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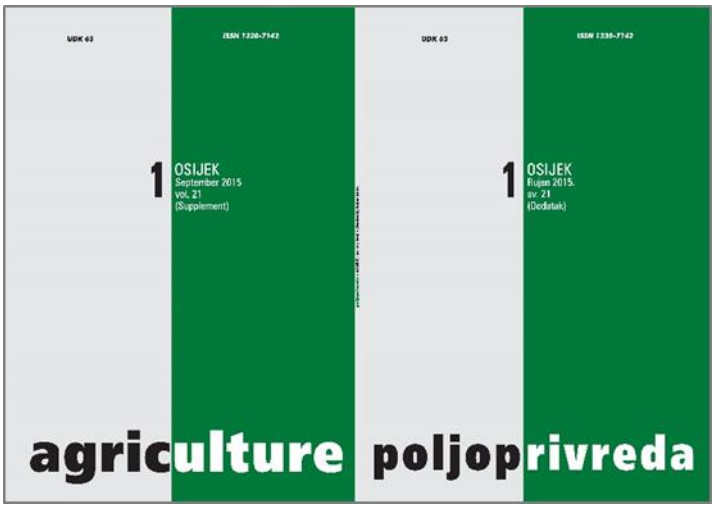

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EFFECT OF β-ALANINE AND L-HISTIDINE ON CONCENTRATION OF CARNOSINE IN MUSCLE TISSUE AND OXIDATIVE STABILITY OF CHICKEN MEAT

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Original scientific paper

SUMMARY

This paper presents the results of two separate experiments, each involving 75 chickens of Cobb 500 provenience, divided into three experimental groups. During the last three weeks of fattening, chickens were fed finisher diets supplemented with amino acids β-alanine (0%, 0.5% and 1%) and L-histidine (0%, 0.3% and 0.5%) in different portions. After chickens have been slaughtered, 10 samples of breast tissue were taken from each group for carnosine content determination in muscle tissue and lipid oxidation expressed as TBARS. Analysis of THE results referring to carnosine concentration in breast muscle proved that supplementation of 0.5% L-histidine affected the carnosine concentration increase in breast muscles from 941.58 µg/g of tissue (H1) to 1186.06 µg/g of tissue (H3), while supplementation of 1% β-alanine influenced the increase in carnosine concentration from 756.15 µg/g of tissue (A1) to 911.01 µg/g of tissue (A3). Supplementation of amino acids did not have effects on TBARS values, but oxidation values decreased along with the supplementation of higher amounts of amino acids to diets, which was particularly expressed in samples stored for 60 days at -20°C. The experimental group H3 (0.5% L-histidine) exhibited 30.54% lower value of lipid oxidation than the control one H1 (0% L-histidine), while the group with 1% β-alanine (A3) had lipid oxidation value by 17.65% lower than the control group A1 (0% β-alanine).

Key-words: β-alanine, L-histidine, carnosine, TBARS, chicken, oxidative stability

INTRODUCTION

Carnosine is a dipeptide produced by synthesis of amino acids β-alanine and L-histidine with help of carnosine synthetase enzyme in brain and skeletal muscle cells (Hipkiss, 1998). Concentration of carnosine in skeletal muscles depends on animal species and animal age, and it is also affected by feeding treatment, types of muscles (white muscle of chickens contains higher concentration of carnosine than dark muscle) (Maikhunthod, 2003). Carnosine is present in high concentrations in breast muscle of chickens (Kralik et al., 2010). Carnosine has antioxidative activity probably resulting from its ability to bond metal ions and to eliminate several types of free radicals (Kralik et al., 2012). As a dipeptide, it exhibits stronger antioxidative activity than some amino acids as its compounds (Maikhunthod, 2003). Concentration of carnosine in animal, as well as in human tissue, can be modified by supplementation of the amino acids being compounds of carnosine or their combination in feed (Dunnet and Harris, 1999; Nagasawa et al., 2001; Tomonaga et al., 2005; Haug et al., 2008; Boldyrev et al., 2013). The research objective was to determine effects of different concentrations of β-alanine and L-histidine supplemented in chicken feed on the concentration of carnosine and oxidation of lipids in breast muscle tissue of Cobb 500 chickens.

MATERIAL AND METHODS

Chickens were divided in three experimental groups in two experiments (75 chickens per group) and fattened for 42 days. During fattening, chickens were fed com-

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plete starter diets containing 24.39% crude protein and 13.93 MJ ME/kg (1st experiment), i.e. 22.92% crude protein and 13.41 MJ ME/kg (2nd experiment). From 22nd to 42nd day chickens in the 1st experiment were fed finisher diets containing 20.07% crude protein and 13.15 MJ ME/kg (H1–control group), 20.22% crude protein and 13.20 MJ ME/kg (H2 group) and 19.80% crude protein and 13.09 MJ ME/kg (H3 group), which were supplemented with L-histidine in various amounts (H1 0%, H2 0.3% and H3 0.5%). In the 2nd experiment, chickens were fed finisher diets containing 18.79% crude protein and 12.10 MJ ME/kg in the A1 group (control), 18.93% crude protein and 12.90 MJ ME/kg in the A2 and 19.46% crude protein and 13.03 MJ ME/kg in the A3 group, which were supplemented with β-alanine (A1 0%, A2 0.5% and A3 1.0%). Chickens were fed *ad libitum*. Composition of diets is overviewed in the Table 1.

Table 1. Composition of the chicken diets

Ingredient, %	Diet day 1-21	Diets day 22-42					
		1st experiment			2nd experiment		
		H1	H2	H3	A1	A2	A3
Corn	51.50	61.20	60.90	60.70	62.70	62.20	61.70
Alfalfa	2.50	3.00	3.00	3.00	3.00	3.00	3.00
Protein gold	2.00	-	-	-	-	-	-
Soybean toasted	9.00	-	-	-	5.00	5.00	5.00
Soybean cake (46%)	29.50	27.80	27.80	27.80	24.00	24.00	24.00
Sunflower oil	0.50	3.00	3.00	3.00	0.30	0.30	0.30
Kuškovit 5% BK+ Kokcisan + phytase	5.00	-	-	-	-	-	-
Kuškovit 5% BK+ phytase	-	5.00	5.00	5.00	5.00	5.00	5.00
L-histidine	-	-	**0.30**	**0.50**	-	**-**	**-**
β-alanine	-	-	**-**	**-**	-	**0.50**	**1.00**
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

H1 0% L-histidine, H2 0.3% L-histidine, H3 0.5% L-histidine, A1 0% β-alanine, A2 0.5% β-alanine, A3 1% β-alanine

Composition per 1 kg of premix „Kuškovit": vit. A 300,000 IUs; vit. D3 40,000 IUs; vit. E 600 mg; vit. K3 40 mg; vit. B1 20 mg; vit. B2 120 mg; vit. B6 40 mg; vit. B12 300 mcg; vit. C 300 mg; niacin 800 mg; pantothenic acid 240 mg; folic acid 10 mg; biotin 2.00 mg; choline chloride 10,000 mg; iodine 12 mg; iron 500 mg; copper 75 mg; manganese 1600 mg; zinc 1000 mg; cobalt 3 mg; selenium 3 mg; antioxidant 2000 mg; calcium min. 38 g; sodium min. 23 g; methionine 55,000 mg; lysine 24,000 mg

The study was conducted in accordance with the Regulations of the Republic of Croatia (Regulations on the animals protection during transport and related operations NN 12/11, Regulations on the Protection of animals at slaughter and killing NN 39/08, Regulations on the protection of animals at the time of killing NN 83/11). After 42 days of fattening and 12-hour fasting, chickens were slaughtered and carcasses were processed as „ready for grill". Breast muscle tissues were sampled for determination of carnosine content and the lipid oxidation values (fresh tissue and tissue kept for 60 days at -20°C). In order to perform analysis of carnosine content in muscle tissue, there were 10 chickens from each group randomly selected from the mixed sample. Samples of tissues were prepared by the method described by Aristoy and Toldra (2004), and concentration of carnosine was determined by the HPLC device (Varian Prostar, USA) equipped with fluorescent detector and Zorbax column ODS, 4.6 x 250 mm (Agilent, USA). The sample was derivatized before injecting with OPA reagent by the method described by Interpichet and Maikhunthod (2005). Oxidation of lipids in breast muscle tissue was determined by the methods of Vyncke (1970) and Lemon (1975). The research results were analyzed by statistical software Microsoft Office Excel (2007). Significance of differences between groups was determined by analysis of variance (ANOVA). The calculated F value was compared with the theoretical F value at a significance level (5%, $P<0.05$). Significance of differences between mean values was determined by the t-test.

RESULTS AND DISCUSSION

Concentration of carnosine in breast muscles

The Table 2 presents the values referring to concentrations of carnosine in breast muscle tissue of Cobb 500 chickens (mixed sample) fed diets supplemented with L-histidine and β-alanine. The data indicate that supplementation of L-histidine in feed influenced the increase of carnosine concentration in chicken breast muscle tissue. If compared to the control group, supplementation of 0.3% L-histidine in diet affected the increase of carnosine concentration in breast tissue by 8.88% whereas supplementation of 0.5% L-histidine affected the increase of carnosine concentration by 25.96%. Statistical analysis was used to determine significant differences in the content of carnosine between the groups. Supplementation of 1% ß-alanine in diets during the last three weeks of fattening resulted in 20.48% higher concentrations of carnosine in breast muscles of A3 group if compared to the control. Supplementation of 0.5% ß-alanine did not influence the increase of carnosine concentrations. The group A3 fed diets with 1% ß-alanine had statistically higher value of carnosine content in breast muscle than the groups A1 and A2.

Table 2. Concentrations of carnosine (μg/g of tissue) in chicken breast muscle tissue (mixed sample) with dietary supplementation of L-histidine and β-alanine

	Groups			P-value
	H1	H2	H3	
1st experiment	941.58 ± 59.72[c]	1025.22 ± 101.18[b]	1186.06±73.75[a]	***0.001***
	A1	A2	A3	
2nd experiment	756.15 ± 118.56[b]	753.29 ± 85.35[b]	911.01 ± 118.81[a]	***0.003***

H1 0% L-histidine, H2 0.3% L-histidine, H3 0.5% L-histidine, A1 0% β-alanine, A2 0.5% β-alanine, A3 1% β-alanine; Values within a column with different superscript letters a, b, c were significantly different (P<0.05)

Haug et al. (2008) performed the experiment of enriching chicken meat with carnosine by supplementing different amounts of L-histidine (1 g/kg, 2 g/kg, 3 g/kg) in chicken feed. Supplementation of L-histidine in 1g/kg of feed already affected the increase of carnosine concentration in breast muscle from 9.96 μmol/g to 16.15 μmol/g, which represented an increase of 62% if compared to the control group of chickens that were not fed histidine. Kai et al. (2015) found out that the chicken meat can be enriched with carnosine if histidine is added in feed in the amount of 200% of NRC norms during 10 days of feeding. Nagasawa et al. (2001) carried out the research to determine whether histidine in feed could affect the content of carnosine in muscle tissue of rats. Supplementation of 2% histidine in feed had positive influence on synthesis of carnosine in muscle tissue and significantly influenced the increase of carnosine concentration in muscle (from 6.48 mmol/g muscle in control group to 9.30 mmol/g muscle in the group fed 2% histidine). Amend et al. (1979) determined that histidine was indispensable in feeding of adult roosters and that the portion of 0.11% in feed was sufficient to keep appropriate live weights and normal concentrations of hemoglobin and to prevent decrease in concentration of histidine dipeptides and free histidine in muscles and in brain tissue. It is assumed that, since then, the needs for histidine increased due to development of new hybrids, shortening of fattening periods, etc. However, other researches proved that the speed of carnosine synthesis in muscles was more affected by β-alanine than by histidine, i.e. β-alanine was the limiting factor in synthesis (Dunnet and Harris, 1999; Harris et al., 2006). Consequently, recent researches are focused on the supplementation of β-alanine in animal feed and water. Tomonaga et al. (2005) performed the experiment within which chickens were given 22 mmol/kg β-alanine orally from 2nd to 6th day of age. Twelve hours after the last dose of β-alanine, chickens were sacrificed and the right breast muscle was taken for analysis of carnosine content. Chicken group that was given β-alanine exhibited significant increase in carnosine concentration from 5459 nmol/g tissue to 8774 nmol/g tissue, which represented an increase of 60.7%. Tomonaga et al. (2012) found out that the addition of β-alanine in water increases the concentration of carnosine in chicken brain and *Musculus pectoralis superficialis*. Dunnet and Harris (1999) investigated the effect of continuous usage of β-alanine in feed on the concentration of carnosine in muscle fibres of *gluteus medius* in horses. Over a period of 30 day, horses were given three times a day β-alanine in the amount of 100 mg/kg of body weight and histidine in the amount of 12.5 mg/kg of body weight. Histidine was also supplemented to feed in order to provide for the appropriate amount of amino acids for biosynthesis of carnosine. Analysis results proved that in muscle fibres that were successfully isolated, concentration of carnosine was higher at the end of the experiment than at the beginning, i.e. availability of β-alanine affected significantly the speed of carnosine synthesis. Chicken meat is sensitive to oxidative changes, which negatively influence taste, smell and meat preservation. Therefore, due to its antioxidative traits, carnosine is especially interesting as a factor in achieving longer meat preservation time.

Indicators of lipid oxidation in meat

Table 3 presents the TBARS values of lipid oxidation from the experiments 1 and 2, shown as mg MDA/kg tissue, measured on fresh breast tissue and on breast tissue stored for 60 days at -20°C. Groups of chickens fed diets with L-histidine exhibited quite equal TBARS values of fresh samples, so there were no significant differences determined between the groups. The sample stored for 60 days at -20°C also did not exhibit statistically significant differences. However, there was a trend of decrease of TBARS values determined in the experimental groups H2 (0.338 mg MDA/kg_{tissue}) and H3 (0.282 mg MDA/kg_{tissue}), if compared to the control group H1 (0.406 mg MDA/kg_{tissue}). In the group with 0.5% L-histidine (H3), TBARS value was lower by 30.54% than in the control group. The group H3 exhibited the highest concentration of carnosine, so it was assumed that the favorable trend of the TBARS value decrease was a consequence of carnosine antioxidative activity. Statistically significant difference was determined only within the group H1, where the storing of samples at -20°C for 60 days resulted in significant increase of TBARS values. The values referring to fat oxidation in breast muscle tissue of chickens fed diets supplemented with β-alanine proved balanced values of TBARS in fresh samples. However, samples of breast tissue stored for 60 days at -20°C exhibited higher values of TBARS in the group A1 (0.425 mg MDA/kg_{tissue}),

while the groups A_2 and A_3 had slightly lower values (0.352 mg MDA/kg_{tissue} and 0.350 mg MDA/kg_{tissue}, respectively). Still, the statistical data analysis did not prove significant differences between the groups.

Table 3. Products of lipid oxidation expressed as TBARS values (mg MDA/kg_{tissue}) in chicken breast muscle tissue samples (fresh and stored for 60 days at -20°C) with dietary supplementation of L-histidine and β-alanine

Storage time	1st experiment			*P-value*
	H1	H2	H3	
Fresh	0.275±0.05[b]	0.298±0.07	0.256±0.02	0.216
60 days	0.406±0.15[a]	0.338±0.11	0.282±0.04	0.056
P-value	***0.015***	0.355	0.084	-
	2nd experiment			
	A1	A2	A3	
Fresh	0.359±0.05	0.341±0.06	0.399±0.04	0.078
60 days	0.425±0.08	0.352±0.07	0.350±0.09	0.103
P-value	0.063	0.745	0.144	-

H1 0% L-histidine, H2 0.3% L-histidine, H3 0.5% L-histidine, A1 0% β-alanine, A2 0.5% β-alanine, A3 1% β-alanine

Although it was determined that duration of storing muscle tissue did not affect TBARS values, more intensive oxidation was found out in group A_1 with the increase in TBARS by 15.52%, while the increase in fat oxidation in group A_2 was small, being only 3.13%. It is important to point out that during the storage time, fat oxidation was lowered in the group A_3 by 14%. Considering the fact that the muscle tissue in the group A_3 had the highest concentration of carnosine, it was expected that antioxidative activity of carnosine would have positive effect on meat preservation. Kralik et al. (2010; 2012) also pointed out that carnosine demonstrated an antioxidant effect in the cell which is amplified by the addition of vitamin E. This fact was also confirmed by Hu et al. (2009), as well as by Djenane et al. (2004).

CONCLUSION

On the basis of the obtained research results, it is concluded that supplementation of particular amino acids, L-histidine and β-alanine, affected the increase of carnosine content in breast muscle tissue of Cobb 500 chickens. Higher average values of carnosine were observed in the study where the chickens received L-histidine in the feed, but in both studies an increase in the content of carnosine in the tissues was observed by increasing the use of amino acids in feed. Dietary supplementation of 0.5% L-histidine (H3) affected the increase of carnosine content by 25.96% if compared to the control group (H1). Dietary supplementation of 1% β-alanine (A3) resulted in 20.48% higher content of carnosine in muscles than in the control group (A1). Although the results referring to lipid oxidation values did not prove significant differences between the groups, it was determined that dietary supplementation of amino acids in higher portions affected the lowering of TBARS values, being particularly expressed in samples kept for 60 days at -20°C. Therefore, it can be concluded that carnosine as a natural dipeptide was a desirable antioxidant used for meat and meat products preservation for a longer period of time.

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