# Effects of Grazing and Stall-Feeding Fattening on Plasma and Muscle Amino Acid Composition in Cashmere Goats: Postprint

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## Abstract

This study investigated the effects of grazing and stall-feeding fattening on the amino acid composition in blood and muscle tissues (longissimus dorsi, triceps brachii, biceps femoris, and gluteus muscles) of adult Albas white cashmere goats and weaned lambs. A 2×2 factorial experimental design was adopted. Sixty 4-month-old weaned Albas white cashmere lambs [(20.36±0.32) kg] and sixty 5-year-old adult goats [(40.38±0.84) kg] with similar body weight, conformation, and good health were selected and divided into 4 groups with 30 animals each. Factor 1 was fattening method: two types, grazing fattening and stall-feeding fattening (fed total mixed ration), designated as PF and SF, respectively. Factor 2 was age: two stages, adult goats and lambs, designated as AG and KG, respectively. The adult goats and lambs were fattened for 60 and 90 days, respectively. The results showed that plasma concentrations of non-essential amino acids (NEAA) and flavor amino acids (DAA) in the PF group were significantly lower than those in the SF group ($P<0.05$), while concentrations of essential amino acids (EAA), functional amino acids (FAA), and limiting amino acids (LAA) were significantly higher than those in the SF group ($P<0.05$). Plasma concentrations of NEAA, FAA, and DAA in the KG group were significantly higher than those in the AG group ($P<0.05$), while concentrations of EAA, branched-chain amino acids (BCAA), and LAA were significantly lower than those in the AG group ($P<0.05$). Overall, the PF group had lower contents of muscle crude protein (CP), EAA, NEAA, total amino acids (TAA), BCAA, FAA, and DAA than the SF group. The KG group had higher contents of muscle CP, EAA, NEAA, TAA, BCAA, LAA, FAA, and DAA than the AG group. It can be concluded that the muscle amino acid composition of stall-fed cashmere goats was superior to that of grazing goats, and the muscle amino acid composition of weaned lambs was superior to that of adult goats.

## Full Text

# Effects of Pasture Fattening and Stall Fattening on Amino Acid Profile in Plasma and Muscle of Cashmere Goats

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### Abstract

This experiment investigated the effects of pasture fattening and stall fattening on amino acid composition in blood and muscle tissues (Longissimus dorsi, arm triceps, biceps femoris, and gluteus) of adult and weaned kid Albas white cashmere goats. A 2×2 factorial design was employed, utilizing sixty healthy 4-month-old weaned kids [(20.36±0.32) kg] and sixty 5-year-old adult goats [(40.38±0.84) kg] with similar body weight and conformation. The animals were divided into four groups of thirty each. Factor 1 was fattening method: pasture fattening (PF) and stall fattening with total mixed ration (SF). Factor 2 was age: adult goats (AG) and kid goats (KG). Fattening duration was 60 days for adults and 90 days for kids. Results showed that plasma concentrations of non-essential amino acids (NEAA) and delicious amino acids (DAA) were significantly lower in PF than SF groups ($P<0.05$), whereas essential amino acids (EAA), functional amino acids (FAA), and limiting amino acids (LAA) were significantly higher ($P<0.05$). The KG group exhibited significantly higher plasma NEAA, FAA, and DAA concentrations than the AG group ($P<0.05$), but significantly lower EAA, branched-chain amino acids (BCAA), and LAA concentrations ($P<0.05$). Overall, muscle crude protein (CP), EAA, NEAA, total amino acids (TAA), BCAA, FAA, and DAA contents were lower in PF than SF groups. The KG group showed higher muscle CP, EAA, NEAA, TAA, BCAA, LAA, FAA, and DAA contents than the AG group. These findings indicate that stall fattening yields superior muscular amino acid profiles compared to pasture fattening, and weaned kids exhibit better muscular amino acid profiles than adult goats.

**Keywords:** fattening method; age; cashmere goat; amino acid

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China ranks first globally in both goat population and mutton production, playing a pivotal role in world sheep and goat husbandry. With rising living standards, consumer demand for goat meat products and meat quality requirements continue to increase. Consequently, improving mutton quality and flavor has become a key research focus. Muscle amino acid composition and content are primary indicators of protein nutritional value, particularly regarding human requirements for essential amino acids (EAA) and non-essential amino acids (NEAA). Higher EAA content and EAA/NEAA ratios indicate superior nutritional value. Beyond serving as crucial metrics for protein quality assessment, amino acids generate volatile compounds through Maillard reactions and Strecker degradation, directly influencing meat palatability and flavor. The main delicious amino acids (DAA) include aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), alanine (Ala), arginine (Arg), methionine (Met), and cysteine (Cys), with acidic amino acids Asp and Glu playing dominant roles in meat umami. However, mutton amino acid composition is influenced by numerous factors including breed, sex, feeding regimen, dietary composition, animal species, and age.

Albas white cashmere goat is an excellent dual-purpose breed from the desert steppes of Ordos, Inner Mongolia, prized for its tender, non-tainted meat with high protein and low fat/cholesterol content, rich in various amino acids. Traditional fattening relies on pasture grazing, but limited grassland area, ecological constraints, and increasing meat demand have made stall fattening increasingly important, particularly for the two primary production age groups—weaned kids and culled adults. However, research comparing the effects of stall versus pasture fattening on amino acid composition in cashmere goat meat, and differences between adult and kid goats, remains scarce. This study, considering goat digestive physiology and utilizing local feed resources, formulated typical fattening total mixed rations (TMR) to investigate whether natural pasture grazing versus TMR-based stall fattening affects meat quality and flavor in adult and kid goats, providing reference for understanding protein metabolism and optimizing fattening protocols through dietary modulation.

### 1.1 Experimental Design and Diets

A 2×2 factorial design was implemented using sixty 4-month-old weaned kids [(20.36±0.32) kg] and sixty 5-year-old adult does [(40.38±0.84) kg] with similar weight and conformation, divided into four groups (n=30). Factor 1 was fattening method: pasture fattening (PF) and stall fattening with TMR (SF). Factor 2 was age: adult goats (AG) and kid goats (KG). TMRs for SF groups were formulated according to *Feeding Standard of Meat-Producing Sheep and Goats* (NY/T 816-2004), fed twice daily ad libitum with free access to water. Environmental conditions and management were consistent across groups. TMR composition, nutrient levels, and amino acid profiles are presented in . PF groups grazed separate natural pastures daily from 07:00 to 18:00. Pasture nutritional levels and amino acid composition are shown in . Adult goats were fattened for 60 days (phase 1: days 1-30, August; phase 2: days 31-60, September). Kids were fattened for 90 days (phase 1: days 1-30, August; phase 2: days 31-60, September; phase 3: days 61-90, October). Initial body weights were recorded for all groups.

### 1.2 Sample Collection and Pre-treatment

For PF groups, forage consumed by grazing goats was tracked and collected for two consecutive days weekly. Monthly forage samples were air-dried, mixed uniformly, and composited by fattening phase for nutritional and amino acid analysis. For SF groups, TMR samples were collected during each fattening phase, air-dried, and analyzed.

Seven days before trial conclusion, ten fasting adult and kid goats per group were selected for jugular blood collection. Plasma was separated by centrifugation. At trial completion, six adult and kid goats per group were slaughtered. Left-side Longissimus dorsi, arm triceps, biceps femoris, and gluteus samples were wrapped in foil and stored at -20°C for analysis.

### 1.3 Test Indicators and Methods

Gross energy (GE), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium, and phosphorus in forage and TMR were determined according to Zhang Liying' s *Feed Analysis and Feed Quality Detection Technology*. Digestible energy (DE) was calculated from GE and digestibility. Digestibility trials were conducted during each phase with six goats per group using the acid-insoluble ash (AIA) method as internal marker. Feces were collected via fecal bags during a 5-day collection period following a 7-day adaptation. Fecal and feed samples were stored at -20°C for DM and AIA determination.

Dietary GE digestibility (%) = 1 - [dietary AIA (%) × fecal GE (%)] / [fecal AIA (%) × dietary GE (%)]. DE (MJ/kg) = dietary GE (MJ/kg) × dietary GE digestibility (%).

Seventeen individual amino acids in forage, TMR, plasma, and muscle samples were determined by acid hydrolysis according to GB/T 5009.124-2003 using an L-8900 amino acid analyzer. For plasma, 750 L was mixed with equal volume of 8% sulfosalicylic acid, refrigerated at 4°C overnight, then centrifuged at 17,968×g for 20 min at 4°C. Supernatant was filtered through 0.22 m membrane for analysis.

Forage, TMR, and muscle samples were dried at 65°C and ground. Lipids were extracted with ether for >24 h. Fifty mg of defatted muscle was hydrolyzed with 15 mL 6 mol/L HCl under nitrogen for 24 h at (110±1)°C. Hydrolysate was filtered, diluted to 25 mL, and 0.5 mL aliquots were dried under nitrogen at 60°C. Residues were reconstituted in 2.5 mL 0.02 mol/L HCl, filtered (0.22 m), and analyzed.

### 1.4 Statistical Analysis

Data were analyzed using two-way ANOVA in SAS 9.0. Differences were considered significant at $P<0.05$ and trends at 0.05 $P<0.10$.

### 2.1 Effects of Fattening Method and Age on Plasma Amino Acid Profile

As shown in , SF group exhibited significantly higher plasma Leu, Phe, His, Gly, Ala, and Tyr concentrations than PF group ($P<0.05$), while Thr, Met, Ile, Lys, Arg, Asp, and Glu were significantly lower (P<0.05). Plasma NEAA and DAA concentrations were significantly higher in SF versus PF (P<0.05), whereas EAA, FAA, and LAA concentrations and EAA/NEAA and EAA/TAA ratios were significantly lower (P<0.05). The AG group showed significantly higher plasma Thr, Val, Leu, and Lys than KG (P<0.05), but lower Met, Ile, Phe, Arg, Asp, Glu, Gly, Cys, and Tyr (P<0.05). Plasma EAA, BCAA, and LAA concentrations and EAA/NEAA and EAA/TAA ratios were significantly higher in AG versus KG (P<0.05), while NEAA, FAA, and DAA were significantly lower (P<0.05). Total plasma TAA did not differ between ages (P>0.05).

Significant interactions (P<0.05) between fattening method and age were observed for plasma Thr, Val, Met, Ile, Leu, Lys, His, Arg, Asp, Glu, Ala, Cys, Tyr, BCAA, LAA, DAA, and EAA/TAA. The PF-AG group showed highest Thr, Met, Lys, Arg, Glu, and LAA, but lowest Leu, His, Ala, Cys, Tyr, NEAA, and DAA. PF-KG had highest Ile, Asp, Cys, and FAA. SF-AG exhibited highest Val, Leu, His, Ala, and BCAA, but lowest Thr, Met, Ile, Phe, Arg, Asp, Glu, and FAA. SF-KG showed highest Phe, Tyr, and DAA, but lowest Val, Lys, BCAA, and LAA.

### 2.2 Effects of Fattening Method and Age on Longissimus dorsi Amino Acid Profile

shows SF group had significantly higher Val, Met, Ile, Leu, Phe, Lys, His, Asp, Ser, and Tyr contents in Longissimus dorsi than PF (P<0.05), with Thr and Pro tending to be higher (0.05 P<0.10). SF group also exhibited significantly higher CP, EAA, NEAA, TAA, and BCAA (P<0.05), with EAA/NEAA, EAA/TAA, and LAA tending higher (0.05 P<0.10). FAA and DAA were numerically increased but not significantly different (P>0.05). The AG group showed significantly lower Val, Met, Leu, Phe, Lys, His, Arg, Asp, Ser, Gly, Cys, Tyr, and Pro than KG (P<0.05), with Thr tending lower (0.05 P<0.10) but Glu significantly higher (P<0.05). CP, EAA, TAA, BCAA, LAA, EAA/NEAA, and EAA/TAA were significantly lower in AG versus KG (P<0.05).

Significant interactions (P<0.05) were found for Gly and Cys, with Lys and EAA/NEAA tending toward significance (0.05 P<0.10). PF-AG had lowest Gly, while SF-AG had highest Gly. SF-KG and PF-KG showed highest Cys contents.

### 2.3 Effects of Fattening Method and Age on Arm Triceps Amino Acid Profile

indicates SF group had significantly higher Val, Met, Leu, His, Cys, Tyr, and Pro in arm triceps than PF (P<0.05). CP, BCAA, FAA, DAA, and EAA/TAA were significantly higher in SF versus PF (P<0.05), with EAA/NEAA tending higher (0.05 P<0.10). The AG group exhibited significantly lower Thr, Val, Ile, Phe, Lys, His, Arg, Asp, Ser, Glu, Gly, Ala, and Tyr than KG (P<0.05), but higher Pro (P<0.05). EAA, NEAA, TAA, BCAA, LAA, and DAA were significantly lower in AG versus KG, with CP, FAA, and EAA/TAA tending lower (0.05 P<0.10).

Significant interactions (P<0.05) affected Ser and EAA, with NEAA and TAA tending toward significance (0.05 P<0.10). PF-KG showed highest Ser and EAA, while PF-AG had lowest values.

### 2.4 Effects of Fattening Method and Age on Biceps femoris Amino Acid Profile

demonstrates SF group had significantly higher Thr, Val, Met, Ile, Leu, Phe, Lys, His, Arg, Asp, Ser, Glu, Ala, Tyr, and Pro in biceps femoris than PF (P<0.05). CP, EAA, NEAA, TAA, BCAA, FAA, LAA, DAA, EAA/NEAA, and EAA/TAA were all significantly higher in SF versus PF (P<0.05). The AG group showed significantly lower Thr, Val, His, Arg, Asp, Ser, Glu, and Ala than KG (P<0.05), but higher Met, Cys, Tyr, and Pro (P<0.05). EAA, TAA, BCAA, FAA, DAA, EAA/NEAA, and EAA/TAA were significantly lower in AG versus KG (P<0.05), with NEAA tending lower (0.05 P<0.10).

Significant interactions (P<0.05) affected Thr, Met, Leu, Phe, Lys, His, Arg, Asp, Ser, Glu, Ala, Tyr, EAA, NEAA, TAA, FAA, LAA, DAA, EAA/NEAA, and EAA/TAA. PF-AG showed lowest Arg, Ser, Glu, Ala, EAA, NEAA, TAA, FAA, DAA, and ratios. PF-KG had lowest Met, Leu, Phe, Lys, His, Asp, Tyr, and LAA. SF-AG exhibited highest Met, Asp, and Tyr. SF-KG showed highest Thr, Leu, Phe, Lys, His, Arg, Ser, Glu, Ala, EAA, NEAA, TAA, FAA, LAA, DAA, and ratios.

### 2.5 Effects of Fattening Method and Age on Gluteus Amino Acid Profile

reveals SF group had significantly higher Met and His in gluteus than PF (P<0.05), significantly lower Thr (P=0.04), and Arg and Ala tending lower (0.05 P<0.10). EAA/TAA tended higher in SF versus PF (0.05 P<0.10), while CP was significantly lower (P<0.05). The AG group showed significantly lower Thr, Val, Met, Ile, Leu, Phe, Lys, His, Arg, Ser, Glu, Gly, Ala, and Pro than KG (P<0.05). CP, EAA, NEAA, TAA, BCAA, FAA, LAA, and DAA were significantly lower in AG versus KG (P<0.05).

Significant interactions (P<0.05) affected gluteus CP, Thr, Met, Ile, Leu, Phe, Lys, Arg, Ser, Glu, Gly, Ala, Cys, Pro, EAA, NEAA, TAA, BCAA, FAA, and DAA, with His, Asp, and LAA tending toward significance (0.05 P<0.10). PF-AG showed lowest CP, Thr, Met, Ile, Leu, Phe, Lys, Arg, Ser, Glu, Ala, Cys, EAA, NEAA, TAA, BCAA, FAA, and DAA. PF-KG exhibited highest values for these same parameters. SF-AG had highest Met and Pro, while SF-KG showed lowest Gly and Pro.

### 3.1 Effects of Fattening Method and Age on Meat Amino Acid Composition

Meat nutritional value depends on protein quantity and quality, particularly its ability to meet human EAA requirements. Fattening method is a primary factor affecting mutton protein quality. Traditional Albas cashmere goat fattening uses pasture grazing, but stall fattening has become predominant for ecological protection. This study utilized local feed resources (sunflower plate powder and corn straw pellets) to formulate typical fattening TMR according to Chinese standards, investigating whether natural pasture versus TMR affects meat amino acid composition. Results showed SF increased muscle CP, EAA, NEAA, and TAA contents, indicating superior protein quality in stall-finished goats. Dietary analysis revealed SF TMR had superior protein levels and amino acid profiles compared to PF pasture. Previous studies from our group demonstrated that stall fattening significantly increased CP intake and digestibility in both adult and kid goats. Increased digestible CP intake would alter blood amino acid concentrations. Feng Tao reported that lambs fed 17% CP diets deposited richer EAA in muscle than those fed 13% or 15% CP. Du Minqing found that lactating sows fed 1.2% Lys showed higher plasma single amino acids, EAA, NEAA, and TAA, suggesting lower utilization and consequently poorer performance. In this trial, SF significantly reduced plasma EAA, FAA, and LAA concentrations, partially explaining the improved amino acid utilization and muscle deposition in SF goats. Additionally, SF significantly increased plasma aromatic amino acids Phe and Tyr. Elevated serum Tyr increases brain Tyr and catecholamines like norepinephrine, which regulates feed intake, potentially explaining increased consumption in SF goats. Thus, stall fattening improved protein intake, digestibility, and utilization, enhancing both quantity and quality of deposited muscle protein. Beyond CP, other nutrients including energy, minerals, and vitamins also influence meat amino acid composition. The SF TMR contained higher levels of energy, Ca, P, and vitamins similar to NRC standards, contributing to improved protein efficiency, though further research is needed.

Exercise levels differed between systems. López-Bote et al. found that exercised pigs (versus sedentary stall-fed) and pastured pigs (primarily grazing) had significantly reduced muscle BCAA compared to stall-fed pigs, attributing this to physical activity. Martins et al. reported higher Longissimus dorsi CP in pastured versus stall-fed pigs, also related to exercise. Zhao et al. demonstrated that moderate and high-intensity exercise increased mTOR signaling pathway phosphorylation in rat skeletal muscle, promoting protein synthesis. However, exercise effects on protein metabolism have been studied primarily in monogastrics, rodents, and humans, with limited research in ruminants. This trial involved substantial free exercise for PF goats versus minimal activity for SF goats, warranting further investigation into exercise effects on muscle protein content. Additionally, differences in light exposure and temperature between systems may also affect protein metabolism and require further study.

Dietary BCAA supplementation improves exercise coordination, endurance, and longevity in rodents by regulating mTOR/eNOS pathways, mitochondrial biogenesis, energy metabolism, and ROS scavenging. This trial found significantly higher BCAA in arm triceps and biceps femoris of SF goats, suggesting health benefits. Increased BCAA may relate to reduced expression of branched-chain amino acid transaminase 2 (BCAT2) and branched-chain -keto acid dehydrogenase (BCKDH), requiring further investigation. Leu activates mTOR signaling in monogastric muscle, and mTOR pathway regulates muscle protein synthesis through downstream S6K1 and 4EBP1 phosphorylation. Sales et al. reported that twin lambs had lower intramuscular Leu than singletons, suppressing mTOR and reducing muscle protein. This trial's significantly higher Val and Leu in SF goat arm triceps and biceps femoris suggests higher protein content may relate to mTOR activity, necessitating deeper study.

Age also affects amino acid synthesis and protein nutritional value. Kimball et al. reported highest muscle protein synthesis rates in newborn piglets, declining with age—consistent with our findings. The KG group showed higher CP, EAA, NEAA, TAA, BCAA, and FAA in all muscles than AG, indicating superior protein synthesis and nutritional value in kids. At slaughter, kids were 7 months old versus 5-year-old adults. Before one year, animals prioritize bone and muscle growth with strong protein, Ca, and P deposition and different protein turnover rates, explaining higher feed protein utilization and muscle deposition in kids. Plasma amino acid concentrations showed KG had significantly higher NEAA and FAA but lower Val, Leu, BCAA, EAA, and LAA than AG, confirming higher protein utilization and deposition in kids. Studies report lower protein synthesis rates in older individuals, consistent with Dillon et al. and our results. Additionally, our previous findings showed adult goats had higher serum insulin but lower growth hormone and IGF-I than kids, indicating higher protein synthesis rates in kids. Zhou et al. demonstrated that dietary Cys supplementation increased plasma IGF-I, phosphorylated mTOR, 4EBP1, and S6K1, promoting protein synthesis.

### 3.2 Effects of Fattening Method and Age on Delicious Amino Acids (DAA) in Mutton

Albas white cashmere goat is a world-renowned dual-purpose breed. Though pasture-finished goat meat is considered tender and flavorful, related research is scarce. Dietary factors significantly affect mutton flavor. Ecological limitations have made stall fattening predominant. Compared to pasture grazing, TMR-based stall fattening substantially alters dietary composition and nutrient levels, potentially affecting meat quality and flavor. DAA such as Gly, Arg, Asp, Ala, and Glu are essential precursors for meat aroma, directly related to umami taste, with Glu being dominant. Higher DAA content indicates better flavor. Met and Cys, sulfur-containing amino acids, generate sulfur heterocyclic compounds during thermal degradation that contribute to meat aroma.

This study compared amino acid composition between natural pasture and TMR diets to provide references for optimizing cashmere goat meat flavor. Results showed SF significantly increased Arg, Asp, Glu, Ala, and DAA in arm triceps and biceps femoris, with Glu accounting for >35% of DAA. SF also significantly increased muscle Met, suggesting improved umami. From an amino acid perspective, stall-finished meat showed better flavor than pasture-finished, likely because TMR contained higher DAA and total amino acid intake than natural pasture. Additionally, SF significantly reduced plasma Met, Arg, Asp, and Glu concentrations, explaining higher muscle contents of these amino acids. Mechanisms underlying flavor differences warrant further investigation at gene, protein, and signaling pathway levels. The KG group also showed higher muscle DAA, Arg, and Asp than AG, suggesting superior kid meat flavor, though mechanisms remain unclear. This trial only addressed amino acid composition; fatty acid composition also critically affects mutton flavor, requiring comprehensive future research.

## Conclusion

Stall fattening increases muscle EAA, NEAA, BCAA, TAA, and DAA contents in cashmere goats compared to pasture fattening. Weaned kid goats exhibit higher muscle EAA, NEAA, BCAA, FAA, and DAA contents than adult goats.

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