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Effect of Amino Acids on Growth Performance, Carcass Characteristics, Meat Quality, and Carnosine Concentration in Broiler Chickens

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The aim of this research was to investigate the deposition of carnosine in broiler muscles by feeding treatments comprising β-alanine, L-histidine, and magnesium oxide in various concentrations. The research was carried out on 120 Cobb 500 broilers divided into four groups. From weeks four to six, broilers were fed finisher mixtures as follows: P1, control group; P2, 0.5% β-alanine + 0.24% MgO; P3, 0.25% L-histidine + 0.24% MgO; and P4, 0.20% β-alanine + 0.10% L-histidine + 0.24% MgO. This paper presents the weights of broilers and their carcasses, portions of main parts of carcasses, technological quality of breast muscles, and concentrations of carnosine in breast and thigh muscles. The following traits of muscle tissue quality were measured: initial and final pH value (45 min after slaughtering pH_1, and 24 h after cooling pH_2), drip loss, color (Minolta colorimeter, expressed as CIE L*, CIE a*, and CIE b* values), meat softness, and cooking loss. Data on relative concentration of protein carbonyl (nmol/mg protein) in the muscles of breasts and thighs and levels of thiobarbituric acid-reactive substances (TBARS) in fresh and frozen breasts muscles (nmol/mg of tissue) are presented. Statistical analysis proved that feeding treatments had an effect on the live weight of broilers in the 4th, 5th, and 6th weeks of fattening ($P<0.05$), as well as on the carcass quality at slaughter ($P<0.05$; except the portion of wings), pH_1 value ($P=0.035$), CIE a* indicator ($P=0.007$), drip loss ($P=0.002$), and meat texture ($P=0.008$). Compared to the control group, synthesis and deposition of carnosine were increased in breast muscles in groups P2, P3, and P4 by 7.51%, 10.62%, and 62.93%, respectively, and in thigh muscles by 61.05%, 78.95%, and 89.52%, respectively. It was also confirmed that feeding treatments influenced the level of TBARS in frozen broiler breast muscles ($P=0.014$).

Key words: β-alanine, broiler, carnosine, L-histidine, meat quality, performance

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Introduction

Functional food components have beneficial effects on certain functions in humans; they improve the body's condition and reduce the risk of some diseases. Carnosine plays an important role in human physiological functions; it regulates intracellular pH, prevents oxidation, and assures normal neurotransmission (Chan and Decker, 1994, Wu and Shiau, 2002). Boldyrev *et al*. (2013) reported that carnosine influenced skeletal muscle, brain, and cardiovascular system functions. It also slows down the aging process (anti-aging factor), and increases the capacity and intensity of an athlete's activities. Carnosine content can be affected by the type of muscle tissue (white or dark meat) and animal species (cattle, sheep, rabbits, and poultry), as well as by breed (autochthonous breeds or hybrids), sex, age, and breeding (Abe and Okuma, 1995; Boldyrev *et al*., 2013). For example, the muscles of some fish (salmon, trout) contain anserine only and human muscles contain carnosine only, but muscles of birds contain both dipeptides (Boldyrev, 2006). It is considered that the poor quality of meat stored in a refrigerator is caused by lipid oxidation, which causes changes in the color, taste, and odor. Poultry meat is highly susceptible to oxidative processes that cause pigment loss, and intensive oxidation can be caused by a high content of polyunsaturated fatty acids. Many studies have shown that lipid oxidation in meat products can effectively be controlled by means of antioxidants (vitamins E and C, selenium, carnosine). Carnosine is a dipeptide composed of β-alanine and L-histidine; due to its physiological role in an organism, it can be considered a bioactive ingredient of food. As precursors of dipeptide, β-alanine and L-histidine are important in the synthesis of carnosine (β-alanine–L-histidine), homocarnosine (γ-glutamine–L-histidine), and anserine (β-alanine–1-methylhistidine). These dipeptides are deposited in some

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animal tissues, such as mice brains, mammal skeletal muscles, and marine fish skeletal muscles (Neidle and Kandera, 1974; Abe, 2000; Martinez *et al*., 2005). The availability of β-alanine and L-histidine in an organism is probably a limiting factor for carnosine synthesis. Carnosine synthetase promotes the condensation of β-alanine with L-histidine in a neutral medium in the presence of magnesium ions and adenosine triphosphate (ATP). It is presumed that this biosynthesis proceeds in two steps.

The first step can be described by the following schema: β-alanine + ATP + carnosine synthetase ⇆ carnosine synthetase-β-alanine-AMP^{Mg2+}+ pyrophosphate

The second step would be: carnosine synthetase-β-alanine-AMP + L-histidine ⇆ L-carnosine + carnosine synthetase + AMP, where AMP = adenosine monophosphate (Kalyankar and Meister, 1959; Stenesh and Winnick, 1960).

A higher content of carnosine is found in the breast muscles, and a lower content is found in the drumstick and thigh muscles of chicken (Plowman and Close, 1988; Intarapichet and Maikhunthod, 2005; Kralik *et al*., 2010a, 2010b, 2014, 2015). Amend *et al*. (1979), Haug *et al*. (2008), Hu *et al*. (2009), Auh *et al*. (2010), Kopec *et al*. (2013a, 2013b), Kralik *et al*., (2014, 2015) investigated the possibilities of enriching broiler meat with carnosine through the application of various feeding treatments.

Since there is no carnosine-enriched broiler meat available on the market, and since human health concerns and dietary supplements are becoming increasingly popular, the authors are convinced that a food product designed for this purpose could be well accepted by consumers, especially by athletes. The aim of this research was to verify the hypothesis that enhanced carnosine synthesis could be achieved by supplementing broiler diets with certain concentrations of β-alanine and L-histidine, with MgO as a carnosine synthetase catalyst.

Materials and Methods

Animals – Feeding and Housing

The research was carried out on 120 Cobb 500 broilers. Broilers were divided into four groups, each containing 30 broilers (P1, P2, P3, and P4, each group comprised three subgroups with 10 broilers). From the day one to 21, broilers were fed a starter mixture that contained 21% crude protein and 13.5 MJ/kg metabolizable energy (ME). At the beginning of week four of the fattening period (day 22), broilers were randomly divided into four groups. From the day 22–42, broilers were fed pelleted finisher mixtures with different concentrations of supplemented amino acids in the presence of magnesium oxide. The P1 group was the control, with 0.0% supplemented β-alanine, L-histidine, and MgO, while other groups had diets that were supplemented as follows: P2, 0.5% β-alanine + 0.24% MgO; P3, 0.25% L-histidine + 0.24% MgO; and P4, 0.20% β-alanine + 0.10% L-histidine + 0.24% MgO.

Feeding and watering was *ad libitum* and automatically regulated. Compositions of feeding mixtures are presented in Table 1. Diets were composed on the basis of physiological needs of the Cobb 500 chickens, and the experiment was performed according to technological standards, upon having obtained the approval of the Ethical Committee. Broilers were kept in groups on the floor (17 broilers/m^2). During the experiment, ambient temperature was gradually reduced from 32 to 20℃. The relative humidity in the room varied from 65% to 75%. The lighting program was 24 h/per day for the first eight days, and after that it included 3 h of darkness per day. The conversion of feed was calculated as a ratio of consumed feed and weight gain. The control of weights and conversion was performed in weeks three, four, five, and six of fattening. After completing the fattening period, broilers were sacrificed on day 42, and the carcasses were processed according to Commission Regulation (EC) No. 543/2008, which regulates marketing standards for poultry meat. Broiler carcasses (n = 20/group: 10 males + 10 females) were cut into breasts, drumsticks with thighs, backs, and wings, the values of which are presented in absolute (g) and relative parts (%).

Meat Quality

Referring to the technological indicators of meat quality, the following was measured: pH_1 value (45 min after slaughter) and pH_2 value (24 h after slaughter and cooling at 4℃) at the *pectoralis major* muscle by using a digital pH meter (MP120 Basic Portable, Mettler Toledo GmbH, Giessen, Germany). Drip loss from breast muscles was determined by the bag method of Honikel, 1998, as follows: a sample of breast muscle, 2 cm in diameter and 3 cm high, was weighed and put into a plastic bag to rest for 48 h at 4℃ in a refrigerator, and the drip loss was calculated as follows:

Drip loss (%) = initial weight of tissue (g) − final weight of tissue (g)/initial weight of tissue (g) × 100

Meat color was determined on samples of breast muscles 24 h after cooling by using a CR-300 Chroma Meter (Konica Minolta, INC., Tokyo, Japan). The color was read in three values: CIE L* for paleness, CIE a* for redness, and CIE b* for yellowness. Calibration of the device was performed by using a standard white plate (Referential No. 16733047, C, Y = 93.0, x = 0.3134, and y = 0.3195; D_{65}, Y = 93.0, x = 0.3159, and y = 0.3324). Before measuring the color, there was fresh vertical cut made in the middle of the breast muscle. The sample was rested for 15 min at 21–22℃ in order to stabilize the color, upon which the muscle tissue color was defined.

Instrumental tenderness was measured on the left half of the breast muscle. Before processing, the meat was defrosted for 24 h at a temperature of 4℃. The samples were then sealed in cooking bags and heat-treated in a water bath to reach a temperature of 77℃ in the center of the meat. After cooking, the samples were cooled to 21–22℃. There were at least three sub-samples cut from the central part of the breast muscle parallel to the muscle fibers in dimensions 3 cm × 1.9 cm × 1.9 cm. Then, each subsample was cut perpendicularly to the direction of the muscle fibers by using a Warner-Bratzler blade attached to a TA.XT*plus* Texture Analyser (Stable Micro Systems, Godalming, Surrey, England), and their average value was expressed as instrumental tenderness

Table 1. **Composition of feeding mixtures for broilers**

Ingredient (%)	Mixtures 22nd–42nd day			
	P1	P2	P3	P4
Corn	60.78	60.04	60.29	60.24
Animal wheat flour	5.00	5.00	5.00	5.00
Soybean cake	23.85	23.85	23.85	23.85
Animal yeast	2.50	2.50	2.50	2.50
Methionine DL	0.12	0.12	0.12	0.12
Lysine 99%	0.12	0.12	0.12	0.12
Sunflower oil	3.70	3.70	3.70	3.70
Salt	0.25	0.25	0.25	0.25
Na-bicarbonate	0.20	0.20	0.20	0.20
Limestone	1.22	1.22	1.22	1.22
Monocalcium phosphate	1.61	1.61	1.61	1.61
Organic acids	0.40	0.40	0.40	0.40
Premix with phytase without coccidiostats	0.25	0.25	0.25	0.25
β-alanine	—	0.50	—	0.20
L-histidine	—	—	0.25	0.10
MgO	—	0.24	0.24	0.24
Total	100.00	100.00	100.00	100.00
*Chemical analysis of mixtures				
Water (g/kg)	109	110	112	114
Ash (g/kg)	57	53	53	38
Crude protein (g/kg)	191.7	190.6	182.0	171.1
Fat (g/kg)	47	52	28	28
Crude fiber (g/kg)	38	39	40	32
Calcium (g/kg)	9.7	9.1	9.6	5.1
Phosphorus (g/kg)	6.9	5.8	5.9	4.3
Sodium (g/kg)	2.1	2.0	1.9	1.0
Sugar	46.6	47.7	39.0	39.6
Starch	371.6	367.9	401.8	442.1
ME (MJ/kg)	12.98	12.98	12.98	12.98

Treatments: P1=control; P2=0.5% β-alanine+0.24% MgO; P3=0.25% L-histidine+0.24% MgO; P4=0.20% β-alanine+0.10% L-histidine+0.24% MgO; ^{a,b}, $P<0.05$; SEM=standard error of the treatment.

Composition of premix in 1 kg of mixture: vit. A (E672) 13,000 IE; vit D3 (E671) 3,000 IE; vit E (DL-α tocopherol) 35 mg; vit K3 2.5 mg; vit B1 3 mg; vit B2 6 mg; nicotinamide 40 mg; calcium pantothenate 12 mg; vit. B6 5 mg; vit. B12 0.02 mg; biotin 0.18 mg; choline chloride 500 mg; folic acid 1 mg; iodine (E2 potassium iodide) 1 mg; iron (E1) 50 mg; copper (E4 copper sulfate-pentahydrate) 15 mg; manganese (E5, manganese sulfate, hid.) 80 mg; zinc (E6 zinc sulfate, hid.) 60 mg; cobalt 0.1 mg; selenium (E8 sodium selenite) 0.2 mg; antioxidant BHT (E321) 100 mg; phytase Ronozyme P (E1614 I) 500 FYT; organic acids 3,000 mg.

* Chemical analysis of mixtures was performed by following referential methods: M-2 (HRN ISO 6496:2001), M-3 (HRN ISO 5984:2004), M-4 (HRN EN ISO 5983-2:2010), M-5 (HRN ISO 6492:2001), M-6 (HRN EN ISO 6865:2001), M11, M12 (HRN ISO 6491:2001), and M-13 (HRN ISO 7485:2001).

or Warner–Bratzler Shear Force (WBSF, N).

Cooking loss was determined on the samples of breast muscle by using the following equation:

Cooking loss (%)={[sample weight before cooking (g) −sample weight after cooking (g)]/sample weight before cooking (g)}×100.

Determination of Carnosine Concentration

Analysis of carnosine in muscle tissue was performed on five male and five female randomly selected broilers. Samples of tissues were prepared according to the method described by Aristoy and Toldra (2004), and the concentration of carnosine was defined with a high-performance liquid chromatography (HPLC) device Varian Prostar (Varian Inc., Palo Alto, CA, USA) equipped with a fluorescent detector and a Zorbax ODS (Agilent, Santa Clara, CA, USA), 4.6×250 mm column. Before injection, the sample was derivatized with OPA reagent according to the method of Intarapichet and Maikhunthod (2005).

Table 2. **Effect of feeding treatments on broiler live weight (g) and feed conversion (kg) according to each week of fattening**

Indicators (n=30)	Treatments - experimental group P1	P2	P3	P4	SEM	P-value
Age (weeks)			Broiler weight			
3	854	859	880	868	4.98	0.268
4	1392[b]	1371[b]	1493[a]	1453[a]	11.48	***<0.001***
5	1988[b]	2006[b]	2113[a]	2021[ab]	17.02	***0.042***
6	2506[c]	2651[b]	2800[a]	2744[ab]	25.56	***<0.001***
Weeks of fattening			Feed conversion			
4	1.71[b]	1.82[a]	1.68[bc]	1.61[c]	0.021	***<0.001***
5	1.92[bc]	1.90[c]	1.96[b]	2.04[a]	0.013	***<0.001***
6	2.16[c]	2.28[a]	2.21[b]	2.02[d]	0.008	***<0.001***
4–6	2.04[a]	2.01[b]	1.96[c]	1.90[d]	0.007	***<0.001***
1st day–6th week	1.67[b]	1.76[a]	1.70[b]	1.66[b]	0.013	***0.003***

Treatments: P1, control; P2, 0.5% β-alanine+0.24% MgO; P3, 0.25% L-histidine+0.24 % MgO; P4, 0.20% β-alanine+0.10% L-histidine+0.24% MgO; [a, b], $P<0.05$; SEM, standard error of treatment.

Determination of Protein Carbonyl Concentration and Level of TBARS

The concentration of protein carbonyl (PC, nmol/mg protein) was determined in breast and thigh muscles by using the method of Levine (2002) modified by Stepić *et al*. (2012), and the level of reactive substances of thiobarbituric acid (TBARS, nmol/g tissue) were determined by the method of Buege and Aust (1978) in fresh and frozen (60 days at −20 ℃) breast muscles.

Statistical Analysis

The research results were analyzed by using the statistical software Statistica 13.2. (Dell Inc., 2016). Referring to the statistical parameters, the following was presented: arithmetic mean ($\bar{x}$) and standard error of the mean (SEM). Differences between the groups were determined by variance analysis (ANOVA) using the GLM procedure. Statistically significant effects of the treatment were determined to be ones for which the calculated value of P for ANOVA was less than 0.05 ($P<0.05$). Fisher's least square difference (LSD) test was used to calculate the differences between groups at $P<0.05$. Statistically significant differences between the experimental groups are marked by different letters (a, b, c, and d).

Results

Effect of the Feeding Treatments on Broiler Performances

Live weights of broilers per group are shown in Table 2. Only one broiler, in the P4 group, died, on the third day of the experiment. The effect of the feeding treatments on weight was obvious after only one week of feeding finisher mixtures, and remained so until the end of the experiment. From the fourth week of fattening, broilers in the P3 group weighed more ($P<0.05$) than broilers in groups P1, P2, and P4. At the end of experiment, broilers in the P3 group were the heaviest (2,800 g), followed by broilers in groups P4 (2,744 g), P2 (2,651 g), and P1 (2,506 g). These results prove that feeding treatments had a significant effect on weights of fattening broilers in the fifth week ($P=0.042$), as well as in the fourth and sixth weeks ($P<0.001$).

Statistical analysis proved that feeding treatments (P1, P2, P3, and P4) had a significant effect on feed conversion in the fourth, fifth and sixth weeks of fattening, as well as in the period from the fourth to sixth week ($P<0.001$). The achieved feed conversion for the whole fattening period was the most favorable in the P4 group (1.66 kg), followed by group P1 (1.67 kg), and then groups P3 and P2 (1.70 kg and 1.76 kg, respectively).

The effect of the feeding treatments on the quality of broiler carcasses is overviewed in Table 3. Weights of carcasses and main parts (breasts, drumsticks with thighs, backs, and wings) were corresponding to live weights of broilers in the experiment. Therefore, group P3 had heavier ($P<0.05$) carcasses, breasts, drumsticks with thighs, and backs than groups P1, P2, and P4. Groups P1 and P3 had better dressing percentages ($P<0.05$) than groups P2 and P4. There were no statistically significant differences determined for the wing weights between experimental groups ($P>0.05$), although there were higher average weights measured in group P3 (heavier carcasses) than in the other groups.

Unlike absolute values, relative portions of breasts, drumsticks with thighs, and wings in carcasses did not differ between the groups ($P>0.05$), i.e., they were not affected by the feeding treatments. The portion of breasts in the carcass ranged from 36.07% (P2) to 37.21% (P4), the portion of drumsticks with thighs ranged from 28.23% (P1) to 29.12% (P2 and P4), and the portion of wings ranged from 9.96% (P3) to 10.33% (P1). A statistically significant effect of the treatments ($P=0.010$) was determined for portions of backs in carcasses, compared to the control.

Table 3. **Effect of feeding treatments on carcass weight, dressing percentage, and absolute and relative portions of main parts in broiler carcass**

Characteristic	Treatments - experimental group				SEM	P-value
(n=20)	P1	P2	P3	P4		
	Carcass weight, dressing percentage, and absolute portions of main parts in carcass					
Live weight (g)	2518[c]	2678[bc]	2848[a]	2709[ab]	32.06	***0.003***
Carcass (g)	1817[b]	1878[b]	2034[a]	1896[b]	23.75	***0.001***
Dressing percentage (%)	72.09[a]	70.15[b]	71.41[a]	69.99[b]	0.22	***0.001***
Breasts (g)	670[b]	677[b]	736[a]	705[ab]	8.62	***0.026***
Drumsticks with thighs (g)	513[b]	547[b]	591[a]	553[ab]	8.21	***0.008***
Backs (g)	447[b]	463[b]	505[a]	444[b]	6.85	***0.004***
Wings (g)	187	192	202	193	2.18	0.087
	Relative portions of main parts in carcass					
Breasts (%)	36.90	36.07	36.21	37.21	0.18	0.076
Drumsticks with thighs (%)	28.23	29.12	29.01	29.12	0.15	0.108
Backs (%)	24.54[a]	24.58[a]	24.82[a]	23.46[b]	0.16	***0.010***
Wings (%)	10.33	10.23	9.96	10.21	0.06	0.132

Treatments: P1, control; P2, 0.5% β-alanine+0.24% MgO; P3, 0.25% L-histidine+0.24% MgO; P4, 0.20% β-alanine+0.10% L-histidine+0.24% MgO; [a, b], $P<0.05$; SEM, standard error of treatment.

Table 4. **Effect of feeding treatments on the technological quality of breast muscles of broilers**

Characteristic	Treatments - experimental group				SEM	P
(n=20)	P1	P2	P3	P4		value
pH_1	6.10[a]	6.02[ab]	6.07[a]	5.97[b]	0.02	***0.035***
pH_2	5.80	5.79	5.81	5.85	0.01	0.502
CIE L*	55.35	54.91	53.88	54.39	0.30	0.342
CIE a*	1.20[a]	0.86[b]	0.63[b]	0.79[b]	0.06	***0.007***
CIE b*	7.98	7.46	7.44	6.63	0.21	0.136
Drip loss (%)	2.03[a]	1.48[b]	1.45[b]	1.64[b]	0.06	***0.002***
Cooking loss (%)	19.70	20.02	19.33	18.99	0.23	0.421
WBSF (N)	31.56[b]	37.20[a]	37.91[a]	35.07[ab]	0.73	***0.008***

Treatments: P1, control; P2, 0.5% β-alanine+0.24% MgO; P3, 0.25% L-histidine+0.24% MgO; P4, 0.20% β-alanine+0.10% L-histidine+0.24% MgO; [a, b], $P<0.05$; SEM, standard error of treatment.

Effect of Feeding Treatments on the Breast Muscle Quality

The effect of feeding treatment on the quality of breast muscles is presented in Table 4. Among the meat quality indicators, the highest importance is attributed to the pH value because it affects traits such as color, water holding capacity, taste, and tenderness. As presented in Table 4, it is visible that the feeding treatments had a statistically significant effect on the pH value of breast muscles measured 45 minutes after slaughter, where the lowest pH_1 value of breast muscles was measured in the P4 group (5.97), which was statistically significantly lower ($P<0.05$) than those in groups P1, P2, and P3. However, 24 h after slaughter, there were no differences determined in pH values between broiler groups ($P=0.502$). Values of pH_2 were quite even, ranging from 5.79 (P2) to 5.85 (P4).

Although no statistically significant differences in the CIE L* values were found between the feeding treatments, it can be seen in Table 4 that the group P1 had the highest CIE L* values, and their breast muscle was redder (CIE a*=1.20, $P<0.05$) compared to the other groups (P2, P3, and P4). Additionally, it is clear that supplementation with β-alanine or L-histidine and their combinations had a favorable effect on drip loss. Drip loss from the breast muscles of the P1 group was statistically significantly higher ($P<0.05$) than in other groups. Cooking loss was not related to feeding treatments of the investigated broiler groups ($P=0.421$). The highest cooking loss was determined in breast muscles of the P2 group (20.02%) and the lowest was in breast muscles of the P4 group (18.99%). However, the composition of diets had a significant effect ($P=0.008$) on the meat

Table 5. **Effect of treatments on concentration of carnosine (mg/kg) in breasts and thigh muscles of broilers**

Part of carcass	Treatments - experimental groups				SEM	P
(n=10)	P1	P2	P3	P4		value
Breast	665.47[b]	715.45[b]	736.17[b]	1084.25[a]	53.83	***0.013***
Thighs	261.19[b]	420.64[a]	467.40[a]	495.01[a]	23.62	***0.002***

Treatments: P1, control; P2, 0.5% β-alanine+0.24% MgO; P3, 0.25% L-histidine+0.24 % MgO; P4, 0.20% β-alanine+0.10% L-histidine+0.24% MgO; [a, b], $P<0.05$; SEM, standard error of treatment.

Table 6. **Effect of the treatment on relative concentration (%) of protein carbonyl (nmol/mg protein) in breast and thigh muscles of broilers**

Muscle	Treatments - experimental groups				SEM	P-value
(n=10)	P1	P2	P3	P4		
Breast	37.89	21.43	22.75	26.17	2.51	0.067
Thigh	8.48	6.47	5.81	9.67	0.67	0.150

Treatments: P1, control; P2, 0.5% β-alanine+0.24% MgO; P3, 0.25% L-histidine +0.24% MgO; P4, 0.20% β-alanine+0.10% L-histidine+0.24% MgO; [a, b], $P<$ 0.05; SEM, standard error of treatment.

Table 7. **Effect of the treatments on levels of TBARS (nmol/g tissue) in fresh and frozen breast muscles of broilers**

Breast muscle	Experimental group				SEM	P-value
(n=10)	P1	P2	P3	P4		
Fresh	18.49	14.38	17.99	19.47	1.05	0.183
Frozen	33.30[a]	30.68[a]	30.04[a]	24.34[b]	0.72	**0.014**

Treatments: P1, control; P2, 0.5% β-alanine+0.24% MgO; P3, 0.25% L-histidine +0.24% MgO; P4, 0.20% β-alanine+0.10% L-histidine+0.24% MgO; [a, b], $P<$ 0.05; SEM, standard error of treatment.

texture (WBSF, N). As determined by WBSF, the experimental groups P2 and P3 had significantly higher ($P<0.05$) WBSF values than group P1.

Effect of Feeding Treatments on the Carnosine Content

The effect of treatments on the carnosine content (mg/kg) in white and dark broiler meat is shown in Table 5. The carnosine contents (mg/kg tissue) were twice as high in breast muscles as in thighs for all broiler groups. Different feeding treatments of broilers had a statistically significant effect ($P<0.05$) on the carnosine contents in the breasts and thighs. The P4 group had a significantly higher ($P<0.05$) carnosine content in breasts than groups P1, P2, and P3. Compared to the control group P1, the analysis of results regarding the enrichment of breast muscles with carnosine showed that carnosine synthesis was increased by 7.51% in group P2, 10.62% in P3, and 62.93% in P4. The results indicated that broilers in the P4 group had the most efficient feeding treatment, in which both supplemented amino acids, although in smaller amounts, were supported by MgO as a catalyst to give better results.

In comparison to the control P1 group, carnosine synthesis in the thigh muscles was increased by 61.05% in the P2 group, 78.95% in P3, and 89.52% in P4.

Effect of Feeding Treatments on Oxidation Processes

Table 6 presents the effect of treatments on the relative concentration of PC (nmol/mg protein) in the breast and thigh muscles. The breast muscles had higher average values of PC concentration than thigh muscles. Contents of PC in the breast muscles ranged from 21.43 (P2) to 37.89 (P1) nmol/mg protein. The lowest PC value was exhibited in the thighs of the P3 group (5.81 nmol/mg protein), and the highest was observed in those of the P4 group (9.67 nmol/mg protein). The feeding treatments had no statistically significant effect ($P>0.05$) on the relative concentration of PC (nmol/mg protein) in both types of muscles tested.

Effect of the treatments on the level of TBARS (nmol/g tissue) in fresh and frozen breast muscle is overviewed in Table 7.

Levels of TBARS (nmol/g tissue) were lower in fresh breast muscle than in frozen. In the fresh breast muscle, the treatments did not have a statistically significant effect ($P > 0.05$) on measured levels of TBARS. The lowest level of TBARS in fresh breast muscle was measured in the P2 group (14.38), and the highest was measured in the P4 group (19.47 nmol/g tissue). However, the treatments did have a statistically significant effect ($P < 0.05$) on the level of TBARS in frozen breast muscles. The lowest level of TBARS in frozen breast muscles was observed in the P4 group (24.34 nmol/g tissue), which was statistically significantly lower ($P < 0.05$) than in other groups (P1, P2, and P3).

Discussion

As presented in this paper, the feeding treatments of broilers (supplements in P2, 0.5% β-alanine + 0.24% MgO; P3, 0.25% L-histidine + 0.24% MgO; and P4, 0.20% β-alanine + 0.10% L-histidine + 0.24% MgO) had statistically significant effects on live weights of broilers during the fourth, fifth, and sixth weeks of fattening ($P < 0.05$). The research results of Ogata (2002) and Kasaoka *et al.* (2004) also confirmed that the supplementation of L-histidine, β-alanine, or carnosine to diets significantly boosted broiler growth. Kopec *et al.* (2013b) stated that the supplementation of 4% spray-dried blood cells (SDBC), with or without Zn, with 0.22% of histidine to broiler feed, influenced broilers' performance and the antioxidative status of meat. The research results of Hu *et al.* (2009) showed that broiler performances and feed conversion were not influenced by supplementation of carnosine to feed. By applying certain treatments, the positive effect of MgO on performances of chickens was reported by Namgung *et al.* (2010). As opposed to Hu *et al.* (2009), Kralik *et al.* (2015) determined that feeding treatments (L-histidine at 0.1, 0.2, and 0.3%, $P = 0.040$) had an effect on broiler live and carcass weights. They did not confirm the effects of the dressing percentage and portions of main parts in chicken carcasses ($P > 0.05$). Kopec *et al.* (2013a) achieved higher broiler weights and a better feed conversion when feeding broilers mixtures that contained forages as sources of histidine and β-alanine, compared to those fed a diet based on soybean flour as a source of protein, although they did not establish the relationship between amino acids in feed and in meat. The research proved that feeding treatments affected the weight of the breasts, drumsticks with thighs, and backs ($P < 0.05$), which is in accordance with the results reported by Hu *et al.* (2009), as well as with our previous results (Kralik *et al.*, 2014, 2015). In this research, feeding treatments only had a significant effect on the relative portions of backs ($P < 0.01$). Hu *et al.* (2009) also stated that weights of breasts and drumsticks of broilers in their research were influenced by the carnosine content in the broilers' feed ($P < 0.05$).

The results of testing the technological quality of breast muscle showed that feeding treatments affected the values of pH_1, CIE a*, drip loss, and meat texture. When resting, live muscle has a pH value of 7.0–7.2. After life functions stop, the oxygen supply stops and the ATP must be created by the anaerobic degradation of glycogen. This process results in the formation of lactic acid, which results in acidification and lowering of the pH value. It is known that the phosphate group, protein, and dipeptides (carnosine and anserine) act as proton donors, i.e., proton acceptors, mainly at a neutral pH, producing a buffer capacity. The pH1 values were similar in groups P1 and P3 (6.10 and 6.07, respectively) while in the P4 group this value was lower (5.97). The pH1 value of the P2 group was not statistically significantly different from the other investigated groups. Kralik *et al.* (2014, 2015) reported that pH_1 and pH_2 values of the breast muscle did not depend on concentrations of L-histidine and β-alanine in feed. Hu *et al.* (2009) stated that supplementation with 0.5% carnosine in broiler feed also had no influence on pH values in breast and thigh muscles. Haug *et al.* (2008) also did not find statistically significant differences in the pH values between broiler groups that had histidine supplemented to feed and those that did not.

Meat color is a characteristic that significantly determines meat quality, because it is the first visual criterion for consumers to estimate the appearance and attractiveness of meat. It is known that low pH values affect the biochemistry of meat by causing a higher degree of paleness and redness. Fresh breast muscle should be pink, and any deviation from this nuance is not acceptable for consumers (Baker and Bruce, 1989). In the present research, the breast muscles had a lower degree of redness compared to the control, as was also determined in our previous research (Kralik *et al.*, 2015) with the addition of L-histidine in feed. Hu *et al.* (2009) determined a higher degree of redness for experimental groups fed with 0.5% carnosine. The pH values of the muscle are associated with the color of the meat. It is known that the ultimate pH (pH_2) has an effect on the structure of myofibrils and, consequently, on the color of meat. As there was no statistically significant difference in the pH_2 value between the groups in our study, we can assume that the pH did not influence the statistically significant differences in CIE a* values. The color of meat (CIE a*) was influenced by feeding treatments ($P = 0.007$). Similar to Hu *et al.* (2009), feeding treatments did not affect the CIE L* and CIE b* values determined by the Minolta CR-300 ($P = 0.342$ and $P = 0.136$). Hu *et al.* (2009) demonstrated that carnosine supplemented to feed improved broiler meat quality. The water holding capacity of meat represents its capacity to retain water in spite of external influences, such as heating or compression, and it is defined as the ability of the muscle to retain moisture. It is considered as one of the most important technological indicators of meat quality, as it determines meat appearance, thermal processing duration, production properties, and edibility. Drip loss ($P = 0.002$) in the experimental groups was statistically significantly lower than in the control group, as was already been established in our previous research (Kralik *et al.*, 2014).

Feeding treatments affected the muscle fiber tenderness ($P = 0.008$). Experimental groups P2 and P3 had statistically higher WBSF (N) values than the control, which was not the case in our previous research (Kralik *et al.*, 2015). However,

it was observed that the values of WBSF in the P4 group did not differ statistically from either group in the experiment. The assumption is that such a result was influenced by the values of drip loss and cooking loss. The above values for P4 group are in between the values of other groups. Musa *et al*. (2006) observed statistically significantly lower WBSF values in female broilers, while Souza *et al*. (2011) and Abdullah *et al*. (2010) confirmed statistically significantly higher WBSF values in male broilers. In their research, Cong *et al*. (2017) state that carnosine supplemented to broiler diets statistically significantly ($P<0.05$) affects the reduction of drip loss, cooking loss, and shear force (WBSF). Their results are consistent with ours for drip loss and shear force.

Since carnosine has an antioxidant activity, it can be assumed that its increased content in the muscles of chickens in experimental groups positively influenced the metabolic processes in the muscles, as well as the sensory indicators of meat quality (color, texture, taste), which depend on the mechanism of the oxidative processes in the muscles. By observing the values of drip loss, cooking loss, shear force, and color of meat, it can be said that the values in the experimental groups are still within the limits of "normal" chicken meat, according to Van Laack *et al*. (2000).

The present research, as well as our previous research (Kralik *et al*., 2010a, 2010b, 2014, 2015), proved that there was a difference in carnosine concentration in the breast and thigh muscles ($P<0.05$). If comparing carnosine concentrations in experimental groups and in the control, then the depositions of carnosine in the breast muscles of the experimental groups were higher in groups P2, P3, and P4, at 49.98 mg/kg, 70.70 mg/kg, and 418.78 mg/kg ($P=0.013$), respectively. The thigh muscles also presented higher concentrations for groups P2, P3, and P4, with 159.45 mg/kg, 206.21 mg/kg, and 233.82 mg/kg ($P=0.002$), respectively. Kralik *et al*. (2014) supplemented feed with 0%, 0.5%, and 1% β-alanine during the last three weeks of fattening. The authors concluded that 1% β-alanine the increased concentration of carnosine in breast muscles of female broilers by 19.11%, and of male broilers by 21.86%. The supplementation of β-alanine was more efficient in forming carnosine in thigh muscles, thus increasing its concentration in female broilers by 39.62%, and in male broilers by 49.96%, and reducing the collagen content in both. Kralik *et al*. (2015) also investigated the effects of histidine, hybrids, and sex on the quality of meat and on carnosine concentrations. They confirmed the influence of all three factors on the deposition of carnosine in broiler muscles. The best results were achieved by supplementing with 0.3% histidine, compared to the control (Cobb 500 broilers: male breast muscles 831.23: 972.04 μg/g). Kopec *et al*. (2013a) stated that the supplementation of different raw materials (fish byproducts meal, porcine blood cells, porcine blood meal, wheat gluten, and fodder yeast), as a source of histidine and β-alanine in broiler feed can moderately affect antioxidant status in the blood and muscles of chickens, mainly as an effect of increased histidine dipeptide concentration. Higher carnosine contents were recorded in the meat of chickens fed porcine blood cells and blood meal. Kopec *et al*. (2013b) used four treatments: control, SDBC, SDBC + Zn, and supplemented L-histidine, and found the following concentrations of carnosine in breast muscles, respectively: 0.891, 1.060, 0.905, and 1.205 mg/g tissue. In comparison to other groups, the best results were obtained in the group with supplemented L-histidine. Tomonaga *et al*. (2012) introduced β-alanine orally through distilled water for five days (0, 0.176, 0.88, 4.4, and 22 mmol/kg). The concentration of carnosine in the brain, muscles, and plasma depended on the supplemented amount of β-alanine, which was not the case with anserine. Kopec *et al*. (2015) investigated the effect of histidine-rich SDBC and pure histidine supplementation on the concentration of carnosine in turkey breast muscle. Supplementation of histidine to feed resulted in an increased 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity in the muscles and blood, but increased histidine concentration was not found in the meat. The weight of turkey and content of isoleucine and valine in the breast muscle was increased. Oxidation processes in meat were identified by the concentration of PC (nmol/mg protein) in fresh muscles and levels of TBARS (mg MDA/kg tissue) in fresh and frozen muscles. Feeding treatments did not affect the relative concentrations of PC ($P>0.05$). Our research proved that supplementation of β-alanine and L-histidine did not affect levels of TBARS in fresh muscles, but it did affect those in frozen ones. Cong *et al*. (2017) pointed out that supplementation with carnosine improved the antioxidative capacity and quality of muscle fibers. Kralik *et al*. (2014) investigated the intensity of lipid oxidation in fresh breast muscle samples and samples kept for 60 days at −20℃ when β-alanine was added to chicken feed (P1, control; P2, 0.5% β-alanine; and P3, 1% β-alanine). They determined that the feeding treatments and meat storage time had no effect on the levels of TBARS. Kopec *et al*. (2013b) confirmed that the source of protein (SDBC) in feed influenced the antioxidative status of muscles to a greater extent than supplemented histidine. Hu *et al*. (2009) determined that supplementation with carnosine to broiler feed reduced the level of TBARS, but increased the total antioxidant capacity (TAOC). The authors concluded that carnosine as a supplement to broiler feed improved the meat quality. The results of Soyer *et al*. (2010) also showed higher levels of TBARS in frozen samples of breast muscles; they observed an increase in level of TBARS (expressed as mg MDA/kg meat) when storing breasts at −18℃ during certain periods. They also reported that the fastest and the most important ($P<0.01$) increase in levels of TBARS occurred in the first two months of storing frozen meat. Pettersen *et al*. (2004) studied oxidative changes in turkey meat, and the research results indicated that the storage duration affected the increase in levels of TBARS in frozen samples. The highest values were determined after six months of storage at −20℃, within which the levels of TBARS were also affected by the method of packaging (in air, in vacuum, or in modified atmosphere). Several authors (Lopez-Bote *et al*., 1998; O'Neill *et al*., 1999; Botsoglou *et*

al., 2003; Smet *et al*., 2008) have suggested supplementation with natural or synthetic antioxidants, among others, as well as carnosine (Hu *et al*., 2009), to broiler feed or meat before freezing in order to improve oxidative stability during storage, either in the fresh or frozen state, all with the purpose of prolonging meat viability. Comparison of our research results with those of previous studies confirmed that, in most cases, broiler meat could be enriched with carnosine by supplementing with β-alanine and/or L-histidine. The successful synthesis and deposition of carnosine depends on the composition of broiler feed, as well as the amount of supplemented amino acids. However, it is necessary to conduct such research in order to optimize the amino acids contents in mixtures for the enrichment of broiler meat with carnosine, without negative impacts on meat quality parameters.

The results of the study showed that the addition of a combination of β-alanine, histidine, and MgO used in the P4 group most efficiently increased the carnosine concentration in the breast and thigh muscles of broilers (62.93% and 89.52 %, respectively). Feeding treatments had an effect on live weight of broilers, as well as on the carcass quality and some parameters of meat quality. The treatment used in the P4 group showed efficacy in maintaining the oxidative stability of the breast muscles during frozen storage. Based on the results of the study, the P4 feed treatment may be recommended as the most optimal for enriching chicken meat with carnosine.

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