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#### Abstract

Diet digestibility can vary based on factors such as the type of ingredients, processing techniques, formulation, fiber content, and nutrient interactions. Unlike proteins and fats, there is no specific carbohydrate requirement, which typically constitutes 30-60% of commercial dried dog foods. Because of the significant proportion of carbohydrates in dog food, this study aimed to evaluate the differences in nutrient digestibility among barley, brown rice, corn, mung bean, and rice, which are common carbohydrate sources in commercial dog foods. All experimental diets had consistent chemical compositions. The digestibility of each carbohydrate source was evaluated using the total feces collection method in four castrated male and four neutered female beagles with an average age of 4.58  $\pm 0.14$  years. The average daily dry matter intake of the five experimental diets was  $203.0 \pm 3.23$  g/day. The percentage of dry matter digestibility of the apparent total tract digestibility (ATTD) was the highest for rice and corn at 92.45% and 92.95%, respectively, followed by brown rice (91.61%), barley (88.81%), and mung beans (80.74%). The percentage of nitrogen-free extract digestibility was also high for rice, corn, and brown rice at 97.08%, 96.14%, and 95.56%, respectively, followed by barley at 90.10% and mung bean at 83.38%. Amino acid digestibility analysis revealed no statistically significant differences between rice, corn, brown rice, and barley, except for methionine, which is an essential amino acid. Although the ATTD and amino acid profile of the mung bean-based diet were less efficient than those of the other test diets, the overall digestibility was satisfactory and there were no significant differences in palatability. The differences in digestibility observed in mung bean-based diets compared to other grain-based diets can be attributed to variations in the starch and fiber content of the raw materials. By leveraging these characteristics, mung bean-based diets may offer strategic benefits for glycemic control and weight management in dogs. Our results may serve as a basis for formulating appropriate diets for dogs.

Keywords: Dogs, Diets, Carbohydrates, Digestibility, Nutrient interactions

## Introduction

Energy is important for sustaining the life of dogs. Protein, fat, and carbohydrates are considered the major energy sources, and energy requirements should be met according to individual needs. Among them, carbohydrates provide energy in the form of glucose and offer various health benefits, including supporting a healthy digestive tract, maintaining the gut microbiota, and facilitating effective weight management. In commercial dog feed, carbohydrates constitute 30–60% of dry food and > 30% of wet food [1, 2]. Most of these carbohydrates are starch, with digestibility varying based on factors such as carbohydrate ingredients, granule size, amylose-to-amylopectin ratio, microstructure, diet form, processing methods, degree of heat treatment, and other components within the recipe [3-6].

Carbohydrate sources in pet foods include grains (e.g., corn, sorghum, rice, and wheat), legumes (e.g., peas and lentils), tubers (potatoes and tapioca), and by-products or fractions of these ingredients [6]. Digestible carbohydrates are absorbed in the small intestine, whereas indigestible carbohydrates can be fermatable and non/poorly fermentable in the large intestine. As a digestible carbohydrate, starch provides energy after absorption. Moreover, the starch concentration is related to the quality of pet food by increasing the expansion and binding properties of the food matrix during the manufacturing process, which affects the durability and formulation of pet food. Indigestible carbohydrates can be insoluble or soluble. Fibers are insoluble and fermentable and are used by the gut microbiota to modulate microbiota and gut health. Carbohydrates have recently gained attention because of their positive physiological effects on health. However, there is not enough information about the in vivo utilization of carbohydrates as much as that of proteins in dogs.

Corn, rice, brown rice, barley, and mung beans are frequently used as carbohydrate sources in commercial dry dog foods [7]. To gain insight into nutritional characteristics, a comparison of the in vivo digestibility of these carbohydrate sources is essential. Therefore, in this study, we aimed to compare the digestibility of commonly used carbohydrate sources and evaluate the nutrient utilization efficiency of these carbohydrate sources in dogs, which would provide a foundation for designing feeding matrices that strategically and appropriately meet nutrient requirements.

## **Materials and Methods**

#### Animals and experimental design

The animal study was approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science (NIAS), Korea (approval number: NIAS2022-0584). In this experiment four spayed and four castrated beagle dogs (aged  $4.58 \pm 0.14$  years) were used. The dogs were individually housed in a room (170 cm  $\times$  210 cm) with a consistent room temperature ( $22 \pm 1^{\circ}$ C) and relative humidity ( $60 \pm 10\%$ ) throughout the experimental period. Water was provided *ad libitum*. The feeding test comprised a three day acclimatization period followed by a four day adaptation period, and fecal collection was conducted over four days. This process was repeated for each diet group. The dog's health was monitored daily and cared for by a veterinarian as needed.

#### **Experimental diets**

The experimental diets were prepared as previously described [8]. The composition of the ingredients was formulated as a completely balanced diet based on the minimum nutrient requirements established by the Association of American Feed Control Officials (AAFCO). Five different carbohydrate sources (barley, corn, rice, mung beans, and brown rice) were used in powdered form and mixed with other ingredients, followed by steaming, molding, cutting, and drying to form pellets. All experimental diets were stored at  $-20^{\circ}$ C and allowed to equilibrate at room temperature for 3 h before feeding. Table 1 shows the composition of the ingredients and the experimental diets. The experimental diet was provided to dogs based on their individual metabolic energy requirement (ME, kcal/day 132 kcal × body weight [BW]<sup>0.75</sup> kg) according to the recommendation of AAFCO.

#### Apparent total tract nutrient digestibility and chemical analysis

Digestibility of the experimental diets was assessed using the whole feces collection method. Fecal samples were collected twice daily at consistent times over 4 days and were subsequently frozen at  $-20^{\circ}$ C until analysis. The diets and fecal samples were dried in a forced-air oven at 75°C and subsequently homogenized for further analysis. The chemical compositions of both diets and fecal

samples were determined using standard methods established by the Association of Official Analytical Chemists [9].

Nutrient digestibility of the experimental diets was calculated using the following equation:

Apparent Digestibility (%) =  $\frac{\text{Nutrient intake (g)} - \text{Nutrient in feces (g)}}{\text{Nutrient intake (g)}} \times 100$ 

#### Statistical analysis

All statistical analyses were performed using R (version 4.2.3). Analysis of variance (ANOVA) was used to evaluate the data, and post-hoc comparisons were performed using Tukey's test. The general linear hypothesis testing (glht) function from the multcomp package was used to identify statistically significant differences between groups. Statistical significance was set at p < 0.05.

## **Results and Discussion**

This study aimed to evaluate the digestibility of commonly used carbohydrate sources in commercial dog food. Carbohydrate sources were selected based on a previous study that reported barley, brown rice, corn, mung bean, and rice as the most commonly used carbohydrate ingredients in dog diets [2]. The experimental diets with barley, brown rice, corn, mung bean, and rice were formulated to be equivalent and it was confirmed that they exhibited no significant differences in chemical composition based on the proximate composition analysis of carbohydrate ingredients and nutritional requirements (Table 1). The NFE contents of the carbohydrate ingredients were 40.12% for corn, 38.39% for brown rice, 36.98% for rice, 36.46% for barley, and 35.64% for mung beans (Table 2). Crude fiber (CF) content was highest in mung bean (1.12%), followed by brown rice (0.44%), barley (0.37%), rice (0.25%), and corn (0.03%). NFE represents the soluble carbohydrate, and crude fiber represents the insoluble carbohydrate. Corn contained the highest NFE and lowest CF. Mung beans served as both carbohydrate and protein sources, exhibiting the lowest NFE content and the highest crude fiber (CF) content (1.12%).

The digestibility of dry matter (DM) was 92.95% for corn, 92.45% for rice, and 91.61% for brown rice, whereas barley and mung beans had lower digestibility values of 88.81% and 80.74%, respectively,

as determined by ATTD analysis (Table 4). Crude protein (CP) digestibility was the highest in rice (92.43%), followed by corn (92.07%) and barley (91.87%). No significant differences were observed in the digestibility of crude ash. The ether extract digestibility was highest in barley (96.52%) and lowest in corn (93.62%). Compared to the chemical composition of the carbohydrate sources, DM was the highest in corn, and the digestibility of DM was also the highest in the corn diet. CP content was the highest in mung beans. However, CP digestibility was higher in diets containing rice, corn, and barley. The mung bean diet showed the lowest digestibility in dogs.

NFE digestibility was higher for rice (97.08%), corn (96.14%), and brown rice (95.56%). The digestibility of barley (90.10%) was lower than that of rice, corn, and brown rice but significantly higher than that of mung beans (83.38%). Interestingly, the NFE content was higher in barley than in brown rice, and the digestibility of NFE was higher in brown rice than in barley. These findings are consistent with those of previous studies. Murray et al. [10] compared the digestibility of rice, corn, and barley, and found DM digestibility to be 83.9% for rice, 85.4% for corn, and 82% for barley. The authors found that while the DM digestibility of rice and corn was similar, barley exhibited significantly lower DM digestibility than rice and corn [10]. Rice supplementation did not affect the digestibility of the mung bean diet. In a wheat-based diet, substituting brown rice at 15% and 30% resulted in a gradual increase in the digestibility of DM, OM, acid-hydrolyzed fat, energy digestibility, ME, and GE [11]. NFE is mostly composed of starch, which is the main source of energy in diets. Although carbohydrate ingredients have a high starch content, their in vivo digestibility does not necessarily correspond to the amount of starch contained in the ingredients that affects the utilization of energy in vivo.

Mung beans are primarily considered a protein source; they also contain various complex carbohydrates and are rich in dietary fiber [6, 12, 13], serving as both a source of carbohydrates and proteins. The mung bean diet was supplemented with 15% rice to achieve 50% NFE because of its higher CP content. The difference in NFE digestibility between rice (97.08%) and mung beans (83.38%) was 13.6%. Based on the proportion of rice supplemented, the digestibility of the mung bean diet was adjusted to that of the rice diet. Table 4 shows the adjusted daily intake and digestibility.

A legume crop, mung beans contain crude protein around 20.0–28.50%, but they also include various complex carbohydrates, with a starch content of 40.6–48.9% and fiber content of 3.21–4.18% [14, 15].

Legume lentils and peas are also classified as legume crops and have lower DM digestibility than rice and corn, similar to mung beans [2]. Specifically, the DM digestibility was 74.5% for lentils and 76.1% for peas, in contrast to 82.4% for rice and 78.6% for corn. Additionally, starch digestibility was 98.8% and 98.7% for lentils and peas, respectively, compared to 99.3% and 99.1% for rice and corn, respectively. Faba beans also have a lower digestibility of DM, OM, and CP than rice [16], and the ATTD decreases linearly with the inclusion of whole faba beans compared to a diet containing a mix of rice and corn [17]. Therefore, the lower digestibility of the diet with mung beans could be explained by the fact that legumes are generally less digestible than cereals in dogs because of their high fiber and low starch content.

OM digestibility was highest in rice (95.45%) and lowest in mung beans (84.93%). ME digestibility was highest in rice (94.67%), followed by corn (94.22%), brown rice (93.63%), barley (91.15%), and mung beans (83.71%). Additionally, brown rice and barley diets showed higher digestibility of amino acids than the mung bean diet, except for methionine (Table 5). The digestibility of methionine was the highest in corn and barley at 96.75% and 95.19%, respectively. Rice and brown rice had 92.23% and 91.49%, respectively, whereas mung beans had the lowest digestibility at 85.68%. Both essential and non-essential amino acids were lowest in mung beans.

Currently, grain-free diets are being developed and offered as a more appropriate nutritional strategy. Despite its high economic value and adequacy as a carbohydrate source in dog food, corn has been devalued and associated with negative perceptions because of its potential to cause allergies and the rising popularity of premium grain- and gluten-free diets [18, 19]. Conversely, commercial pet food formulations that replace grains with legumes or tubers as primary carbohydrate sources are gaining popularity [16, 20]. However, scientific evidence supporting this assertion is lacking [21]. In this study, rice, corn, and brown rice showed the highest digestibility for CP, NFE, OM, and ME. Moreover, when the proportion of chicken breast was reduced to equalize the protein levels of the other experimental diets, accounting for the protein content of mung beans, the digestibility of CP was significantly reduced with mung beans. In addition, the ATTD digestibility of DM, NFE, OM, and ME also decreased when mung bean was used in dog food as a carbohydrate source, similar to the results of previous studies. However, diets containing legums may be more beneficial for glycemic control than grain-based diets

[2]. Among the various carbohydrate sources (rice, barley, corn, and peas), a pea-based diet in adult dogs has been reported to reduce oxidative stress and protect the cardiovascular system by lowering the glycemic response [22].

In commercial dry dog food, carbohydrates constitute the largest portion of nutrients; however, carbohydrate content is not required to be listed in the guaranteed analysis [23, 24]. This study showed the effects of different carbohydrate sources on in vivo digestibility. Rice, corn, and brown rice had higher digestibility of energy such as CP, NFE, and ME. If dogs require a higher energy intake, rice, corn, and brown rice would be the proper carbohydrate source. However, if dogs need to control their body weight, it would be better to choose dog food with barley or mung beans.

There are multiple factors to be considered when people choose dog foods, not only as nutritional values but also as functional values. However, it is not easy to choose pet food from a vast number of choices. Our results contribute to our understanding of the digestibility of five carbohydrate sources (barley, brown rice, corn, mung beans, and rice) in healthy adult dogs to develop more appropriate dietary strategies for dogs..

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# **Tables and Figures**

Items	Barley	Brown rice	Corn	Mung bean	Rice
Ingredient (%)					
DM	88.73	87.88	91.09	89.10	86.33
СР	8.91	7.93	8.06	25.65	6.56
EE	1.33	1.72	0.03	1.41	0.31
CF	0.70	1.21	0.09	5.23	0.14
СА	0.75	1.09	0.34	3.81	0.55
NFE	77.04	75.93	82.57	53.00	78.77
Calories (kcal/kg)	4041	3917	4017	4011	3608
Ingredient composition (%)			<		
Barley powder	37.35		-		
Brown rice powder	-	37.57			-
Corn powder			35.93	-	-
Mung bean powder	-		-	25.48	-
Rice powder		-		15.00	36.13
Lard	1.44	1.46	1.70	3.04	1.48
Water	35.00	35.00	35.00	35.00	35.00
Salt	0.20	0.20	0.20	0.20	0.20
Vitamin and mineral premix <sup>1</sup>	0.40	0.40	0.40	0.40	0.40
Calcium phosphate	1.00	1.02	1.01	1.15	0.95
Calcium carbonate	0.74	0.72	0.76	0.67	0.75
Potassium citrate	1.00	1.00	1.00	1.00	1.00
Tryptophan	0.01	0.02	0.01	0.04	0.01
Cabbage powder	1.00	1.00	1.00	1.00	1.00
Green laver	1.00	1.00	1.00	1.02	1.00
Yolk powder	8.00	8.00	8.00	8.00	8.00
Chicken breast	12.86	12.61	13.99	8.00	14.08

#### Table 1. Ingredient formulations and compositions of experimental diets.

Values are expressed as means. DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; CA, crude ash; NFE, nitrogen-free extract; ME, metabolizable energy. <sup>1</sup> Vitamin and mineral premix supplied per kg of diets: 3500 IU vitamin A; 250 IU vitamin D<sub>3</sub>; 25 mg vitamin E; 0.052 mg vitamin K; 2.8 mg vitamin B<sub>1</sub>(thiamine); 2.6 mg vitamin B<sub>2</sub> (riboflavin); 2 mg

vitamin B<sub>6</sub> (pyridoxine); 0.014 mg vitamin B<sub>12</sub>; 6 mg Cal-d-pantothenate; 30 mg niacin; 0.4 mg folic acid; 0.036 mg biotin; 1,000 mg taurine; 44 mg FeSO<sub>4</sub>; 3.8 mg MnSO<sub>4</sub>; 50 mg ZnSO<sub>4</sub>; 7.5 mg CuSO<sub>4</sub>; 0.18 mg Na<sub>2</sub>SeO<sub>3</sub>; 0.9 mg Ca(IO<sub>3</sub>)<sub>2</sub>.

Table 2	. Analyzed	chemical	composition	of	experimental	diets	based	on	various	carbohydrate
ingredie	nts									

Item (%)	Barley	Brown rice	Corn	Mung bean	Rice
DM	70.09	70.97	70.63	74.21	70.15
СР	21.46	20.64	21.73	21.28	21.37
EE	7.86	7.45	5.63	10.92	7.50
CF	0.37	0.44	0.03	1.12	0.25
CA	3.94	4.05	3.12	5.25	4.05
NFE	36.46	38.39	40.12	35.64	36.98
ME (kcal/kg)	3577	3799	3886	4017	3637

Values are expressed as means. DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; CA, crude ash; NFE, nitrogen-free extract.

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 Table 3. Average daily intake, metabolic energy, and body parameters of dogs fed with various

 carbohydrate sources diets

Items (unit)	Barley	Brown rice	Corn	Mung bean	Rice	F value	<b>Pr(&gt;F)</b>
ADFI (g/day) <sup>1</sup>	$292.0 \pm 4.76^{a}$	$283.1 \pm 4.67 \ ab$	$289.1 \pm 4.77$ <sup>a</sup>	$269.9\pm4.43^b$	$291.9 \pm 4.73$ <sup>a</sup>	3.96	<0.01
ME intake (kcal/day) <sup>2</sup>	$787.0\pm12.84$	$764.2\pm12.60$	$764.2\pm12.60$	$788.1\pm12.92$	$782.2\pm12.67$	0.89	0.480
Body weight (kg)							
Initial	$12.1\pm0.94$	$12.6\pm1.10$	$12.4\pm0.98$	$12.6\pm1.20$	$11.7\pm0.72$	1.19	0.331
Final	$12.4\pm0.98$	$12.6\pm1.20$	$12.6\pm1.10$	$12.6\pm1.12$	$12.1\pm0.94$	0.28	0.887
	$300.0 \ \pm$		137.5 ±	1			
BWG (g)	98.20 <sup>ab</sup>	$25.0 \pm 75.00^{b}$	83.32 <sup>ab</sup>	$25.0 \pm 61.96^{b}$	$462.5 \pm 93.90^{a}$	5.15	< 0.01
FCR (ADFI/BWG)	-0.4	0.8	1.3	0.5	0.6	0.56	0.694

Values are expressed as the mean  $\pm$  SEM. ADFI, average daily feed intake; ME, metabolizable energy; BWG, body weight gain; FCR, feed conversion ratio; SEM, standard error of the mean. <sup>1</sup> ADFI values were calculated based on dry matter. <sup>2</sup> ME was calculated using the following equation: ME (kcal/d) = (crude protein  $\times$  3.5) + (ether extract  $\times$  8.5) + (nitrogen-free extract  $\times$  3.5). Different letters (a and b) indicate significant differences between means. The letters a and ab or ab and b indicate non-significant differences. Additionally, if the ANOVA was not statistically significant, no letters were displayed in the table.

	Barley	Brown rice	Corn	Mung bean (correction)	Rice	SEM	F value	Pr(>F)
Daily DM intake (g)								
DM	204.66	200.93	204.21	200.27	204.75	1.44	0.43	0.786
СР	62.66 <sup>b</sup>	58.44 <sup>ac</sup>	62.83 <sup>b</sup>	57.43 <sup>c</sup>	62.37 <sup>ab</sup>	0.56	6.81	< 0.001
EE	22.95 <sup>a</sup>	21.09 <sup>b</sup>	16.28 <sup>c</sup>	29.47 <sup>d</sup>	21.89 <sup>ab</sup>	0.70	162.00	< 0.001
СА	11.50 <sup>a</sup>	11.47 <sup>ac</sup>	9.02 <sup>b</sup>	14.17 <sup>c</sup>	11.82 <sup>ab</sup>	0.27	90.67	< 0.001
NFE	106.46 <sup>a</sup>	108.69 <sup>ac</sup>	116.00 <sup>b</sup>	96.18 <sup>c</sup>	107.94 <sup>ab</sup>	1.26	16.36	< 0.001
ОМ	192.08	188.22	195.10	183.08	192.20	1.48	2.22	0.087
ME (kcal/kg) <sup>1</sup>	1044.48 <sup>a</sup>	1075.59 <sup>ab</sup>	1123.54 <sup>b</sup>	1084.09 <sup>ab</sup>	1061.55 <sup>ab</sup>	8.60	2.81	0.040
ATTD (%)								
DM	88.81 <sup>b</sup>	91.61 <sup>ab</sup>	92.95 <sup>a</sup>	80.74 <sup>c</sup> (70.14)	92.45 <sup>a</sup>	0.79	46.09	< 0.001
СР	91.87 <sup>a</sup>	91.56 <sup>ab</sup>	92.07 <sup>a</sup>	82.65 <sup>b</sup> (73.79)	92.43 <sup>a</sup>	0.66	39.49	< 0.001
EE	96.52 <sup>a</sup>	94.80 <sup>ab</sup>	93.62 <sup>b</sup>	94.40 <sup>ab</sup> (92.91)	96.06 <sup>ab</sup>	0.31	3.84	0.011
СА	51.61	54.42	63.12	56.80 (60.90)	52.26	1.69	1.62	0.192
NFE	90.10 <sup>b</sup>	95.56 <sup>a</sup>	96.14 <sup>a</sup>	83.38 <sup>c</sup> (70.99)	97.08 <sup>a</sup>	0.92	36.47	< 0.001
ОМ	91.44 <sup>b</sup>	94.24 <sup>a</sup>	94.62 <sup>a</sup>	84.93 <sup>c</sup> (75.40)	95.45 <sup>a</sup>	0.67	45.31	< 0.001
ME	90.15 <sup>b</sup>	93.63 <sup>a</sup>	94.22 <sup>a</sup>	83.71 <sup>°</sup> (73.80)	94.67 <sup>a</sup>	0.71	53.66	< 0.001

 Table 4. Nutrient intake and apparent total trace nutrient digestibility in dogs fed with various

 carbohydrate sources diets

Values are expressed as means. Different letters (a, b, c, and d) above the number indicate significant differences between means. If the same letters, such as a and ab or ab and b, are present, it indicates an insignificant difference. Additionally, if the ANOVA was not statistically significant, no letters were displayed in the table. DM, dry matter; CP, crude protein; EE, ether extract; CA, crude ash; NFE, nitrogen-free extract; OM, organic matter; ME, metabolizable energy; ATTD, apparent total tract nutrient digestibility. <sup>1</sup> ME was calculated using the following equation: ME (kcal/kg) = (CP × 3.5) + (EE × 8.5) + (NFE × 3.5).

 Table 5. Apparent total trace nutrient digestibility of amino acids in various carbohydrate sources diets

 in dogs

Amino acid (%)	Barley	Brown rice	Corn	Mung bean (correction)	Rice	SEM	F value	Pr(>F)
Essential amino acid				×				
Arginine	93.51 <sup>a</sup>	94.17 <sup>a</sup>	93.91 <sup>a</sup>	85.69 <sup>b</sup> (78.19)	93.98 <sup>a</sup>	0.61	26.29	< 0.001
Histidine	91.36 <sup>a</sup>	91.87 <sup>a</sup>	91.66 <sup>a</sup>	79.13 <sup>b</sup> (67.81)	91.64 <sup>a</sup>	0.87	46.10	< 0.001
Isoleucine	92.37 <sup>a</sup>	92.38 <sup>a</sup>	92.71 <sup>a</sup>	82.93 <sup>b</sup> (74.18)	92.61 <sup>a</sup>	0.71	26.35	< 0.001
Leucine	93.13 <sup>a</sup>	91.99 <sup>a</sup>	94.10 <sup>a</sup>	83.03 <sup>b</sup> (74.14)	92.85 <sup>a</sup>	0.75	25.86	< 0.001
Lysine	91.74 <sup>a</sup>	92.71 <sup>a</sup>	92.00 <sup>a</sup>	81.55 <sup>b</sup> (71.55)	92.60 <sup>a</sup>	0.77	34.39	< 0.001
Methionine	95.19 <sup>ac</sup>	91.49 <sup>b</sup>	96.75 <sup>°</sup>	85.68 <sup>d</sup> (79.75)	92.23 <sup>ab</sup>	0.70	26.81	< 0.001
Phenylalanine	92.80 <sup>a</sup>	90.90 <sup>a</sup>	92.45 <sup>a</sup>	82.03 <sup>b</sup> (73.16)	91.84 <sup>a</sup>	0.76	22.42	< 0.001
Threonine	89.17 <sup>a</sup>	90.32 <sup>a</sup>	90.29 <sup>a</sup>	74.28 <sup>b</sup> (59.33)	90.80 <sup>a</sup>	1.17	28.13	< 0.001
Tryptophan	88.86 <sup>a</sup>	89.87 <sup>a</sup>	90.65 <sup>a</sup>	76.81 <sup>b</sup> (64.34)	90.60 <sup>a</sup>	1.15	10.62	< 0.001
Valine	91.06 <sup>a</sup>	91.03 <sup>a</sup>	91.38 <sup>a</sup>	79.69 <sup>b</sup> (69.09)	91.41 <sup>a</sup>	0.86	2.72	< 0.001
Nonessential amino acid								
Alanine	90.18 <sup>a</sup>	90.87 <sup>a</sup>	91.51 <sup>a</sup>	79.45 <sup>b</sup> (68.93)	91.08 <sup>a</sup>	0.85	26.30	< 0.001
Aspartic acid	89.10 <sup>a</sup>	90.75 <sup>a</sup>	90.34 <sup>a</sup>	78.98 <sup>b</sup> (68.54)	90.51 <sup>a</sup>	0.82	30.05	< 0.001
Cysteine	84.24 <sup>a</sup>	78.76 <sup>a</sup>	87.47 <sup>a</sup>	59.85 <sup>b</sup> (41.73)	79.88 <sup>a</sup>	1.81	22.43	< 0.001
Glutamic acid	93.57 <sup>a</sup>	91.55 <sup>a</sup>	91.31 <sup>a</sup>	82.35 <sup>b</sup> (73.80)	91.80 <sup>a</sup>	0.74	35.47	< 0.001
Glycine	88.05 <sup>a</sup>	89.36 <sup>a</sup>	88.91 <sup>a</sup>	73.71 <sup>b</sup> (59.46)	89.46 <sup>a</sup>	1.08	39.03	< 0.001
Proline	92.49 <sup>a</sup>	89.27 <sup>a</sup>	91.85 <sup>a</sup>	74.84 <sup>b</sup> (60.87)	90.27 <sup>a</sup>	1.16	38.60	< 0.001
Serine	82.68 <sup>a</sup>	83.54 <sup>a</sup>	84.63 <sup>a</sup>	70.95 <sup>b</sup> (60.93)	82.02 <sup>a</sup>	1.04	12.64	< 0.001
Tyrosine	90.51 <sup>a</sup>	88.70 <sup>a</sup>	92.28 <sup>a</sup>	77.99 <sup>b</sup> (67.13)	89.99 <sup>a</sup>	0.97	20.29	< 0.001

Values are expressed as means. Different letters (a, b, c, and d) above the number indicate significant differences between means. If the same letters, such as a and ab or ab and b, are present, this indicates an insignificant difference. Additionally, if the ANOVA was not statistically significant, no letters were displayed in the table. SEM: standard error of the mean.