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Effects of Supplementary Blood Meal on Carnosine Content in the Breast Meat and Laying Performance of Old Hens

N. Namgung, D. H. Shin, S. W. Park and I. K. Paik*

Department of Animal Science and Technology, Chung-Ang University, Ansung-Si, Kyounggi-Do, 456-756, Korea

ABSTRACT : The objective of this research was to evaluate the effects of dietary supplementation of blood meal (BM) as a source of histidine, and magnesium oxide (MgO) as a catalyst of carnosine synthetase, on carnosine (L-Car) content in the chicken breast muscle (CBM), laying performance, and egg quality of spent old hens. Four hundred eighty laying hens (*Hy-Line*® Brown), 95wk old, were allotted randomly into five replicates of six dietary treatments: T1; 100% basal diet, T2; 100% basal diet+MgO, T3; 97.5% basal diet+ 2.5% BM, T4; 97.5% basal diet+2.5% BM+MgO, T5; 95% basal diet+5% BM, T6; 95% basal diet+5% BM+MgO. Magnesium oxide was added at 0.3% of diets. The layers were fed experimental diets for 5wk. There were no significant differences in the weekly L-Car content in CBM among all treatments during the total experimental period, but some of the contrast comparisions showed higher L-Car in CBM of T6. The L-Car contents linearly decreased ($p<0.01$ or $p<0.05$) as the layers got older except in T4 ($p>0.05$). There were significant differences in egg weight ($p<0.01$) and soft and broken egg ratio ($p<0.05$). The control (T1) was highest in egg weight and T6 was lowest in soft and broken egg ratio. Among the parameters of egg quality, there were significant differences in eggshell strength ($p<0.01$) and egg yolk color ($p<0.05$). Magnesium oxide supplementation increased the eggshell strength and BM tended to decrease egg yolk color. Eggshell color, eggshell thickness, and Haugh unit were not influenced by BM and MgO. In conclusion, BM and MgO did not significantly influence the L-Car in CBM of spent layers. The L-Car content rapidly decreased as the layers became senescent. Eggshell strength was increased by MgO supplementation. (**Key Words :** Blood Meal, Carnosine, Chicken Breast Muscle, Spent Layer, Magnesium Oxide, Eggshell Strength)

INTRODUCTION

Carnosine, β-alanyl-L-histidine (L-Car) is dipeptide that was discovered in mammalian skeletal muscle (Boldyrev and Severin, 1990). Carnosine has many beneficial roles as an antioxidant (Bogardus and Boissonneault, 2000; Nagasawa et al., 2001), antiglycation molecule (Hipkiss, 2005; Alhamdani et al., 2007), anti-cross-linker (Hobart et al., 2004) and anti-aging agent (Wang et al., 2000; Hipkiss et al., 2001). Many researches on L-Car has focused on its physiological roles (Boldyrev et al., 1997; Begum et al., 2005) and therapeutic activities (Gariballa and Sinclair, 2000). Although L-Car is biosynthesized endogenously in the animal body, little is known about the nutritional manner of enrichment of L-Car in the animal body. A few papers have reported that the supplementation of L-histidine (L-His) (Amend et al., 1979; Haug et al., 2008) or β-alanine (β-Ala) (Tomonaga et al., 2005) increases L-Car in the chicken muscle. White muscle tissue has a higher L-Car content than red muscle tissue (Plowman and Close, 1988; Boldyrev et al., 2004). Among the white muscle tissue animals, chicken is a representative animal that has high L-Car content (Boldyrev and Severin, 1990). Chicken breast muscle (CBM) has more L-Car content than other parts of the chicken (Intarapichet and Maikhunthod, 2005).

Produce of L-Car enriched chicken as a functional animal product in a niche market will be a viable option. In order to biosynthesize L-Car, L-His and β-Ala are needed as constituent elements of L-Car, and Mg is needed as a catalyst of L-Car synthetase (Kalyankar and Meister, 1959). Beta-alanine, however, is not present in natural feedstuffs. In contrast, L-His is widely contained in the natural feed ingredients as an essential amino acid (Bonfanti et al., 1999). Blood meal (BM) is the richest source of L-His among the common feed ingredients (NRC, 1994). Producing L-Car enriched broiler CBM was successful (Namgung et al., 2010). The economic lifespan of layer is about 24 month old when the egg production reached about 65 to 70%. The old senescent layers which reach the end of

* Corresponding Author: I. K. Paik. Tel: +82-31-670-3028, Fax: +82-31-676-2196, E-mail: ikpaik@cau.ac.kr
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economic lifespan cannot be used for edible meat source. If L-Car content in CBM of old spent layers can be increased, they can be used as an inexpensive source of L-Car in many applicable food industry.

This research was conducted to determine whether supplementary BM as L-His source, and MgO as a catalyst, can affect the L-Car content in the CBM and egg quality of old spent layers.

MATERIALS AND METHODS

Experimental design and diets

For this experiment, 480 layers (*Hy-Line*® Brown) of 95 wk old were housed in stainless cages (A shape, 2-tier, 30×40×30 cm in size). All layers were allotted randomly into 6 dietary treatments T1; 100% basal diet, T2; 100% basal diet+MgO, T3; 97.5% basal diet+2.5% BM, T4; 97.5% basal diet+2.5% BM+MgO, T5; 95% basal diet+5% BM, T6; 95% basal diet+5% BM+MgO. Magnesium oxide (60% Mg) was used as a catalyst of L-Car synthetase at the level of 0.3% of diets to provide an extra 1,800 mg of Mg, which is three times that of the NRC (1994) recommendation. Each treatment had five replications (that is, 16 layers were allotted for a replication). The BM was spray dried pig blood and the general composition and amino acids profile of BM (Fine Inc., Ansung-Si, Kyonggi-Do, Korea) are shown in Table 1. Ingredients and composition of experimental diets are shown in Table 2. The composition of general and amino acids of BM and diets were determined using AOAC (1990) methods. The layers were fed for 5wk. Water and diets were supplied *ad libitum*, and lighting system was regulated as 16 L:8 D during the entire experimental period.

Table 1. General and amino acid composition of blood meal (BM)

	Compositions[1]
Amino acids composition (g/100 CP)	
Aspatic acid	10.47
Threonine	3.45
Serine	4.57
Glutamine	9.10
Proline	3.52
Glycine	4.26
Alanine	7.33
Cystine	0.56
Valine	6.86
Methionine	0.22
Isoleucine	0.79
Leucine	11.43
Tyreonine	2.49
Phenylalanine	6.11
Histidine	5.76
Lysine	7.83
Arginine	3.78
Total	88.53
General compositions	
Moisture (%)	6.92
DM (%)	93.08
CP (%)	91.85
ME[2] (kcal/kg)	3,000
C.fat (%)	0.09
C.fiber (%)	0.06
C.ash (%)	1.06
Na (%)	1.11

[1] Compositions were analyzed using AOAC (1990) methods.
[2] ME was calculated based on NRC (1994).

Preparation of CBM and egg sample

At the end of each wk, one hen from each replication was sacrificed by cervical dislocation, a method approved by Animal Care Committee of Chung-Ang University. Preparation and assay of samples followed the method of Aristoy et al. (2004) in principle. About 10 g of CBM was taken from each hen and frozen immediately at -4°C in a freezer. Frozen CBM was freeze-dried until completely dry (about 72 h). The dried CBM was finely ground, and 1 g of the ground CBM was mixed with 24 ml of distilled water in a centrifuge tube (Falcon®). The tubes were shaken for 2 h at room temperature. Subsequently, the tubes were centrifuged under the conditions of 11,000×g and 4°C for 20 min. Three hundred μl of the supernatant were deproteinized by adding 900 μl of MeOH and then kept in a -4°C freezer for 12 h. After coagulation, protein was centrifuged under the conditions of 16,000×g and 4°C for 3 min. Finally, the supernatant was filtered through membrane syringe filters (MCE type, Pore size 0.45 μm, ADVANTEC MFS, Inc. Japan).

Eggs (five eggs per each replication, 25 eggs per treatment) were collected for evaluation of overall egg qualities. Eggshell strength was tested by Texture Analyzer (Stable Micro System, UK). Egg shell thickness was measured by Dial Pop Guage (Model 7360, Mitutoyo Co., Kawasaki213, Japan). Eggshell and egg yolk color were measured by the Roche Shell and Yolk color Fan (Switzerland), respectively, within the range of 7 to 12. Haugh unit (HU) was calculated by 100 log (H-(1.7×$W^{0.37}$) +7.57), where H and W indicate egg white height and egg weight, respectively.

HPLC system

The HPLC system consisted of two pumps (GILSON Model 305, France) and a fluorescence detector (GILSON Model 121, France). The separation was achieved by a CLIPEUS-C18 (250×4.5 mm, 5 μm) column (Higgins

Table 2. Ingredients and composition of experimental diets with different level in blood meal (BM) and magnesium oxide (MgO)

	Treatment[1]					
	T1	T2	T3	T4	T5	T6
Ingredients (%)						
Corn (USA, No 3)	54.80	54.80	53.43	53.43	52.06	52.06
Soybean meal (44%)	22.43	22.43	21.87	21.87	21.30	21.30
Corn gluten (61%)	2.52	2.52	2.45	2.45	2.40	2.40
Animal fat	2.50	2.50	2.44	2.44	2.37	2.37
Full-fat soy	5.00	5.00	4.88	4.88	4.75	4.75
Dicalcium-P	1.72	1.72	1.68	1.68	1.64	1.64
Limestone	9.68	9.68	9.43	9.43	9.19	9.19
Salt	0.25	0.25	0.24	0.24	0.24	0.24
Cholin 50%	0.05	0.05	0.05	0.05	0.05	0.05
D.S-Met-99%	0.95	0.95	0.93	0.93	0.90	0.90
Vitamin and mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10
BM(90% CP)	0.00	0.00	2.50	2.50	5.00	5.00
MgO	-	+	-	+	-	+
Total	100.00	100.00	100.00	100.00	100.00	100.00
Compositions						
ME[3] (kcal/kg)	2,800	2,800	2,805	2,805	2,810	2,810
C.P (%)	18.00	18.00	19.85	19.85	21.69	21.69
Ca (%)	4.00	4.00	3.90	3.90	3.80	3.80
P. available (%)	0.40	0.40	0.40	0.40	0.39	0.39
L-His (%)	0.42	0.42	0.55	0.55	0.68	0.68
Mg (%)	0.14	0.32	0.15	0.33	0.15	0.33

[1] T1 = 100% basal diet; T2 = 100% basal diet+MgO; T3 = 97.5% basal diet+2.5% BM; T4 = 97.5% basal diet+2.5% BM+MgO; T5 = 95% basal diet+5% BM; T6 = 95% basal diet+5% BM+MgO.

[2] Contains per kg: Vit A, 12,000,000 IU; Vit D_3, 2,500,000 IU; Vit E, 20,000 IU; Vit K_3, 1,800 mg; Vit B_1, 2,000 mg; Vit B_2, 6,000 mg; Vt B_6, 3,000 mg; Vit B_{12}, 20 mg; Ca-pantothenic acid, 10,000 mg; Folic acid, 1,000 mg; Oxyzero, 6,000 mg; Niacin, 25,000 mg; Biotin, 50 mg; I, 1,000 mg; Fe, 50,000 mg; Mn, 65,000 mg; Zn, 65,000 mg; Cu, 5,000 mg; Co, 250 mg; Se, 150 mg.

[3] Claculated composition.

Analytical, Inc. USA). Twenty μl of the sample solution was reacted with 100 μl of the OPA solution for 2 min before being injected into HPLC (OPA pre-column derivation method). The mobile phase consisted of the following: Phase A (50 mM acetic acid, pH 4.37) and Phase B (MeCN:MeOH:THF = 70%:25%:5% respectively). The flow rate was 1 ml/min for 50 min duration. The linear regression of standard was prepared using crystalline L-Car with distilled water in the range of 50 to 200 ppm. Chemicals for HPLC analysis were obtained as follows: standard L-carnosine (99% purity) solution from Sigma Inc. (Switzerland), acetonitrile (MeCN) and methanol (MeOH) from J.T. Baker (China, USA, respectively), tetrahydrofuran (THF) from Sigma Inc. (Germany), O-phthaldialdehyde (OPA) from Sigma Inc. (Austria) and 2-Mercaptoethanol (MCE) from SIGMA-ALDRICH.

Statistical analysis

All experimental data were analyzed by ANOVA using the GLM procedure (SAS Institute, 1995). Significant differences of treatment means of L-Car content and layer performance, and egg qualities were verified using Duncan's Multiple Range Test. To confirm the change of L-Car content from 1 to 5 wk, linear, and quadratic responses were tested. And the orthogonal contrasts for the effect of BM and MgO were performed. A replication was the experimental unit.

RESULTS AND DISCUSSION

Carnosine content of CBM

Carnosine in egg was not detected via analysis of present experiment, and there was no report about the L-Car in eggs. Therefore, the result of the L-Car content of CBM only was analyzed and shown in Table 3. There were no significant differences among treatments in all experimental period. However, contrast analysis showed that T6 (Basal diet 95%+BM5%+MgO) was significantly ($p<0.05$) higher than T4 (Basal diet 97.5%+BM2.5%+MgO) in L-Car content of CBM in the 1st wk. In the 2nd wk, differences were significant ($p<0.05$) in T4 vs. T6, T5 vs. T6, and T1 vs. T6 where T6 (Basal diet 95%+BM5%+MgO) was always

Table 3. The effect of blood meal (BM) and magnesium oxide (MgO) on the carnosine content in chicken breast muscle (CBM) of spent laying hens (mg/g DM)

wk	Treatments[1]						SEM (n = 5)
	T1	T2	T3	T4	T5	T6	
1*	32.23	31.44	32.64	28.06	35.16	35.11	2.305
2*	25.33	27.61	27.67	24.55	24.15	30.23	1.650
3	24.36	27.72	24.92	24.03	25.34	26.12	1.963
4*	23.55	24.89	22.81	21.59	24.40	25.60	1.263
5	21.80	24.36	26.05	24.84	20.57	22.71	1.930
Mean	25.45	27.20	26.82	24.61	25.92	27.95	1.824
Linear	<0.001	0.021	0.006	NS	<0.0001	<0.0001	
Quadratic	NS	NS	0.023	NS	0.013	NS	

[1] T1 = 100% basal diet; T2 = 100% basal diet+MgO; T3 = 97.5% basal diet+2.5% BM; T4 = 97.5% basal diet+2.5% BM+MgO; T5 = 95% basal diet+5% BM; T6 = 95% basal diet+5% BM+MgO.

NS : Not significant.

* Contrast : 1st wk; T4 vs. T6, p = 0.044. 2nd wk; T4 vs. T6, p = 0.030, T5 vs. T6, p = 0.027, T1 vs. T6, p = 0.018. 4th wk; T4 vs. T6, p = 0.050.

higher in L-Car content. In the 4th wk, contrast of T4 vs. T6 was also significant ($p<0.05$). Other comparisons did not show any significant differences. Weekly change (95 wk to 100 wk of age) of L-Car in CBM showed significantly linear decrease in T1 ($p<0.01$), T2 ($p<0.05$), T3 ($p<0.01$), T5 ($p<0.01$), and T6 ($p<0.01$). There were also significant ($p<0.05$) quadratic decrease in T3 and T5. Although, BM and MgO treatments did not show significant differences in linear comparison, the results indicate that BM level influences L-Car in CBM in certain weeks (wk 1, 2 and 4). The BM 5% treatment showed higher L-Car than BM 2.5% and BM 0% treatments. Effect of MgO was shown only in 2nd week at BM 5% treatment. It was shown that L-Car in CBM rapidly decrease as senescence proceeds in old layers. Although direct comparison between old layer and human may not the proper, Stuerenburg and Kunze (1999) reported that the L-Car concentration of skeletal muscle of neuromuscular disease patients showed negative concentrations by increase of age, and the response of skeletal and heart muscle of rats were similar to those of human beings. As the result of the present study, production of L-Car enriched CBM by treating BM and MgO may not be practical in old spent layers.

Layer performance and overall egg quality

The results of performance and egg quality are shown in Table 4. In case of layer performance, there were no significant differences in hen-day and hen-house egg production, feed intake, feed conversion, and mortality except egg weight and soft and broken egg ratio. Egg weight was highest ($p<0.01$) in T1 (Control) among other treatments, and soft and broken egg ratio was lowest ($p<0.05$) in T6 (Basal 95+BM5+MgO). In case of overall egg quality, there were no significant differences in eggshell color, eggshell thickness, and Haugh unit. Magnesium oxide supplemented treatments (T2, T4, and T6), however, showed significantly higher ($p<0.01$) eggshell strength (kg/cm^3) than those without MgO supplementation. Egg yolk color index was significantly higher in T1 (Control) and T2 (Control+MgO) than in other BM supplemented treatments. Soft and broken egg ratio of T6 (Basal 95+BM5+MgO) was the lowest.

In this experiment, MgO was used as a catalyst of carnosine synthetase. Interestingly, eggshell strength of MgO-treated treatments were significantly ($p<0.01$) stronger than those without MgO. Inorganic portion of eggshell is composed of Ca (98.2%), Mg (0.9%) and phosphorus (0.9%) (Romanoff and Romanoff, 1949). The occurrence of Mg in calcite has been extensively studied from the point of view of the Mg/Ca ratio (Cusack et al., 2003). Hess and Britton (1997) reported that the effects of excess dietary magnesium on eggshell of white Leghorn hens decreased. Stillmak and Sunde (1971) reported that eggshell quality was not influenced in hens fed excess Mg for the entire laying cycle. McWard (1967) reported that the Mg content of the grower rations had no effect on rate of lay and egg weight or eggshell thickness. In the present study, however, supplementation of Mg at the level of 0.18% (0.3% as MgO) significantly increased eggshell strength in old laying hens.

CONCLUSION

Generally, supplementation of BM and MgO did not siginificantly influenced L-Car content in CBM of old layers. However, BM 5% treatments and MgO supplementation showed higher L-Car content in some contrast analysis.

The level of L-Car in CBM rapidly decreased as senescense proceeds in old layer.

Supplementation of MgO to old layer diet increased eggshell strength.

Table 4. The effect of blood meal (BM) and magnesium oxide (MgO) on the laying hen performance and egg quality in spent laying hens

Parameter	Treatments[1]						SEM
	T1	T2	T3	T4	T5	T6	(n = 5)
	---------- Layer performance ----------						
Hen-day egg production[2] (%)	66.74	64.74	62.63	61.50	64.29	64.28	2.787
Hen-House egg production[3] (%)	66.72	64.74	62.63	60.47	64.29	64.22	2.777
Egg weight	71.45^{A}	69.02^{C}	70.69^{AB}	69.69^{BC}	70.13^{ABC}	71.00^{AB}	0.494
Feed intake (g/d)	117.84	116.24	115.88	116.33	113.20	114.66	1.795
Feed conversion (g/100 g egg mass)	2.47	2.57	2.65	2.73	2.56	2.60	0.120
Soft and broken egg (%)	0.85^{ab}	1.43^{a}	1.09^{a}	1.37^{a}	0.46^{b}	0.33^{b}	0.206
Mortality (%)	1.25	-	-	1.25	-	2.50	0.955
	---------- Overall egg quality ----------						(n = 25)
Eggshell strength* (kg/cm^{3})	1.83^{B}	2.16^{A}	1.94^{AB}	2.17^{A}	1.80^{B}	2.18^{A}	0.097
Eggshell color	10.26	10.20	10.09	10.37	10.15	10.19	0.126
Egg thickness (mm)	0.40	0.39	0.39	0.39	0.39	0.40	0.002
Egg yolk color	9.87^{a}	9.85^{a}	9.68^{ab}	9.56^{ab}	9.59^{ab}	9.47^{b}	0.106
Haugh unit[4]	76.21	77.47	76.39	76.41	78.58	78.44	1.048

$^{a-c, A-C}$ Within a row, means without a common superscript differ at $p<0.05$ or $p<0.01$.

[1] T1 = 100% basal diet; T2 = 100% basal diet+MgO; T3 = 97.5% basal diet+2.5% BM; T4 = 97.5% basal diet+2.5% BM+MgO; T5 = 95% basal diet+5% BM; T6 = 95% basal diet+5% BM+MgO.

[2] Total number of eggs/number of live layers×100

[3] Total number of eggs / number of layers housed×100

[4] Haugh unit = 100 log (H-(1.7×W$^{0.37}$+7.57)), where H refers to albumen height (mm) and W refers to measurements of egg weight (g).

*Contrast: No MgO treatments (T1, T3, and T5) vs. MgO treatments (T2, T4, and T6), p = 0.0001.

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