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Original Research Articles

# A comparative analysis of muscles nutrient composition between cultured male and female black-spotted frogs (*Pelophylax nigromaculatus*)

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This study compared growth traits and hind-limb muscle nutritional composition between male and female cultured black-spotted frogs (*Pelophylax nigromaculatus*) to evaluate the influence of sex on production performance and product nutritional value. A total of 141 adult frogs (77 males, 64 females) were weighed at a farm in Changde, Hunan, and ~7 males and ~7 females were randomly selected for carcass and hind-limb weight measurement and muscle sampling. Three muscle samples per sex were analyzed for proximate composition, amino acid profile, and fatty acid composition; essential amino acid index (EAAI), amino acid score (AAS), and chemical score (CS) were calculated according to FAO/WHO and reference protein models. Female frogs had significantly greater body weight, carcass weight and hind-limb muscle mass than male frogs ($P < 0.05$). No significant sex differences were found in basic proximate components (moisture, crude fat, ash). Amino-acid analysis indicated that males generally had higher amino-acid contents, with several amino acids (alanine, histidine, arginine, isoleucine, leucine, valine, etc.) significantly higher in males than in females ($P < 0.05$). Despite these individual amino-acid differences, composite protein-quality indices (EAAI, AAS, CS) showed no clear differences between sexes; fatty-acid profiles also revealed no adverse or significant sex-related differences. Overall, sex had a significant effect on yield in black-spotted frogs but a limited effect on overall muscle nutritional composition. Except for certain amino acids, the nutritional value of meat from both sexes was similar; therefore, sex can be considered as a factor to improve yield and economic efficiency in breeding and farming practices.

## 1. INTRODUCTION

With rising consumer demand for high-protein, low-fat and functional aquatic products, aquaculture increasingly emphasizes improving product nutritional quality and production efficiency through optimized husbandry and feed formulation.[1,2] *Pelophylax nigromaculatus* (black-spotted frog) is widely farmed in parts of Asia for its rapid growth, high fecundity, hardiness, and tender meat, and thus holds significant economic and dietary value.[3,4] Previous studies have shown that dietary supplementation with plant functional components (e.g., ginseng polysaccharides and ginsenosides) can significantly enhance growth rate and immune status in black-spotted frogs, providing practical evidence for nutritional interventions to improve farming outcomes.[5]

Sexual dimorphism is widespread among many aquaculture and amphibian species, profoundly affecting growth patterns, energy allocation, and body composition.[6,7] Typically, females accumulate greater nutrient and energy reserves to support ovarian development and spawning, leading in some species to larger body size and higher lipid stores; males, by contrast, may allocate more resources to muscle development and reproductive behaviors (e.g., courtship, calling, territoriality) to increase mating success, resulting in sex-specific specialization in muscle quality or particular organs.[8-10] In amphibians, such sex differences extend beyond body size to muscle fiber type, metabolic pathways, energy utilization efficiency, and endocrine reg-

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ulation.[11,12] For example, *Xenopus laevis* exhibits distinct amino acid and fatty acid profiles relative to fish and also shows sex-specific differences, and other studies have reported sex-related variation in muscle metabolism and fiber composition that influence locomotor performance and energy storage.[13]

However, systematic data on amino acid and fatty acid composition of black-spotted frog muscle—particularly comparative analyses between sexes—remain scarce, limiting refined management and processing strategies for this species. The study quantifies sex-specific differences in growth traits and hind-limb muscle nutrient composition (proximate composition, amino acid profile, and fatty acid profile) in farmed black-spotted frogs. The results provide empirical support for implementing sex-differentiated husbandry, optimizing feed formulations, and developing selective-breeding strategies, and they establish a foundation for subsequent mechanistic research and validation with larger sample sizes.

## 2. MATERIALS AND METHODS

### 2.1. ANIMALS AND SAMPLING COLLECTION

A total of 141 adult black-spotted frogs (77 males and 64 females) were sampled from a single pond at a commercial aquaculture farm in Changde, Hunan, China. All frogs were reared under the farm's routine conditions and fed a commercial diet (TONGWEI CO., LTD., Hunan, China). Body weight was recorded for each individual. From this cohort, approximately seven males and seven females were randomly selected for slaughter to determine carcass weight and hind-limb weight. Biochemical analyses (proximate composition, amino-acid profile, and fatty-acid profile) were performed on individual samples: hind-limb muscle was taken from 3 females and 3 males. All procedures complied with institutional animal care and use guidelines (Approval No. JSDX-2024-005). Frogs were anaesthetized with MS-222 (tricaine methane sulfonate; Sigma-Aldrich, Germany) at 100 mg/L and euthanized prior to sampling.

### 2.2. CHEMICAL COMPOSITION ANALYSIS

Moisture, crude lipid, crude protein, and ash contents of hind limb muscles in frogs were assayed by AOAC (2023).[14] Moisture was measured by an electrothermal constant temperature drying box (202-OAB, Taisite Instrument, Tianjin, China) at 105 °C until at a constant weight; crude protein (Nitrogen × 6.25) was determined by the micro-Kjeldahl method using an Auto Kjeldahl System (K9840, Hanon Technologies, Shandong, China). Crude lipid was determined by Solvent extraction using fat meter (SZF-06G, Shanghai Cany Precision Instrument Co. Ltd., Shanghai, China). Ash was determined gravimetrically until reaching a constant weight in a muffle furnace at 550 °C.

### 2.3. AMINO ACID ANALYSIS

The amino acid compositions in hind limb muscles of frogs were determined using HPLC amino acid analysis, referring to the method described by Tumbarski et al.[15] The samples (Muscle freeze-dried powder) were totally hydrolyzed up to free amino acids with HCL of 6 mol/L. The hydrolysates were then analyzed using Agilent 1260 Infinity II LC System (CA, USA) equipped with DAD detector and Spolar C18 S5 column (5 μm, 4.6 × 250 mm, Shiseido Company, Kyoto, Japan). Amino acid content was analyzed by the external standard method using the Agilent amino acid standard (Agilent Technologies Inc.).

### 2.4. FATTY ACID ANALYSIS

The fatty acid distribution in hind limb muscles of frogs was analyzed by gas-liquid chromatography as previously described by Chiu et al.[16] Briefly, fatty acid methyl esters were prepared by direct trans-esterification and separated by Thermo Trace 1310 gas chromatograph coupled to an ISQ single-quadrupole mass spectrometer (ThermoFisher Scientific, MA, USA). Individual fatty acids were identified by comparing their peak retention times with those of known pure certified standards (Sigma-Alrich, Missouri, USA), Dionex™ Chromeleon™ CDS software (ThermoFisher Scientific, MA, USA) was used for data acquisition and processing. The level of each individual fatty acid represents the percentage of the total area of the identified fatty acids.

### 2.5. NUTRITIONAL EVALUATION

Amino acid composition was evaluated using the essential amino acid index (EAAI). The relative nutrition index was calculated using the formula provided by the FAO/WHO.[17] Furthermore, the egg protein model proposed by the Chinese Academy of Preventive Medicine's Institute of Nutrition and Food Hygiene for nutritional evaluation was used to calculate the amino acid score (AAS) and chemical score (CS) in accordance with the Dietary Protein Quality Evaluation in Human Nutrition[18] as follows:

Essential amino acid index (EAAI): $EAAI = \sqrt[n]{\frac{a_1}{a_1'} \times \frac{a_2}{a_2'} \times \ldots \times \frac{a_n}{a_n'}} \times 100$

where $a_1, a_2, \ldots, a_n$represent the content of essential amino acids in the test protein, $a_1', a_2', \ldots, a_n'$represent the corresponding essential amino acid content in the reference protein (whole egg protein), and $n$is the number of essential amino acids.

Amino acid score (AAS): $AAS = \frac{\text{Content of amino acid in test protein (mg/g)}}{\text{Content of the same amino acid in reference protein (mg/g)}} \times 100$

Chemical score (CS): $CS = \frac{\text{Content of limiting amino acid in test protein (mg/g)}}{\text{Content of the same amino acid in reference protein (mg/g)}} \times 100$

The reference protein used was whole egg protein with the following essential amino acid pattern (mg/g protein): Ile 54, Leu 86, Lys 70, Met+Cys 57, Phe+Tyr 93, Thr 47, Trp 17, Val 66, His 22.

### 2.6. STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of the mean (SEM). SPSS 25.0 software was used to analyze all data collected in this study. The difference in samples was analyzed

**Table 1. Growth performance and carcass characteristics of male and female cultured black-spotted frogs**

| Items | Male | female |
|---|---|---|
| Body weight (BW, g) | 30.48±7.46 $^{B}$ | 39.29±11.52$^{A}$ |
| Carcass weight (CW, g) | 28.28±3.29 $^{b}$ | 33.85±4.71$^{a}$ |
| Hind limbs weight (HLW, g) | 9.99±1.05 $^{B}$ | 13.81±1.7 $^{A}$ |
| CW/BW (%) | 87.68±1.45$^{A}$ | 61.54±2.36$^{B}$ |
| HLW/BW (%) | 29.90±1.01$^{A}$ | 25.16±1.35$^{B}$ |

Note: Data are presented as mean ± SEM (n = 7). Different lowercase letters indicate significant differences ($P$< 0.05), and different capital letters indicate extremely significant differences ($P$ < 0.01).

**Table 2. Comparison of general nutritional components in cultured male and female frog legs muscles (wet weight basis, g/100g)**

| Items | Male | Female |
|---|---|---|
| Crude protein | 19.07±0.7 | 18.6±1.41 |
| Crude fat | 1.60±0.30 | 1.67±0.35 |
| Moisture | 76.87±0.87 | 78.20±1.18 |
| Ash | 1.07±0.24 | 0.93±0.14 |

Note: Data are presented as mean ± SEM (n = 3).

using independent samples t test, with a significance level of $P$ < 0.05, and a highly significant level of $P$ < 0.01.

## 3. RESULTS

### 3.1. GROWTH PERFORMANCE COMPARISON

The comparison of growth performance revealed significant sex-related differences in body and carcass traits of cultured black-spotted frogs (**Table 1**). Females had a significantly greater body weight (BW) at 39.29 ± 11.52 g compared with males at 30.48 ± 7.46 g ($P$<0.01). Absolute carcass weight (CW) and hind-limb weight (HLW) were also significantly higher in females (CW: 33.85 ± 4.71 g vs 28.28 ± 3.29 g, $P$<0.05; HLW: 13.81 ± 1.70 g vs 9.99 ± 1.05 g; $P$<0.01). However, males outperformed females in relative indices: males exhibited a markedly higher carcass yield (CW/BW) of 87.68±1.45 % versus 61.54 ± 2.36 % in females ($P$<0.01), and a higher hind-limb yield (HLW/BW) of 29.90±1.01 % compared with 25.16±1.35 % in females ($P$<0.01).

### 3.2. PROXIMATE COMPOSITION COMPARISON

Analysis of proximate composition in hind-limb muscle showed no significant differences between sexes (**Table 2**). Males had slightly higher crude protein content than females (19.07 ± 0.7 % vs 18.6 ± 1.41 %), while females had higher moisture content (78.2±1.18 % vs 76.87±0.87 %). Crude fat and ash contents were similar between sexes.

### 3.3. AMINO ACID COMPOSITION COMPARISON

Analysis of amino acid composition in hind-limb muscle revealed that males had significantly higher contents of several amino acids compared to females (**Table 3**). Total amino acids (TAA) were higher in males (20.74±0.87%) than in females (19.35±0.03%). Essential amino acids (EAA) were significantly higher in males than in females (8.25±0.31% vs 7.70±0.19%, $P$<0.05). Individual amino acids including alanine, histidine, arginine, isoleucine, leucine, and valine were significantly higher in males ($P$<0.05). However, the ratios of EAA/TAA and flavor amino acids to total amino acids (FAA/TAA) showed no significant differences between sexes, indicating similar amino acid composition patterns.

### 3.4. AMINO ACID SCORING ANALYSIS

Evaluation of black-spotted frog muscle protein based on the FAO/WHO essential amino acid pattern revealed minimal differences between sexes (**Table 4**). In amino acid scoring (AAS), lysine had the highest score (1.33 for males, 1.32 for females), while methionine + cystine was the limiting amino acid with the lowest score (0.31 for both sexes). Chemical scoring (CS) showed lysine scores exceeding 1.70, phenylalanine + tyrosine and isoleucine scores above 1.10, while methionine + cystine remained the lowest (0.55). Essential amino acid index (EAAI) was 75.55 for males and 75.2 for females, showing negligible difference.

### 3.5. FATTY ACID COMPOSITION ANALYSIS

Analysis of fatty acid composition in hind-limb muscle of black-spotted frogs revealed overall similar fatty acid profiles between sexes with some compositional differences (**Table 5**). Linoleic acid ($C_{18}$:$_2\omega_6$c) was the predominant fatty acid, accounting for 33-36% of total fatty acids, followed by oleic acid ($C_{18}$:$_1\omega_9$c) at 21-23%. Polyunsaturated fatty acids (PUFA) had the highest content, with females slightly higher than males (43.67±2.23 % vs 42.35±7.40 %), primarily contributed by linoleic acid. Monounsaturated fatty acids (MUFA) content was similar between sexes (ap-

**Table 3. Amino acid content in hind-limb muscle of cultured male and female black-spotted frogs (g/100 g wet weight)**

| Amino acids | Male | Female |
|---|---|---|
| **Non-essential amino acids** | | |
| Aspartic acid# | 2.17±0.11 | 2.02±0.03 |
| Glutamic acid# | 3.38±0.17 | 3.19±0.05 |
| Serine | 0.94±0.04 | 0.88±0.01 |
| Glycine# | 0.98±0.06 | 0.93±0.12 |
| Alanine# | 1.23±0.05$^{a}$ | 1.15±0.02$^{b}$ |
| Proline | 0.78±0.04 | 0.75±0.07 |
| Tyrosine | 0.73±0.07 | 0.70±0.02 |
| **Semi-essential amino acids** | | |
| Histidine | 0.83±0.05$^{a}$ | 0.69±0.03$^{b}$ |
| Arginine | 1.45±0.01$^{A}$ | 1.35±0.02$^{B}$ |
| **Essential amino acids** | | |
| Isoleucine* | 1.05±0.03$^{a}$ | 0.98±0.02$^{b}$ |
| Leucine* | 1.77±0.04$^{a}$ | 1.63±0.04$^{b}$ |
| Phenylalanine* | 0.86±0.03 | 0.80±0.01 |
| Lysine* | 2.17±0.10 | 2.03±0.06 |
| Threonine* | 0.93±0.06 | 0.88±0.01 |
| Valine* | 1.03±0.04$^{a}$ | 0.95±0.01$^{b}$ |
| Methionine* | 0.45±0.05 | 0.42±0.04 |
| Cystine | N.D. | N.D. |
| **Summary indices** | | |
| Total amino acids (TAA) | 20.74±0.87 | 19.35±0.03 |
| Essential amino acids (EAA) | 8.25±0.31$^{a}$ | 7.70±0.19$^{b}$ |
| Flavor amino acids (FAA) | 7.75±0.38 | 7.29±0.06 |
| EAA/TAA (%) | 39.81±0.19 | 39.77±0.93 |
| FAA/TAA (%) | 37.37±0.31 | 37.65±0.36 |

Note: Data are presented as mean ± SEM (n = 3).
N.D. = Not detected.
Different lowercase letters indicate significant differences ($P$< 0.05), and different capital letters indicate extremely significant differences ($P$ < 0.01).
* Represented essential amino acid. # FAA, flavor amino acids

**Table 4. Comparative nutritional indices of cultured male and female black-spotted frog muscle and the adult essential amino acid (EAA) model recommended by FAO/WHO**

| Amino acids | AAS | | CS | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Isoleucine | 0.86 | 0.85 | 1.14 | 1.13 |
| Leucine | 0.89 | 0.88 | 1.09 | 1.07 |
| Lysine | 1.33 | 1.32 | 1.72 | 1.71 |
| Methionine + cystine* | 0.31 | 0.31 | 0.55 | 0.55 |
| Phenylalanine + tyrosine | 0.76 | 0.77 | 1.13 | 1.14 |
| Threonine | 0.86 | 0.87 | 1.00 | 1.01 |
| Valine | 0.68 | 0.67 | 0.90 | 0.88 |
| EAAI | | | 75.55 | 75.2 |

Note: *Shoulder markers indicate restricted amino acids.
AAS, the amino acid score; CS, chemical score; EAAI, essential amino acid index.

proximately 28-29%), while saturated fatty acids (SFA) had the lowest content with no significant difference between sexes (approximately 18%). Total essential fatty acids were higher in females than males (36.57±1.50 % vs 34.09±4.92

**Table 5. Fatty acid composition and nutritional evaluation of hind-limb muscle lipids in cultured male and female black-spotted frogs (g/100 g)**

| Fatty acids | Male | Female |
|---|---|---|
| **Saturated fatty acids** | | |
| Pentadecanoic acid ($C_{15:0}$) | N.D. | 0.24±0.08 |
| Palmitic acid ($C_{16:0}$) | 12.45±2.15 | 13.05±0.45 |
| Stearic acid ($C_{18:0}$) | 5.51±1.13 | 4.65±0.40 |
| Lignoceric acid ($C_{24:0}$) | 0.45±0.10 | 0.62±0.15 |
| **Monounsaturated fatty acids** | | |
| Palmitoleic acid ($C_{16:1}$) | 2.98±1.43 | 3.16±1.06 |
| Oleic acid ($C_{18:1}\omega_{9}c$) | 21.61±2.88 | 22.78±4.51 |
| Gondoic acid ($C_{20:1}$) | 0.57±0.08 | 0.56±0.06 |
| Erucic acid ($C_{22:1}\omega_{9}$) | 2.53±0.64 | 2.48±0.81 |
| Nervonic acid ($C_{24:1}$) | 0.31±0.10 | 0.34±0.16 |
| **Polyunsaturated fatty acids** | | |
| Linoleic acid ($C_{18:2}\omega_{6}c$) | 33.17±4.81 | 35.72±1.49 |
| α-Linolenic acid ($C_{18:3}\omega_{3}$) | 0.92±0.12 | 0.85±0.02 |
| Eicosadienoic acid ($C_{20:2}$) | 0.43±0.11 | 0.49±0.15 |
| Dihomo-γ-linolenic acid ($C_{20:3}\omega_{6}$) | 0.57±0.13 | 0.60±0.19 |
| Arachidonic acid ($C_{20:4}\omega_{6}$) | 1.16±0.43 | 1.00±0.41 |
| EPA ($C_{20:5}\omega_{3}$) | 0.91±0.23 | 0.83±0.30 |
| DHA ($C_{22:6}\omega_{3}$) | 5.19±1.64 | 4.18±0.80 |
| **Summary** | | |
| Total fatty acids | 88.77±13.17 | 91.54±4.30 |
| Saturated fatty acids | 18.41±2.78 | 18.56±1.05 |
| Monounsaturated fatty acids | 28.01±4.34 | 29.31±4.55 |
| Polyunsaturated fatty acids | 42.35±7.40 | 43.67±2.23 |
| Essential fatty acids | 34.09±4.92 | 36.57±1.50 |
| EPA+DHA | 6.10±1.87 | 5.01±1.06 |

Note: Data are presented as mean ± SEM (n = 3).
N.D. = Not detected.

%), whereas EPA+DHA content was slightly higher in males (6.10±1.87 % vs 5.01±1.06 %).

## 4. DISCUSSION

This study systematically compared growth performance and muscle nutritional composition between male and female black-spotted frogs, revealing that females exhibited significantly greater body weight, carcass weight, and hind limb muscle mass than males. These findings are consistent with the widespread phenomenon of sexual size dimorphism (SSD) in amphibians, where females are typically larger than males. Such patterns are generally attributed to the higher reproductive demands of females, as increased body size provides greater abdominal capacity for carrying and producing more eggs, thus enhancing reproductive success.[19,20] Similar observations have been reported in other anuran species, such as the Asiatic toad (*Bufo gargarizans*) and *Kaloula rugifera*, where females are generally larger, while males may exhibit specialized features in limb structure or muscle mass to facilitate behaviors like amplexus, calling, or territorial competition.[21]

The muscle nutrition analyses showed that, although several individual amino acids (alanine, histidine, arginine, isoleucine, leucine, valine, etc.) were significantly higher in males ($P < 0.05$), total amino-acid content and crude protein only displayed a slight, non-significant male tendency. More importantly, composite protein-quality indices (EAAI, AAS, CS) did not differ markedly between sexes, indicating that despite differences in certain amino acids, the balance of essential amino acids and overall protein nutritional value of hind-limb muscle are comparable between males and females.[22,23] Biologically, this pattern is consistent with sex-specific resource allocation: females tend to accumulate somatic and reproductive reserves, resulting in significantly greater body weight, carcass weight and hind-

limb muscle mass, while males' advantage in certain amino acids may reflect subtle functional adjustments of muscle related to locomotion or reproductive behaviors.[24-26] Because the sample size for nutritional assays was limited, the observed differences are presented as qualitative trends; resolving causal links among sex, nutrition, and phenotype will require larger, multi-site, and multi-timepoint sampling combined with muscle histology, metabolic enzyme activity assays, and endocrine profiling.

Fatty-acid profiles were essentially equivalent in male and female frogs, with no clear sex-related differences in major SFA, MUFA, PUFA, or n-3/n-6 balance. This similarity suggests that muscle lipid composition in farmed specimens is driven primarily by diet and systemic lipid metabolism rather than by sex per se.[27,28] Nutritionally, the absence of adverse sex differences implies that lipid-related health attributes of the meat are effectively comparable between sexes under the examined conditions.

In conclusion, females provided higher yield (body, carcass, and hind-limb muscle mass), whereas intrinsic muscle nutritional composition (protein quality and fatty-acid profile) was similar between sexes. For production optimization, sex should be considered to maximize yield; for nutritional quality, meat from either sex can be treated as broadly equivalent. Further studies incorporating muscle histology, metabolic markers, and multi-site sampling would help elucidate the physiological basis of the slight amino-acid tendencies observed.

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AUTHOR CONTRIBUTIONS

Formal analysis: Rong-Hua Wang. Data curation: Rong-Hua Wang. Funding acquisition: Rong-Hua Wang. Writing – original draft: Rong-Hua Wang, Qing Tan. Investigation: Si-han Luo, Jing-ye Ma, Lan-jun Cui, Hui Tang. Methodology: Si-han Luo, Jing-ye Ma, Lan-jun Cui. Validation: Qing Tan, Ke-jun Liu. Supervision: Ke-jun Liu. Writing – review & editing: Jin-Long Wang.

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki, and all procedures used in this experiment were approved by the Hunan University of Arts and Science Institutional Animal Care and Use Committee and were performed in accordance with approved protocols, protocol code: JSDX-2024-005 and date of approval: 2024-03-10.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

The data that has been used is confidential.

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