Postprint: Exploration of the Limiting Order and Requirement Model of Four Essential Amino Acids in Weaned Lambs

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Abstract

This experiment aimed to investigate the limiting order and requirement model of four essential amino acids in weaned lambs using the amino acid deletion method. One hundred 50-day-old weaned Hu ram lambs were randomly divided into 5 groups with 4 replicates per group and 5 lambs per replicate. The control group was the positive control (PC) group fed an amino acid-balanced starter feed, while the four experimental groups were fed starter feeds with lysine (PD-Lys group), methionine (PD-Met group), threonine (PD-Thr group), or tryptophan (PD-Trp group) deleted from the PC diet, respectively, with the remaining amino acid contents kept consistent. The preliminary period was 10 days, and the formal experimental period was 60 days. Body weight was measured at 60, 90, and 120 days of age, and at 120 days of age, 6 lambs were randomly selected from each group for slaughter. The results showed: 1) During 60–120 days and 90–120 days of age, the average daily gain (ADG) of lambs in the PD-Met group was significantly lower than that in the other groups ($P < 0.05$), and the feed conversion ratio (F/G) was significantly higher than that in the other groups ($P < 0.05$); at 120 days of age, the pre-slaughter live weight, empty body weight, carcass weight, loin eye area, head weight, hoof weight, and skin weight of the PD-Met group were significantly lower than those of the PC, PD-Thr, and PD-Trp groups ($P < 0.05$), but there were no significant differences in dressing percentage (DP), GR value, and blood weight among the five groups ($P > 0.05$). 2) When maximum ADG and F/G were used as evaluation indices, the limiting order of amino acids during 60–90 days and 90–120 days of age was Met, Lys, Thr, and Trp; whereas when maximum DP was used as the evaluation index, the limiting order of amino acids at 120 days of age was Trp, Thr, Lys, Met. 3) When maximum ADG was used as the evaluation index, the appropriate ratios of Lys, Met, Thr, and Trp during 60–90 days and

90–120 days of age were 100:44:44:8 and 100:42:38:12, respectively; when maximum F/G was used as the evaluation index, the appropriate ratios of Lys, Met, Thr, and Trp during 60–90 days and 90–120 days of age were 100:54:45:7 and 100:47:39:12, respectively; and when maximum DP was used as the evaluation index, the appropriate ratio of Lys, Met, Thr, and Trp during 60–120 days of age was 100:34:38:8. In conclusion, deficiency of dietary essential amino acids (Lys, Met, Thr, and Trp) seriously affected the growth performance, slaughter performance, and organ indices of weaned Hu lambs, with Met having the most pronounced effect, followed by Lys, while Thr and Trp had weaker effects; the limiting order and requirement model of Lys, Met, Thr, and Trp differed depending on the evaluation indices (ADG, F/G, and DP) and growth stages.

Full Text

Exploration of Requirement Models and Limiting Sequence of Four Essential Amino Acids in Weaned Lambs

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Abstract: This study investigated the limiting sequence and requirement models of four essential amino acids in weaned lambs using the amino acid partial deduction method. One hundred male Hu lambs weaned at 50 days of age were randomly allocated into five groups, each consisting of four replicates with five lambs per replicate. The control group received a starter diet with balanced amino acids (PC group), while four experimental groups were fed starters with partial deductions of lysine (PD-Lys), methionine (PD-Met), threonine (PD-Thr), or tryptophan (PD-Trp) based on the PC formulation, with all other amino acids held constant. The pretrial period lasted 10 days, followed by a 60-day formal trial. Lambs were weighed at 60, 90, and 120 days of age, and six lambs per group were selected for slaughter at 120 days. The results showed: (1) During 60–120 days and 90–120 days of age, the PD-Met group exhibited significantly lower average daily gain (ADG) and significantly higher feed-to-gain ratio (F/G) compared to all other groups ($P < 0.05$). At 120 days, the PD-Met group had significantly lower live weight before slaughter, empty body weight, carcass weight, ribeye area, head weight, feet weight, and skin/wool weight compared to the PC, PD-Thr, and PD-Trp groups ($P < 0.05$), though no significant differences were observed among the five groups in dressing percentage (DP), GR value, or blood weight ($P > 0.05$). (2) Using maximum ADG and F/G as evaluation criteria, the limiting amino acid sequence for both 60–90 days and 90–120 days was Met, Lys, Thr, and Trp; however, using maximum DP as the criterion, the limiting sequence at 120 days was Trp, Thr, Lys, and Met. (3) When maximum ADG served as the evaluation index, the optimal ratios of

Lys:Met:Thr:Trp were 100:44:44:8 for 60–90 days and 100:42:38:12 for 90–120 days. When maximum F/G was used, the optimal ratios were 100:54:45:7 for 60–90 days and 100:47:39:12 for 90–120 days. When maximum DP was the criterion, the optimal ratio for 60–120 days was 100:34:38:8. In conclusion, dietary deficiency of essential amino acids (Lys, Met, Thr, and Trp) severely impairs growth performance, slaughter performance, and organ indices in weaned Hu lambs, with Met deficiency having the most pronounced effect, followed by Lys, while Thr and Trp had weaker effects. The limiting sequences and requirement models for Lys, Met, Thr, and Trp varied depending on the evaluation index (ADG, F/G, or DP) and growth stage.

Keywords: Hu sheep; weaned lamb; starter; amino acid model; lysine; methionine; tryptophan

Introduction

Essential amino acids (EAA) are nutrients that animals cannot synthesize themselves and are required for building the protein framework of the animal body [1]. A balanced amino acid profile facilitates the synthesis of growth-related bioactive substances and is crucial for animal growth and development [2]. Currently, research on amino acid balance patterns for post-weaning lambs remains limited, and establishing such patterns for this stage is important for improving production performance, immune function, feed efficiency, and reducing environmental pollution from waste [3]. Zhang et al. [4] and Wang et al. [5] successfully obtained lysine, methionine, and threonine balance patterns for calves at different growth stages using the amino acid partial deduction method. Kerr et al. [6] and Zhang et al. [7] demonstrated that appropriate dietary levels of lysine, methionine, threonine, and tryptophan can improve growth performance in growing pigs. Since the 1950s, limiting amino acid sequences have been extensively studied in pig and poultry diets. Wang et al. [5] identified lysine, methionine, and threonine as the first, second, and third limiting amino acids in calves [8], though NRC (2012) and other researchers have not established limiting amino acid sequences for lambs [9]. As amino acid nutrition research advances, studying limiting amino acids can reveal their sequence at different physiological stages and further determine the degree of limitation on lamb growth. Few studies have reported amino acid requirements for weaned lambs, and research on dietary amino acid composition and proportions—ideal amino acid patterns—remains scarce. It is necessary to investigate amino acid requirements and ideal patterns to develop nutritional standards suitable for lamb growth, thereby improving economic benefits for farmers and environmental conditions [10]. Therefore, this study aimed to investigate the limiting sequence and appropriate amino acid patterns in starter diets for Hu lambs aged 60–120 days using the partial deduction method, employing feeding and slaughter trials to establish optimal amino acid models in starters for achieving maximum ADG, F/G, and dressing percentage (DP), providing a theoretical basis for scientific feeding.

1.1 Experimental Time and Location

The trial was conducted from October 7 to December 7, 2015, at Hailun Sheep Industry Co., Ltd. in Taizhou City, Jiangsu Province. Nutritional level analyses were performed at the Laboratory of Livestock Nutrition and Feed, Feed Research Institute, Chinese Academy of Agricultural Sciences.

1.2 Experimental Design

Based on the amino acid partial deduction method described by Wang et al. [11], this study employed a single-factor completely randomized design. One hundred male Hu lambs weaned at 50 days of age with an average body weight of approximately 11 kg were divided into five groups according to similar body weight and age, with four replicates per group and five lambs per replicate. Using the partial deduction method, lambs were assigned to an amino acid-balanced control (PC) group or groups with 30% deductions of Lys (PD-Lys), Met (PD-Met), Thr (PD-Thr), or Trp (PD-Trp).

1.3 Experimental Diets

Based on previous research from our laboratory [12], the PC group starter was formulated with a protein level of 15.0%. The methionine requirement for the lamb amino acid balance model was set at 0.38% (Met as 6.4% of CP) [13]. The lysine requirement was calculated according to the Lys:Met ratio of 3.1:1.0 proposed by Storm et al. [14], and threonine and tryptophan requirements were calculated using the lamb amino acid balance model (Lys:Thr:Trp = 100.0:50.5:14.3) proposed by Diao [1]. The PC starter contained 1.16% Lys, 0.38% Met, 0.58% Thr, and 0.16% Trp. Experimental group starters were formulated by adjusting rumen-protected amino acid supplementation levels. Rumen-protected lysine was provided by Degussa GmbH, rumen-protected methionine by CJ CheilJedang Corporation, and rumen-protected threonine and tryptophan by Kangdequan Technology Co., Ltd. Each product contained approximately 65% lysine hydrochloride, methionine hydrochloride, threonine hydrochloride, or tryptophan hydrochloride, with a rumen bypass rate $ $80%. The amino acid contents, composition, and nutrient levels of the starters are shown in Table 1 and Table 2 .

Table 1 Amino acid contents of starters (dry matter basis)
Items Lysine, Methionine, Threonine, Tryptophan
Groups PC, PD-Lys, PD-Met, PD-Thr, PD-Trp
Calculated values

Table 2 Composition and nutrient levels of starters (dry matter basis)
Items
Ingredients: Corn, Wheat bran, Soybean meal, Alfalfa meal, Premix[1], RP-Lys, RP-Met, RP-Thr, RP-Trp, Total
Nutrient levels[2]: DM, CP, ME/(MJ/kg), EE, Ash, NDF, ADF, Ca, TP,

Essential amino acids (EAA) - Lys, Phe, Met, Thr, Leu, Ile, Val, Trp, Non-essential amino acids (NEAA) - His, Cys, Arg, Tyr, Gly, Ser, Glu, Pro, Asp, Ala, Total amino acids (TAA)

[1]The premix provided per kg of starter: VA 15,000 IU, VD 5,000 IU, VE 90 IU, Cu 12 mg, Fe 90 mg, Mn 56 mg, Zn 100 mg, I 0.8 mg, Se 0.3 mg, Co 0.5 mg.
[2]ME was a calculated value; all other nutrient levels were measured values. Nutrient levels were determined according to *Feed Analysis and Feed Quality Analysis Technics* [15].

1.4 Management Practices

Lambs were housed in pens (200 cm × 375 cm) at five lambs per pen. Starter feeding training began at 50 days of age. From 60 to 120 days of age, daily starter feeding amounts were provided at 4% of body weight as proposed by McMillan [16]. Starter was fed twice daily (08:00 and 16:00), and water was available ad libitum. Each lamb had approximately 1.50 m^2 of activity space. All pens were disinfected once every two weeks using 2.0% sodium hydroxide solution, 0.5% povidone-iodine solution, and 0.2% chloroisocyanuric acid solution in rotation. All experimental lambs followed the farm' s normal vaccination program.

1.5 Measurements

1.5.1 Starter Nutritional Levels Gross energy was determined using a Parr-6400 oxygen bomb calorimeter. Crude protein content was measured with a KDY-9830 automatic Kjeldahl nitrogen analyzer. Dry matter, crude fat, crude ash, calcium, and phosphorus contents were determined according to *Feed Analysis and Feed Quality Analysis Technics* [15]. Amino acid contents were analyzed using an automatic amino acid analyzer (L-8800, Hitachi, Japan). Samples were ground to pass through a 60-mesh sieve, hydrolyzed with 10 mL of 6 mol/L HCl in hydrolysis tubes under vacuum at (110$±$1)°C for 24 h, cooled, filtered, evaporated to dryness in a desiccator, dissolved in 1 mL of 0.02 mol/L HCl, and held in air for 30 min before analysis. For tryptophan determination, samples were hydrolyzed with 5 mol/L NaOH solution [17].

1.5.2 Growth Performance **ADG:** Lambs were weighed before morning feeding at 60, 90, and 120 days of age to calculate ADG for 60–90 days and 90–120 days.
Feed intake: Daily feed offered and refusals were recorded accurately for each pen, with feeding amounts adjusted every 30 days based on body weight starting at 60 days.
F/G: Calculated from ADG and feed intake for each period.

1.5.3 Slaughter Performance and Organ Indices At 120 days of age, six healthy lambs near the average body weight were selected from each group, fasted for 16 h, and slaughtered. Live weight before slaughter (LWBS) was

recorded at 08:00 on the slaughter day. After exsanguination, lambs were skinned, and heads, feet, and viscera were removed to obtain carcass weight. According to *Livestock Anatomy and Histology*, internal organs were separated and weighed fresh (heart, liver, lungs, spleen, kidneys) to calculate organ percentages of LWBS. The gastrointestinal tract was emptied and cleaned, and weights of rumen, reticulum, omasum, abomasum, large intestine, and small intestine were recorded to calculate percentages of LWBS and total stomach compartment weight (TCSW) [18].

Formulas:
Empty body weight (EBW, kg) = LWBS -total gastrointestinal content weight
Carcass weight (CW, kg) = LWBS -weight of skin, head, feet, reproductive organs and surrounding fat, and viscera (kidneys and surrounding fat retained)
DP (%) = 100 × CW / LWBS
Organ weight percentage of LWBS (%) = 100 × organ weight / LWBS
Individual stomach compartment percentage of TCSW (%) = 100 × individual compartment weight / total weight of four compartments

After slaughter, the outline of the longissimus dorsi muscle between the last two ribs was traced on sulfuric acid paper, and the area was determined using a planimeter (CS- , Wuxi Surveying and Mapping Instrument Factory, Jiangsu Province) to obtain ribeye area. GR value was measured as tissue thickness 11 cm from the dorsal midline between ribs 12 and 13 using a vernier caliper, with three replicate measurements averaged per lamb.

1.5.4 Calculation of Amino Acid Sequence and Proportion Calculations of limiting amino acid sequence and optimal proportions followed methods described by Wang et al. [5] and Wang et al. [11].

1.6 Data Processing Data were initially processed using Excel 2013, followed by one-way ANOVA using SAS 9.2 statistical software. Differences were considered significant at $P < 0.05$.

Results

2.1 Effects of Amino Acid Partial Deduction on Growth Performance

As shown in Table 3 , during 60–90 days of age, ADG in PD-Lys and PD-Met groups was significantly lower than in PD-Trp ($P < 0.05$). During 90–120 days, ADG in PD-Met was significantly lower than in PC, PD-Lys, PD-Thr, and PD-Trp groups ($P < 0.05$). Over the entire 60–120 day trial, PD-Met had the lowest ADG, significantly lower than PC, PD-Lys, PD-Thr, and PD-Trp ($P < 0.05$), while PD-Lys was significantly lower than PC and PD-Trp ($P < 0.05$). The importance order of amino acids for ADG was Met > Lys > Thr > Trp. No significant differences in feed intake were observed among groups during 60–90 days, 90–120 days, or 60–120 days ($P > 0.05$). No significant differences in F/G were found during 60–90 days ($P > 0.05$), but during 90–120 days, F/G in

PD-Met was significantly higher than in PC, PD-Thr, and PD-Trp ($P < 0.05$). Over 60–120 days, PD-Met had significantly higher F/G than all other groups ($P < 0.05$). The importance order of amino acids for F/G was Met > Lys > Thr > Trp.

Table 3 Effects of amino acids partial deduction in starter on growth performance of Hu weaned lambs
Items: ADG (g/d) for 60–90 days, 90–120 days, 60–120 days; Feed intake (g/d) for 60–90 days, 90–120 days, 60–120 days; F/G for 60–90 days, 90–120 days, 60–120 days
Groups: PC, PD-Lys, PD-Met, PD-Thr, PD-Trp, P-value

In the same row, values with different small letter superscripts indicate significant difference ($P < 0.05$). The same applies below.

2.2 Effects of Amino Acid Partial Deduction on Slaughter Performance

As shown in Table 4 , at 120 days of age, no significant differences were observed among groups in DP, backfat thickness, GR value, or blood weight ($P > 0.05$). The PD-Met group had significantly lower LWBS, EBW, CW, ribeye area, head weight, feet weight, and skin/wool weight compared to PC, PD-Thr, and PD-Trp ($P < 0.05$), though PD-Met did not differ significantly from PD-Lys ($P > 0.05$), and PC did not differ significantly from PD-Thr or PD-Trp ($P > 0.05$). The importance order of amino acids for DP was Trp > Thr > Lys > Met.

Table 4 Effects of amino acids partial deduction in starter on slaughter performance of Hu weaned lambs
Items: LWBS (kg), EBW (kg), CW (kg), DP (%), Ribeye area (cm^2), GR value (mm), Backfat thickness (mm), Blood weight (g), Head weight (g), Feet weight (g), Skin and wool weight (g)
Groups: PC, PD-Lys, PD-Met, PD-Thr, PD-Trp, P-value

2.3 Effects of Amino Acid Partial Deduction on Internal Organ Indices

As shown in Table 5 , at 120 days, heart and liver weights in PD-Lys, PD-Met, PD-Thr, and PD-Trp groups were significantly lower than in PC ($P < 0.05$). The percentage of liver weight to LWBS was significantly lower in PD-Met, PD-Thr, and PD-Trp than in PC ($P < 0.05$). Kidney weight in PD-Lys and PD-Met was significantly lower than in PC ($P < 0.05$). No significant differences were observed between experimental and PC groups in spleen and lung weights or their percentages of LWBS, or in kidney weight percentage of LWBS ($P > 0.05$).

Table 5 Effects of amino acids partial deduction in starter on internal organ indices of Hu weaned lambs
Items: Heart weight (g) and % of LWBS; Liver weight (g) and % of LWBS; Spleen weight (g) and % of LWBS; Lung weight (g) and % of LWBS; Kidney

weight (g) and % of LWBS
Groups: PC, PD-Lys, PD-Met, PD-Thr, PD-Trp, P-value

2.4 Effects of Amino Acid Partial Deduction on Gastrointestinal Development

As shown in Table 6 , at 120 days, the percentage of abomasum weight to TCSW in PD-Lys and PD-Trp was significantly lower than in PC, PD-Met, and PD-Thr ($P < 0.05$). No significant differences were observed among PC, PD-Lys, PD-Met, PD-Thr, and PD-Trp in weights of rumen, reticulum, omasum, abomasum, or small intestine ($P > 0.05$), nor in percentages of rumen, reticulum, or omasum weight to TCSW or LWBS, or in small intestine weight percentage of LWBS ($P > 0.05$).

Table 6 Effects of amino acids partial deduction on gastrointestinal tract development of Hu weaned lambs
Items: Rumen, Reticulum, Omasum, Abomasum, Small intestine, Large intestine -weight (g), % of TCSW, % of LWBS
Groups: PC, PD-Lys, PD-Met, PD-Thr, PD-Trp, P-value

2.5 Amino Acid Sequence and Proportion for Maximum ADG, F/G, and DP

The calculated sequences and proportions of Lys, Met, Thr, and Trp for maximum ADG and F/G are presented in Table 7 . Using maximum ADG as the criterion, the slopes (S) for PD-Lys, PD-Met, PD-Thr, and PD-Trp were 0.230, 0.490, 0.128, and 0.220 at 60–90 days, and 0.498, 0.958, 0.098, and 0.745 at 90–120 days, yielding optimal Lys:Met:Thr:Trp ratios of 100:44:44:8 and 100:42:38:12, respectively. Using maximum F/G as the criterion, the slopes were -0.185, -0.584, -0.120, -0.177 at 60–90 days and -0.510, -1.263, -0.125, -0.764 at 90–120 days, resulting in optimal ratios of 100:54:45:7 and 100:47:39:12, respectively.

Table 7 Sequence and proportion of amino acids to reach maximum ADG and F/G of lambs at different stages
Groups: PD1-Lys, PD1-Met, PD1-Thr, PD1-Trp (60–90 days); PD2-Lys, PD2-Met, PD2-Thr, PD2-Trp (90–120 days)
Criteria: ADG, F/G

S: slope, representing the effect of limiting amino acid intake on ADG, F/G, and DP. P: amino acid content in experimental groups required to maintain the same ADG, F/G, and DP as PC group, expressed as a proportion of PC group. C: amino acid content [mg/(kg $BW^{0 \cdot 75} \cdot d$)] required to maintain the same ADG, F/G, and DP as PC group. R: all amino acid proportions relative to lysine. The same applies below.

The calculated sequences and proportions for maximum DP are presented in Table 8 . Using maximum DP as the criterion, the slopes for PD-Lys, PD-Met,

PD-Thr, and PD-Trp were -0.087, -0.100, -0.017, and -0.106 at 60–120 days, yielding an optimal Lys:Met:Thr:Trp ratio of 100:34:38:8.

Table 8 Sequence and proportion of amino acids to reach maximum DP of lambs at different stages
Groups: PD1-Lys, PD1-Met, PD1-Thr, PD1-Trp
Criterion: DP

Discussion

3.1 Effects on Growth Performance

Amino acids are the building blocks of body protein, and providing amino acid-balanced starters promotes protein accretion. Supplementing balanced essential amino acids in low-protein starter diets for weaned lambs significantly affects growth performance. Wang et al. [19] reported that lysine deduction in 2–4-week-old calves substantially impacted ADG, with the lysine-deducted group showing 65.25%, 5.41%, and 28.19% lower ADG than control, methionine-deducted, and threonine-deducted groups, respectively. In contrast, our study found that methionine deduction in weaned lambs during 60–90 days and 90–120 days significantly affected ADG, with PD-Met reducing ADG by 29.54%, 14.74%, 25.36%, and 27.07% compared to PC, PD-Lys, PD-Thr, and PD-Trp groups, respectively, over 60–120 days. This discrepancy suggests that methionine deficiency in lambs causes growth retardation and impaired development, while lysine, though growth-promoting, may not be the first-limiting amino acid at this stage. El-Tahawy et al. [20] reported that adding 3.30 g/kg methionine to concentrate mixtures significantly improved ADG compared to unsupplemented controls, consistent with our findings. Animals have different amino acid deficiencies at various growth stages, with the most severe deficiency termed the first-limiting amino acid. Wang et al. [5] identified lysine as the first-limiting amino acid in calves, followed by methionine and threonine. Our study, using ADG as the criterion, found methionine to be first-limiting in lambs, followed by lysine, threonine, and tryptophan, indicating species differences in amino acid limitation.

Feed-to-gain ratio is a crucial tool for measuring protein conversion efficiency and production efficiency. Improving F/G while maintaining growth reduces fecal excretion and benefits environmental protection. Our results showed methionine deduction had the greatest impact on F/G, followed by lysine, threonine, and tryptophan. El-Tahawy et al. [20] and Nolte et al. [21] similarly identified methionine as the first-limiting amino acid for growing lambs, consistent with our findings. However, Nolte et al. [22] suggested lysine was not limiting in lambs, possibly due to experimental methodology and rumen amino acid infusion imbalances. In our study, F/G differences were not significant during 60–90 days but became pronounced with age, suggesting an initial adaptation period to the starter and slower gastrointestinal development. Wang et al. [5] found lysine remained the first-limiting amino acid for calves at 0–2 months,

contrasting with our lamb results where PD-Met showed the poorest F/G, being 25.05%, 17.37%, 22.67%, and 24.68% lower than PC, PD-Lys, PD-Thr, and PD-Trp, respectively. Thus, supplementing the first-limiting amino acid can optimize protein nutrition absorption, reduce metabolic burden, improve growth rate, and enhance feed utilization efficiency.

3.2 Effects on Slaughter Performance

Slaughter performance evaluation facilitates assessment of developmental status at different stages and is important for improving meat quality. Our study showed that amino acid deduction groups had lower slaughter performance indices than PC, except for DP, backfat thickness, GR value, and blood weight. Storm et al. [14] found that infusing lysine, methionine, threonine, and tryptophan at 9.0%, 4.1%, 5.6%, and 2.0% in 2-month-old lambs enabled rapid attainment of slaughter weight with adequate nutrition. Ferreira et al. [23] similarly observed that blood weight did not change significantly with growth in Merino sheep, consistent with our results. Partial deductions of lysine and methionine did not significantly affect blood weight but significantly impacted carcass, head, feet, and skin/wool weights, possibly because methionine is the first-limiting amino acid for wool keratin synthesis, and its supplementation promotes wool growth and developmental status [24]. Interestingly, tryptophan was identified as the first-limiting amino acid for maximum DP in our study, differing from the sequences for maximum ADG and F/G, a finding not previously reported. This discrepancy may stem from the lack of nitrogen balance trials for deeper investigation, potentially limiting accuracy in determining amino acid limitation. Therefore, reducing dietary protein while adding rumen-protected amino acids to achieve balanced proportions not only promotes carcass and wool growth but also accelerates time to slaughter weight in Hu lambs. Further research combining nitrogen balance trials is needed to thoroughly investigate slaughter performance in weaned lambs.

3.3 Effects on Organ Indices

Organ development directly affects subsequent growth and immune status in Hu sheep, making organ index measurement important for assessing developmental status. Ferreira et al. [23] found that lung, kidney, and spleen weights did not change significantly with growth, similar to our results, indicating partial amino acid deduction does not substantially affect organs. Bouyeh [25] reported that adding 1.10% lysine and 0.50% methionine to broiler diets increased heart and liver weights compared to unsupplemented diets, with high lysine and methionine levels reducing feed intake, decreasing fat content, and significantly improving economic returns. Zhang et al. [26] found that partial deductions of lysine, methionine, and threonine did not significantly affect immune organ weights or indices in calves, though all experimental groups had lower immune organ weights than PC, with threonine deduction affecting immune function. In our study, PC had significantly higher heart, liver, and kidney weights than

amino acid-deducted groups. Although spleen weight differences were not significant, PD-Thr showed a trend toward lower immune organ indices, suggesting threonine deduction may not have been sufficient to significantly impact immune organs. Other experimental groups had immune organ indices similar to PC, indicating threonine can enhance immune function and pathogen resistance at certain levels, and appropriate threonine supplementation in starters ensures healthy growth.

3.4 Effects on Gastrointestinal Development

Rumen and abomasum development in lambs directly affects digestive capacity and growth performance [1]. By 8 weeks of age, rumen development approaches adult size. Lv et al. [28] found that a dietary protein level of 15.01% with Lys/Met ratio of 3.1 optimized stomach and rumen weight gain and promoted gastrointestinal development. Our results showed that PD-Trp and PD-Lys had significantly lower abomasum weight percentages of TCSW than other groups, while PC tended to have higher stomach and rumen weights. Small intestine weight followed the pattern PC > PD-Lys > PD-Met > PD-Thr > PD-Trp, consistent with Li [29], who reported that increasing lysine in milk replacer improved gastrointestinal development, suggesting lysine content influences rumen development. The small intestine is the primary site of nutrient digestion and absorption in weaned lambs, and its normal development is key to efficient nutrient utilization. Although small intestine weights did not differ significantly among groups, PC showed 2.85%, 10.80%, 7.21%, and 7.60% greater growth than PD-Lys, PD-Met, PD-Thr, and PD-Trp, respectively. Our study demonstrates that reducing dietary protein while adding amino acids benefits digestive organ development and allows lambs to reach their growth potential.

3.5 Amino Acid Sequence and Proportion for Maximum ADG, F/G, and DP

The concept of optimal amino acid proportions originates from the classic "barrel theory," which states that optimal growth requires each EAA to meet animal needs—the amount that maximizes production performance. Goodband et al. [30] proposed an ideal amino acid pattern for 20-kg piglets of Lys:Met:Thr:Trp = 100:(27–28):62:18. Lv et al. [28] found that Tibetan lambs achieved maximum ADG and optimal stomach development with a dietary Lys/Met ratio of 3.1. Ferreira et al. [23] determined an optimal pattern of 100:55:72 for Lys:Met:Thr in growing South African Mutton Merino carcasses. Our study using the deduction method found optimal Lys:Met:Thr:Trp ratios of 100:44:44:8 for 60–90 days and 100:42:38:12 for 90–120 days for maximum ADG, with methionine and tryptophan levels lower than those for piglets and Lys/Met ratios of 2.27–2.38. No previous reports exist for optimal F/G ratios; our study found 100:54:45:7 for 60–90 days and 100:47:39:12 for 90–120 days. For maximum DP, the optimal ratio was 100:34:38:8, with methionine lower than for maximum ADG and F/G. This suggests that although ruminant

digestive systems resemble monogastric animals initially, substantial differences exist in nutrient digestion and absorption at later stages, possibly related to breed, environment, and diet. Using carcass amino acid composition for diet formulation may be inaccurate and cause wasteful excesses. These findings further demonstrate that genetic differences among species result in substantial variations in amino acid proportions.

Conclusions

1. In weaned lamb diets, the limiting amino acid sequence for maximum ADG and F/G across different growth stages is Met > Lys > Thr > Trp. Using DP as the criterion, the limiting sequence is Trp > Thr > Lys > Met.

2. Balanced amino acid supply directly affects lamb growth, development, and slaughter performance, while amino acid deduction can influence internal organ development.

3. Different evaluation criteria yield different optimal proportions of the four amino acids for 60–120-day-old weaned lambs: for maximum ADG, 100:44:44:8 at 60–90 days and 100:42:38:12 at 90–120 days; for maximum F/G, 100:54:45:7 at 60–90 days and 100:47:39:12 at 90–120 days; and for maximum DP, 100:34:38:8 at 60–120 days.

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Note: Figure translations are in progress. See original paper for figures.