

## Macromineral Requirements for Maintenance and Growth of 20-35 kg Dorper × Hu F1 Male Lambs (Postprint)

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### Abstract

This study employed the comparative slaughter method to determine the maintenance and growth requirements of macro minerals [calcium (Ca), phosphorus (P), potassium (K), sodium (Na), and magnesium (Mg)] in Dorper × Hu (Dorper-Hu) F1 crossbred male lambs during the 20-35 kg body weight stage. Thirty-five Dorper-Hu F1 male lambs [initial body weight (19.20±\$0.36) kg] were selected as experimental animals. Seven lambs were randomly selected and slaughtered at approximately 20 kg body weight to determine the initial macro mineral content of the empty body. Another seven lambs were randomly selected, fed a total mixed pellet diet ad libitum (AL), and slaughtered at approximately 28 kg body weight. The remaining 21 lambs were randomly divided into three groups: AL group, 70% AL group, and 40% AL group, with seven lambs per group. When the AL group reached approximately 35 kg body weight, these three groups were slaughtered. After slaughter, the macro mineral content of the empty body (head + feet, skin, viscera + blood, and carcass) was determined. The results showed that during the 20-35 kg body weight stage, the maintenance requirements for calcium, phosphorus, potassium, sodium, and magnesium [based on empty body weight (EBW)] were 24.01, 11.70, 3.20, 6.60, and 1.20 mg/kg EBW, respectively, and the growth requirements [based on empty body weight gain (EBWG)] were 11.55-11.41, 5.82-5.77, 1.47-1.69, 0.42-0.44, and 0.98-0.88 g/kg EBWG, respectively. In conclusion, the determination of macro mineral requirements for 20-35 kg Dorper-Hu F1 lambs will facilitate the formulation of rational diets for this growth stage and benefit the improvement of lamb growth performance.

## Full Text

### Macroelement Requirements for Maintenance and Growth of Dorper×Hu F1 Male Lambs Weighing 20 to 35 kg

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**Abstract:** A comparative slaughter trial was conducted to estimate the macroelement [calcium (Ca), phosphorus (P), potassium (K), sodium (Na) and magnesium (Mg)] requirements for maintenance and growth of Dorper×Hu F1 male lambs weighing 20 to 35 kg. Thirty-five male lambs [initial body weight (BW) was  $(19.20 \pm 0.36) \text{ kg}$ ] were used as experimental animals. Seven lambs were randomly chosen and slaughtered. The remaining 28 male lambs in the growth phase of 20 to 35 kg BW and improvement of growth performance of lambs.

**Keywords:** comparative slaughter method; Dorper×Hu F1 male lambs; maintenance requirement; growth requirement; macroelement

## Introduction

Macroelement requirements have received considerable attention because excess or deficiency of any macroelement can interfere with the absorption or utilization of other macroelements, thereby impairing animal health, growth performance, and even reducing survival rate. Furthermore, accurately predicting macroelement requirements can reduce their excretion and environmental pollution [?]. Minerals are not only important components of animal tissues but also play crucial roles in biochemical processes within the body. Both mineral deficiency and excess can severely affect animal health and growth performance [?]; therefore, in-depth research on mineral requirements is particularly important for the sustainable and healthy development of the sheep industry. Previous studies have investigated mineral requirements for various sheep breeds and weight ranges, including 20–35 kg Dorper×Small-tailed Han lambs [?], 35–50 kg Dorset×Small-tailed Han lambs [?], German Merino lambs (18–55 kg) [?], and Saanen dairy goats (20 and 25 kg) [?, ?]. Although these studies have explored mineral requirements for meat sheep in various countries, the mineral requirements for 20–35 kg Dorper×Hu F1 male lambs have not been reported.

The Hu sheep is a famous local breed in China, characterized by early maturity, year-round estrus, two litters per year, multiple births per litter, good milk performance, rapid growth and development, desirable meat production performance after improvement, and tolerance to high temperature and humid-

ity [?, ?]. This breed is mainly distributed in the Taihu Lake region of China and is well recognized by local consumers. The Dorper sheep, originally from South Africa, is known for its cold tolerance, early maturity, and rapid growth [?], and has been introduced to China to improve the growth performance and carcass traits of Hu sheep. Consequently, the Dorper×Hu cross has become one of the primary meat sheep production systems in many regions of China. However, due to the lack of corresponding nutrient requirement standards, most feed formulations during production can only refer to foreign standards. Since there are certain differences in meat sheep breeds, growth characteristics, and feed ingredients between China and other countries, directly applying foreign nutritional standards may lead to problems such as feed resource waste caused by excessive nutrient supply or failure to meet animal requirements due to insufficient nutrient supply. Our research group has previously studied the energy and protein requirements of Dorper×Hu crossbred lambs at different ages [?], but mineral requirements remain unknown, creating difficulties for scientific and precise feeding. The comparative slaughter technique (CST) is a precise and direct method that has been widely used to establish requirements for protein, energy, minerals, and trace elements [13-16]. This study employed the comparative slaughter method to determine the macroelement maintenance and growth requirements of Dorper×Hu F1 male lambs during the early fattening stage (20-35 kg), providing basic data for improving China's meat sheep feeding standard system.

### 1.1 Experimental Location

The trial was conducted at the Haimen Goat Research and Development Center of Nanjing Agricultural University in Nantong City, Jiangsu Province. Ambient temperature was maintained between 15.5-26.5°C, with an average humidity of 61.2%. The entire experimental procedure complied with the Animal Welfare Management Regulations of the College of Animal Science and Technology, Nanjing Agricultural University.

### 1.2 Experimental Design

Thirty-five Dorper×Hu F1 male lambs with an average initial body weight (BW) of  $(19.20 \pm 0.36)$  kg and approximately 65 days of age were selected as experimental animals. To determine initial body weight, seven lambs were randomly selected and slaughtered as a baseline (BL) group. Another seven lambs were randomly selected, offered a pelleted mixed diet ad libitum (AL), and slaughtered as an intermediate slaughter group (IM) when they reached approximately 28 kg BW. Simultaneously, the remaining 21 lambs were randomly allocated to three groups: AL, 70%AL, and 40%AL groups, with seven lambs per group. According to NRC (2007) [?], the expected average daily gain (ADG) for these feeding levels would be 300, 200, and 0 g/d, respectively. When the AL group lambs reached approximately 35 kg BW, all groups were slaughtered after 16 h of feed and water deprivation [?].

### 1.3 Basal Diet and Feeding Management

The basal diet was a total mixed pellet consisting primarily of corn, soybean meal, and soybean straw, with a concentrate-to-forage ratio of approximately 51:49. The composition and nutrient levels are shown in Table 1. The use of pelleted feed minimized waste and facilitated accurate measurement of feed intake. A 10-day pre-trial period was conducted before the formal experiment, during which all lambs were dewormed with ivermectin at 0.2 mg/kg BW. Each lamb was housed in an individual stainless steel pen (3.2 m long × 0.8 m wide) equipped with an automatic waterer and feed trough. Lambs were fed once daily at 08:00 h with AL access to feed and water. The feed supply for the AL group was adjusted each morning to ensure approximately 10% orts (on a dry matter basis). The feed allowance for restricted groups was also adjusted daily based on the dry matter intake (DMI) of the AL group on the previous day. Daily feed samples and orts (approximately 10% of total orts) were collected and stored at -20°C. These samples were later pooled, dried at 55°C for 72 h, ground, and stored for analysis. Body weight was measured at 16:00 h on the day before slaughter.

### 1.4 Slaughter Procedure

Lambs were anesthetized and exsanguinated, with blood collected and weighed. The weights of internal organs, hide, wool, head, feet, carcass, and adipose tissues were recorded. Gastrointestinal tract contents (rumen, reticulum, omasum, abomasum, small intestine, and large intestine) were removed and weighed to obtain empty body weight (EBW), which was calculated as BW minus the weight of gastrointestinal contents and bladder. All body components were frozen at -6°C, then cut with a stainless steel saw, ground, mixed, and a 500 g sample was taken for analysis. These samples were thawed, and a 100 g subsample was freeze-dried for 72 h, then ground and mixed with a stainless steel blender. The sampling procedure followed Galvani et al. [?, ?] with appropriate modifications. Briefly, the head and carcass were split along the dorsal midline. The right half of the carcass, head, forelimbs, and hindlimbs were separated into muscle, fat, and bone. Bone tissue was crushed with a bone grinder, while muscle and fat tissues were cut into small pieces. A 500 g sample from each body component was stored at -20°C.

### 1.5 Chemical Analysis

**1.5.1 Diet and Orts Samples** Dry matter (DM) and crude ash content in diet and orts samples were determined according to AOAC (1990) [?]. Calcium (Ca), phosphorus (P), potassium (K), sodium (Na), and magnesium (Mg) contents were determined by atomic emission spectrometry [?, ?].

**1.5.2 Body Tissue Macroelement Analysis** Except for wool, 100 g of each body tissue was freeze-dried to determine DM content [?]. All samples

(including wool) were analyzed for macroelement content by atomic emission spectrometry.

## 1.6 Calculation Methods

**1.6.1 Calculation of Initial Body Macroelement Content** The macroelement content in the empty body of lambs in the BL group was determined as the initial value. A linear regression equation between EBW and BW was established based on data from the BL group:

$$EBW = a + b \times BW$$

where EBW and BW are in kg. The contents of water, crude ash, and macroelements in the empty body were calculated using this equation.

### 1.6.2 Calculation of Macroelement Maintenance Requirements

Macroelement maintenance requirements were calculated using the comparative slaughter method [?]. Macroelement retention in the animal body was calculated as the difference between final and initial body contents. Macroelement loss was calculated as the difference between intake and retention. The maintenance requirement was obtained from the linear regression relationship between macroelement retention (mg/kg EBW) and macroelement intake (mg/kg EBW). The negative intercept when macroelement intake was 0 mg/(kg EBW · d) represents endogenous metabolic loss of macroelements, which indicates the maintenance requirement of the animal, expressed as mg/(kg EBW · d).

### 1.6.3 Calculation of Macroelement Growth Requirements

ARC (1980) [?] reported that body macroelement content could be established in an allometric logarithmic model with EBW, from which macroelement body content at different EBW could be derived:

$$\text{Log } y = a + b \times \text{Log } x$$

where y is the amount of macroelement in the body (g) after removing gastrointestinal contents; a is the intercept; b is the regression coefficient; and x is EBW (kg).

Equation (2) was transformed and derived to obtain the following equation for predicting macroelement growth requirements at different EBW:

$$y = b \times 10^a \times x^{(b-1)}$$

where  $y$  is the amount of macroelement required per unit empty body weight gain (EBWG) (g/kg EBWG);  $x$  is EBW (kg); and  $a$  and  $b$  values are obtained from equation (2).

The calculation of macroelement requirement per unit live weight gain required conversion using the ratio of pre-slaughter BW to EBW.

### 1.7 Statistical Analysis

Data were analyzed using SAS 9.0 software with a completely randomized design model. Linear regression analysis was performed using the PROC MIXED procedure. Dry matter intake (DMI), crude protein intake (CPI), metabolizable energy intake (MEI), body macroelement content, ADG, and EBW at different feeding levels were analyzed using the PROC MIXED procedure. Outliers were identified as values exceeding the mean  $\pm$  2.5 standard deviations. Differences were compared using Duncan's method, with significance declared at  $P < 0.05$ .

## Results

### 2.1 Growth Performance and Body Macroelement Content

As shown in Table 2, DMI, CPI, MEI, EBW, ADG, and EBWG of lambs increased significantly with increasing feeding level ( $P < 0.05$ ). Body Ca, P, Na, K, and Mg contents decreased significantly with increasing feeding level ( $P < 0.05$ ).

### 2.2 Macroelement Intake and Retention

As shown in Table 3, macroelement intake, retention, and retention rate of Ca, P, Na, K, and Mg increased significantly with increasing feeding level ( $P < 0.05$ ).

### 2.3 Macroelement Maintenance Requirements

As shown in Table 4, there was a high correlation between macroelement retention and macroelement intake. Therefore, linear regression equations between macroelement retention and macroelement intake were established. Theoretically, the negative intercept when macroelement intake is 0 mg/kg EBW represents the maintenance requirement of the animal under maintenance conditions, expressed as mg/(kg EBW  $\cdot$  d).

### 2.4 Body Macroelement Content and Growth Requirement Prediction

As shown in Table 5, the linear relationship between BW and EBW was:  $EBW = 0.0507 + 0.859 \times BW$  [ $R^2 = 0.96$ , root mean square error (RMSE) = 1.11,  $P <$

0.01]. The allometric logarithmic equations established between body macroelement content and EBW were significant ( $P < 0.01$ ), with  $R^2$  ranging from 0.88 to 0.93.

As shown in Table 6, prediction equations for macroelement growth requirements were established based on the allometric equations in Table 5 to calculate macroelement growth requirements per unit EBWG.

### 2.5 Macroelement Growth Requirements for Live ADG of 20–35 kg Dorper×Hu F1 Male Lambs

As shown in Table 7, to calculate macroelement growth requirements for live ADG, the macroelement growth requirements for empty body ADG were divided by the corresponding BW/EBW ratio. The BW/EBW ratios at 20, 25, 30, and 35 kg were 1.14, 1.15, 1.15, and 1.17, respectively. With increasing BW, the growth requirements for Ca, P, and Na per unit live ADG decreased, while those for K and Mg increased.

## Discussion

### 3.1 Macroelement Maintenance Requirements

The factorial method is commonly used to determine macroelement requirements for different animal species. This method yields a dynamic nutritional requirement model that estimates maintenance and growth requirements at different BW [?]. Currently, the factorial model has been adopted in reports by NRC (2007) [?], NRC (2001) [?], NRC (2000) [?], and Suttle [?]. This model divides animal mineral requirements into two components: maintenance and growth, with the formula:  $GR = (P + M)/A$  (where GR is total requirement; P is growth requirement; M is maintenance requirement; and A is nutrient absorption and utilization rate). Therefore, accurate determination of mineral growth and maintenance requirements is a crucial step in establishing mineral requirement models.

Calcium and P constitute a large proportion of animal bones and teeth and are important components that enhance enzyme activity and promote nerve excitability transmission. In practice, Ca and P are macroelements added in relatively large amounts and must be carefully considered when formulating diets [?]. The results of this study showed that the Ca maintenance requirement for Dorper×Hu F1 male lambs during the early fattening stage was 20.69 mg/kg BW. This value is higher than the Ca maintenance requirement for sheep (16 mg/kg BW) reported by ARC (1980) [?] using the endogenous fecal loss method. Fernandes et al. [?] reported Ca maintenance requirements of 27.4 and 16.1 mg/kg BW for Boer crossbred male goats (20–35 kg BW) using comparative slaughter and minimum endogenous loss (MEL) methods, respectively. These differences are mainly due to variations in methodology and animal breeds.

Phosphorus plays a very important role in animal growth and development.

In practice, P is excreted from animals through feces, urine, and other waste products, causing environmental pollution. Therefore, researchers have paid considerable attention to P requirements. Reports on P maintenance requirements in ruminants vary widely. This study showed that the P maintenance requirement for 20–35 kg Dorper×Hu F1 male lambs was 10.09 mg/kg BW, which is lower than values reported by Fernandes et al. [?], Ji et al. [?], and NRC (1985) [?]. Additionally, ARC (1965) [?] reported a P maintenance requirement of 42.5 mg/kg BW for sheep, which was reduced to 14 mg/kg BW by ARC (1980) [?] based on the endogenous loss method. Endogenous loss of P is affected by factors such as feed intake and diet quality [?]. Salivary P also plays an important role in P metabolism [?]. Furthermore, P loss can occur when hair and dander are shed from the animal body. These factors may explain the large differences in reported P maintenance requirements.

Sodium and K, as electrolytes in animal tissues, play important roles in maintaining osmotic pressure and controlling water metabolism. This study calculated Na and K maintenance requirements for 20–35 kg Dorper×Hu F1 male lambs using the comparative slaughter method. These values differ from previous reports [?, ?]. Meschy [?] reported Na and K maintenance requirements for finishing sheep of 15 and 50 mg/kg BW, respectively. ARC (1980) [?] reported a K maintenance requirement of 38 mg/kg BW for finishing sheep using the endogenous fecal-urinary method, while NRC (2007) [?] reported a Na maintenance requirement of 10.8 mg/kg BW. These reported values are significantly higher than those obtained in this study. In addition to fecal and urinary excretion, Na and K are also lost through skin sweating, especially in hot and humid climates [?]. These factors may contribute to different results among researchers.

Magnesium constitutes a large proportion of animal tissues and plays important physiological roles in constituting and activating related enzymes and ensuring appropriate nerve and muscle excitability. The Mg content in forage varies considerably, making Mg deficiency and excess common in practice [?]. Many factors affect Mg absorption and requirements in diets, including K, Ca, P, Al, Fe, Na, protein, fat, organic acids, carbohydrate types, Mg status, and feeding frequency [?]. This study showed that the Mg maintenance requirement for 20–35 kg Dorper×Hu F1 male lambs was 1.03 mg/kg BW, which is lower than the Mg maintenance requirement of 3 mg/kg BW for finishing sheep reported by NRC (2007) [?]. Few reports exist on Mg maintenance requirements for finishing sheep, making extensive comparisons difficult, and further research is needed to explain the different results.

### 3.2 Macroelement Growth Requirements

Animal growth and development is relatively complex, and research perspectives determine the content of the study. From a biochemical perspective, animal growth is a physiological process of continuous accumulation of nutrients such as protein, energy, minerals, and water in the body [?]. Therefore, under conditions

that meet different growth rates, the deposition requirement of nutrients is the corresponding growth requirement. NRC (2007) [?] reported macroelement growth requirements for sheep as: Ca 11 g/kg EBWG, P 6.0 g/kg EBWG, Na 1.1 g/kg BWG (body weight gain), K 1.8 g/kg BWG, and Mg 0.41 g/kg BWG. In this study, the growth requirements for 20-35 kg male lambs were: Ca 11.55-11.41 g/kg EBWG, P 5.82-5.77 g/kg EBWG, K 1.47-1.69 g/kg EBWG, Mg 0.42-0.44 g/kg EBWG, and Na 0.98-0.88 g/kg EBWG. The results of this study showed that for male lambs, the growth requirement for Ca was higher than that reported by NRC (2007) [?]; the growth requirements for Na, P, and K were lower than those reported by NRC (2007) [?]; while the growth requirement for Mg was similar to that reported by NRC (2007) [?]. Under the conditions of this experiment, these differences indicate the specificity of macroelement requirements for Dorper×Hu F1 lambs in China.

## Conclusions

1. The macroelement maintenance requirements for 20-35 kg Dorper×Hu F1 male lambs were: Ca 24.01 mg/kg EBW, P 11.70 mg/kg EBW, Na 3.20 mg/kg EBW, K 6.60 mg/kg EBW, and Mg 1.20 mg/kg EBW.
2. The macroelement growth requirements for 20-35 kg Dorper×Hu F1 male lambs were: Ca 11.55-11.41 g/kg EBWG, P 5.82-5.77 g/kg EBWG, K 1.47-1.69 g/kg EBWG, Mg 0.42-0.44 g/kg EBWG, and Na 0.98-0.88 g/kg EBWG.

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