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CARNOSINE CONTENT AND MUSCLE OXIDATIVE STABILITY OF MALE AND FEMALE BROILER CHICKENS

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SUMMARY

Carnosine is a dipeptide with antioxidative effects in broiler muscles. Its anti-ageing effect has also been determined recently, which is especially important for human health and vitality preservation. The research investigated concentration of carnosine in breast and thigh muscles of Cobb 500 broilers. It was carried out on 20 male and female broilers that were conventionally fattened for 42 days. Carnosine concentrations and TBARS values were measured on fresh breast and thigh muscles with respect to broiler sex. Content of carnosine was slightly higher in female broiler breast muscles than in male's (1079.85 : 1012.66 μg/g tissue; P>0.05). Female broiler thigh muscle tissue also contained higher carnosine values than male's (464.69 : 404.97 μg/g tissue; P>0.05). The research proved that carnosine was more deposited in breast muscle tissue than in thigh muscle tissue, regardless of broiler sex. Lipid peroxidation products measured as TBARS values (mg MDA/kg tissue) did not statistically differ according to broiler sex or muscle type (P>0.05). Further research needs to be directed towards control of peroxidation products during meat storage.

Key-words: carnosine, broiler muscles, gender, TBARS

INTRODUCTION

One of the natural antioxidants that can be used to extend the shelf life of meat is carnosine. Carnosine is a dipeptide, composed of L-histidine and β-alanine, which can be considered as bioactive food component due to its physiological function. Lipid oxidation is one of the main factors causing quality deterioration of meat and meat products (Kennedy et al., 2005). During the recent years there has been a great pressure to the foods industry to reduce the use of artificial food additives, whose task is to increase the stability of food products. As a result there was a need for the use of natural antioxidants in both production and processing of various foods. Morrissey et al. (1998.) reported that supplementation of carnosine in combination with vitamin E improves the stability of lipids which is reflected by improved viability of meat products. Carnosine content depends on the type of muscle tissue (white or dark meat) and type of animal (cattle, sheep, rabbit, poultry), but also breed (breeds and hybrids), gender, age and breeding method (Abe and Okuma, 1995). Carnosine is concentrated in a muscle tissue. In muscles of poultry higher content of carnosine was found in a white compared to dark meat (Intarapichet and Maikhunthod, 2005; Plowman and Close, 1988). However, there is a very limited amount of information related to the effect of addition of carnosine in feed on the oxidative stability and quality of poultry meat. Because of that topic of carnosine as an antioxidant becomes very interesting to scientists worldwide.

MATERIAL AND METHODS

Chickens were fattened for 42 days. During the first three weeks of the experiment chickens consumed the starter mixture, and for the last three weeks they were fed commercial finisher mixture (Table 1.). After the fattening period, 20 Cobb provenance broilers were sacrificed and their carcasses chilled for 24 hours at +4°C. After cooling, carcasses were weighed and processed

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according to the procedure laid down by Commission Regulation (EC) no. 543/2008, which describes the rules relating to market standards for poultry meat. In order to determine meat quality, values of pH_1 (45 minutes after slaughtering) and pH_2 (24 h after slaughtering) were measured in breast muscle samples, using a digital pH-meter "Mettler" MP120-B.

Table 1. The composition of diets fed to chickens from the 1st-42nd day of fattening

Tablica 1. Sastav krmnih smjesa od 1.-42. dana tova pilića

Ingredient (%)	Raw materials in diets (%)	
	Starter	Finisher
	1st-21st days	22st-42st days
Corn	51.50	62.70
Soybean cake	29.5	24.00
Toasted soybean	9.00	5.00
Protein gold	2.00	-
Kuškovit 5% BK+phytase	-	5.00
Kuškovit 5%+Kokcisan+ phytase	5.00	-
Alfaalfa	2.50	3.00
Oil	0.50	0.30
Total:	100	100
Chemical composition of diets		
Moisture, g/kg	96	100
Crude protein, g/kg	243.9	200.7
Crude fat, g/kg	47	57
Crude fiber, g/kg	44	43
Ash, g/kg	60	56
Calcium, g/kg	11.1	10.6
Phosphorus, g/kg	4.8	4.3
Sodium, g/kg	1.9	1.8
Metabolic energy, MJ/kg	15.46	15.27

*Chemical analysis of food was made according to reference 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), M-13 (HRN ISO 7485:2001).

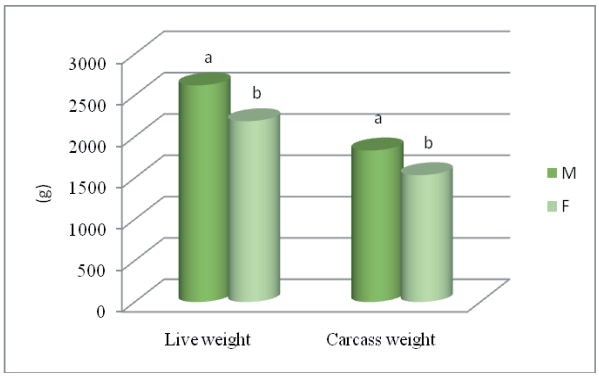
Furthermore, after cutting the carcasses on basic parts, each part was weighed separately and the share of the basic parts in carcass was calculated. Breasts and drumstick with thighs were deboned. Breast muscles samples were taken for determination of carnosine content and lipid oxidation. Samples for determination of carnosine content have been prepared according to the method described by Aristoy and Toldra (2004), and carnosine content was determined by use of HPLC unit (Varian Prostar, USA) with fluorescent detector and ZORBAX ODS column, 4.6 x 250 mm (Agilent, USA). Each sample was derivatized before injection with the OPA reagent prepared according to Intarapichet and Maikhunthod (2005). Lipid oxidation values in breast and thigh muscles (TBARS) were determined using the method of Vyncke (1970) and Lemon (1975). Colour was measured at the thickest part of breasts using a device Minolta Camera Co. Ltd., Model CR-300. Colour is represented by three values: CIE L* for the degree of fading, CIE a* for the degree of redness and CIE b* for the degree of yellowness. Calibration of the device was performed using a standard white tile (reference No. 16,733,047, CY = 93.0, x =. 3134 and y =. 3195; D65 Y = 93.0, x =. 3159, y =. 3324). Releasing of water from the pectoral muscle tissue was determined by the drip loss method described by several authors (Lundstrom and Malmfors, 1985; Barton-Gade et al., 1994), where drip loss value was calculated by the following formula:

Drip loss (%) = š(initial value (g) - final value (g)) / initial value (g)ć x 100.

Results of the research were analyzed using the statistical program Microsoft Office Excel (2007). The significance of differences between groups was determined by analysis of variance (ANOVA). The calculated F value was compared with the theoretical critical F value at three levels of significance (5 % $P < 0.05$; 1 % $P < 0.01$ and 0.1 % $P < 0.001$). The significance of differences between mean values was determined using t-test.

RESULTS AND DISCUSSION

Final weight of broilers (10 male and 10 female carcasses) are shown in Figure 1. From the data it is evident that male broilers after a fattening period of 42 days, reached 2622 g and female broilers 2188 g ($P<0.001$). After sacrificing broilers were measured in carcass weight male broilers had significantly higher average carcass weight of 1834 g out of 1535 g of female broilers ($P<0.001$).



M = male broilers, F = female broilers: a,b P<0.001

Figure 1. Live weight and carcass weight of broilers

Slika 1. Živa masa i masa trupa brojlera

Table 2 shows the proportion of the basic parts of the carcass, which in both sexes were equal. Thus fortified portions of breast in male and female broilers were 35.09 % : 35.20 %, drumsticks with thigh 29.82 % : 29.71%, wing 10.83 % : 10.85 % and back 24.26 % : 24.24 % (P>0.05). The results of the basic parts of the carcasses similar to ours showed Kralik et al. (2006) in the work of assessing the quality of broiler carcasses and meat on the Croatian market.

Table 2. Shares of the main parts in carcass (%)

Tablica 2. Udjeli osnovnih dijelova u trupu (%)

Parts of the carcasses	Male broilers $\bar{x}$ ± s	Female broilers $\bar{x}$ ± s	P values
Breast	35.09 ± 1.34	35.20 ± 2.35	0.907
Drumsticks with thigh	29.82 ± 1.07	29.71 ± 0.75	0.801
Wing	10.83 ± 0.46	10.85 ± 0.56	0.949
Loin	24.26 ± 1.00	24.24 ± 1.40	0.986

$\bar{x}$ = mean; s = standard deviation; n.s. P>0.05

Parameters of technological properties of the pectoral muscle in relation to gender are shown in Table 3. PH_1 values were consistent in both sexes of broilers (6.33 vs 6.34), whereas male broilers reached less pH_2 values in relation to female broilers (5.87 vs 5.97, P=0.198). Furthermore, the pectoral muscles of female broilers were laid off over the water (drip loss) in relation of breast muscles of male broilers (2.78 % and 1.89 %; P=0.198). The color of muscle tissue of female had a brighter pectoral muscle in relation to the male, but the difference was not statistically significant (P>0.05). Intense degree of redness, CIE a* and yellowness b* CIE of pectoral muscle tissue had male broilers as compared to female broilers (CIE a* 2.10 and 1.82, and CIE b* 2.82 or 2.56; P>0.05). The colour of muscle tissue is an important parameter according to which consumers evaluate the quality of meat. Therefore, producers must take care of the factors that could adversely affect the parameters describing the meat quality (Qiao et al., 2002). The colour of muscle tissues of the pelvic tissues can be categorized into three groups: lighter than normal, normal and darker than normal. Different authors quote different limits in the classification of breast muscle tissue by colour. Thus, Soares et al. (2007) classified muscles of broiler breasts in the following groups according to the values of L (L>53 brighter than normal or PSE; L<44 darker than normal or DFD and 48 ≤ L ≤ 53 is considered normal). Compared with the mentioned authors, broiler breasts in the conducted experiments are classified as „normal" broiler. Salakova et al. (2009) mentioned that sex of broilers has a statistically significant effect on the colour of the pectoral muscle using of L* value. The authors mention that brighter muscle tissue (L*) have female broilers in relation to male broilers (P<0.01), which is consistent with the values of the conducted experiments.

Table 3. Influence of gender on the technological properties of the breast muscle

Tablica 3. Utjecaj spola na tehnološka svojstva prsnog mišićnog tkiva

Trait	Male $\bar{x}$ ± s	Female $\bar{x}$ ± s	P value
pH_1	6.33 ± 0.23	6.34 ± 0.25	0.927
pH_2	5.87 ± 0.15	5.97 ± 0.25	0.233
Drip loss (%)	1.89 ± 1.28	2.78 ± 1.68	0.198
CIE L*	51.46 ± 2.79	52.25 ± 4.20	0.626
CIE a*	2.10 ± 0.83	1.82 ± 0.66	0.404
CIE b*	2.82 ± 1.18	2.56 ± 2.19	0.747

$\bar{x}$ = mean; s = standard deviation; n.s. P>0.05

Table 4 shows the oxidation products of lipids measured in fresh tissues of breast and thigh. Noticeable is a uniform oxidation of lipids in breast muscles in both sex (0.462 mgMDA/kg of tissue or 0.464 mgMDA/kg of tissue; P=0.996). Furthermore, the sex of broilers had no effect on differences in the oxidation of lipids of thigh (P=0.331). Also there were no statistically significant differences determined by type of muscles between male and female broilers (P>0.05).

Table 4. Products of lipid oxidation measured as TBARS values (mg MDA/kg of tissues) in fresh tissue

Tablica 4. Produkti oksidacije lipida mjereni kao TBARS vrijednosti (mgMDA/kg$_{tkiva}$) na svježim tkivima

Muscle tissue	Male $\bar{x}$ ± s	Female $\bar{x}$ ± s	Effects of sex
Breast	0.462 ± 0.091	0.464 ± 0.132	0.996
Thigh	0.430 ± 0.042	0.451 ± 0.051	0.331
Effects of type meat	0.314	0.726	P – value

$\bar{x}$ = mean; s = standard deviation; n.s. P>0.05

The content of carnosine in muscle tissue of breast and thighs is shown in Table 5. Higher values of carnosine were determined in the breast tissue of female broilers (1079.85 μg/g tissue), compared to male broilers (1012.85 μg/g tissue), but the difference was not statistically significant (P=0.374). Also, a higher content of carnosine was found in muscle tissue of female broiler thighs in relation to male broilers (464.69 μg/g tissue or 404.97 μg/g tissue; P=0.321). In the comparison between the carnosine content of tissue, significantly higher (P<0,001) content was found in a breast muscle in relation to the thigh muscle in both sexes of broilers. Smaller values of carnosine in the breast muscle tissue of broilers of both sexes (female = 971.37 μg/g muscle tissue or male = 932.84 μg/g muscle tissue) indicate Kralik et al. (2010a). Content of carnosine in thigh muscle tissue of chickens of both sexes was also smaller in previous research of Kralik et al. (2010b). Authors stated that female thigh tissue contained 339,28 μg carnosine/g tissue in comparison with male thigh tissue carnosine content of 319,29 μg/g tissue, but the difference was not statistically significant (P>0,05). Intarapichet and Maikhunthod (2005) in a work on the content of carnosine in muscle tissue in relation to genotype and sex as well as antioxidant activity in white and dark muscle tissue of broilers reported that gender and genotype of the animals have a significant statistical impact on the content of carnosine in tissues (white meat of female =1200.05 μg/g, and male =684.82 μg/g, while the dark muscle of female = 304.88 μg/g, and male =279.57 μg/g). Although the values of these authors are slightly higher for the white meat of female broilers and lower for the male broilers, while they are lower for the dark meat in both sexes in relation to our values, the content of carnosine in relation to gender and type of tissue was consistent in both surveys. Furthermore the same authors state that the addition of carnosine in feed for broilers reduces the formation of TBARS (lower lipid oxidation in muscle tissue).

Table 5. The concentration of carnosine in muscle tissue of chicken (μg/g tissue)

Tablica 5. Koncentracija karnozina u mišićnom tkivu pilića (μg/g tkiva)

Muscle tissue	Male $\bar{x}$ ± s	Female $\bar{x}$ ± s	Effects of sex
Breast	1012.66 ± 195.64^{A}	1079.85 ± 127.31^{A}	0.374
Thigh	404.97 ± 137.76^{B}	464.69 ± 123.59^{B}	0.321
Effects of type meat	P<0.001	P<0.001	P - value

$\bar{x}$ = mean; s = standard deviation; n.s. P>0.05 effect of sex; A,B, P<0.001 effects of type meat

CONCLUSION

Based on the research conducted on the carnosine content in white and dark broiler meat of different sexes it can be concluded that the female broiler meat by the carnosine content is more complexed than the male broiler meat. We also found out that the white meat is richer in carnosine than the dark meat. Research should continue in the direction of enrichment of broiler meat with carnosine and determine the sustainability of such meat as compared to conventional meat composition.

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SADRŽAJ KARNOZINA I OKSIDATIVNA STABILNOST MIŠIĆA MUŠKIH I ŽENSKIH BROJLERSKIH PILIĆA

SAŽETAK

Karnozin je dipeptid koji ima antioksidativno djelovanje u mišićima pilića. U posljednje vrijeme pripisuje mu se antiageing effect, što je posebno značajno u održavanju zdravlja i vitalnosti ljudi. U radu se istražuje koncentracija karnozina u prsnim mišićima i mišićima zabataka Cobb 500 brojlerskih pilića. Istraživanje je provedeno na 20 muških i ženskih brojlera tovljenih 42 dana na konvencionalan način. Na svježim mišićima prsa i zabataka izmjerene su koncentracije karnozina, kao i TBARS vrijednosti prema spolu pilića. Sadržaj karnozina u mišićnome tkivu prsa bio je neznatno veći kod ženskih nego kod muških pilića (1079,85 : 1012,66 µg/g tkiva; P>0,05). Veće vrijednosti karnozina utvrđene su, također, i u mišićnome tkivu zabataka kod ženskih u odnosu na muške piliće (464,69 : 404,97 µg/g tkiva; P>0,05). Kod oba spola primijećeno je veće odlaganje karnozina u prsnom mišićnom tkivu, u odnosu na tkivo zabataka. Produkti oksidacije lipida, mjereni kao TBARS vrijednosti (mg MDA/kg tkiva), nisu se statistički razlikovali niti prema vrsti mišića, niti prema spolu (P>0,05). Istraživanja je potrebno nastaviti u pravcu kontrole produkata oksidacije tijekom čuvanja mesa pod određenim kondicijama.

Ključne riječi: karnozin, mišići brojlera, spol, TBARS

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