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ARTICLE

Effect of Dietary Supplementation of Blood Meal and Additional Magnesium on Carnosine and Anserine Concentrations of Pig Muscles

Se Won Park, Chan Ho Kim, Jong Woong Kim, Hye Seong Shin, In Kee Paik, and Dong Yong Kil*
Department of Animal Science and Technology, Chung-Ang University, Anseong 456-756, Korea

Abstract

The objective of this study was to investigate the effect of dietary supplementation of blood meal as a source of L-histidine, and the addition of magnesium (Mg) as a catalyst of carnosine synthetase for the carnosine and anserine concentrations of pig muscles (*longissimus dorsi*, LD and *vastus intermedius*, VI). A total of twenty-four pigs with an average body weight of 60.2±4.2 kg were randomly allotted to one of three dietary treatments (eight replicates), during 56 d of the feeding trial. Dietary treatments included: (1) Basal: basal diet; (2) BM: 95% basal diet + 5% blood meal; and (3) BM+Mg: 94.8% basal diet + 5% blood meal + 0.2% MgO (60% Mg). Results indicated that drip loss in the LD was less (p<0.05) for meat with BM+Mg treatment than that with Basal treatment, but the values for BM treatment did not differ from those of the other two treatment groups. The concentrations of carnosine in the LD were increased by 10.0% in both BM and BM+Mg treatment groups over the Basal treatment group (significance not verified). The concentrations of carnosine and anserine in the VI were not affected by the dietary treatments. Inclusion of additional Mg in diets had no effect on carnosine and anserine concentrations in the LD and VI. In conclusion, dietary supplementation of blood meal could be a potential method of fortifying the pork with carnosine. Inclusion of additional Mg in the diets containing blood meal had no benefit on carnosine and anserine depositions in pig muscles.

Key words: anserine, carnosine, *Longissimus dorsi*, pigs, *Vastus intermedius*

Introduction

Carnosine (β-alanyl-L-histidine) is a cytoplasmic dipeptide present in various animal tissues such as brain, skeletal muscle, and heart (Boldyrev and Severin, 1990) and is synthesized from L-histidine and β-alanine by carnosine synthetase with Mg as a catalyst (Kalyankar and Meister, 1959). Anserine is a methylated form of carnosine (Boldyrev and Severin, 1990). It has been reported that carnosine and anserine have beneficial roles such as antioxidants, antiglycation molecules, and anti-aging agents (Bogardus and Boissonneault, 2000; Nagasawa *et al.,* 2001), and therefore, they have been considered as functional bioactive compounds for human consumption (Guiotto *et al*., 2005).

There have been many efforts to produce animal products fortified with carnosine and anserine, particularly by a dietary means. It has been reported that dietary supplementation of blood meal high in L-histidine that is a direct source of carnosine synthesis increased carnosine and anserine concentrations in chicken breast (Auh *et al*., 2010; Park *et al*., 2013). However, there has been limited information about the pork by feeding the diets containing blood meal to pigs. In addition, Mg is an essential cofactor of carnosine synthetase, and therefore, inclusion of additional Mg in the diets containing blood meal may be effective to promote carnosine and anserine synthesis in animal products. This hypothesis has been tested in chickens previously (Namgung *et al*., 2010; Park *et al*., 2013), but it has not been verified in pigs.

The objective of the current experiment, therefore, was to determine the effect of dietary supplementation of blood meal with or without additional Mg on the concentrations of carnosine and anserine in pig muscles (*longissimus dorsi*, LD and *vastus intermedius*, VI).

Materials and Methods

Experimental design and diets

A total of 24 pigs [(Landrace × Yorkshire) × Duroc] were used in this experiment. Average initial body weight of

*Corresponding author: Dong Yong Kil, Department of Animal Science and Technology, Chung-Ang University, Anseong 456-756, Korea. Tel: 82-31-670-3028, Fax: 82-31-676-2196, E-mail: dongyong@cau.ac.kr

pigs was 60.2±4.2 kg. All pigs were randomly allotted to 1 of 3 dietary treatments and each treatment had 8 replicated pigs (4 barrows and 4 gilts). Three dietary treatments included: (1) basal diet (Basal), (2) 95% basal diet + 5% blood meal (BM), and (3) 94.8% basal diet + 5% blood meal + 0.2% MgO (60% Mg; BM+ Mg). All diets were formulated to meet or exceed the estimated requirements for all nutrients (NRC, 1998). Energy concentrations were similar across all 3 dietary treatments. The nutrient compositions of experimental diets were presented in Table 1. A commercial-type blood meal was purchased from a local company (Arirang BNS, Korea) and analyzed for lysine (1.48%), methionine (2.32%), threonine (4.33%), valine (6.20%), and histidine (6.33%). The diets and water were supplied to pigs as ad libitum basis for 56 d to reach average market body weight of approximately 105 kg. All animal protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University.

Sample preparation

On the last day of the experiment, all pigs were fasted for 12 h and were then transported to a commercial abattoir near the experimental station. After arrival, pigs were kept in holding pens for 1 to 3 h, and then slaughtered by electrical stunning, lifted off the floor, and exsanguinated. Carcasses were eviscerated, split, and placed in a chilling room at 5 °C for 12 h. The samples for the LD between 9th and 13th rib and the VI were collected from all pigs and were kept in an ice box during transporting to the laboratory. The LD and VI was frozen at -60°C before the analyses of nutrients, carnosine, and anserine. The pH, drip loss, and meat color in the LD were determined at 38 h after slaughter.

Sample analysis

Amino acid compositions in 3 diets and blood meal were determined by the method of Baker *et al*. (2011). Nutrient concentrations in diets and muscles were analyzed by AOAC methods (AOAC, 1990). The drip loss was measured according to the method of Honikel (1998). In short, a 4 cm thick slice of the LD (approximately 100 g) were trimmed, placed in a plastic container, and suspended from a fish hook for 24 h at 4°C. Drip loss was calculated by comparing the final and initial weight of the LD. The pH of the LD was measured by a pH meter (Model HI99163, Hanna Instruments, Romania). Meat color for CIE L* (lightness), a* (redness), and b* (yellowness) of the LD was measured by a Minolta chromameter (Model CR-400,

Table 1. Formula and composition of experimental diets

	Dietary treatments[a]		
	Basal	BM	BM+Mg
Ingredient, %			
Corn	37.70	35.69	35.49
Wheat	17.00	16.15	16.15
Soybean meal	11.10	10.55	10.55
DDGS[b]	10.00	9.50	9.50
Lupin kernel	7.00	6.65	6.65
Blood meal	0.00	5.00	5.00
Tallow	4.00	3.80	3.80
Tapioca	3.15	2.99	2.99
Molasses	2.50	2.38	2.38
Rapeseed meal	2.00	1.90	1.90
Limestone	1.31	1.31	1.31
Palm kernel meal	1.00	0.95	0.95
Rice bran	0.85	0.81	0.81
Corn gluten meal	0.81	0.77	0.77
Salt	0.40	0.38	0.38
Wheat bran (domestic)	0.30	0.29	0.29
$_{L}$-Lysine-HCl-78%	0.28	0.28	0.28
Sugar	0.15	0.15	0.15
$_{L}$-Threonine-98%	0.05	0.05	0.05
Phytase	0.05	0.05	0.05
NSP enzyme	0.05	0.05	0.05
MgO	-	-	0.20
Vitamin premix[c]	0.15	0.15	0.15
Mineral premix[d]	0.15	0.15	0.15
Total	100.00	100.00	100.00
Energy and Nutrient composition[e]			
ME, kcal/kg	3,350	3,331	3,331
Crude protein, %	15.97	20.23	21.29
Crude fat, %	7.39	7.67	7.26
Crude ash, %	5.96	6.02	6.11
Lysine, %	1.76	1.50	1.51
Methionine, %	2.73	2.24	2.33
Threonine, %	3.23	3.46	3.52
Valine, %	1.50	1.50	1.42
Histidine, %	4.37	5.92	5.54
Ca, %	0.62	0.62	0.62
Mg, %	0.16	0.17	0.28

[a]Basal, the basal diets; BM, 95.0% Basal+5.0% blood meal; BM+ Mg, 94.8% Basal+5.0 blood meal+0.2% MgO

[b]Corn Distillers Dried grains with Solubles

[c]Provided per kilogram of the complete diet: vitamin A (from vitamin A acetate), 9,000 IU; vitamin D_3, 1,800 IU; vitamin E (from DL-α-tocopheryl acetate), 25 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3 mg; vitamin B_{12}, 20 mg; calcium pantothenate, 15 mg; folic acid, 0.5 mg; biotin, 0.1 mg; niacin, 24 mg; pyridoxine, 2 mg; ethoxquine, 6.6 mg

[d]Provided per kilogram of the complete diet: Fe (as $FeSO_4 \cdot 7H_2O$), 66.67 mg; Zn (as $ZnSO_4 \cdot H_2O$), 36.67 mg; Mn (as $MnSO_4 \cdot H_2O$), 34.4 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 66.67 mg, Se (as Na_2SeO_3), 0.12 mg; I (as $Ca(IO_3)_2 \cdot H_2O$), 0.7 mg

[e]Analyzed values for all nutrients except for ME and Ca (NRC, 1998)

Minolta Co. Ltd., Japan). The color measurements were performed 3 times with different surfaces of the samples and the average values were calculated.

The concentrations of carnosine and anserine in the LD and VI were analyzed by the methods of Aristoy and Toldra (2004). Briefly, approximately 10 g frozen samples of the LD and VI were freeze-dried to a constant weight and finely ground. The ground samples were mixed with 24 mL of distilled water in a centrifuge tube. The tubes were shaken for 2 h at room temperature and centrifuged under the conditions of 11,000×*g* at 4°C for 20 min. The supernatant was filtered using membrane filter (MCE type, Pore size 0.45 μm, ADVANTECMFS, Inc., Japan). Three hundred μL of the supernatant were de-proteinated with 900 μL methanol. After the coagulation of protein, the samples were centrifuged again under the conditions of 16,000 ×*g* at 4°C for 3 min and diluted 100 folds for HPLC analysis.

The HPLC analysis was performed using Varian 920-LC (Varian Medical Systems, 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, USA), and 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°C, flow rate was 1 mL/min, and running time was 13 min. The linear regression of standard was prepared using crystalline carnosine and anserine with distilled water in the range of 50 to 150 ppm. Detailed information for chemicals used for HPLC analysis was reported previously (Auh *et al*., 2010).

Statistical analysis

All data were analyzed by ANOVA according to a completely randomized design using the Proc Mixed procedure of SAS (SAS Inst., Inc., USA). Individual pig was an experimental unit. Outlier data were identified by the UNIVARIATE procedure of SAS, but no outliers were found. Least squares means were calculated and the means among treatments were compared by the PDIFF option with the Tukey's adjustment. Significance was set at $p<0.05$.

Results and Discussion

All pigs remained healthy and easily consumed their respective diets throughout the experiment. There were no differences in body weight gain, feed intake, and feed efficiency during 56 d of the feeding trial (Data were not shown). For nutrient contents in pig muscles (Table 2), the concentrations of dry matter (DM), crude ash, and crude protein in the LD and VI were not different among dietary treatments. The concentrations of crude fat in the LD were less ($p<0.01$) for BM and BM+Mg treatment groups than for Basal treatment. The reason for this observation is likely associated with both a greater amount of crude protein in the diets containing blood meal than the basal diet and less energetic efficiency of crude protein for de novo lipogenesis in the body than other energy substrates such as carbohydrates and fats (Kil *et al.*, 2013). However, the concentrations of crude fat in the VI did not differ among dietary treatments. Differences in the amounts of potential fat depositions between the LD and VI may be the reason for this observation.

Drip loss was less ($p<0.05$) for BM+Mg treatment than Basal treatment, whereas no significant differences were

Table 2. Effect of dietary supplementation of blood meal and additional Mg on the proximate analysis of *longissimus dorsi* (LD) and *vastus intermedius* (VI) in pigs

Items	Dietary treatments[a]			SEM	*p*-value
	Basal	BM	BM+Mg		
Longissimus dorsi (%)					
DM	22.6	23.8	23.6	0.85	0.58
Crude ash	0.99	0.99	0.99	0.02	0.99
Crude fat	3.17[x]	2.70[y]	2.63[y]	0.06	<0.01
Crude protein	24.0	24.3	24.0	0.61	0.93
Vastus intermedius (%)					
DM	21.9	22.7	23.4	0.78	0.76
Crude ash	0.99	0.99	0.99	0.03	1.00
Crude fat	1.96	1.99	1.87	0.06	0.43
Crude protein	22.7	22.9	22.8	0.24	0.84

[x,y]Values in the same row with no common superscripts are different ($p<0.05$)

[a]Basal, the basal diets; BM, 95.0% Basal+5.0% blood meal; BM+Mg, 94.8% Basal+5.0 blood meal+0.2% MgO

observed for BM treatment as compared to other 2 treatment groups (Table 3). This result is likely a consequence of high amounts of Mg in the diets containing blood meal and additional Mg. It has been reported that inclusion of additional Mg in diets fed to growing-finishing pigs decreased drip loss in the pork, possibly due to the amelioration of stress responses in pigs (D'Souza *et al.*, 1998; Kietzman and Jablonski, 1985). Although there was no significant difference, the drip loss was also reduced by 25.0% in BM treatment as compared to Basal treatment. This observation may be related with increased concentrations of carnosine in the LD as observed in this experiment and its antioxidant effects on the lipid membrane of muscle cells. Ma *et al*. (2010) reported that dietary supplementation of carnosine improved antioxidant capacity and decreased drip loss in the pork. However, in other measurements for determining pork quality, the pH and meat color (CIE L*, a*, and b*) in the LD were not influenced by dietary treatments.

The concentrations of carnosine in the LD were increased numerically by approximately 10.0% in both BM and BM+Mg treatment groups than Basal treatment although we failed to detect the significance (Table 4). Similar results were also observed by the previous experiments using broiler chickens. Auh *et al*. (2010) and Park *et al*. (2013) reported that feeding the diets containing 5.0% blood meal to broiler chickens increased the concentrations of carnosine in the breast meat. These results suggest that blood meal high in L-histidine can be a useful dietary ingredient in pig diets for producing the carnosine-enriched pork as observed in the chicken breast. However, the concentrations of anserine, a methylated form of carnosine, in the LD were not affected by dietary treatments. This result may be associated with little activity of carnosine methylation to anserine in pig muscles (Boldyrev and Severin, 1990). On the contrary, the concentrations of carnosine and anserine in the VI did not differ among dietary treatments. The reason for the differences in carnosine deposition between the LD and VI may be the fact that carnosine depositions are greater for white muscle type than for red muscle type and the LD contains a greater amount of white muscle fiber than the VI (Beecher *et al.*, 1965; Plowman and Close, 1988).

Inclusion of additional Mg in the diets containing blood meal had no positive effects on carnosine and anserine depositions in the LD and VI. The Mg is a cofactor of carnosine synthetase (Kalyankar and Meister, 1959), and therefore, it is likely expected that dietary supplementation of additional Mg may promote carnosine synthesis in animal products (Park *et al.*, 2013). However, the current ex-

Table 3. Effects of dietary supplementation of blood meal and additional Mg on drip loss, pH, and meat color of *longissimus dorsi* (LD) in pigs

Items	Dietary treatments[a]			SEM	*p*-value
	Basal	BM	BM+Mg		
Drip loss, %	2.8[x]	2.1[xy]	1.7[y]	0.29	<0.05
pH	5.74	5.77	5.75	0.02	0.62
Meat color[b]					
CIE L*	49.8	49.1	50.7	0.80	0.38
CIE a*	4.7	5.1	5.8	0.42	0.19
CIE b*	6.9	7.0	7.6	0.22	0.07

[x,y]Values in the same row with no common superscripts are different ($p<0.05$)
[a]Basal, the basal diets; BM, 95.0% Basal+5.0% blood meal; BM+Mg, 94.8% Basal+5.0 blood meal+0.2% MgO
[b]CIL L* (lightness), black (0) to white (100) color scale; CIE a* (redness), red (+) to green (−) color scale; CIE b* (yellowness), yellow (+) to blue (−) color scale

Table 4. Effects of dietary supplementation of blood meal and additional Mg on carnosine and anserine concentrations of pig muscles

Items	Dietary treatments[a]			SEM	*p*-value
	Basal	BM	BM+Mg		
Longissimus dorsi (LD, mg/100g DM)					
Carnosine	312.7	347.5	348.2	14.90	0.19
Anserine	10.8	11.8	16.2	2.69	0.36
Vastus intermedius (VI, mg/100g DM)					
Carnosine	291.3	306.3	296.3	15.47	0.79
Anserine	15.8	16.0	18.2	1.26	0.37

[a]Basal, the basal diets; BM, 95.0% Basal+5.0% blood meal; BM+Mg, 94.8% Basal+5.0 blood meal+0.2% MgO

periment and previous experiments using broiler chicken (Park *et al*., 2013) failed to find significant effects on the concentrations of carnosine in animal products. Therefore, it is most likely that inclusion of additional Mg in diets fed to animals may have no beneficial effects on carnosine synthesis in the animal body.

In conclusion, blood meal may be a potential ingredient in pig diets for enhancing carnosine concentrations in the pork. Inclusion of additional Mg in the diets containing blood meal decreases drip loss, but has no benefit on increasing concentrations of carnosine and anserine in the pork.

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