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≪Research Note≫

Effects of Dietary Supplementation of Histidine, β-Alanine, Magnesium Oxide, and Blood Meal on Carnosine and Anserine Concentrations of Broiler Breast Meat

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An experiment was conducted to evaluate the effect of dietary supplementation of histidine, β-alanine, blood meal (BM), magnesium oxide (MgO), and their combination on carnosine and anserine concentrations of broiler breast meat. A total of 210 1-d-old broiler chicks were randomly allotted to 3 replicates of 7 dietary treatments: (1) Control diet (C), (2) C＋histidine (His), (3) C＋β-alanine (β-Ala), (4) C＋histidine＋β-alanine (His＋β-Ala); (5) C ＋histidine＋β-alanine＋MgO (His＋β-Ala＋MgO), (6) C＋5% BM (BM), and (7) C＋5% BM＋MgO (BM＋ MgO). Histidine, β-alanine, and MgO were supplemented to diets at 2.9, 3.7, and 4.0 g/kg diet, respectively. The broilers were fed experimental diets for 32 d and the concentrations of carnosine and anserine in breast meat were measured weekly and at 32 d of the age. Results indicated that the concentrations of carnosine, anserine, and their sum in breast meat were increased (linear, $P<0.01$) with age in all treatments. There were significant differences in carnosine concentrations among treatments in all weeks. At 32 d, all 3 histidine-supplemented treatments (His, His＋ β-Ala, and His＋β-Ala＋MgO) and BM＋MgO treatment had greater ($P<0.05$) carnosine concentrations than the control. The anserine concentrations differed ($P<0.01$) among treatments at 7, 14, and 21 d. The sum of carnosine and anserine concentrations was the greatest for His＋β-Ala＋MgO treatment, but the least for β-Ala treatment at 21 d. In conclusion, dietary supplementation of histidine alone or with β-alanine may increase carnosine concentrations, but not anserine concentrations of broiler breast meat. Dietary supplementation of additional MgO in diets containing His, β-Ala, and/or BM has little effect on carnosine and anserine concentrations in broiler breast meat.

Key words: anserine, β-alanine, blood meal, carnosine, histidine, broiler breast meat

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Introduction

Carnosine (β-alanyl-L-histidine) is a dipeptide synthesized from β-alanine and histidine by carnosine synthetase, whereas anserine (β-alanyl-1-methyl-L-histidine) is a methylated form of carnosine (Boldyrev and Severin, 1990). High concentrations of carnosine and anserine have been found in the brain and muscles, especially for breast muscle, of mammalian and avian species (Kohen *et al*., 1988; O'Dowd *et al*., 1988; Biffo *et al*., 1990). Recently, carnosine has gained increasing attention as a functional ingredient for human food because of its high antioxidant activity (Mozdzan *et al*., 2005), high buffering capacity to maintain intracellular pH change (Abe, 2000), and anti-glycating and anti-aldehyde effects (Aldini *et al*., 2005; Guiotto *et al*., 2005). Previous experiments indicated that carnosine concentrations in animal products and human body could be increased by dietary manipulation. One possible means has been implicated as the inclusion of β-alanine or histidine in the diet because they are the basic components of carnosine. Harris *et al*. (2006) reported that dietary supplementation of β-alanine elevated carnosine concentrations in human body. Amend *et al*. (1979) and Haug *et al*. (2008) also reported that dietary supplementation of 0.1% histidine increased carnosine and anserine concentrations of chicken muscle. However, there has been limited information about the effect of dietary supplementation of β-alanine, histidine or their combination on carnosine and anserine concentrations in chicken meat. Moreover, Mg is a cofactor for carnosine synthetase (Kalyankar and Meister, 1959), such that increased supplementation of Mg in the diet containing additional β-alanine and histidine

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may further promote carnosine synthesis in chicken meat; however, this hypothesis has not been verified. In addition, our previous experiment reported that feeding the diet containing 5% blood meal, which contains high amounts of L-histidine, to broiler chicken increased carnosine concentrations in the breast meat (Auh *et al*., 2010). Therefore, it is also hypothesized that supplementation of additional Mg in the diet containing blood meal may have synergistic effects on increasing concentrations of carnosine in the breast meat of chicken.

The objective of this experiment, therefore, was to determine the effect of dietary supplementation of L-histidine, *β*-alanine, blood meal (BM), magnesium oxide (MgO), and their combination on carnosine and anserine concentrations of broiler breast meat.

Materials and Methods

Experimental Design and Diets

All animal protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University. A total of 210 1-d old Ross® broiler chicks were purchased from a local hatchery (Yang-Ji Hatchery, Pyeong-Taek, South Korea). The chicks were randomly allotted to 21 wire-floored battery cages with 5 male and 5 female per cage. There were 7 dietary treatments with 3 replicate cages per treatment. Seven dietary treatments included: (1) control diet (C), (2) C+histidine (His), (3) C+*β*-alanine (*β*-Ala), (4) C+histidine+*β*-alanine (His+*β*-Ala), (5) C+histidine+*β*-alanine+MgO (His+*β*-Ala+MgO), (6) C+5% blood meal (BM), and (7) C+5%

Table 1. **Formula and composition of experimental diets fed to broilers (from 1 d to 32 d)**

Ingredients, %	Treatments[1]						
	Control	His	*β*-Ala	His+*β*-Ala	His+*β*-Ala+MgO	BM	BM+MgO
Corn	23.40	23.14	23.07	22.81	22.41	22.31	21.92
Wheat	38.87	38.84	38.83	38.80	38.80	36.93	36.92
Soybean meal	27.10	27.10	27.10	27.10	27.10	25.75	25.75
Blood meal	0.00	0.00	0.00	0.00	0.00	5.00	5.00
Animal fat	4.50	4.50	4.50	4.50	4.50	4.11	4.11
Rape seed meal	3.00	3.00	3.00	3.00	3.00	2.85	2.85
Dicalcium phosphate	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Calcium carbonate	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Lysine-HCl - 78%	0.26	0.26	0.26	0.26	0.26	0.25	0.25
Sodium chloride	0.26	0.26	0.26	0.26	0.26	0.25	0.25
Threonine - 10%	0.25	0.25	0.25	0.25	0.25	0.24	0.24
Sodium bicarbonate	0.20	0.20	0.20	0.20	0.20	0.19	0.19
Broiler premix[2]	0.10	0.10	0.10	0.10	0.10	0.09	0.09
Anticoccidial drug	0.05	0.05	0.05	0.05	0.05	0.04	0.04
NSP enzyme	0.05	0.05	0.05	0.05	0.05	0.04	0.04
Phytase	0.05	0.05	0.05	0.05	0.05	0.04	0.04
MgO	—	—	—	—	0.40	—	0.40
Histidine	—	0.29	—	0.29	0.29	—	—
β-alanine	—	—	0.37	0.37	0.37	—	—
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition							
ME, kcal/kg	3,110	—	—	—	—	3,106	—
Crude protein, %	20.00	—	—	—	—	22.61	—
Calcium, %	0.95	—	—	—	—	0.86	—
Methionine+Cystine, %	0.85	—	—	—	—	1.47	—
Analyzed composition							
Moisture, %	8.33	—	—	—	—	10.55	—
Crude protein, %	19.23	—	—	—	—	23.13	—
Crude fat, %	8.39	—	—	—	—	5.90	—
Crude fiber, %	3.07	—	—	—	—	3.30	—
Crude ash, %	6.06	—	—	—	—	5.60	—

[1] Control=control diet (C); His=C+histidine; *β*-Ala=C+*β*-alanine; His+*β*-Ala=C+histidine+*β*-alanine; His+*β*-Ala+MgO=C+histidine+*β*-alanine+MgO; BM=C (95%)+blood meal (5%); BM+MgO=C (95%)+blood meal (5%)+MgO.

[2] Provides per kg of diet: vitamin A, 9,000IU; vitamin D_3, 2,500IU; vitamin E, 20IU, vitamin K_3, 1.2 mg; thiamin, 1.2 mg; riboflavin, 4 mg; niacin, 20 mg; pyridoxine, 8 mg; choline, 1,200 mg; biotin, 0.5 mg; folic acid, 0.5 mg; Cu, 10 mg; Mn, 105 mg; Se, 0.25 mg; Zn, 75 mg; I, 1 mg; Fe, 50 mg; Co, 0.1 mg.

Table 2. **Analyzed amino acid composition of experimental diets and blood meal used in this experiment**

Amino acids	Treatments[1]							
	Control	His	β-Ala	His+β-Ala	His+β-Ala+MgO	BM	BM+MgO	Blood meal
	% (percentage of total amino acids)							
Aspartic acid	8.83	8.67	8.93	8.87	8.60	9.28	8.75	9.60
Threonine	4.35	4.00	4.00	3.50	3.44	4.05	3.47	4.33
Serine/Glutamine	19.50	18.88	18.72	19.54	19.35	19.41	20.95	12.77
Proline	1.04	1.02	0.95	1.03	1.06	1.05	0.92	1.74
Glycine	11.86	14.80	13.11	13.74	13.17	12.21	13.97	8.84
Alanine	8.24	8.90	10.11	9.11	9.54	8.66	8.83	7.73
Cysteine	4.43	4.32	4.41	4.26	4.26	5.48	4.71	6.20
Valine	3.13	2.97	2.80	2.93	2.89	2.84	2.39	2.39
Methionine	3.21	2.98	3.22	2.97	2.91	2.63	2.32	2.32
Isoleucine	10.20	9.74	10.10	9.62	9.65	10.90	10.20	10.50
Leucine	2.77	2.70	2.76	2.71	2.68	2.67	2.45	4.10
Tyrosine	3.36	3.29	3.36	3.31	3.32	3.74	3.40	7.84
Phenylalanine	2.69	4.38	3.70	4.07	3.87	3.80	3.26	5.67
Histidine	4.85	5.17	4.28	5.13	5.72	6.10	5.94	6.33
Lysine	2.12	2.29	1.95	1.94	1.78	1.66	1.63	1.48
Arginine	2.24	2.10	2.22	2.08	2.04	2.04	1.72	3.73

[1] Control=control diet(C); His=C+histidine; β-Ala=C+β-alanine; His+β-Ala=C+histidine+β-alanine; His+β-Ala+MgO=C+histidine+β-alanine+MgO; BM=C (95%)+blood meal (5%); BM+MgO=C (95%)+blood meal (5%)+MgO.

blood meal+MgO (BM+MgO). Formula and chemical composition of experimental diets are present in Table 1, whereas analyzed compositions of amino acids in diets and BM are present in Table 2. The control diet was a commercial crumble-type broiler feed. The BM diet was formulated to be iso-caloric to the control diet. Crystalline histidine and β-alanine were purchased from Sigma-Aldrich Korea (Model No. H8125 and A9920). The blood meal (*i.e.,* spray dried porcine blood) was purchased from Arirang BNS, Inc. (Anseong-si, Gyeonggi-do, South Korea). The supplementation level of histidine and β-alanine for His, β-Ala, His +β-Ala, and His+β-Ala+MgO treatment groups were calculated based on the concentrations of histidine and alanine in 5% BM. According to the supplier, the concentrations of histidine and β-alanine in BM were 5.76 and 7.33%, respectively. Therefore, supplementation level was 2.9 g for histidine and 3.7 g for β-alanine per kg diet. As β-alanine is an intermediate in the catabolic metabolism, and is generally absent in BM, β-alanine was assumed to have the same potency as α-alanine in BM. As a cofactor of carnosine synthetase, Mg was supplemented to the diet at the level of 2,400 mg/kg diet, which was greater by 4 times than NRC (1994) recommendation, in the form of reagent-grade MgO (60% Mg). The experimental period was 32 d and feeds were supplied to birds as *ad libitum* basis during the entire experiment. Water was available at all times. The light was provided for 24 h.

Sample Preparation and Analysis

At the end of each week and at 32 d, 6 birds per each treatment (2 birds per replication) were selected based on the mean body weight and then sacrificed by cervical dislocation for breast meat sampling. Amino acid composition in 5 diets and BM were determined by the method as previously described by Baker *et al*. (2011). Breast samples for carnosine and anserine analysis was prepared by the method of Aristoy and Toldra (2004). Briefly, approximately 10 g of broiler breast was taken from each bird and frozen immediately at −4℃ in a freezer. Frozen broiler breast was freeze-dried for 72 h. The dried broiler breast was finely ground, and 1 g of the ground sample was mixed with 24 m*l* of distilled water in a centrifuge tube (BD Biosciences, San Jose, CA, USA). The tubes were shaken for 2 h at room temperature. The tubes were then centrifuged with the conditions of 11,000×g at 4℃ for 20 min. The supernatant was filtered using membrane filter (MCE type, Pore size 0.45 μm, ADVANTEC Inc., Tokyo, Japan). Three hundred μL of the supernatant were deproteinated with 900 μ*l* of methanol (MeOH) in a 1.5 m*l* micro-tube and were kept in −4℃ freezer for 12 h. After the coagulation of protein was confirmed, it was centrifuged under the conditions of 16,000×g at 4℃ for 3 min. The samples used in the present experiment were diluted 100 folds for HPLC injection.

All samples were analyzed using Varian 920-LC (Varian Medical Systems, Palo Alto, CA, USA) equipped with UV-detector, automatic injector, and dipeptide materials were separated with ZORBAX Eclipse Plus C-18 column (250 mm ×4.5 mm, 5 μm, Aglient Technologies, Santa Clara, CA, USA), and L-carnosine was detected most precisely at 210

nm. The mobile phase used isocratic HPLC method (5 mM sodium 1-heptane-sulfonate, pH 2.3, by 85% phosphoric acid with MeOH, 65%:35%, respectively). Column temperature was held at 30℃, flow rate was 1 ml/min, and running time was 13 min. The linear regression of standard was prepared using crystalline L-carnosine and L-anserine with distilled water in the range of 50 to 150 ppm.

Statistical Analysis

All experimental data were analyzed by the MIXED procedure in SAS (SAS Institute Inc., Cary, NC). The experimental unit was the individual bird. The model included dietary treatments as a fixed effect. Least squares means were calculated and the means were compared by PDIFF option with a Tukey adjustment in SAS. An α-value of 0.05 was used to assess significance among means. To confirm the change of carnosine and anserine concentrations with age, orthogonal polynomial contrast test was also performed.

Results

All birds remained healthy and easily consumed their respective diets throughout experiment. There was no difference in feed intake among treatments. Carnosine and anserine concentrations of broiler breast meat are shown in Table 3. Concentrations of carnosine, anserine, and carnosine+anserine were linearly increased ($P<0.01$) as the birds became mature in all treatment groups. Carnosine concentrations differed among treatments in all age ($P<0.01$ at 7, 14, 21 and 28 d, while $P<0.05$ at 32 d). In general, carnosine concentrations were greater for histidine-supplemented treatment groups (His, His+β-Ala, and His+β-Ala+MgO) and BM-supplemented treatments (BM and BM+MgO) than for the control and β-Ala treatment groups. At 32 d of the age, carnosine concentrations was increased by 23.1% for His treatment, 29.9% for His+β-Ala treatment, 31.4% for His+β-Ala+MgO treatment, 20.0% for BM treatment, and 24.7% for BM+MgO treatment as compared to the control, although the significance was not detected between the control and BM treatment. The β-Ala treatment had no significant effect on carnosine concentrations of broiler breast meat as compared to the control during the entire experiment. Dietary supplementation of Mg that was used as a cofactor of carnosine synthetase increased ($P<0.01$) carnosine concentrations only in BM treatment at the age of 28 d. Anserine concentrations of breast meat differed ($P<0.01$) at 7, 14 and 21 d among treatments, but no significant differences were observed at 28 and 32 d of the age. At 21 d, anserine concentrations were greater for His+β-Ala+MgO treatment than for the control, but other treatments had no significant

Table 3. **Carnosine and anserine concentrations of broiler breast meat**

Age, d	Treatments[1]							*P*-value[2]	SEM
	Control	His	β-Ala	His+ β-Ala	His+ β-Ala+ MgO	BM	BM+MgO		
Carnosine (mg/g, DM)									
7	5.72^{C}	6.65^{C}	5.73^{C}	10.63^{A}	10.07^{AB}	7.73^{BC}	5.84^{C}	<0.01	0.840
14	8.42^{D}	10.37^{CD}	9.96^{CD}	13.18^{A}	13.02^{AB}	10.45^{C}	11.12^{BC}	<0.01	0.690
21	12.26^{DE}	13.38^{CD}	11.62^{E}	16.86^{A}	15.15^{B}	14.36^{BC}	14.10^{BC}	<0.01	0.561
28	13.08^{BC}	13.89^{BC}	14.04^{BC}	17.53^{A}	14.82^{BC}	12.87^{C}	15.15^{B}	<0.01	0.773
32	13.85^{c}	17.47^{ab}	14.78^{bc}	18.72^{a}	19.01^{a}	16.93^{abc}	17.76^{ab}	<0.05	1.110
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
Anserine (mg/g, DM)									
7	18.78^{AB}	20.29^{AB}	18.01^{B}	21.39^{A}	21.20^{A}	14.50^{C}	17.64^{B}	<0.01	0.942
14	25.94^{AB}	27.34^{A}	25.62^{AB}	26.29^{AB}	24.59^{BC}	24.77^{BC}	22.68^{C}	<0.01	0.771
21	26.40^{BC}	28.19^{AB}	25.66^{C}	25.34^{C}	30.34^{A}	24.65^{C}	25.82^{BC}	<0.01	0.794
28	27.97	27.39	26.71	25.98	27.98	29.26	26.43	0.23	0.938
32	36.10	33.53	37.94	37.58	37.74	30.25	33.40	0.27	2.444
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
Carnosine+Anserine (mg/g, DM)									
7	24.49^{BC}	26.94^{B}	23.73^{C}	32.02^{A}	31.26^{A}	22.23^{C}	23.48^{C}	<0.01	1.032
14	34.36^{C}	37.71^{AB}	35.57^{BC}	39.46^{A}	37.61^{AB}	35.22^{BC}	33.80^{C}	<0.01	0.927
21	38.66^{CD}	41.57^{B}	37.27^{D}	42.19^{B}	45.49^{A}	39.01^{CD}	39.93^{BC}	<0.01	0.863
28	41.05	41.28	40.75	43.51	42.80	42.12	41.57	0.29	0.880
32	49.96	51.00	52.72	56.30	56.75	47.18	51.16	0.07	2.154
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		

[1] Control=control diet (C); His=C+histidine; β-Ala=C+β-alanine; His+β-Ala=C+histidine+β-alanine; His+β-Ala+MgO=C+histidine+β-alanine+MgO; BM=C (95%)+blood meal (5%); BM+MgO=C (95%)+blood meal (5%)+MgO.

[2] *P*-values for main effect (*i.e.,* dietary treatment effect).

[A–E] Values in the same row with no common superscripts are different ($P<0.01$).

[a–c] Values in the same row with no common superscripts are different ($P<0.05$).

effect on anserine concentrations of breast meat. Accordingly, the sum of carnosine and anserine concentrations were different ($P<0.01$) only at 7, 14 and 21 d. Histidine-supplemented treatments (His, His+β-Ala, and His+β-Ala+MgO) had greater concentrations of the sum of carnosine and anserine concentrations than for the control at 14 and 21 d, but there was no such an effect for BM-supplemented treatments (BM and BM+MgO) and β-Ala treatment.

Discussion

The observation for increased concentrations of carnosine and anserine in chicken breast meat with age in this experiment agrees with Auh *et al*. (2010) who reported that carnosine and carnosine+anserine concentrations of broiler breast meat were increased from 1 wk to 5 wk of age, regardless of dietary treatments.

Gariballa and Sinclair (2000) reported that histidine-deficient diet reduced carnosine concentrations of skeletal muscle in rats, which indicates that the histidine is the essential component for carnosine synthesis. Amend *et al*. (1979) and Haug *et al*. (2008) reported that supplementation of 0.1% histidine increased carnosine concentrations by 64% and anserine concentrations by 10% in chicken meat. However, Tamaki *et al*. (1977) reported that the histidine-supplemented diet increased the carnosine concentrations of the gastrocnemius muscle of rats, but had no effect on its anserine concentrations. Dunnet and Harris (1999) observed a significant increase in the carnosine concentrations of horse middle gluteal muscle when the diet was supplemented with β-alanine and histidine for 30 d. However, Tomonaga *et al*. (2005) found that the carnosine concentrations of broiler breast muscles were increased but the anserine concentrations were slightly decreased by feeding the diet containing 0.38% supplemental β-alanine. In the other experiment of Tomonaga *et al*. (2006), up to 2% β-alanine supplementation to the broiler diets was reported to increase carnosine and anserine concentrations of the brain, but not of the breast muscle. These results may implicate that dietary supplementation of histidine is more effective than that of β-alanine for the purpose of increasing carnosine concentrations of broiler breast meat. These results are also supported by our findings of this experiment. Likewise, inclusion of BM in diets as a source of histidine increased carnosine concentrations in broiler breast meat as comparable to crystalline histidine supplementation. Increased carnosine concentrations as a result of feeding the diet containing BM were also observed by our previous experiment (Auh *et al*., 2010).

Based on our observation in this experiment, moreover, there may be potential synergistic effect of dietary histidine in the presence of β-alanine on increasing carnosine concentrations. Previous experiment reported that dietary BM high in histidine increased carnosine concentrations of chicken meat with no effects on anserine concentrations (Auh *et al*., 2010). Similar results were also observed in this experiment, but significance was not verified.

Magnesium is known as a cofactor of carnosine synthetase (Kalyankar and Meister, 1959), and therefore, it was hypothesized that supplementation of additional Mg in the diet may accelerate carnosine synthesis in chicken meat. In this experiment, however, the results were inconsistent with age (i.e., feeding duration). Dietary Mg supplementation increased carnosine concentrations only for broilers fed the diet containing BM at 28 d, whereas no positive effects were observed in other ages. Inconsistent results of dietary Mg supplementation were also observed by Namkung *et al*. (2010) who reported that Mg supplementation in diets containing 5% BM increased carnosine concentrations of breast meat of laying hens only at 2 weeks during whole 5 weeks feeding trial. The reason for these variable results with age is not clear because there has been the lack of data pertaining to the relationship among carnosine synthetase activity, Mg as a cofactor, and age. Based on our results, however, it appears that supplementation of additional Mg in diets may not be effective to promote carnosine synthesis in breast meat of chicken. Further research is required to verify the age-dependent effects of dietary Mg on carnosine synthesis in animals.

In conclusion, dietary supplementation of histidine alone or with β-alanine increases carnosine concentrations, but has little impact on anserine concentrations of broiler breast meat. Dietary supplementation of additional Mg has no benefit on increasing concentrations of carnosine and anserine concentrations of broiler breast meat.

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