Effects of Natural Grazing and Grazing with Supplementary Feeding for Fattening on Amino Acid Composition in Plasma and Muscle of Mutton Sheep (Postprint)

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Abstract

This study investigated the changes in amino acid (AA) composition in plasma and muscle tissues (longissimus dorsi, triceps brachii, and biceps femoris) of Hulun Buir (HL) lambs and first-generation crossbreds of Hulun Buir and Dorper sheep (HZ) under two different fattening methods: natural grazing (NG) and grazing with supplementary feeding (GS). A $2\times2 completely randomized experimental design was adopted, selecting sixty healthy 4-month-old weaned male HL lambs and sixty HZ lambs with similar body weight and condition. Factor 1 was fatteni day fattening period. The results showed that: 1) In plasma, the NG group had significantly lower contents of esse 0.05), while taste-active amino acids (DAA) content tended to be lower than the GS group (0.05 $P <$ 0.10). 2) Compared with the GS group, crude protein (CP) and LAA contents in longissimus dorsi were significantl 0.05). 3) Compared with the GS group, LAA and DAA contents in triceps brachii were significantly lower in the NG g 0.05), and EAA content tended to be lower (0.05 $P<0.10). 4) In biceps femoris, the NG group had significantly lower contents of CP, EAA, non-essential amino acids (NEAA), TAA, LAA, branched-chain amino acids (BCAA), and DAA than the GS group ($P<0.05). 5) Compared with the HL group, plasma EAA in the HZ group, LAA in longissimus dorsi, BCAA in triceps brachii, and EAA content in biceps femoris were significantly higher or tended to be higher ($P<0.10). It can be concluded that the protein nutritional value of lamb meat in the grazing with supplementary feeding group was superior to that in the natural grazing group, and the protein nutritional value of HZ lamb meat was superior to that of HL lambs.

Full Text

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Abstract: This study investigated changes in amino acid (AA) composition in plasma and muscle tissues (longissimus dorsi, triceps brachii, and biceps femoris) of Hulunbeier lambs (HL) and first-generation hybrids of Hulunbeier and Dorper sheep (HZ) under two fattening regimes: natural grazing (NG) and grazing with supplementary feeding (GS). A $2\$\times2 factorial design was employed, with sixty healthy 4-month-old weaned male lambs of each breed selected for similar body weight and condition. Factor 1 comprised fattening m day fattening trial yielded the following results: (1) Plasma contents of essential amino acids (EAA), limiting am 0.05), while delicious amino acid (DAA) contents showed a tendency to be lower (0.05 $P <$ 0.10). (2) Compared with the GS group, the NG group exhibited significantly higher crude protein (CP) and LAA co 0.05). (3) The NG group showed significantly lower LAA and DAA contents in the triceps brachii compared with the 0.05), with EAA contents also tending to be lower (0.05 $P<0.10). (4) In the biceps femoris, the NG group had significantly lower CP, EAA, non-essential amino acid (NEAA), TAA, LAA, branched-chain amino acid (BCAA), and DAA contents than the GS group ($P<0.05). (5) Compared with the HL group, the HZ group showed significantly or near-significantly increased plasma EAA, longissimus dorsi LAA, triceps brachii BCAA, and biceps femoris EAA contents ($P<0.10). These findings indicate that grazing with supplementary feeding produces lamb meat with superior protein nutritional value compared to natural grazing, and that hybrid HZ lambs exhibit better protein nutritional value than pure HL lambs.

Keywords: Hulunbeier sheep; natural grazing; grazing with supplementary feeding; amino acids

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Introduction

The mutton sheep industry constitutes a vital component of China' s livestock sector. Rising living standards have increased both consumer demand for mutton and expectations for meat quality. Examining differences in lamb muscle amino acid composition across fattening regimes and breeds provides a theoretical foundation for understanding the metabolic mechanisms underlying AA metabolism and for improving mutton nutritional quality. Essential amino acid composition and content serve as primary indicators of mutton protein quality.

The Maillard reaction represents a crucial pathway for meat flavor formation, generating aromatic compounds through reactions between flavor-associated amino acids and reducing sugars. Amino acids related to flavor include aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), alanine (Ala), arginine (Arg), methionine (Met), and cysteine (Cys).

Multiple factors influence mutton AA content. Previous research comparing AA profiles across sheep breeds found that Tar-Dorset crossbred lambs contained 20 amino acids, with EAA content ranking highest in Tar-Dorset crosses, followed by Tibetan sheep, Small-tailed Han sheep, Duolang sheep, Toksun sheep, and Kirgiz sheep. Studies on feeding conditions have demonstrated that greenhouse-reared sheep exhibit significantly higher valine and isoleucine contents than conventionally housed animals.

Hulunbeier sheep (HL) represent an excellent local mutton breed developed through long-term natural selection and artificial breeding in Inner Mongolia, characterized by delicious, non-tainted meat with good texture and rich nutrition. The Hulunbeier-Dorper first-generation hybrid (HZ) combines HL with Dorper genetics. Traditionally, both HL and HZ lambs are weaned in July and marketed between late September and early October. However, recent extensive grassland degradation and desertification in the Hulunbeier region have substantially reduced pasture quality, particularly during summer and autumn, failing to meet the nutritional demands of rapidly growing weaned lambs. Consequently, implementing reasonable grazing with supplementary feeding (GS) for HL and HZ lambs has become an effective measure to increase daily weight gain, slaughter weight, and lean meat percentage while shortening the finishing period and improving herders' economic returns. Nevertheless, whether GS significantly affects lamb meat AA composition and content compared with natural grazing (NG), and whether differences exist between HL and HZ lambs, remain unreported.

This study therefore aimed to investigate differences in AA content and composition in plasma and muscle tissues of HL and HZ lambs under NG and GS regimes, providing a theoretical basis for understanding how fattening mode and breed affect AA metabolism and meat quality improvement.

1.1 Experimental Design and Diets

A $2\$\times 2 factorial design was employed, selecting sixty healthy 4-month-old weaned male lambs each of HL [(32.58\pm 0.12) kg] and HZ [(43.83\pm\$0.78)$ kg] with similar body weight and condition. Factor 1 comprised two fattening modes: NG and GS. Factor 2 comprised two breeds: HL and HZ. Lambs were divided into four groups of 30 animals each. The 60-day fattening trial consisted of an early period (days 1–30) and a late period (days 31–60). The NG group grazed on natural pasture (August–October), with pasture nutrient and AA levels shown in Table 1 . The GS group received concentrate supplementation in addition to grazing, with daily allowance increasing from

0.27 kg/head during the first month to 0.53 kg/head during the second month. Concentrate composition and nutrient levels are presented in Table 2 . All lambs were weighed before the trial, with ad libitum access to feed and water, and consistent housing and management conditions across groups.

1.2 Sample Collection and Pretreatment

One week before the trial' s conclusion, five fasting lambs from each group were selected in the morning for jugular blood collection and plasma separation via centrifugation. At the trial' s end, five lambs per group were slaughtered, and left-side longissimus dorsi, triceps brachii, and biceps femoris muscle samples were collected, wrapped in aluminum foil, and stored at -20 °C for analysis.

1.3 Test Indicators and Methods

Measurements included 17 AA contents in plasma, longissimus dorsi, triceps brachii, and biceps femoris, as well as AA intake: Asp, threonine (Thr), serine (Ser), Glu, Gly, Ala, cysteine (Cys), valine (Val), Met, isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), lysine (Lys), histidine (His), Arg, and proline (Pro). AA content was determined via acid hydrolysis following GB/T 5009.124-2003 using an L-8900 automatic AA analyzer. Routine nutrient analysis of forage and concentrate followed methods described in "Feed Analysis and Quality Detection Technology" edited by Zhang Liying.

Plasma samples (750 L) were mixed with an equal volume of 8% sulfosalicylic acid and refrigerated at 4 °C overnight, then centrifuged at 17,968×g for 20 min at 4 °C. Supernatants were filtered through 0.22 m membranes into sample vials. Muscle samples were dried at 65 °C, pulverized, and defatted with ether for over 24 h. Fifty mg of defatted muscle was hydrolyzed with 15 mL of 6 mol/L HCl in a sealed tube under nitrogen for 2 min, then incubated at (110$±$1) °C for 24 h. After cooling, the hydrolysate was filtered, diluted to 25 mL, and 0.5 mL aliquots were dried under nitrogen at 60 °C. The residue was reconstituted in 2.5 mL of 0.02 mol/L HCl, sonicated, and filtered through 0.22 m membranes.

AA intake was calculated based on dry matter intake (DMI) and dietary AA content. DMI was determined using total fecal collection combined with an indigestible marker method. Six lambs per group were selected at the trial' s start and end, fitted with fecal collection bags during a 7-day adaptation and 5-day collection period. Feces were collected four times daily, with 50 g samples frozen at -20 °C along with forage samples for DM and acid-insoluble ash (AIA) analysis. DMI calculations were: DMI (g) = daily fecal DM output (g) × fecal DM AIA (%) / forage DM AIA (%) for NG; and DMI (g) = daily concentrate DMI (g) × concentrate AIA (%) / forage AIA (%) + daily concentrate DMI (g) -daily fecal DM output (g) × fecal AIA (%) for GS. AA intake (g/kg W^0.75) = DMI (g) × dietary AA content (%) / metabolic body weight (kg W^0.75).

1.4 Statistical Analysis

Data were analyzed using SAS 9.0 software for significance testing and multiple comparisons. Differences were considered significant at $P<0.05$ and tending toward significance at $0.05 \leq P<0.10$.

2.1 Amino Acid Intake of Lambs

Amino acid intake during early (days 1–30) and late (days 31–60) fattening periods is shown in Tables 3 and 4 , respectively. During the early period, GS lambs exhibited significantly higher intake of all 17 AAs, as well as LAA, EAA, NEAA, DAA, FAA, BCAA, and TAA, compared with NG lambs ($P<0.05$). HZ lambs also showed significantly higher intake of all 17 AAs and these AA categories than HL lambs ($P<0.05$). Similar patterns were observed during the late period.

The interaction between fattening mode and breed significantly affected intake of all 17 AAs and AA categories during the early period ($P<0.05$). Except for Lys intake (highest in GS-HZ, lowest in GS-HL) and LAA intake (highest in GS-HL, lowest in NG-HL), all other AA intakes were highest in GS-HZ and lowest in NG-HL. During the late period, the interaction significantly or near-significantly affected 15 of 17 AAs and all AA categories ($P<0.10$), with GS-HZ showing the highest values and NG-HL or NG-HZ showing the lowest.

2.2 Effects of Fattening Mode and Breed on Plasma AA Composition

As shown in Table 5 , compared with the GS group, the NG group had significantly lower plasma contents of Thr, Ser, Ala, Met, Leu, Phe, Lys, His, Arg, and Pro ($P<0.05$), but significantly higher Gly and Cys contents ($P<0.05$). Plasma EAA, LAA, FAA, and TAA contents were significantly lower in NG lambs ($P<0.05$), while DAA content tended to be lower ($0.05 \leq P<0.10$). Compared with HL lambs, HZ lambs showed significantly higher plasma contents of Thr, Glu, Gly, Ala, Met, Tyr, and Pro ($P<0.05$), with Cys content tending to be higher ($0.05 \leq P<0.10$). Plasma EAA content was significantly higher in HZ lambs ($P<0.05$), while the EAA/TAA ratio was significantly lower ($P<0.05$).

The interaction between fattening mode and breed significantly affected plasma Asp and Ser contents ($P<0.05$). Asp content was highest in GS-HL and lowest in GS-HZ, while Ser content was highest in GS-HL and lowest in NG-HL.

2.3 Effects of Fattening Mode and Breed on Longissimus Dorsi AA Composition

Table 6 presents the effects on longissimus dorsi AA composition. Compared with GS, NG lambs showed significantly higher Asp content ($P<0.05$), with Lys and Arg contents tending to be higher ($0.05 \leq P<0.10$), but significantly

lower Cys and His contents (P<0.05). The NG group also had significantly higher CP and LAA contents in longissimus dorsi (P<0.05). Compared with HL, HZ lambs exhibited significantly lower Ile and His contents (P<0.05), with Pro content tending to be lower (0.05≤P<0.10), but significantly higher Cys content (P<0.05).

The interaction between fattening mode and breed significantly or near-significantly affected Ile, Tyr, and Pro contents (P<0.10), with NG-HL showing the highest and NG-HZ the lowest values. Significant interactions were also observed for Gly, EAA, NEAA, TAA, LAA, and DAA contents (P<0.10), with NG-HL highest and GS-HL lowest.

2.4 Effects of Fattening Mode and Breed on Triceps Brachii AA Composition

As shown in Table 7 , compared with GS, NG lambs had significantly lower contents of Asp, Thr, Gly, Cys, Ile, Leu, Phe, and His in triceps brachii (P<0.05). LAA and DAA contents were significantly lower in NG lambs (P<0.05), while EAA content tended to be lower (0.05≤P<0.10). Compared with HL, HZ lambs showed significantly higher Ile content (P<0.05) but significantly lower Thr and Tyr contents (P<0.05). BCAA content was significantly higher in HZ lambs (P<0.05).

The interaction between fattening mode and breed significantly affected Thr, Glu, Leu, Phe, and His contents (P<0.05), with GS-HL highest and NG-HL lowest. Ser content was highest in NG-HZ and lowest in NG-HL, while Tyr content was highest in GS-HL and lowest in GS-HZ. Pro content was highest in GS-HZ and lowest in NG-HZ.

2.5 Effects of Fattening Mode and Breed on Biceps Femoris AA Composition

Table 8 shows the effects on biceps femoris AA composition. Compared with GS, NG lambs had significantly lower contents of Glu, Gly, Val, Met, Ile, Leu, Tyr, Phe, and His (P<0.05), with Asp and Lys contents tending to be lower (0.05≤P<0.10). CP, EAA, NEAA, TAA, LAA, BCAA, and DAA contents were all significantly lower in NG lambs (P<0.05). Compared with HL, HZ lambs showed significantly higher Met, Ile, and Phe contents (P<0.05), with Gly and Pro contents tending to be higher (0.05≤P<0.10). EAA content in biceps femoris tended to be higher in HZ lambs (0.05≤P<0.10).

The interaction between fattening mode and breed significantly affected Val content (P<0.05), with GS-HL highest and NG-HL lowest. CP content was highest in GS-HZ and lowest in NG-HZ.

3.1 Effect of Breed on Mutton Protein Nutritional Value

Breed represents a primary factor influencing muscle AA content. Previous studies comparing AA profiles across sheep breeds found that Tan sheep had significantly higher total EAA content than other groups, indicating superior nutritional value. Research on different sheep breeds showed Tar-Dorset crossbreds contained 20 amino acids, with EAA content ranking highest in Tar-Dorset crosses, followed by Tibetan, Small-tailed Han, Duolang, Toksun, and Kirgiz sheep. Reports on breed differences in mutton AA composition remain limited, with most research focusing on pigs and poultry. Our results demonstrated that compared with HL, HZ lambs showed significantly or near-significantly increased LAA in longissimus dorsi, BCAA in triceps brachii, and EAA in biceps femoris. Thus, from an AA nutrition perspective, HZ lambs exhibited slightly superior meat protein nutritional value compared with HL lambs.

No previous studies have compared AA content between HL and HZ lambs. Our plasma AA results showed that HZ lambs had significantly or near-significantly higher contents of Thr, Glu, Gly, Ala, Cys, Met, Tyr, and Pro than HL lambs, with only Ser tending to be lower. This may partially explain the observed differences, though underlying mechanisms require further investigation. Dietary AA intake represents a primary factor affecting plasma AA composition, and our results confirmed that HZ lambs had significantly higher AA intake per unit metabolic body weight than HL lambs.

BCAA serve as important energy-supplying AAs that accelerate gluconeogenesis to provide energy. The significantly higher BCAA content in HZ triceps brachii and slightly higher BCAA in biceps femoris indicate that HZ muscle tissue can better supply energy and promote protein synthesis, partially explaining the higher protein levels in HZ lambs. Additionally, differences in muscle AA content between breeds may relate to variations in AA utilization, metabolic capacity, and genetic factors, though precise mechanisms warrant further investigation.

3.2 Effect of Fattening Mode on Mutton Protein Nutritional Value

Fattening mode constitutes another critical factor affecting mutton protein quality. In our study, GS lambs consumed increased energy and protein through concentrate supplementation, altering meat AA composition. These effects likely stemmed from differences in dietary energy, protein levels, and AA profiles. Previous research identified dietary protein and energy levels as primary causes of tissue AA differences. Studies in sheep demonstrated that increased dietary energy levels enhanced the rate of AA incorporation into muscle protein, though energy level did not affect Gly, Ile, Leu, Phe, and Ser contents. Other research showed that increasing dietary protein levels significantly elevated muscle AA content in lambs fed medium- and high-energy diets. Our GS group received concentrate supplementation that increased dietary energy, CP, and TAA contents, resulting in higher CP, EAA, NEAA, FAA, TAA, and LAA contents in

triceps brachii and biceps femoris compared with NG.

Nutrient distribution in animals is influenced by feed intake, AA composition, feeding behavior, physiological state, and environmental factors. Feed intake and dietary protein and AA levels directly affect plasma protein and AA contents. Our results showed GS lambs had significantly higher AA intake per unit metabolic body weight than NG lambs. Plasma AA analysis revealed that GS lambs had significantly higher contents of Thr, Ser, Ala, Met, Leu, Phe, Lys, His, Arg, Pro, EAA, TAA, LAA, and FAA than NG lambs, with only Gly and Cys being lower. This partially explains why most AA contents were higher in GS triceps brachii and biceps femoris.

Dietary AAs promote protein synthesis, potentially by increasing blood insulin levels. Research indicates that dietary BCAA enhances protein synthesis by activating AA transporters and inhibiting energy sensor-activated protein kinase activity. Leucine regulates protein synthesis through mTOR signaling; increased Leu content phosphorylates and inactivates 4E-binding protein 1 (4E-BP1), causing dissociation of eukaryotic initiation factor 4 (eIF4) and promoting protein translation. Our dietary AA analysis showed that supplemented concentrate contained higher BCAA and Leu levels than pasture, suggesting GS lambs had greater protein synthesis capacity, which may explain their higher muscle protein levels. However, this study did not examine related hormones and signaling pathways, necessitating further investigation into protein metabolism regulation.

Notably, NG lambs had higher CP, EAA, NEAA, FAA, and TAA contents in longissimus dorsi than GS lambs, indicating superior meat quality in this muscle. This demonstrates significant tissue-specific differences in muscle AA composition, with GS effects varying by muscle location. Different AA contents across longissimus dorsi, biceps femoris, and triceps brachii were also observed. Research in cattle reported that longissimus dorsi had significantly higher Glu, Ile, Leu, Lys, Ser, Tyr, and Val contents than gluteal muscle, but lower Arg, Cys, Gly, His, and Thr contents. These tissue differences likely arise because different tissues have specialized functions, leading to differential nutrient distribution. Studies on Inner Mongolia white cashmere goats demonstrated that blood AA flux represents the sum of metabolic processes across tissues, with AAs allocated to muscle deposition, skin, and hair growth. Thus, the significant differences in muscle AA composition between NG and GS lambs likely reflect complex interactions among multiple factors, requiring further investigation to provide a basis for dietary modulation of muscle protein quality under grazing supplementation systems.

Mutton AA composition and content affect not only protein nutrition but also flavor. Mutton flavor develops primarily through the combination of umami AA Glu with 5’ -inosine monophosphate disodium. Previous studies identified high Glu content (30.2%) as a primary reason for the delicious taste of Duolang sheep meat, far exceeding the reported 10.4% in other breeds. High Met content in Tan sheep meat, which produces sulfur-containing aromatic compounds through

thermal degradation, was also identified as a key flavor contributor. Our results showed varying contents of flavor-related AAs (Glu, Gly, Ala, Cys, Met) across breeds and fattening modes, potentially affecting mutton flavor. However, fatty acid content and composition also critically influence mutton flavor, necessitating comprehensive studies integrating fatty acid and carbohydrate metabolism to fully characterize flavor differences across fattening regimes and breeds.

Grazing with supplementary feeding yields lamb meat with superior protein nutritional value compared to natural grazing, and hybrid Hulunbeier-Dorper lambs produce meat with better protein nutritional value than pure Hulunbeier lambs.

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