Characterizing Sections of Elk Velvet Antler by pH and Mineral, Fatty Acid, and Amino Acid Composition

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Abstract This study was conducted to determine the composition change in different sections of elk velvet antler at 90 days. The following parameters were analyzed: moisture, crude protein, crude fat, crude ash, crude fiber, pH, minerals (Ca, P, K, Mg, Fe, Mn, Zn, Cu, and Pb), amino acids, and fatty acids. Dry matter, crude fiber, and crude ash contents were higher in the base of the antlers and lower in the tip. In contrast, crude protein and crude fat contents were highest in the tip. Moisture content was high in the upper and medium sections of the antlers, but the difference was not significant. Calcium (Ca), phosphorus (P), and magnesium (Mg) contents were high in the base of the antlers, potassium (K) content was high in the tip of the antlers, and zinc (Zn) content showed no difference between antler sections. Saturated fatty acid content was highest in the base of the antlers, whereas unsaturated fatty acid content was highest in the tip. Among unsaturated fatty acids, monounsaturated fatty acid content was high in the tip of the antler, whereas polyunsaturated fatty acid content was high in the upper section of the antler. Essential amino acid content was highest in the upper section of the antler, whereas non-essential amino acid content tended to be high in the tip of the antler, and essential amino acid and polyunsaturated fatty acid contents tended to be high in the upper section of the antler. The tip of the antler had the highest nutrient content. In order to prevent cardiovascular disease, consumption of a certain amount of polyunsaturated fatty acids and amino acids from the upper section of the antler could be beneficial in terms of pharmacological efficacy.

Keywords: deer, velvet antler, section, fatty acid, amino acid

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1. Introduction

Deer are nomenclaturally subcategorized into the following five subfamilies: Hydropotinae, represented by water deer, Muntiacininae and Cervinae, represented by Formosan deer and elk, Odocolinae, represented by reindeer, and Moschiniæ; however, deer have also been classified differently according to different perspectives [1,2,3]. In Korea, Formosan deer have been imported from Japan and Taiwan since the 1950s, followed by import of red deer and elk from New Zealand and North America, which initiated deer breeding in Korea [3]. According to data aggregated in 2018, there are approximately 1,700 deer farms in Korea, with a breeding scale of more than 26,000 deer, representing a gradual decreasing trend [4]. The most bred deer species in Korea are the Formosan deer, red deer, and elk, among which elks produce a large number of antlers and their breeding frequency is currently increasing.

A wide range of research has been conducted on deer, including research on their ecology [5], phylogenetics [6], venison [7,8], and velvet antlers [9,10]. Studies on velvet antlers have investigated their regenerative capability [11,12], efficacy [13,14], and components [10]. Moreover, velvet antlers have long been used for medicinal purposes and have even been recorded in the Donguibogam (Principles and Practice of Eastern Medicine). Known uses of velvet antlers include prevention of osteoporosis [15,16], increased immune activation [17], wound healing [18], suppression of oxidative action [13,14,19], and suppression of platelet aggregation [20]. Velvet antler is also used as a functional food, and research is being conducted on the specific components of velvet antler. Velvet antler contains minerals, fatty acids, and amino acids, as well as crude protein, crude fat, and crude ash; however, the component content is thought to differ by antler section, with more crude fat, crude protein, essential fatty acids, and essential amino acids in the tip and upper part of the antler, and more crude ash, Ca, and P in the base of the antler [14,21,22].
Amino acids are a basic unit of proteins; a lack of essential amino acids in food may adversely affect the physiological functions of the human body. Supplements of essential amino acids have been shown to improve depression symptoms, muscular dysfunction, and daily activities in geriatric patients, ultimately enhancing their quality of life [23]. Fatty acids play various roles, including gene regulation [24], and they are the main component of the bilayer in the cell membrane [25]. In addition to being an important source of energy, polyunsaturated fatty acids are effective at preventing cardiovascular disease [26].

As the component content and efficacy may depend on the section of velvet antler, additional analysis is required to determine the component content in each section of velvet antler. Such data could ensure more effective use of velvet antler as a functional food and medicine and provide more information to consumers. Therefore, this study was conducted to analyze the mineral, amino acid, and fatty acid content and to determine general components, such as moisture, crude fat, crude protein, crude fiber, crude ash, and pH, in different sections of elk velvet antler.

2. Materials and Methods

2.1. Collection of Velvet Antlers

In order to collect velvet antler samples, four elks (Cervus canadensis) were bred at the Animal Genetic Resources Research Center, National Institute of Animal Science (NIAS), Korea. The individual elks whose antlers were to be cut at 90 days after casting were selected, and the experiment was conducted on the applicable date after casting. The velvet antler was cut according to a modified previously described method [22]. The cut velvet antler was washed immediately with water, packed in plastic wrap, and kept in a freezer at a temperature of -20°C. The main stem and lateral branch were separated by slicing, and the main stem was quadrisected into tip, upper, middle, and base sections. The tip was the uppermost part, the upper section extended from the bottom of the tip section to the part where the third branch comes out, the middle section extended from the second branch to the part where the third branch comes out, and the base extended from the main branch to the bottom of the part where the second branch comes out. The samples were then collected prior to conducting the experiment. Each section of velvet antler was sliced to 1-1.5 mm thickness with a cutter without removing the hairs (skin) from the velvet antler. The sliced samples were freeze-dried and kept in a freezer at a temperature of -20°C. This study did not use lateral branches, in accordance with a previous study [22].

2.1. Velvet Antler Component Analysis

2.2.1. Proximate Composition

To obtain the proximate composition, the moisture content was analyzed using the normal pressure drying method by heating at 105°C, the crude fat content was analyzed using the Soxhlet extraction method, the crude ash content was analyzed using the dry ashing method at 550°C, the crude protein content was analyzed using the Kjeldahl method, and the crude fiber content was analyzed using a fiber analyzer (ANKOM A200 Technology) according to the AOAC method (1995). Dry matter indicated the content of dry matter after freeze-drying.

2.2.2. pH

The pH was measured with a pH meter (Orion Star A211; Thermo Fisher Scientific, Inc., Waltham, MA, USA) after homogenizing 1 g of sample with 9 mL of distilled water (PolyTron® PT-2500E; Kinematica, Malters, Switzerland).

2.2.3. Minerals

The mineral components were analyzed using an inductively coupled plasma spectrometer (OPTIMA 7300 DV; PerkinElmer, Shelton, CT, USA) after pretreating samples through the nitric acid wet technique.

2.2.4. Fatty Acids

The fatty acid composition was analyzed according to the method used in a previous study [8]. Folch solution (chloroform:methanol = 2:1) was used to extract fat, and samples were collected in a test tube. Then, 1.5 mL of 0.5 N NaOH methanol solution was added, briefly vortexed, and heated at 100°C for 5 min. The solution was then cooled in cold water and 2 mL of 10% BF₃-methanol solution (Supelco, Bellefonte, PA, USA) was added. After vortexing, the solution was reheated at a temperature of 100°C for 2 min and then cooled. To extract fatty acid methyl ester (FAME), 2 mL of iso-octane was added and vortexed for 1 min. One milliliter of saturated NaCl solution was added to the solution, which was then vortexed for 1 min and layer-separated with a centrifugal separator (2,000 rpm, 3 min, 15°C). The supernatant was used for subsequent gas chromatography analysis.

2.2.5. Amino Acids

The amino acid content was analyzed by the ninhydrin method using an amino acid analyzer after decomposing the specimen with nitric acid and hydrochloric acid and diluting the filtered liquid sample. Forty milliliters of 6N HCl was added to the sample and nitrogen gas was injected. The sample was capped and after hydrolysis at 100°C for 24 h transferred to an evaporation flask. Hydrochloric acid was removed with a rotary evaporator at 50°C; once evaporation was completed, the bottle was washed with distilled water and its content was transferred to the evaporation flask and evaporated again three times. Subsequently, a buffer solution or distilled water for sample dilution was added in small quantities to the evaporation flask and dried. The amino acids were then dissolved and filtered through No 5 B filter paper to obtain 50 mL of sample. Sulfur-containing amino acids were treated with performic acid before acid hydrolysis and transformed into cysteic acid and methionine sulfone. After hydrolysis with 6N HCl, 20 mL of performic acid was added to the bottle, and the bottle was placed in a freezer at less than 5°C. The following morning, the bottle was connected to the rotary evaporator and all volatile substances except the sample were vaporized at a temperature of 50°C. Thereafter, 6N HCl was added and the sample was hydrolyzed at a temperature of 110°C for 24 h, similar to other amino acid analyses.
2.3. Statistical Analysis

All results were analyzed using one-way ANOVA to obtain the value of each treatment plot. The Tukey test was used to conduct significant difference tests at the 5% level (Statistics Analytical System, SAS program, v. 9.4.; SAS Institute Inc., Cary, NC, USA 2008). The analysis results are indicated as the mean ± SD.

3. Results and Discussion

3.1. Proximate Composition

The results of the general components of elk velvet antler at 90 days after casting are shown in Table 1. Dry matter content was highest (41.25%) in the base and lowest (21.99%) in the tip. Moisture was highest (3.59%) in the middle and lowest (1.88%) in the base; however, the difference was not significant, and the moisture content was lower than that reported by [21]. Crude protein content was high (67.48%) in the tip and low (49.18%) in the base. However, the difference was not significant between sections. Significant differences were observed in dry matter, crude protein, crude fat, and crude fiber content between sections, consistent with these previous findings; significant difference was observed between sections.

Table 1. Proximate Composition and Dry matter of Elk Velvet Antler

| Component (%)       | Tip          | Upper        | Middle       | Base         | p-value |
|---------------------|--------------|--------------|--------------|--------------|---------|
| Moisture            | 2.31 ± 0.98  | 3.50 ± 0.87  | 3.59 ± 1.26  | 1.88 ± 0.55  | 0.06    |
| Crude protein       | 67.48 ± 2.105 | 64.30 ± 2.045 | 56.66 ± 2.425 | 49.18 ± 0.455 | <0.01   |
| Crude fat           | 5.04 ± 1.635 | 2.32 ± 0.255 | 2.55 ± 0.225 | 2.51 ± 0.205 | <0.01   |
| Crude fiber         | 1.32 ± 0.555 | 2.43 ± 0.690 | 2.56 ± 0.550 | 3.85 ± 0.300 | <0.01   |
| Crude ash           | 17.02 ± 3.845 | 26.85 ± 2.800 | 36.09 ± 1.935 | 44.66 ± 0.695 | <0.01   |
| Dry matter          | 21.99 ± 4.025 | 30.46 ± 2.135 | 38.86 ± 4.635 | 41.25 ± 5.805 | <0.01   |

Data are means ± SD (N = 4).
** Different letters in a row indicate significant differences (p < 0.05).

3.2. pH

The pH results of elk velvet antler 90 days after casting are shown in Table 2. The pH range was 7.76-7.87, and no significant difference was observed between sections.

Table 2. pH Values of Elk Velvet Antler

| pH     | Tip          | Upper        | Middle       | Base         | p-value |
|--------|--------------|--------------|--------------|--------------|---------|
|        | 7.76 ± 0.15  | 7.85 ± 0.13  | 7.86 ± 0.07  | 7.87 ± 0.11  | 0.53    |

Data are means ± SD (N = 4).

3.3. Minerals

The mineral content in elk velvet antler 90 days after casting is shown in Table 3. The Ca content was 45.98 g/kg in the tip, 105.72 g/kg in the upper section, 135.07 g/kg in the middle section, and 140.83 g/kg in the base (p < 0.05). The P content was 26.45 g/kg in the tip, 55.94 g/kg in the upper section, 72.08 g/kg in the middle section, and 71.99 g/kg in the base (p < 0.05). Ca and P were the most abundant minerals in velvet antler, similar to the results of [14], and their content was high in the base, similar to other studies [10,22,32]. However, the high Ca and P contents in the middle section was not reported in previous research. The K content was 8.29 g/kg in the tip, 4.20 g/kg in the upper section, 2.65 g/kg in the middle section, and 1.39 g/kg in the base. Unlike Ca and P, K content was higher in the tip than in other sections (p < 0.05), which was similar to the results of [14]. The Mg content was 1.46 g/kg in the tip, 2.17 g/kg in the upper section, 2.82 g/kg in the middle section, and 2.95 g/kg in the base. The Mg content increased toward the base, showing a similar tendency to that observed for Ca and P. The Fe content was 217.63 mg/kg in the tip, 689.56 mg/kg in the upper section, 412.09 mg/kg in the middle section, and 65.34 mg/kg in the base. The relative Fe content was similar to that reported in previous studies [10,14]; however, Fe content was lower in the tip than in the upper and middle sections. Zn and Mn contents exhibited no significant difference between sections. Pb was not detected, and Cu was detected in only some individuals. Ca and P showed a pattern similar to the ossification process of mammal cartilage reported in a previous study [33], which is attributed to the fact that Ca and P, which are key elements for the ossification process, increase in content toward the base. Ca and vitamin D intake and regular body activity are important for preventing osteoporosis [34,35]; therefore, a high Ca content in velvet antler sections is considered to be beneficial for preventing osteoporosis.
3.4. Fatty Acids

The results of the fatty acid composition analysis of elk velvet antler 90 days after casting are shown in Table 4. In the tip, the palmitic acid content was 29.27%, the oleic acid content was 24.08%, the stearic acid content was 13.26%, the linoleic acid content was 7.14%, and the arachidonic acid content was 8.72%. Fatty acid content was highest in the tip, similar to other studies [21,22,31]. Furthermore, the unsaturated fatty acid content was high in the tip, whereas the saturated fatty acid content was lowest in the tip and high in other sections, which showed a different tendency from that reported in [22] and [36]. Unsaturated fatty acids are divided into monounsaturated fatty acids with one double bond and polyunsaturated fatty acids with more than two double bonds; the content of the former was high (35.35%) in the tip, whereas the content of the latter was high (22.78%) in the upper section. Among the analyzed fatty acids, eicosapentaenoic acid (EPA) was detected in only some individuals and ω-linolenic acid, eicosenoic acid, and docosahexaenoic acid were not detected. Among linolenic acids, ω-linolenic acid was not detected, whereas α-linolenic acid was detected in all sections. A previous study [37] compared the fatty acid content at 40 and 60 days and reported a similar trend to the one observed here at 40 days, i.e., there was no difference between saturated fatty acid and unsaturated fatty acid contents. However, at 60 days, the saturated fatty acid content had increased but the unsaturated fatty acid content had decreased, resulting in a considerably higher relative saturated fatty acid content [37]. According to [26], replacing saturated fatty acids with polyunsaturated fatty acids rather than with monounsaturated fatty acids or carbohydrates is effective for preventing coronary artery disease (CAD). Therefore, the velvet antler sections most appropriate for preventing cardiovascular disease are considered to be the tips, where the saturated fatty acid content is low and the unsaturated fatty acid content is high, and the upper section, which has higher polyunsaturated fatty acid and lower saturated fatty acid content compared to the middle and base sections.

### Table 3. Mineral Composition of Elk Velvet Antler

| Component   | Section | Tip          | Upper         | Middle        | Base          | p-value |
|-------------|---------|--------------|---------------|---------------|---------------|---------|
| Ca (g/kg)   |         | 45.98 ± 16.50a | 105.72 ± 8.07a | 135.07 ± 14.44a | 140.83 ± 53.88a | <0.01   |
| P (g/kg)    |         | 26.45 ± 13.34a | 55.94 ± 3.92a  | 72.08 ± 8.33a  | 71.99 ± 36.18a  | 0.02    |
| K (g/kg)    |         | 8.29 ± 1.44a   | 4.20 ± 0.14a   | 2.65 ± 0.37a   | 1.39 ± 0.08a    | <0.01   |
| Mg (g/kg)   |         | 1.46 ± 0.41a   | 2.17 ± 0.23a   | 2.82 ± 0.44a   | 2.95 ± 0.88a    | <0.01   |
| Fe (mg/kg)  |         | 217.63±105.57b | 689.56±88.03b  | 412.09±183.89b | 65.34±26.19b    | <0.01   |
| Zn (mg/kg)  |         | 44.44±24.76    | 47.53±5.80     | 50.03±5.41     | 43.96±26.73     | 0.96    |
| Mn (mg/kg)  |         | 1.19±0.48      | 1.19±0.22      | 1.17±0.06      | 0.73±0.52       | 0.27    |
| Pb (mg/kg)  |         | 0.00           | 0.00           | 0.00           | 0.00           | 0.74    |
| Cu (mg/kg)  |         | 0.69±1.37      | 1.44±2.87      | 0.92±1.85      | 0.00           |         |

Data are means ± SD (N = 4). Different letters in a row indicate significant differences (p < 0.05).

### Table 4. Fatty Acid Composition of Elk Velvet Antler

| Component (%) | Section | Tip | Upper | Middle | Base | p-value |
|---------------|---------|-----|-------|--------|------|---------|
| CI4:0 (Myristic acid) |         | 3.58 ± 0.82a | 5.41 ± 0.35a | 6.20 ± 1.43a | 7.51 ± 2.11a | 0.01    |
| CI6:0 (Palmitic acid)   |         | 29.27 ± 1.64a | 34.87 ± 1.41a | 39.92 ± 3.29a | 43.77 ± 3.74a | <0.01   |
| CI6:1n7 (Palmitoleic acid) |     | 3.97 ± 0.79a | 1.33 ± 0.25a  | 1.66 ± 0.65a  | 1.36 ± 0.54a  | <0.01   |
| CI8:0 (Stearic acid)     |         | 13.26 ± 0.62  | 15.75 ± 0.71  | 17.06 ± 2.09  | 16.76 ± 4.09  | 0.09    |
| CI8:1n9 (Oleic acid)     |         | 24.08 ± 2.43a | 16.74 ± 1.42a | 15.96 ± 3.59a | 13.13 ± 3.74a | <0.01   |
| CI8:1n7 (Vaccenic acid)  |         | 7.30 ± 0.66a  | 3.13 ± 0.20a  | 2.81 ± 0.42a  | 2.56 ± 0.45a  | <0.01   |
| CI8:2n6 (Linoleic acid)  |         | 7.14 ± 0.59a  | 8.41 ± 0.18a  | 6.62 ± 1.15a  | 5.56 ± 0.72a  | <0.01   |
| CI8:3n6 (ω-linolenic acid) |    | 0.00           | 0.00           | 0.00           | 0.00           |        |
| CI8:3n6 (ω-linolenic acid) |    | 0.53 ± 0.19a   | 0.45 ± 0.10a   | 0.35 ± 0.17a  | 0.39 ± 0.17a  | 0.48    |
| CI20:1n9 (Eicosanoic acid) |    | 0.00           | 0.00           | 0.00           | 0.00           |        |
| CI20:4n6 (Arachidonic acid) |    | 8.72 ± 0.55a   | 10.47 ± 0.61a  | 7.09 ± 1.28a  | 6.01 ± 1.75a  | <0.01   |
| CI20:5n3 (EPA*)          |         | 0.20 ± 0.41a   | 0.31 ± 0.62a   | 0.37 ± 0.75a  | 0.40 ± 0.80a  | 0.98    |
| CI22:4n6 (Adrenic acid)  |         | 1.95 ± 0.40a   | 3.14 ± 0.48a   | 1.95 ± 0.77a  | 1.64 ± 0.74a  | 0.02    |
| CI22:6n3 (DHA*)          |         | 0.00           | 0.00           | 0.00           | 0.00           |        |

| SFA*         | 46.11 ± 2.75a | 56.03 ± 1.12a | 63.18 ± 2.92a | 68.95 ± 2.15a | <0.01   |
| UFA*         | 53.89 ± 2.75a | 43.97 ± 1.12a | 36.82 ± 2.92a | 31.05 ± 2.15a | <0.01   |
| MUFA*        | 35.35 ± 3.66a | 21.19 ± 1.38a | 20.43 ± 4.43a | 17.06 ± 4.64a | <0.01   |
| PUFA*        | 18.54 ± 1.26a | 22.78 ± 0.36a | 16.39 ± 3.45a | 13.99 ± 3.33a | <0.01   |
| MUFA/SFA     | 0.77 ± 0.12a  | 0.38 ± 0.03a  | 0.33 ± 0.08a  | 0.25 ± 0.07a  | <0.01   |
| PUFA/SFA     | 0.40 ± 0.02a  | 0.41 ± 0.01a  | 0.26 ± 0.06a  | 0.20 ± 0.05a  | <0.01   |

* Different letters in a row indicate significant differences (p < 0.05).
* EPA: Eicosapentaenoic acid; UFA: Unsaturated fatty acid; DHA: Docosahexaenoic acid; MUFA: Monounsaturated fatty acid; SFA: Saturated fatty acid; PUFA: Polyunsaturated fatty acid.
and highest in the base. This is similar to previous research on velvet antlers of elk [31] and Formosan deer upper section to the base. Moreover, the amino acids that lowest in the base, glycine content was lowest in the tip [22]. Unlike the amino acid content in velvet antler, which amino acid content was higher than the essential amino acid content was high in the tip and polyunsaturated fatty acid contents, which are essential for preventing osteoporosis, and the upper section exhibited high essential amino acid and polyunsaturated fatty acid contents, which are essential for the body and effective for preventing cardiovascular disease, respectively. Moreover, the tip harbored the most nutrients. The results of this study provide basic data on the composition of velvet antlers by their section. Therefore, among the different velvet antler sections, the base exhibited high Ca content, which is effective for preventing osteoporosis, and the upper section exhibited high essential amino acid and polyunsaturated fatty acid contents, which are essential for the body and effective for preventing cardiovascular disease, respectively. Moreover, the tip harbored the most nutrients. The results of this study provide basic data on the composition of velvet antlers by their section.

### 3.5. Amino Acids

The results of amino acid composition analysis 90 days after casting are shown in Table 5. Amino acids were the most abundant component of velvet antler, and included glycine, proline, glutamic acid, methionine, and alanine. Arginine and tyrosine contents were high in the tip (3.78% and 1.12%, respectively), lysine (4.34%), leucine (5.40%), and methionine (5.46%) contents were high in the upper section, and glycine (8.33%) and proline contents (5.89%) were high in the base. The essential amino acid content was 22.26% in the tip, 27.82% in the upper section, 22.11% in the middle section, and 13.83% in the base, whereas the non-essential amino acid content was 33.39% in the upper section, 32.23% in the middle section, and 29.71% in the base. In contrast, crude fiber and crude ash content increased toward the base; conversely, the K content increased between sections. Ca, P, and Mg contents increased increased toward the base. pH showed no difference difference between sections. The saturated fatty acid content tended to decrease and that of glycine increased increased toward the base. Among unsaturated fatty acids, monounsaturated fatty acid content decreased toward the base. The dry matter content increased toward the base, whereas the crude fat and crude protein contents decreased toward the base. pH showed no difference between sections. Ca, P, and Mg contents increased toward the base; conversely, the K content increased toward the tip. The Zn content showed no significant difference between sections. The saturated fatty acid content increased toward the base, whereas the unsaturated fatty acid content decreased toward the base. Among unsaturated fatty acids, monounsaturated fatty acid content was high in the tip and polyunsaturated fatty acid content was high in the upper section. The amino acid content tended to decrease and that of glycine increased toward the base. Among the amino acids, the essential amino acid content was high in the upper section of the antlers. Therefore, among the different velvet antler sections, the base exhibited high Ca content, which is effective for preventing osteoporosis, and the upper section exhibited high essential amino acid and polyunsaturated fatty acid contents, which are essential for the body and effective for preventing cardiovascular disease, respectively. Moreover, the tip harbored the most nutrients. The results of this study provide basic data on the composition of velvet antlers by their section. Additional studies should examine other components such as vitamins and their pharmacological efficacies.

#### Table 5. Amino Acid Composition of Elk Velvet Antler

| Component (%) | Tip       | Upper     | Middle    | Base      | p-value |
|---------------|-----------|-----------|-----------|-----------|---------|
| Lys           | 3.59±0.82a | 4.34±0.33a | 3.50±0.51b | 2.32±0.17a | <0.01   |
| Leu           | 4.03±1.11b | 5.40±0.58b | 4.23±0.78b | 2.37±0.21a | <0.01   |
| Met           | 4.68±1.04a | 5.46±0.30a | 4.47±0.64a | 3.08±0.19a | <0.01   |
| Val           | 2.63±0.66b | 3.50±0.28a | 2.64±0.56b | 1.63±0.13a | <0.01   |
| Ile           | 1.36±0.42a | 0.92±0.03a | 0.85±0.03b | 0.75±0.04b | <0.01   |
| Thr           | 2.36±0.62a | 2.61±0.19a | 2.15±0.26b | 1.44±0.09a | <0.01   |
| Phe           | 2.27±0.50b | 3.17±0.13a | 2.51±0.41a | 1.52±0.13a | <0.01   |
| His           | 1.36±0.44a | 2.44±0.29a | 1.75±0.47b | 0.72±0.14a | <0.01   |
| Gly           | 7.06±1.18a | 7.38±0.25a | 7.95±0.49b | 8.33±0.35a | 0.08    |
| Cys           | 0.86±0.08a | 0.56±0.03a | 0.40±0.06a | 0.34±0.04a | <0.01   |
| Arg           | 3.78±0.36b | 3.66±0.09b | 3.52±0.07a | 3.21±0.15b | 0.01    |
| Pro           | 5.53±0.55a | 5.63±0.08a | 5.83±0.19a | 5.89±0.25a | 0.37    |
| Tyr           | 1.12±0.45b | 0.97±0.06b | 0.77±0.10b | 0.50±0.04b | 0.02    |
| Asp           | 0.62±0.06b | 0.44±0.02b | 0.37±0.02a | 0.32±0.03b | <0.01   |
| Ser           | 2.81±0.60a | 2.94±0.18a | 2.53±0.22a | 1.93±0.10a | <0.01   |
| Glu           | 7.34±1.38b | 6.61±0.22a | 6.04±0.27b | 5.20±0.22a | 0.01    |
| Ala           | 4.26±0.05a | 5.21±0.26a | 4.82±0.28a | 3.99±0.15a | <0.01   |
| Essential amino acids | 22.26±5.57 | 27.82±2.11 | 22.11±3.52 | 13.83±1.03 | <0.01   |
| Non-essential amino acids | 33.36±1.72 | 33.39±0.67 | 32.23±0.71 | 29.71±1.21 | <0.01   |

Data are means ± SD (N = 4).

* Different letters in a row indicate significant differences (p < 0.05).
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Statement of Competing Interests

The authors have no competing interests.

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