

## Postprint: Nutritional and Muscle Fiber Histological Characteristics of Hu Sheep

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### Abstract

To investigate the nutritional characteristics and histological properties of muscle fibers in Hu sheep, conventional nutritional components (moisture, crude protein, crude fat, crude ash, calcium, phosphorus), 18 amino acid contents in muscles of Hu sheep at different ages (newborn, weaned, adult), and histological characteristics of muscle fibers (muscle fiber diameter, muscle fiber density) in different muscle parts (triceps brachii, biceps femoris, longissimus dorsi) were determined. The results showed that with increasing age, muscle moisture content decreased significantly ( $P < 0.05$ ), while crude fat, crude protein, crude ash, calcium, and phosphorus contents increased gradually, with significant differences between adult sheep and newborn and weaned lambs ( $P < 0.05$ ). The contents of threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, and arginine in Hu sheep muscle increased significantly with age ( $P < 0.05$ ), and non-essential amino acid contents showed a general increasing trend with age. Among all amino acids in Hu sheep muscle, glutamic acid had the highest content, followed by aspartic acid. Muscle fiber diameter in Hu sheep at different ages showed the pattern: longissimus dorsi < biceps femoris < triceps brachii, while muscle fiber density showed the pattern: longissimus dorsi > biceps femoris > triceps brachii, with significant differences in muscle fiber density among the three parts in same-age Hu sheep ( $P < 0.05$ ). For the same muscle part, muscle fiber diameter increased with age, while muscle fiber density decreased with age, with significant differences among different ages (except for triceps brachii between newborn and weaned lambs) ( $P < 0.05$ ). In conclusion, Hu sheep muscle is rich in nutrients with a complete amino acid profile, and histological characteristics of muscle fibers differ among different ages and parts; weaned lamb meat is more nutritious and possesses better characteristics such as flavor and tenderness.

## Full Text

### Nutritional Characteristics and Muscle Fiber Histological Properties of Hu Sheep Muscle

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**Abstract:** This study investigated the nutritional characteristics and muscle fiber histological properties of Hu sheep muscle across different age groups (newborn, weaned, and adult). We measured conventional nutritional components (moisture, crude protein, crude fat, ash, calcium, and phosphorus), 18 amino acids, and histological characteristics (muscle fiber diameter and density) in three muscle regions (arm triceps, biceps femoris, and longissimus dorsi). The results demonstrated that moisture content decreased significantly with age ( $P < 0.05$ ), while crude fat, crude protein, ash, calcium, and phosphorus contents increased gradually, with significant differences between adult sheep and both newborn and weaned lambs ( $P < 0.05$ ). Essential amino acids including threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, and arginine increased significantly with age ( $P < 0.05$ ), while non-essential amino acids showed a general upward trend. Glutamic acid was the most abundant amino acid, followed by aspartic acid. Across all age groups, muscle fiber diameter followed the pattern: arm triceps > biceps femoris > longissimus dorsi, while muscle fiber density showed the inverse pattern: longissimus dorsi > biceps femoris > arm triceps, with significant differences among the three anatomical sites within each age group ( $P < 0.05$ ). For the same muscle region, fiber diameter increased with age while fiber density decreased, with significant differences between age groups (except for arm triceps between newborn and weaned lambs) ( $P < 0.05$ ). These findings indicate that Hu sheep muscle is nutritionally rich with a complete amino acid profile, and that muscle fiber histological properties vary significantly with age and anatomical location, with weaned lamb meat being particularly nutritious and characterized by superior flavor and tenderness.

**Keywords:** Hu sheep; muscle; nutrient components; muscle fiber histological characteristics

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## Introduction

As living standards improve, public awareness of health maintenance has grown substantially, with food nutrition becoming a primary focus. Mutton ranks

among China's three major meat products and represents a highly nutritious food source. Chinese people have consumed mutton for six to seven thousand years, valuing its tender texture, rich flavor, high nutritional value, and low fat and cholesterol content that facilitates digestion and absorption. Particularly valued as a winter tonic, sheep consume various herbs, earning them the designation "repository of medicines." Li Shizhen noted in *Compendium of Materia Medica* that mutton is "sweet and bitter, warm in nature, warms the center and supplements deficiency, boosts qi, invigorates the stomach, benefits fitness, nourishes kidney qi, supports gallbladder function, brightens vision, and treats consumptive cold and various injuries." This illustrates that mutton not only contains abundant nutrients but also possesses medicinal properties, and without religious or cultural prohibitions, it has gained increasing popularity in recent years [1].

China is a major mutton producer, accounting for one-third of global production in 2014, yet it is not a mutton processing powerhouse. Over 90% of products remain fresh meat, primarily due to insufficient systematic research on mutton quality and processing characteristics, and a lack of carcass cutting techniques tailored to Chinese breeds, consumption habits, and processing methods [2]. Particularly lacking are comprehensive studies on quality characteristics of commercial mutton from specific breeds at different production stages, preventing the development of high-end products and value-added processing needed for international market competition. Meanwhile, developed sheep-producing nations such as Australia, France, New Zealand, the United Kingdom, and the United States produce lamb meat accounting for over 70% of total mutton output, compared to less than 30% in China [3], indicating that lamb production represents a key direction for China's sheep industry development.

Hu sheep, an excellent local breed in China, are primarily distributed in the Taihu Lake basin region of Zhejiang and Jiangsu provinces. This dual-purpose breed (meat and skin) exhibits strong reproductive capacity, good milk performance, satisfactory meat production, and excellent adaptability, particularly possessing prolificacy genes that provide a theoretical foundation for developing superior multi-lamb breeds. In recent years, with the expansion of the mutton sheep industry, Hu sheep have been raised nationwide either as meat sheep [4] or as dam lines for crossbreeding and new strain development [5-7], with research and applications in superior gene screening and mining [8-10], demonstrating substantial potential for utilization in China's sheep industry, particularly in mutton production. This study investigated the basic nutritional components of Hu sheep muscle at different ages and the histological characteristics of muscle fibers across different ages and anatomical sites, aiming to provide scientific evidence for Hu sheep pure breeding, new breed development, and high-quality mutton product development.

## Materials and Methods

**1.1.1 Sample Collection** Mutton samples were obtained from a Hu sheep population raised at Luoning Nongben Animal Husbandry Technology Development Co., Ltd. in Luoyang, Henan Province. All sheep were raised under identical environmental conditions using phased feeding protocols. Five animals were randomly selected from each age group—newborn lambs (1-3 days), weaned lambs (2.5 months), and adult sheep (2 years)—for a total of 15 animals. Longissimus dorsi muscle samples were collected for nutritional analysis, while arm triceps, biceps femoris, and longissimus dorsi samples were collected for muscle fiber histological analysis at the slaughterhouse.

**1.1.2 Major Equipment** Kjeldahl distillation apparatus, conical flasks, digestion tubes, and nitrogen analyzers (Beijing Zhongke Huanshi Co., Ltd.); Soxhlet extractors (Shanghai Yunyun Instrument Co., Ltd.); muffle furnaces and water baths (Henan Xinke Analytical Instrument Co., Ltd.); L-8900 automatic amino acid analyzer (Hitachi, Japan); UV spectrophotometer, microtome, and microscopic color image analysis equipment (Hubei Xiaogan Kuohai Medical Technology Co., Ltd.).

**1.1.3 Major Reagents** Hydroxylamine hydrochloride, triethanolamine, ethylenediamine, and starch (Shanghai Lingfeng Chemical Reagent Co., Ltd.); malachite green indicator, calcium standard solution, and other indicators (Tianjin Zhengcheng Chemical Products Co., Ltd.); hematoxylin, eosin, and neutral balsam (Tianjin Jinke Fine Chemical Research Institute); all other reagents were domestic analytical grade.

**1.2.1 Determination of Conventional Nutritional Components and Amino Acid Content** Moisture content was determined according to *Meat and Meat Products—Determination of Moisture Content* [11]; crude protein according to GB 5009.5—2010 *Determination of Protein in Foods* [12]; crude fat according to GB/T 9695.7—2008 *Meat and Meat Products—Determination of Total Fat Content* [13]; ash by incineration gravimetric method; calcium according to GB/T 9695.13—2009 *Meat and Meat Products—Determination of Calcium Content* [14]; and phosphorus according to GB/T 9695.4—2009 *Meat and Meat Products—Determination of Total Phosphorus Content* [15]. Amino acid composition and content were determined using an L-8900 automatic amino acid analyzer following the method described in reference [16]: 5 mg of dried meat sample was hydrolyzed with 6 mol/L HCl at 110°C for 24 h, deacidified, diluted to 5 mL with water, and analyzed.

**1.2.2 Muscle Fiber Histological Characteristics** Post-slaughter samples of arm triceps, longissimus dorsi, and biceps femoris were fixed in 10% formaldehyde for 12 h, dehydrated through graded ethanol series, cleared in xylene for approximately 30 min, infiltrated with melted paraffin for about 12 h, embedded, trimmed, and sectioned using a microtome. Sections were stained with

eosin and examined under a microscope to measure muscle fiber diameter and density.

**1.3 Statistical Analysis** All data were analyzed using SPSS 17.0 statistical software, with significance testing performed using LSD method.

## Results

**2.1 Conventional Nutritional Components in Hu Sheep Muscle** As shown in , moisture content in newborn lamb muscle was 77.01%, decreasing to 74.24% in adult sheep. Crude protein content increased significantly with age, from 20.3% in newborn lambs to 22.6% in adult sheep ( $P < 0.05$ ). Additionally, crude fat, ash, calcium, and phosphorus contents increased with age, with significant differences between newborn and adult sheep ( $P < 0.05$ ).

**3.2 Amino Acid Content in Hu Sheep Muscle** Comparison across age groups revealed that adult sheep had the highest amino acid content (19.14% total), followed by weaned lambs (17.92%), with newborn lambs having the lowest (17.51%). Among essential amino acids, lysine was most abundant, comprising 1.70%, 1.78%, and 1.86% in newborn, weaned, and adult sheep respectively, accounting for 9.71%, 9.93%, and 9.72% of total measured amino acids. Leucine was the second most abundant essential amino acid at 1.47%, 1.53%, and 1.58% respectively, representing 8.40%, 8.53%, and 8.25% of the total. Among non-essential amino acids, glutamic acid was highest at 3.06%, 3.16%, and 3.34%, accounting for 17.48%, 17.63%, and 17.45% of the total, followed by aspartic acid at 1.66%, 1.72%, and 1.80% (9.48%, 9.60%, and 9.40% of total). Other amino acids generally increased with age.

**2.3 Muscle Fiber Histological Characteristics of Hu Sheep** As shown in and [Figure 1: see original paper], muscle fiber diameter across all age groups followed the pattern: longissimus dorsi < biceps femoris < arm triceps, though differences among sites were not significant ( $P > 0.05$ ). Conversely, muscle fiber density showed the inverse pattern: longissimus dorsi > biceps femoris > arm triceps, with significant differences observed for newborn and adult sheep, where longissimus dorsi and biceps femoris densities were significantly greater than arm triceps ( $P < 0.05$ ). Muscle fiber diameter increased significantly with age across all sites (newborn < weaned < adult) ( $P < 0.05$ ), while fiber density decreased significantly with age (newborn > weaned > adult), except for arm triceps between newborn and weaned lambs ( $P < 0.05$ ).

## Discussion

**3.1 Conventional Nutritional Components and Meat Quality Traits in Hu Sheep Muscle** Like other livestock meats, mutton contains water, protein, fat, minerals, and vitamins. Lawrie [17] proposed that meat physical characteristics provide intuitive and easily measurable indicators of quality, with

most traits determining eating quality and economic value, making them essential for meat quality research. The adult Hu sheep muscle moisture content of 74.24% measured in this study was higher than that of Balikun sheep (72.57%) [18], Henan Large-tailed Han sheep (71.78%) [19], and Tan sheep (70.94%) [20], indicating tender meat quality. Intramuscular fat deposition increased with age, reaching 4.80% in adult Hu sheep—higher than Suited sheep (3.14%) [21], Small-tailed Han sheep (2.49%) [22], Beichuan White goats (2.20%) [23], Guanzhong dairy goats (4.78%) [24], and Nanjiang Yellow goats (2.50%) [25]. Mutton fat provides both dietary fat and flavor enhancement. Lamb meat's lower fat content results in reduced deposition of flavor precursors and milder odor, representing an important characteristic beyond tenderness. Mutton protein serves as an excellent source of human dietary protein, with Hu sheep muscle containing over 20% protein. Adult Hu sheep reached 22.6% crude protein, exceeding Suited sheep (19.2%) [21], Small-tailed Han sheep (21.6%) [22], Henan Large-tailed Han sheep (22.49%) [19], Tan sheep (20.36%) [26], and Luxi Black-headed mutton sheep (20.02%) [27]. Adult Hu sheep ash content of 1.21% was higher than Henan Large-tailed Han sheep (0.91%) [19] and Tan sheep (1.05%) [28]. Calcium and phosphorus contents were abundant at 5.56 mg/kg and 22.1 mg/kg respectively. These results demonstrate rich nutritional content and superior meat quality in Hu sheep, with variations from other Chinese breeds attributable to differences in breed, sex, age, feeding level, and carcass location.

### 3.2 Amino Acid Content and Meat Quality Traits in Hu Sheep Muscle

Mutton amino acids provide balanced nutrition for humans. Essential amino acid contents in newborn, weaned, and adult Hu sheep were 8.92%, 9.26%, and 9.66% respectively, while non-essential amino acids were 8.69%, 8.66%, and 9.48%. Adult Hu sheep essential and non-essential amino acid contents differed from Chen Xuejun et al.'s [29] values of 8.79% and 11.49%, possibly due to different classification methods (this study classified histidine and arginine, generally considered essential for infants, as essential amino acids based on reference [30]), age differences (2 years vs. 4 years), or different rearing environments. Research indicates that serine, glutamic acid, glycine, isoleucine, leucine, alanine, and proline are flavor precursor amino acids, particularly glutamic acid as the primary umami substance. This study found abundant essential and umami amino acids in Hu sheep muscle, with high contents of key flavor precursors, especially glutamic acid (3.34%) and aspartic acid (1.80%), similar to Chen Xuejun et al.'s [29] results (3.30% and 1.94%) and higher than Small-tailed Han sheep (2.48% and 1.23%) [26]. Although weaned lambs had lower glutamic acid than adults, their proportion of total amino acids was higher than both adult and newborn lambs. Consequently, while newborn lamb is tender, its taste is inferior to weaned lamb, which excels in both tenderness and flavor. Lysine, considered the first limiting amino acid for humans, was highest across all age groups, with the greatest proportion in weaned lambs, demonstrating Hu sheep's unique nutritional properties and potential for producing flavorful meat products, particularly from weaned lambs.

### 3.3 Muscle Fiber Histological Characteristics and Meat Quality Traits in Hu Sheep

Muscle fiber diameter and density are closely related to meat quality, representing important indicators of tenderness and water-holding capacity. Finer fibers with greater density correlate with increased intramuscular fat deposition. As fiber diameter increases, tenderness decreases; generally, smaller diameter and higher density indicate better quality, though sarcomere length, myofibril fragmentation index, and water-holding capacity also influence tenderness. This study found age-related differences in fiber diameter and density at the same anatomical site, with younger animals producing more tender meat due to significantly hardened fibers with age, consistent with findings by Zeng Yongqing et al. [31] and Gao Aiqin et al. [32]. Anatomical site differences existed within the same age group, with longissimus dorsi showing the smallest fiber diameter (26.22  $\mu\text{m}$  in adults), similar to Sun Wei et al.'s [33] measurement (26.77  $\mu\text{m}$ ) and smaller than Suited sheep (42.81  $\mu\text{m}$ ) [21], Small-tailed Han sheep (28.84  $\mu\text{m}$ ) [22], Nanjiang Yellow goats (34.13  $\mu\text{m}$ ) [23], and Guanzhong dairy goats (38.10  $\mu\text{m}$ ) [24]. Thus, Hu sheep exhibit relatively small fiber diameter and high density compared to Suited and Small-tailed Han sheep. China's diverse sheep breeds, climatic conditions, management systems, and feed resources may cause variations even at the same age and anatomical site. Tenderness significantly influences consumer preference, which can guide purchasing decisions based on age and anatomical location.

### Conclusions

1. Hu sheep muscle is rich in nutrients with a complete amino acid profile, with all conventional nutritional components except moisture increasing with age.
2. Analysis of the proportion of key amino acids indicates that weaned lamb meat offers superior nutritional value, umami, and aroma.
3. Under identical feeding and management conditions, Hu sheep muscle nutritional characteristics and fiber properties are influenced by age and anatomical location, with weaned lamb meat being the most tender and flavorful.

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