. The daily feed intake and refusal were recorded and samples of both (10% of the total amount) were collected daily and kept frozen at −20°C until estimating the mineral daily intake for all animals. The lambs were slaughtered at a BW of 49.93 ± 1.03 kg (at an age of 192 days old). Notably, sixty days were required for the completion of this experiment.

**Slaughter and sample collection**

One day prior to the slaughter, the animals were weighed for the shrunk BW (SBW) following a 16 h of water and feed deprivation. The animals were slaughtered by exsanguination after inhalation of CO2 and the blood was gathered and weighed. The mass of the skin, viscera, head, wool, carcase, feet, as well as kidney, pelvic and heart fat (KPH fat) that were taken out from internal organs were recorded. Afterward, the gastrointestinal tract content (CGIT, including the large and small intestines, omasum, reticulum, abomasum and the rumen) was taken out and weighed prior to and following its content elimination. The empty BW (EBW) was calculated by subtracting the weight of both CGIT and the bladder mass from SBW. The carcase was initially frozen at −6°C and then chopped using a stainless steel band saw followed by grinding and homogenisation for sample collection. Samples were collected in accordance with the procedure of Galvani et al. (2008, 2009) with slight modification. Briefly, the carcase, including the head, was separated from the dorsal midline. Then, the right side of the carcase, including the head, posterior and anterior feet, fat, bone and muscle, was separated. The bone was ground using a bone miller (model SGJ-3600; Langfang Huiyong Machinery Plant, Hebei, China), filtered through an 8-mm sieve, and homogenised. Fat and muscle were separately chopped into small pieces, ground using an electric screw grinder (Model-12, Xinmai Machine Plant, Shanghai, China), then were filtered through a 4-mm sieve and homogenised. Finally, samples of 500 g from each body component (muscles, bones, fats, viscera, etc.) of each animal were collected and preserved at −20°C until chemical analysis. Before the chemical analysis, each sample was unfrozen and sub-samples (100 g) were subjected to 72 h of freeze-drying and then were finely ground by a stainless-steel blender (model KFS181; Yichun Kavins Technology Limited Company, Jiangxi, China).

**Chemical analysis**

The feed and orts samples were oven-dried for 72 h at 55°C, ground in Willey mill (Arthur H. Thomas, Philadelphia, Pennsylvania) to pass through a 1-mm screen. The DM content of the feed and orts was determined by drying at 135°C for 2 h (AOAC 1990; method 930.15). The metabolisable energy (ME) was estimated according to NRC (2007). The crude protein (CP) was determined by a Kjeldahl method (AOAC 1997; method 984.13). The ash content was determined by combustion in a muffle furnace at 600°C for 4 h (Myers and Beede 2009). The ash was dissolved in HNO3 (3 mol/L) within a water bath for 10 min. The dissolved ash was filtered and the ash residues remaining over the filter paper were further ashed for 12 h at 450°C followed by additional acid-dissolving and filtration. The final filtered solution contained 0.3 mol/L of HNO3. The levels of Zn, Cu, Mn and Fe in water, orts and feed were determined by the inductively coupled plasma-atomic emission spectrometry (ICP-AES); Unicam PU701 (Thermo Electron GmbH, Dreieich, Germany). The conditions of analysis were as follows: radial observation for plasma; Hildebrand-Grid as the sprayer; sprayer pressure at 34–40 p.s.i.; the power of 1000 W; cooling gas as argon at 11 L/min; sample flow at 1 mL/min, and at every element-specific emission wavelength. The Cu content measured in fat tissues and Mn content in fat, bone and muscle tissues were lower than the detection limit (0.05 mg/L). As a result, the bone was ashed over two stages (at 500°C and 900°C, respectively) and Mn was measured again in the bone sample using the atomic absorption spectrometer (graphite furnace AAS, PerkinElmer [PE 5100 Z]). The measuring parameters were set as follows: the gap width was 0.2 nm; the wavelength was 279.5 nm, the base correction was Zeeman; the cuvette was uncoated; the lamp current was 20 mA; no modifier was used; the injection capacity was 20 μL; the drying was completed in two-stages (at 90°C as well as 120°C); the integration was
area; the atomisation temperature was 2200 °C; and calibration was conducted a range from 1 to 10 μg/L. The flame AAS (Unicam PU 9400; Thermo Electron GmbH, Dreieich, Germany) was used to analyse Cu content in the bone, under the following measuring principles: air-acetylene was used as the flame; gap width was 0.5 nm, and the wavelength was 324.8 nm. The Zn, Cu, Mn and Fe concentrations within the drinking water could not be detected. The certified bovine liver (NIST bovine liver 1577 b) was adopted to control the quality of trace element analyses (Braselton et al. 1997). The recovery rates measured were 98, 103, 102 and 107% for Zn, Mn, Cu and Fe, respectively (Bellof et al. 2006; Bellof and Pallauf 2007).

Body components (muscle, bone, viscera mixed with blood, skin, wool and fat): the 100 g subsamples collected based on every original sample without wool were subjected to 72 h of freeze-drying (Galvani et al. 2009) for determination of the DM content. Subsequently, each subsample that included wool was analysed for the trace element (Mn, Fe, Zn and Cu) as mentioned before.

Data calculation and analysis

Initial whole-body composition

Using the regression equations, the initial composition of the empty body was predicted from the body composition of the baseline animals. Assuming that the trace minerals contents in the whole-body include the total contents in each component (muscle, bone, viscera mixed with blood, skin, wool and fat) of the body, the original trace minerals, ash and water contents of an empty body were estimated (Equation (1)) from baseline animals’ mean whole-body composition and the initial EBW (Fernandes et al. 2007):

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

in which \(a\) is indicative of the intercept, whereas \(b\) represents the regression coefficient.

Trace minerals requirements for maintenance

The CST was adopted to calculate the requirements for maintenance (Lofgreen and Garrett 1968). The trace minerals deposited within the body were calculated as the difference between the initial and final measurements for each animal in the AL, 70 and 40% groups. The total losses of a trace mineral were computed as the difference between intake and deposition of that mineral for each animal. The maintenance net requirements were predicted by the linear regression of the daily deposition to the daily intake of each trace mineral (mg/kg of EBW), with the extrapolation of the daily intake of 0 mg/kg of EBW. The resulting intercept from the regression indicates the inevitable losses of trace minerals which represents the maintenance net requirements (mg/kg EBW/day) (Zhang et al. 2015).

Trace minerals requirements for growth

The logarithm regarding Cu, Mn, Fe or Zn content in the whole body in relation to the log of EBW was regressed to estimate every trace mineral content within the empty body in accordance with ARC (1980):

\[
\log_{10} y = a + \left( b \times \log_{10} x \right) \tag{2}
\]

in which \(\log_{10} y\) stands for logarithm for the total amount of trace minerals in the empty body (g); \(a\) is indicative of the intercept and \(b\) indicates the regression coefficient for the contents of trace minerals based on EBW, while \(\log_{10} x\) represents the EBW (kg) logarithm.

Equation (2) antilog was used, and the EBW-related derivation was adopted for determining trace minerals net requirement for every 1 kg EBW gain (EBWG) every day (Equation (2)):

\[
y' = b \times 10^a \times \text{EBW}^{(b^{-1})} \times x \tag{3}
\]

in which \(y'\) represents gain requirements for every trace mineral; \(b\) stands for the regression coefficient; and \(a\) represents the intercept. The equation was differentiated to compute the estimates of the gain composition at various EBW values. For estimating the trace minerals requirements for the net BW gain, the body composition gain values were divided by the BW-to-EBW ratio factor. Those animals raised with restricted intake levels were eliminated from that prediction equation since they had different growth patterns compared with animals that were raised randomly (Pereira et al. 2019).

Statistical analysis

The SAS (SAS Institute, Cary, North Carolina) was used to analyse data. Analysis of linear regression was carried out with the PROC REG. Analyses of ME intake (MEI), CP intake (CPI), DMI, EBW, ADG and body composition were conducted utilising PROC MIXED for various levels of feeding. The residuals that were plotted against the forecasted values were utilised for examining the model assumptions for error normality, independence and homoscedasticity (Deng et al. 2014). Every data point was regarded as an outlier and was eliminated out of the database when Studentized residual lied beyond those ±2.5 range values (Deng et al. 2014).
Meanwhile, the retained level of Zn was increased with increasing the feed intake level (p < .05). The Mn, Fe, Zn and Cu concentrations in the EBW declined with the increase of the feed intake level (p < .05).

### Results

**Performance parameters and trace mineral content of the EBW**

The performance parameters and the trace mineral content of the EBW are illustrated in Table 2. The CPI, DMI, EBW, MEI, EBWG and ADG increased with the increase of the feed intake level (p < .05). The Mn, Fe, Zn and Cu concentrations in the EBW declined with the increase of the feed intake level (p < .05).

**Metabolism and intake of trace minerals**

The retained Cu, Fe and Mn levels were elevated (p < .05) with increasing the feed intake level (Table 3). Meanwhile, the retained level of Zn was increased (p < .05) in lambs fed 100% compared to those fed 40% of AL intake, but no difference was observed compared with animals fed 70% AL intake.

**Estimates of trace minerals net requirements for maintenance**

The retained trace minerals showed a high correlation (ranging from 0.88 to 0.92) with the respective intake (Table 4). Hence, the linear relationship between the intake and retention of the trace minerals was established for predicting the net requirements for the maintenance through extrapolation of linear regression until the retention of trace minerals intake was 0. This linear regression intercept represented the unavoidable losses of trace minerals which equal the net requirements for the maintenance.

**Trace minerals composition of the whole body and estimates of trace minerals net requirements for growth**

The original EBW for every lamb was calculated based on the original BW (root mean square error (RMSE) = 1.14, \( r^2 = 0.92, \ p < .001, \ n = 7 \): EBW, kg = \(-0.2580 + 0.8325 \times \) BW, kg (Table 5). Logarithmic allometric equations that were utilised for calculating the relationship between the EBW and the total content of trace minerals in the empty body were quite marked (p < .001), which offered one data suiteing \( R^2 \) value ranging from 0.88 to 0.95. Equations for predicting those nutrients per kg EBWG were gained through
deriving the logarithmic regression equations for Mn, Fe, Zn and Cu body contents based on the EBW logarithm (Table 6).

The Cu and Fe contents in the empty body declined as BW increased, while those of Zn and Mn were elevated with the increase in BW (Table 5). The trace minerals deposition within EBWG was identical to that of EBW (Table 6).

Trace minerals net requirements for the live weight gain

For calculating the trace minerals net requirements for the live weight gain (Table 7), the empty weight gain composition values were divided by factors correcting BW/EBW ratio; these factors were 1.21 1.21, 1.20, 1.20 standing for 35, 40, 45 and 50 kg BW, respectively. The net requirements for the live weight gain increased for Mn and Zn but decreased for Cu and Fe with the increase of BW.

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

| Item | $b$ | $a$ | $R^2$ | $p$ Value | Net req, mg/(kg EBW/day) | Net req, mg/(kg BW/day) |
|------|-----|-----|-------|-----------|--------------------------|-------------------------|
| Fe   | 0.0490 | −0.429 | 0.890 | <.0010 | 0.429 | 0.354 |
| Mn   | 0.001 | −0.005 | 0.910 | <.0010 | 0.005 | 0.004 |
| Cu   | 0.316 | −0.048 | 0.880 | <.0010 | 0.048 | 0.040 |
| Zn   | 0.053 | −0.094 | 0.920 | <.0010 | 0.094 | 0.077 |

Retained trace element, mg/kg EBW = $a + b \times$ trace element intake, mg/kg EBW.

EBW: empty body weight; Req: requirement.

**Table 5.** Logarithm equations to estimate body composition of trace elements (Fe, Mn, Cu and Zn) of Dorper × Hu ram lambs.

| Item          | $b$   | $a$   | $R^2$ | RMSE     | $p$ Value | BW, kg | 35 | 40 | 45 | 50  |
|---------------|-------|-------|-------|----------|-----------|--------|----|----|----|-----|
| EBW, kg       | 0.832 | −0.258 | 0.920 | 1.140    | <.0010    | 28.88  | 33.04 | 37.21 | 41.37 |
| Fe, mg/kg EBW | 0.913 | 2.013 | 0.910 | 0.060    | <.0010    | 77.160 | 76.260 | 75.490 | 74.800 |
| Mn, mg/kg EBW | 1.257 | −0.533 | 0.880 | 0.070    | <.0010    | 0.690  | 0.720 | 0.740 | 0.760 |
| Cu, mg/kg EBW | 0.880 | 0.861 | 0.950 | 0.090    | <.0010    | 4.900  | 4.820 | 4.750 | 4.690 |
| Zn, mg/kg EBW | 1.151 | 1.242 | 0.910 | 0.070    | <.0010    | 29.020 | 29.610 | 30.150 | 30.640 |

EBW (kg) = $a + [b \times$ (BW, kg)]. Log$_{10}$ $y = a + [b \times \log_{10} x]$ in which log$_{10}$ $y$ stands for logarithm for the total amount of trace minerals in the empty body (g); $a$ is indicative of the intercept, and $b$ indicates the regression coefficient for the contents of trace minerals based on EBW, while log$_{10}$ $x$ represents the EBW (kg) logarithm.

EBW: empty body weight; RMSE: root mean square error.

**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 ram lambs.

| Item | BW, kg | Equations$^b$ | 35   | 40   | 45   | 50  |
|------|--------|--------------|------|------|------|-----|
| Fe, mg/kg EBWG | 70.41 | 69.65 | 69.02 | 68.40 |
| Mn, mg/kg EBWG | 0.86  | 0.87  | 0.90  | 0.93 |
| Cu, mg/kg EBWG | 4.31  | 4.22  | 4.18  | 4.15 |
| Zn, mg/kg EBWG | 33.46 | 33.98 | 34.46 | 35.20 |

ADG: average daily gain; EBW: empty body weight; EBWG: empty body weight gain.

$^a$In order to calculate the net trace element requirements for ADG, 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 1.21, 1.21, 1.20, 1.20 for ram lambs, and corresponded to animals with BWs of 35, 40, 45 and 50 kg, respectively.

$^b$Component concentration = $b \times 10^a \times$ EBW($b \times \log_{10} x$), in which $a$ and $b$ 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 ram lambs.

| Item          | BW, kg | ADG, g/day | 35 | 40 | 45 | 50  |
|---------------|--------|------------|----|----|----|-----|
| Fe            |        |            | 5.820 | 0.071 | 0.360 | 2.770 |
| Mn            |        |            | 11.640 | 0.142 | 0.720 | 5.540 |
| Cu            |        |            | 17.460 | 0.213 | 0.108 | 8.310 |
| Zn            |        |            | 20.100 | 0.151 |       |       |

ADG: average daily gain; BW: body weight.
Discussion

The ADG, EBWG and EBW were higher with increasing the feed intake level. A similar positive association between feed intake and both EBWG and EBW was observed in Canindé goats of 15–25 kg BW (Silva et al. 2013). Also, our results are in line with those reported by Zhang et al. (2015) in Dorper × Hu lambs of 20–35 kg BW. The Mn, Fe, Zn and Cu concentrations were declined as the level of feed intake increased in this study, which is consistent with Zhang et al. (2015) findings in Dorper × Hu lambs of 20–35 kg BW. In contrast, Silva et al. (2013) reported an increase of micro-minerals in the empty body as the feed intake level increases in castrated Canindé goats reared under the grazing system.

In this study, the Cu and Fe concentrations were declined, whereas those of Mn and Zn were increased as the BW of Dorper × Hu ram lambs increased from 35 to 50 kg. Bellof and Pallau (2007) reported a similar trend of these mineral depositions in relation to the BW change from 30 to 55 kg in German Merino Landsheep. However, the Mn, Fe and Cu concentrations within the body were slightly declined as the BW increased from 20 to 35 kg in male and female Dorper × Hu crossbred lambs; Zn concentration followed the same trend for female but not for male Dorper × Hu crossbred lambs (Zhang et al. 2015). These differences might have been associated with the different growth stages of the Dorper × Hu crossbred lambs. In this study, the retained levels of the trace minerals Cu, Mn, Zn and Fe were significantly elevated with the increase of the feed intake level. Generally, the level of intake influences the absorption; the higher the intake the higher the absorption. In addition, the physiological condition and the relative growth of the bone, muscle, and the adipose tissue may differently influence the trace minerals deposition.

Iron is important for ruminants, for example for performance and fitness; usually Fe supply is not a problem, also because for Fe there is an active system of absorption (NRC 2007). The net Fe requirement for maintenance was estimated from 12.42 to 17.74 mg/day for a BW range of 35–50 kg in this study. These values were far larger than those reported by NRC (2007) which estimated the Fe net requirement for the maintenance as from 0.49 to 0.70 mg/day for a BW range of 35–50 kg. These values were also far larger than those reported by Zhang et al. (2015) who estimated the net Fe requirement for maintenance of Dorper × Hu lambs of 20–35 kg BW to be 2.63–4.60 mg/day for ram lambs and 6.14 to 10.75 mg/day for ewe lambs, respectively.

Manganese is an indispensable dietary nutrient for ruminant animals, which is required for reproductive efficiency as well as skeletal development (Underwood and Suttle 1999; Suttle 2010). The deficiency of Mn is associated with reproductive disorders in ruminants (Hidiroglou 1979a). In ruminant animals, it is hard to define the minimum requirement for Mn as its availability in feed changes with the diet composition, especially for the contents of the P and Ca (NRC 2007). It may be helpful to optimise the assessment for Mn of lambs by measuring the activity of pyruvate carboxylase within the liver (Hidiroglou 1979b). The net Mn requirement for maintenance of Dorper × Hu ram lambs was estimated in this study as 4.2 μg/kg BW/day, which is higher than that of the NRC (2007) value (2 μg/kg BW/day). This value was also higher than that of Zhang et al. (2015) who estimated the net Mn requirements for maintenance within a BW range from 20 to 35 kg to be 2.7 and 3.7 μg/kg BW/day in female and male Dorper × Hu lamb breed, respectively.

Copper is an indispensable trace mineral that plays a significant role in various body biochemical reactions (Underwood and Suttle 1999; Suttle 2010). Nevertheless, the Cu requirements, as well as interaction with other minerals, are not clearly understood and deserve more investigations. The high dietary concentrations of Cu antagonists, like Fe, S and Mo, raise the dietary Cu requirements (McDowell 2003). It has been assumed that the faecal endogenous losses of Cu match its requirements for maintenance (NRC 2007), as faeces are the main route of Cu excretion, and losses from other excretion routes are not necessarily be substituted (Suttle 2010). In our study, the net Cu requirement for maintenance of the Dorper × Hu ram lambs was 40.3 μg/kg BW/day; this value is remarkably higher than that estimated by previous reports (Grace and Clark 1991; NRC 2007; Suttle 2010) which stated a maintenance net requirement of Cu as 4 μg/kg BW/day. Also, Zhang et al. (2015) reported higher values for Dorper × Hu lambs with a BW range of 20–35 kg; the net Cu requirements for maintenance were 36.2 and 23.8 μg/kg BW/day for male and female animals, respectively.

Zinc is an essential trace mineral having a great role in various metalloenzymes involved in modulating the animal’s health and growth (Underwood and Suttle 1999). Moreover, Zn exerts a paramount function in appetite (Suttle 2010) and gene expression (Dreosti 2001) regulation. Nevertheless, the information is largely lacking regarding a factorial model of the net Zn requirement for maintenance (Suttle 2010;
Ji et al. (2014). In this research, the net Zn requirement for maintenance of Dorper × Hu ram lambs was 77.7 μg/kg BW/day. Our estimated value matches the value of 76 μg/kg BW/day that has been approved by the NRC (2007). Similarly, Zhang et al. (2015) reported values of 81.6 and 72 μg/kg BW/day as a net Zn requirement for maintenance of female and male Dorper × Hu lambs (with a BW range of 20–35), respectively. In contrast, these values were lower than the values estimated by Ji et al. (2014) who reported net Zn requirements for maintenance as 165 and 97 μg/kg BW/day for female and male Dorper × thin-tailed Han crossbred lambs (with a BW range of 20–35), respectively.

Using a factorial model, the NRC (2007) recommended net requirements for growth as 0.47 mg Mn, 55 mg Fe, 24 mg Zn and 1.06 μg Cu for each kg of live weight gain. Zhang et al. (2015) observed that the net requirements for growth of Dorper × Hu lambs, in the range of 20–35 kg BW, were declined from 3.00 to 2.40 mg for Cu, from 0.30 to 0.22 mg for Mn and from 56.60 to 49.40 mg for Fe for each kg live weight gain. In contrast, the requirements were declined from 22.30 to 20.50 mg for Zn, from 0.35 to 0.25 mg for Mn, from 24.70 to 17.60 mg for Fe and from 2.30 to 1.70 mg Cu for each kg live weight gain in female, whereas it was elevated from 22.70 to 23.00 mg of Zn for each kg live weight gain in male Dorper × Hu lambs (Zhang et al. 2015). However, in our research, net requirements of the Dorper × Hu ram lambs for the growth were declined from 3.6 to 3.4 mg for Cu and from 58.2 to 56.50 mg for Fe; while it was increased from 0.71 to 0.77 mg for Mn and from 27.70 to 29.10 mg Zn for each kg of live weight gain in the BW range from 35 to 50 kg. Taken together, net Mn, Cu, Zn and Fe requirements for growth of the Dorper × Hu ram lambs in the BW range of 35–50 kg were increased relative to those recommended by NRC (2007) and by Zhang et al. (2015). In the German Merinoland sheep, Bellof and Pallauf (2007) estimated the net Cu, Zn, Mn and Fe requirements for growth as 1.41, 30.0, 1.04 and 26.1 mg, respectively, for each kg of EBWG at the BW of 18–55 kg. These values were lower for Zn, Fe and Cu but higher for Mn compared to our estimated values in this study. The difference could be attributed in part to the feed, management practice, breed, as well as environmental and physiological conditions.

It is important to determine the dietary requirements of trace minerals adopting the factorial models which consider both maintenance and production (NRC 2007). The factorial models allow the calculation of the dietary mineral requirements based on the available DMI and absorption coefficient (ARC 1980). The net requirement of mineral underestimates their total dietary requirements because the ingested minerals not completely absorbed, the underestimation degree is negatively correlated with the mineral’s utilisation efficiency (Suttle 2010). However, if there are credible data of all model components (ARC 1980; White 1996), a factorial method has the major merit that the requirements could be predicted for various circumstances of production. Hence, these estimates recommended of nutrients can serve as a starting point used in diet formulation for meeting the minimum requirements and concentrations (Robert and Van 2006).

**Conclusions**

In conclusion, the maintenance net requirements for Cu, Mn and Fe of the Dorper × Hu ram lambs were higher compared with those recommended by NRC (2007). The net requirement of Zn for the maintenance was similar to NRC (2007) recommendations. The growth net requirements of Mn, Fe, Zn and Cu of Dorper × Hu ram lambs with the BW of 35–50 kg were greater than NRC (2007) recommendations. More studies that use various diets, ages, genders and production systems should be further carried out, so as to shed more light on the changes in those dietary requirements of trace elements.

**Acknowledgments**

The authors thank all the members of the Feng Wang’s laboratory who contributed to sample determination.

**Disclosure statement**

The authors declare that there is no conflict of interest perceived to prejudice the impartiality of the research reported.

**Funding**

The study was funded by the National Natural Science Foundation of China [grant number 31902180], the Research Project of Natural Science Foundation of Jiangsu Province [BK20170488], the China Postdoctoral Science Foundation [2017M610358], and the Su Bei Special Science and Technology Foundation [SZ-HA 2017008].

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