

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

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Abstract

To investigate the nutritional characteristics and muscle fiber histological properties of Hu sheep muscle, conventional nutritional components (moisture, crude protein, crude fat, crude ash, calcium, phosphorus), the contents of 18 amino acids, and muscle fiber histological characteristics (muscle fiber diameter and density) were determined in different muscle locations (triceps brachii, biceps femoris, longissimus dorsi) of Hu sheep at different ages (newborn, weaned, adult). The results showed that with increasing age, the moisture content in muscle decreased significantly ($P < 0.05$), while the contents of crude fat, crude protein, crude ash, calcium, and phosphorus 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 the content of non-essential amino acids 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 of longissimus dorsi < biceps femoris < triceps brachii, while muscle fiber density showed the pattern of longissimus dorsi > biceps femoris > triceps brachii, with significant differences in muscle fiber density among the three locations in sheep of the same age ($P < 0.05$). For the same muscle location, 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 summary, Hu sheep muscle is rich in nutrients with a complete range of amino acids, and muscle fiber histological characteristics differ among different ages and locations. Weaned lamb meat is more nutritious and possesses characteristics of enhanced flavor and tenderness.

Full Text

Preamble

Nutritional Characteristics and Muscle Fiber Histological Characters of Muscle from Hu Sheep

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Abstract: In order to study the nutritional characteristics and muscle fiber histological characters of muscle from Hu sheep, the contents of common nutrition components (moisture, crude protein, crude fat, ash, calcium and phosphorus), eighteen kinds of amino acids and histological traits (muscle fiber diameter and density of three parts from arm triceps, biceps femoris and longissimus dorsi) of Hu sheep with different ages (newborn, weaned and adult) were measured. The results showed as follows: with the age increasing, the moisture content in muscle was significantly decreased ($P < 0.05$), and the crude fat, crude protein, ash, calcium and phosphorus contents were gradually increased, and the differences of those indexes between newborn and weaned lambs and adult sheep were significant ($P < 0.05$). The threonine (Thr), valine (Val), Met (methionine), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), lysine (Lys), histidine (His) and arginine (Arg) contents in muscle of Hu sheep were significantly increased with the age increasing ($P < 0.05$), and the non-essential amino acids had generally upward trend with the age increasing. Among all of amino acids in muscle of Hu sheep, glutamic acid (Glu) content was the highest and the second was aspartic acid (Asp). The muscle fiber diameter of arm triceps in the three ages' sheep was the biggest, followed by the biceps femoris and the longissimus dorsi. The muscle fiber density of longissimus dorsi in the three ages sheep was the biggest, followed by the biceps femoris and the arm triceps, and the difference of muscle fiber density among the three body part muscles of the same age sheep was significant ($P < 0.05$). For the same body part muscle, the muscle fiber diameter was increased and the muscle fiber density was decreased with the age increasing, and the significant differences were existed among different ages (except the arm triceps of newborn and weaned lambs) ($P < 0.05$). It is concluded that Hu sheep muscle has rich nutrition and many kinds of amino acids, and its muscle fiber histological characters are different among different body parts and different ages. Especially, for weaned lamb, the muscle is more nutritious with more sweet and tender.

Keywords: hu sheep; muscle; nutrient components; muscle fiber histological characters

In recent years, with the gradual improvement of living standards, people's

health awareness has become increasingly strong, with food health becoming a key focus. As one of the three major meat products in China, mutton is a highly nutritious food. Chinese people began consuming mutton six to seven thousand years ago. Mutton features tender texture, delicious taste, rich nutrition, low fat and cholesterol content, easy digestion and absorption, and high edible value, making it an ideal food for winter nourishment. Sheep consume various herbs, earning them the reputation of a “treasury of medicines.” Li Shizhen noted in *Compendium of Materia Medica* that “mutton tastes sweet and bitter, with warm properties, can warm the middle-jiao and tonify deficiency, tonify the middle and augment qi, stimulate appetite and strengthen the body, benefit kidney qi, nourish gallbladder and brighten eyes, and treat consumptive cold and various injuries from overexertion.” This demonstrates that mutton not only contains abundant nutrients but also possesses medicinal value. Moreover, without religious or cultural taboos, mutton has gained increasing popularity in recent years. China is a major mutton producer, accounting for one-third of global mutton production in 2014. However, China is not a mutton processing powerhouse, with over 90% of products being fresh mutton. This is due to the lack of systematic research on mutton quality characteristics and processing properties, the absence of mutton cutting techniques suitable for Chinese breeds, consumption habits, and processing methods, and particularly the lack of serialized studies on the quality characteristics of commercial mutton produced by specific breeds at different stages. Consequently, China cannot produce high-end mutton products or increase added value to compete in international markets. Meanwhile, developed sheep-farming countries such as Australia, France, New Zealand, the United Kingdom, and the United States produce lamb meat accounting for over 70% of total mutton output, whereas China produces less than 30%. Therefore, lamb production represents a future direction for China’s sheep industry.

Hu sheep are an excellent local breed in China, primarily distributed in the Taihu Lake basin of Zhejiang and Jiangsu provinces. As a dual-purpose breed for both pelts and meat, Hu sheep exhibit excellent characteristics including high prolificacy, good milk performance, satisfactory meat production, and strong adaptability. Particularly, Hu sheep possess multiple birth genes, providing a theoretical basis for establishing superior prolificacy gene resources in China. In recent years, with the development of the mutton sheep industry, Hu sheep have been raised as meat sheep or as dams for crossbreeding and new strain development across major sheep-producing regions in China. Research has also been conducted on screening and mining superior genes. Hu sheep hold great promise for utilization in China’s sheep industry, particularly in mutton sheep development. This study investigated the basic nutritional components of muscle from Hu sheep of different ages under conventional feeding conditions, as well as the muscle fiber histological characteristics across different ages and body parts, aiming to provide scientific basis for Hu sheep pure breeding, new breed development, and production of premium mutton products.

1.1.1 Sample Collection

Mutton samples were collected from Hu sheep raised at Luoning Nongben Animal Husbandry Science and Technology Development Co., Ltd. in Luoyang, Henan Province. All sheep were raised in the same environment and conditions using phased feeding protocols. Slaughtered sheep were randomly selected from newborn lambs (1–3 days old), weaned lambs (2.5 months old), and adult sheep (2 years old), with five animals from each group, totaling 15 sheep. For nutritional component analysis, longissimus dorsi muscle samples were collected. For muscle fiber histological characteristic analysis, samples were collected from the longissimus dorsi, biceps femoris (leg), and triceps brachii (shoulder) 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 Yunyang 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); other reagents were domestically produced analytical grade.

1.2.1 Determination Methods for Routine Nutritional Components and Amino Acid Content in Muscle

Moisture content determination followed the method specified in “Meat and Meat Products—Determination of Moisture Content.” Crude protein content was determined according to GB 5009.5–2010 “Determination of Protein in Foods.” Crude fat content was determined according to GB/T 9695.7–2008 “Meat and Meat Products—Determination of Total Fat Content.” Ash content was determined using the incineration gravimetric method. Calcium content was determined according to GB/T 9695.13–2009 “Meat and Meat Products—Determination of Calcium Content.” Phosphorus content was determined according to GB/T 9695.4–2009 “Meat and Meat Products—Determination of Total Phosphorus Content.” Amino acid composition and content were determined using an L-8900 automatic amino acid analyzer following the method described in reference [16]. Specifically, 5 mg of dried meat sample was hydrolyzed with

6 mol/L hydrochloric acid at 110°C for 24 hours, deacidified, diluted to 5 mL with water, and analyzed using the L-8900 automatic amino acid analyzer.

1.2.2 Methods for Studying Muscle Fiber Histological Characteristics

After slaughter, triceps brachii, longissimus dorsi, and biceps femoris muscles were collected according to experimental requirements. Samples were fixed in 10% formaldehyde for 12 hours, then dehydrated through an ethanol gradient from low to high concentration, cleared with xylene for approximately 30 minutes, infiltrated with melted paraffin for about 12 hours, and embedded. The embedded tissue blocks were trimmed and sectioned using a microtome. After staining with eosin, muscle fiber diameter and density were measured under a microscope.

1.3 Statistical Analysis

All data were analyzed using SPSS 17.0 statistical software, with significance testing performed using the LSD method.

2.1 Determination Results of Routine Nutritional Components in Hu Sheep Muscle

As shown in Table 1, the moisture content of newborn lamb muscle was 77.01%, decreasing to 74.24% in adult sheep. Crude protein content increased significantly with age, rising 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 lambs and adult sheep ($P < 0.05$).

3.2 Determination Results of Various Amino Acid Contents in Hu Sheep Muscle

Comparison across different ages revealed that adult sheep had the highest amino acid content, with a total of 19.14%, followed by weaned lambs at 17.92%, and newborn lambs at 17.51%. Among essential amino acids, lysine content was highest, accounting for 1.70%, 1.78%, and 1.86% in newborn, weaned, and adult sheep muscle, respectively, representing 9.71%, 9.93%, and 9.72% of total measured amino acids. Leucine ranked second, with contents of 1.47%, 1.53%, and 1.58% in the three age groups, representing 8.40%, 8.53%, and 8.25% of total amino acids. Among non-essential amino acids, glutamic acid was most abundant, reaching 3.06%, 3.16%, and 3.34% in newborn, weaned, and adult sheep, respectively, representing 17.48%, 17.63%, and 17.45% of total amino acids. Aspartic acid ranked second, with contents of 1.66%, 1.72%, and 1.80%, representing 9.48%, 9.60%, and 9.40% of total amino acids. Other amino acids also generally increased with age.

2.3 Determination Results of Muscle Fiber Histological Characteristics in Hu Sheep

As shown in Table 3 and Figure 1 [Figure 1: see original paper], muscle fiber diameter in newborn, weaned, and adult sheep consistently followed the pattern: longissimus dorsi < biceps femoris < triceps brachii, though differences among body parts were not significant ($P > 0.05$). Conversely, muscle fiber density showed the opposite trend: longissimus dorsi > biceps femoris > triceps brachii, with significant differences observed between the longissimus dorsi, biceps femoris, and triceps brachii in newborn and adult sheep ($P < 0.05$). Muscle fiber diameter increased with age across all body parts, following the pattern: newborn lambs < weaned lambs < adult sheep, with significant differences among age groups ($P < 0.05$). Muscle fiber density decreased with age, following the pattern: newborn lambs > weaned lambs > adult sheep, with significant differences among age groups (except for the triceps brachii between newborn and weaned lambs) ($P < 0.05$).

3.1 Routine Nutritional Component Content and Meat Quality Traits of Hu Sheep Muscle

Like other livestock and poultry meats, mutton contains water, protein, fat, minerals, and vitamins. Lawrie [17] considered meat physical traits as relatively intuitive and easily measurable indicators that reflect meat quality, with most traits determining eating quality and holding significant economic importance. Therefore, physical traits constitute an important aspect of meat quality research.

Table 1 shows that adult Hu sheep muscle moisture content was 74.24%, higher than that of Balikun sheep (72.57%) [18], Henan Large-tailed Han sheep (71.78%) [19], and Tan sheep (70.94%) [20], indicating higher moisture content and more tender meat in Hu sheep. Intramuscular fat deposition increased with age, reaching 4.80% in adult Hu sheep, higher than Sunit sheep (3.14%) [21], Small-tailed Han sheep (2.49%) [22], Beichuan White goat (2.20%) [23], Guanzhong dairy goat (4.78%) [24], and Nanjiang Yellow goat (2.50%) [25]. Mutton fat provides necessary dietary fats and enhances meat flavor. Lamb meat contains low fat content, resulting in less deposition of characteristic flavor precursors and milder odor, representing another important feature beyond tenderness. Mutton protein serves as an excellent source of human dietary protein, with Hu sheep muscle containing over 20% protein. Adult Hu sheep showed the highest crude protein content at 22.6%, exceeding Sunit 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-head mutton sheep (20.02%) [27]. Adult Hu sheep muscle ash content was 1.21%, higher than Henan Large-tailed Han sheep (0.91%) [19] and Tan sheep (1.05%) [28]. Adult Hu sheep muscle contained abundant calcium (5.56 mg/kg) and phosphorus (22.1 mg/kg). The measured routine nutritional components were rich, indicating excellent meat quality. Differences compared with most

Chinese mutton sheep breeds arise because main nutritional components vary by breed, sex, age, feeding level, and carcass location.

3.2 Amino Acid Content and Meat Quality Traits of Hu Sheep Muscle

Mutton amino acids provide balanced amino acids for human nutrition. For Hu sheep, essential amino acid contents in newborn, weaned, and adult sheep muscle reached 8.92%, 9.26%, and 9.66%, respectively, while non-essential amino acid contents reached 8.69%, 8.66%, and 9.48%. Adult Hu sheep essential and non-essential amino acid contents showed some differences compared with values of 8.79% and 11.49% measured by Chen Xuejun et al. [29], 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]), different ages of adult sheep (2 years vs. 4 years), or different feeding environments. Numerous studies have shown that serine, glutamic acid, glycine, isoleucine, leucine, alanine, and proline are flavor precursor amino acids, particularly glutamic acid, the main umami substance in meat. This study found abundant essential and umami amino acids in Hu sheep muscle, with high contents of important flavor precursors, especially glutamic acid (3.34%) and aspartic acid (1.80%), similar to Chen Xuejun et al.'s results for Hu sheep (3.30% and 1.94%) [29] and higher than those for Small-tailed Han sheep (2.48% and 1.23%) [26]. Although weaned Hu sheep muscle contained less glutamic acid than adults, its proportion in total amino acids was higher than in adults and newborn lambs. Therefore, while newborn lamb meat is tender, its taste is inferior to weaned lamb meat, which excels in both tenderness and flavor. Lysine, considered the first limiting amino acid for humans, showed the highest content across all ages, with the highest proportion in weaned lambs. This demonstrates the unique nutritional characteristics of Hu sheep meat and indicates that proper processing can produce rich, flavorful meat products, with weaned lamb meat being particularly nutritious.

3.3 Muscle Fiber Histological Characteristics and Meat Quality Traits of Hu Sheep

Muscle fiber diameter and density are closely related to meat quality. These parameters are important indicators affecting muscle tenderness and water-holding capacity. Breeds with finer muscle fibers and higher density show greater intramuscular fat deposition. As muscle fiber diameter increases, tenderness decreases. Generally, smaller fiber diameter and higher density indicate better meat quality. Additionally, sarcomere length, muscle fiber fragmentation index, and water-holding capacity also significantly affect tenderness. This study found differences in muscle fiber diameter and density among ages for the same body part, with younger animals showing better tenderness, as muscle fibers significantly hardened with age, consistent with conclusions from Zeng Yongqing et al. [31] and Gao Aiqin et al. [32] regarding age effects on sheep muscle fibers.

Muscle fiber diameter varied among body parts within the same age group, with

the longissimus dorsi showing the smallest diameter. Adult Hu sheep longissimus dorsi muscle fiber diameter was 26.22 μm , similar to the average value (26.77 μm) measured by Sun Wei et al. [33] for Hu sheep but smaller than Sunit sheep (42.81 μm) [21], Small-tailed Han sheep (28.84 μm) [22], Nanjiang Yellow goat (34.13 μm) [23], and Guanzhong dairy goat (38.10 μm) [24]. Thus, Hu sheep have relatively small muscle fiber diameter and high density compared with Sunit and Small-tailed Han sheep. China's numerous sheep breeds are widely distributed across different climatic conditions, feeding management systems, and forage resources, which may cause differences in muscle fiber histological characteristics even at the same age and body part. Tenderness significantly influences consumer purchasing decisions, allowing consumers to consider factors such as age and body part when selecting ideal mutton products.

In conclusion, Hu sheep muscle is rich in nutrients with complete amino acid composition. All routine nutritional components except moisture increase with age. From the perspective of proportions of important amino acids in total amino acids, weaned lamb meat offers greater nutritional value, umami, and aroma. Under the same feeding and management conditions, the nutritional characteristics and muscle fiber properties of Hu sheep are influenced by age and body part, with different muscle fiber diameters and densities observed across ages and body parts. Overall, weaned lamb meat is more tender and delicious.

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