

CARNOSINE - POLYFUNCTIONAL BIOLOGICALLY ACTIVE INGREDIENT

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Carnosine – a Polyfunctional, Biologically Active Ingredient

Karnozin – polifunkcionalan biološki aktivan sastojak

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CARNOSINE – A POLYFUNCTIONAL, BIOLOGICALLY ACTIVE INGREDIENT

Kralik, G., Kralik, Z., Gvozdanić, K.

Scientific review

Pregledni znanstveni članak

SUMMARY

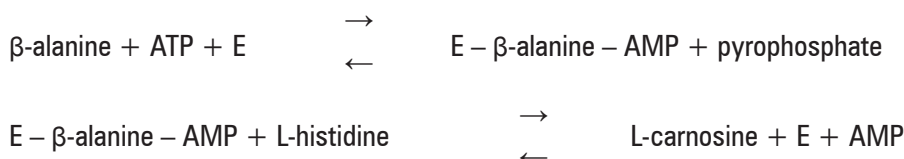
In this paper, the authors summarize the studies referring to the enrichment of breast and thigh muscles with carnosine that has an important function in physiological processes. Research has shown that carnosine improves the quality of chicken meat. By adding the β -alanine and L-histidine amino acids in chickens' feed, carnosine synthesizes in skeletal muscles, brain, heart muscle and olfactory receptor cells. It has been determined that the content of carnosine depends on the type of muscle (white or dark meat), broiler genotype, and sex. Chicken meat is sensitive to the oxidation processes, but lipid oxidation can be efficiently prevented by enriching the meat with carnosine.

Keywords: carnosine, β -alanine, L-histidine, muscles, broilers

CARNOSINE – COMPOSITION AND PHYSIOLOGICAL ROLE

Carnosine is a dipeptide comprised out of β -alanine and L-histidine, and methylated analogs are anserine and ophidine (Boldyrev et al., 2013). Although carnosine

and anserine have been known for many years as the components of skeletal muscles (Kalyankar and Meister, 1959), their biological role and synthesis mechanism are still under consideration. Stenesh and Winnick (1960) described the formation of carnosine in two levels:



Carnosine has an antioxidative property, it is water-soluble and a natural metabolite in cells, where it removes the active oxygen compounds. It is present in brain cells, heart muscle, kidneys, stomach, olfactory receptor cells and skeletal muscles. Carnosine can inhibit the lipid oxidation by catching free radicals and chelation of metals. As for the pH, carnosine and anserine display a significant buffer activity, which can be explained by their biological role (Gariballa and Sinclair, 2000). Medical research shows that carnosine can prolong the life of cells and preserve cellular homeostasis (Boldyrev et al., 2013).

Carnosine has an important function in physiological processes. Carnosine regulates intracellular pH, prevents oxidation, and ensures a normal transmission (Boldyrev et al., 2013). Boldyrev et al. (2013) report that carnosine affects the function of skeletal muscles, brain, and cardiovascular system. Besides that, it slows the aging process (exerting an anti-aging effect). The

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carnosine content depends on the type of muscle (white or dark meat) and the type of animal (cattle, sheep, poultry), as well as on the breed, sex, and husbandry (Boldyrev et al., 2013). Poultry meat is sensitive to the oxidation processes that destroy the pigments and increase the content of polyunsaturated fatty acids. Some studies show that the lipid oxidation in meat can be efficiently controlled by adding the antioxidants like the vitamins E and C, selenium, and carnosine. The quality of chicken meat can be improved by the antioxidants in feed because, subsequent to the digestion, they are incorporated in the cell membranes and protect the tissue from oxidation - that is, from the reactive oxygen

species (Hu et al., 2009, Cong et al., 2017). Manhiani (2011) researched the concentration of carnosine in different tissues of broilers bred in normal conditions, as well as in those exposed to stress. The carnosine level in the breast muscles of chickens bred under stress was ten times higher than of those bred in a control group (17.39: 1.85 mg/g). Tomonaga et al. (2004) reported that adding β -alanine in the chicken feed may reduce the catabolic processes caused by the stress and improve efficiency of feed utilization in chickens.

Figure 1 shows the role of carnosine in tissue metabolism.

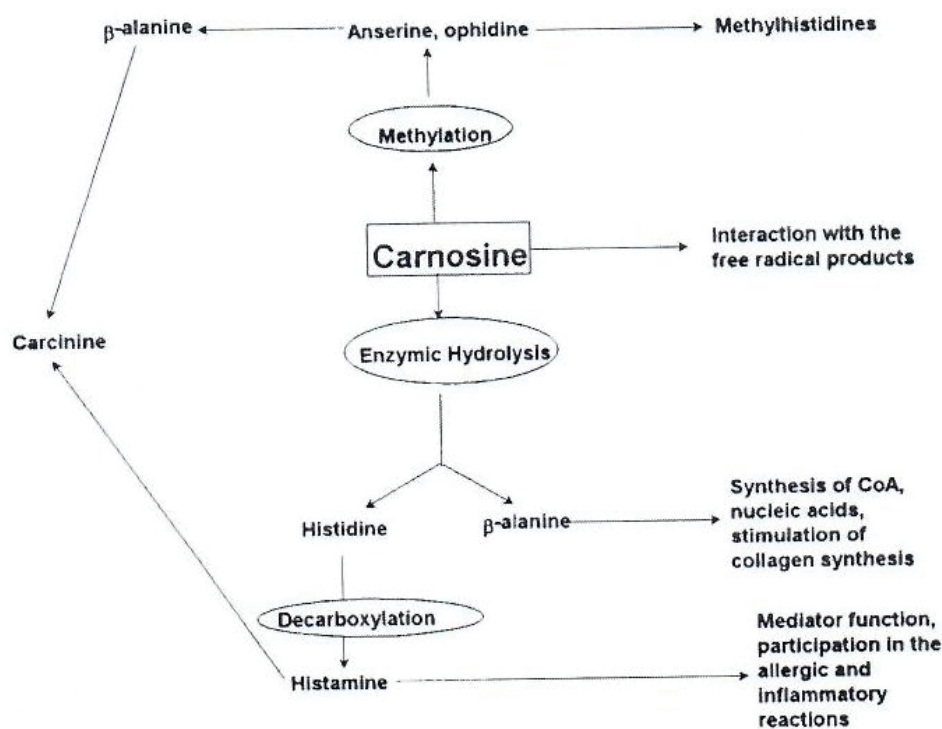


Figure 1 Carnosine metabolism in tissues (Gariballa and Sinclair, 2000)

Grafikon 1. Metabolizam karnozina u tkivu (Gariballa i Sinclair, 2000.)

DIETARY B-ALANINE ENHANCES CARNOSINE IN BROILERS

Tomonaga et al. (2005, 2006) dealt with the problem of enriching chicken meat with carnosine and anserine and obtained different results. In the first experiment, Tomonaga et al. (2005) with oral addition of β -alanine (22 mmol/kg) in the period from the second to the sixth day, they established that the concentration of carnosine increased by 60.7% in the brain and breast muscles. Control group contained 1235 ± 138 mg/kg of carnosine, and the experimental group 1985 ± 154 mg/kg of muscles. In the second experiment, Tomonaga et al. (2006) fed the broilers

during the period of four weeks with a feed in which they added 0.5%, 1%, and 2% of β -alanine. The authors did not achieve an increase in carnosine, anserine, and taurine in the chickens' muscles (*M. pectoralis superficialis*, *M. pectoralis profundus* and *M. biceps femoris*). The authors concluded that the addition of β -alanine into the chickens' feed affects the brain activity but is inefficient in the increase of dipeptides in the muscle tissue. In a later research, Tomonaga et al. (2012) managed to increase the carnosine levels in brain, *M. pectoralis superficialis*, and plasma in chickens by adding β -alanine (0.176, 0.88, 4.4 and 22 mmol/kg) orally two times per day during a five-day period. They did

not find any influence of the added β -alanine on the anserine content. Tomonaga et al. (2012) and Perim et al. (2019) point out that it is necessary to take care of several factors during the investigation of this problem, such as the following: genetic basis, age, sex, muscle type, meal composition, feeding duration and other. Tomonaga et al. (2012) suggest that the addition of β -alanine causes a decrease of taurine in tissue, which consequently causes a physiological disfunction such as stunting. Sturman (1993), Hu et al. (2000), and Perim et al. (2019) all agree with that supposition. Beta-alanine is a limiting factor in the synthesis of skeletal muscles (Perim et al., 2019). The homeostasis of carnosine depends on its synthesis and disintegration. A synthesis reaction of β -alanine and L-histidine is catalyzed by the carnosine synthase (CARNOS), an enzyme found in muscles. Harris et al. (2006) suggest that β -alanine is equally efficient in increasing the contents of muscular carnosine, just as β -alanine and L-histidine are if combined together. Qi et al. (2018) researched the addition of β -alanine (0-control, 250,500 and 2000 mg/kg of feed). The added β -alanine into the chicken feed increased the contents of carnosine in the breast muscle from 3155 $\mu\text{g/g}$ to 3417 $\mu\text{g/g}$. At the same time, the contents of taurine and anserine decreased from 145 $\mu\text{g/g}$ and 8290 $\mu\text{g/g}$ to 142 $\mu\text{g/g}$ and 7918 $\mu\text{g/g}$ in the breast muscles. Kralik et al. (2014) researched the effects of adding 0, 0.5 and 1% of β -alanine into the feed concerning the concentration of carnosine in chickens' muscular tissue. They determined the most efficient synthesis and carnosine deposition in the breast muscles in female and male chickens with the addition of 1% β -alanine (19.11%, that is, 21.86%). In the thigh muscles, the most efficient carnosine synthesis is determined by adding 0.5% of β -alanine (41.39% more in male chickens and 39.62% more in female chickens).

DIETARY HISTIDINE ENHANCES CARNOSINE IN BROILERS

Chicken meat has a high content of histidine-containing dipeptides (HCDs), according to Barbaresi et al. (2019). It is a matter of anserine and carnosine in the breast and thigh muscles. The authors researched the influence of two genotypes of broilers, three production systems, sex, and two different ages at slaughter ($2 \times 3 \times 2 \times 2$). They determined 2.5 times and 1.9 times more anserine than carnosine in the breast and thigh muscles, as well as the differences caused by the genotype, production system, age, and sex of chickens. The breast muscles in male chickens contained from 8.60 to 17.3, and in female chickens from 7.7 to 22.3 mmol/kg of carnosine, depending on the researched factors. The breast muscles contain the higher concentra-

tions of carnosine when compared to the drumstick and thigh muscles (Kralik et al., 2010 a, b). With the intention of increasing the concentration of carnosine in the meat and its oxidation stability, Kralik et al. (2015) added β -alanine (0, 0.5 and 1%) and L-histidine (0, 0.3 and 0.5%) into the chicken feed. They determined a higher carnosine deposition in the breast muscles in cases of higher concentrations of amino acids. Kai et al. (2015) researched the effects of three levels of histidine in feed: 67% (low), 100% (control), and 200% (high) in relation to the NRC recommendations (1994) for the synthesis of carnosine and anserine. The authors determined that the control group contained 1434 ± 86 and 5902 ± 153 $\mu\text{g/g}$ of carnosine breast muscles and anserine breast muscles, respectively. The high levels of histidine in the feed increased the carnosine up to 2464 ± 186 and anserine up to 6652.5 ± 288 $\mu\text{g/g}$ of muscles. The concentration of carnosine in a chicken organism can be modified by adding the amino acids (β -alanine and L-histidine) individually or collectively in the feed (Haug et al., 2008; Boldyrev et al., 2013). According to Harris et al. (2006), the intensity of carnosine synthesis in muscles is more under the influence of β -alanine than L-histidine. Kopec et al. (2013) fed the chickens with a feed containing by-products like fish meal, pig blood cells, blood meal, wheat gluten and yeast, which are all the sources of histidine and β -alanine. They did not find a connection between the amino acids in the feed and the chicken meat (except in the usage of blood by-products: 1.66: 2.06, 210 mg/g of tissue). In the second experiment, the quoted authors used a treatment, with control, containing 4% of blood meal in the chicken feed (SDBC, SDBC treatment + 0.025% ZnO, and a treatment with 0.217% of L-histidine). The muscles of the control group contained 0.891 mg/g, and the muscles of chickens fed with histidine contained 1205 mg/g of carnosine in tissue. A Zn addition increased the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes. Moro et al. (2020) suggest that histidine is a limiting factor in the synthesis of carnosine that has a strong antioxidative effect. Kopec et al. (2013) concluded that the addition of histidine in the chicken feed may increase the contents of carnosine and anserine and improve the meat quality.

THE INFLUENCE OF HISTIDINE AND β -ALANINE SUPPLEMENTATION OF BROILER DIETS ON THE CARNOSINE CONTENT

The research conducted by some authors has shown that the carnosine concentration in broiler meat can be successfully increased by adding β -alanine, L-histidine, or carnosine to the feed during the fattening period. Table 1 illustrates the possibilities of enrichment of chicken meat with carnosine.

Table 1. The influence of adding β -alanine, L-histidine, and carnosine to feed on the carnosine content in broiler meatTablica 1. Utjecaj dodavanja β -alanina, L-histidina i karnozina u hranu na sadržaj karnozina u mesu brojlera

| Author / Autor | Ingredient / Sastojak | Carnosine content / Sadržaj karnozina |
|-----------------------------|--|---|
| Tomunaga et al. (2012) | β - alanine, mmol/kg / β - alanin, mmol/kg | Breast, mmol/kg / Prsni mišić, mmol/kg |
| | 0.18 | 6116 |
| | 4.40 | 9162 |
| Kralik et al. (2015) | L- histidine, % / L- histidin, % | Breast, μ g/g / Prsni mišić, μ g/g |
| | Control / Kontrola | 871-912 |
| | 0.1 | 924-958 |
| | 0.2 | 966-969 |
| Kai et al. (2015) | L- histidine, % / L- histidin, % | Breast, μ g/g / Prsni mišić, μ g/g |
| | 0.14 | 1434 |
| | 4.61 | 2464 |
| Cong et al. (2017) | Carnosine, mg/kg | Thigh, μ g/g / Zabatak, μ g/g |
| | Control / Kontrola | 1509 |
| | 100 | 1539 |
| | 200 | 1560 |
| Qi et al. (2018) | β - alanine, mg/kg / β - alanin, mmol/kg | Breast, μ g/g, / Prsni mišić, μ g/g |
| | Control / Kontrola | 2693 |
| | 0.250 | 3150 |
| | 0.500 | 3903 |
| | 1.00 | 3494 |
| Suwanvichanee et al. (2022) | Amino acid, % / Aminokiselina, % | Breast, μ g/g / Prsni mišić, μ g/g |
| | Control / Kontrola | 2756 |
| | β - alanine, 1 % | 3484 |
| | L- histidine, 0.5 % | 3659 |
| | β - al. + L- hist. | 4212 |

The differences in carnosine content between the individual experiments depending on chickens' genotypes and the amount of added amino acids have been identified. The results showed that carnosine content can be increased by 15.0–71.8 % when adding L-histidine, 26.8–86.0 % when adding β -alanine, and by 6.9 % when adding carnosine.

THE INFLUENCE OF CARNOSINE ON THE QUALITY INDICATORS OF BROILER MEAT

Cong et al. (2017) established that adding carnosine to the chickens' feed (0, 100, 200 or 400 mg/kg⁻¹ over the span of 42 days) affects the manufacturing performances, meat quality, antioxidant capacity, and the characteristics of muscle fibers. Carnosine is absorbed in the gastrointestinal tract and is usually deposited

in the brain and muscular tissue. Adding carnosine to chickens' feed increases pH and redness (CIE a*) and decreases lightness (CIE L*), cooking loss, shear force, and hardness (P<0.05). In general, the pH of muscles is connected to the meat color. The final pH affects the myofibril structure and muscle color. The authors concluded that, if carnosine is added to the chickens' feed, it functions as a growth promoter and an antioxidant, and it positively affects the chicken meat quantity and quality. Kralik et al. (2018) reported on the enrichment of broiler meat with carnosine by using β -alanine, L-histidine, and MgO. The feeding treatments were as follows: P₁ control, P₂ 0.5 % of β -alanine + 0.24 % MgO, P₃ 0.25 % of L-histidine + 0.24 % MgO, P₄ 0.20 % of β -alanine + 0.10 % of L-histidine + 0.24 % of β -alanine. The effect of feeding treatments on the technological qualities of meat is showed in Table 2.

Table 2. The effect of feeding treatments on the technological quality of breast muscles of broilers**Tablica 2. Utjecaj hranidbenoga tretmana na tehnološka svojstva prsnih mišića brojlera**

| Characteristics / Svojstva N = 20 | Treatments / Tretmani | | | | | |
|--|-----------------------|---------------------|--------------------|----------------------|------|---------|
| | P ₁ | P ₂ | P ₃ | P ₄ | SEM | P value |
| pH ₁ | 6.10 ^a | 6.02 ^{a,b} | 6.07 ^a | 5.97 | 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 (%) / gubitak pri cijedenju | 2.03 ^a | 1.48 ^b | 1.45 ^b | 1.64 ^b | 0.06 | 0.002 |
| Cooking loss (%) / gubitak pri kuhanju | 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 ^{a,b} | 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 treatments

The authors found out that the influence of feeding treatments on the carnosine concentration in the breast and thighs muscle, as well as the level of TBARS values in the fresh and frozen meat (Table 3 and Table 4). The data in Table 2 show that the feeding treatments influenced the pH. The lowest pH was recorded in the broilers' breast muscle (5.97), which contained the lowest levels of carnosine (1084.25; 665.47; 715.45 and 736.17; P<0.05). After 24 hours of cooling the breast muscle, no differences in pH₂ (P=0.502) were established. The control group of breast muscle tissue showed the highest CIE L* value (P>0.05), but the CIE a* value was statistically higher (P<0.03) when compared to the other groups. A drip loss value was the highest in the P₁ treatment (P<0.05). The differences in the cooking loss values of muscles between the treatments with different carnosine content were not statistically significant (P=0.421). The WBSF (N) value was the highest in the group that contained the smallest amount of carnosine (P=0.008). The pH of muscles in a living organism varies between 7.0 and 7.2. Oxygen supply is ceased after killing, and the ATP is produced from the anaerobic breakdown of glycogen.

Lactic acid, which reduces the pH of its environment, is also produced. When 0.5% of carnosine was added to the broilers' feeding (Hu et al., 2009), its influence on the pH of meat was not established, which was also the case in the research conducted by Kralik et al. (2014; 2015). Qi et al. (2021) point out that adding histidine reduces redness (CIE a*) and yellowness (CIE b*) values 45 minutes *post-mortem* (P<0.05), but increases the CIE b* and pH in the breast muscles 24 hours *post mortem*. Also, adding β-alanine alone or in a combination with L-histidine significantly (p<0,01) increases the Δ pH in the breast muscles. In general, the authors conclude that adding the aforementioned amino acids may improve the quality of breast meat, as well as antioxidant capacity. Kopec et al. (2020) came to the same conclusion when researching the influence of adding the identical amino acids or carnosine to the chicken feed. Lackner et al. (2021) state that the age and storage life of meat have the main impact on quality parameters. The authors also increased the carnosine levels by adding L-histidine to the breast meat, though in the insignificant quantities.

Table 3. The effect of treatments on the concentration of carnosine (mg/kg) in the broilers' breast and thigh muscles**Tablica 3. Utjecaj tretmana na koncentraciju karnozina (mg/kg) u prsnome mišiću i zabatku brojlera**

| Part of carcass / Dio trupa N = 10 | Treatments / Tretmani | | | | | |
|---------------------------------------|-----------------------|---------------------|---------------------|----------------------|-------|---------|
| | P ₁ | P ₂ | P ₃ | P ₄ | SEM | p value |
| Breast / Prsni mišić | 665.47 ^b | 715.45 ^b | 736.17 ^b | 1084.25 ^a | 53.83 | 0.013 |
| Thighs / Zabatak | 261.19 ^b | 420.34 ^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 treatments

The feeding treatment exerted a statistically significant ($p < 0.05$) effect on the breast muscle's pH value, being lower in P_4 than in the other groups in which the fiber tenderness ($p = 0.008$) of the P_2 and P_3 groups had a higher WBSF value when compared to the control group. These results are in line with

those reported by Soyer et al. (2010) and Cong et al. (2017). The analysis of carnosine content results in the breast muscle tissue proved that the carnosine content increased by 7.0%, 10.62%, and 62.93% in P_2 , P_3 and P_4 groups, respectively, when compared to the P_1 group.

Table 4. The effect of treatment on the level of TBARS (nmol/g tissue) in the broilers' fresh and frozen breast muscles

Tablica 4. Utjecaj tretmana na razinu TBARS-a (nmol/g tissue) u svježem i zamrznutom prsnom mišiću brojlera

| Brest muscle / Prsni mišić N = 10 | Treatments / Tretmani | | | | | |
|--------------------------------------|-----------------------|--------------------|--------------------|--------------------|------|---------|
| | P_1 | P_2 | P_3 | P_4 | SEM | p value |
| Fresh / Svježe | 18.49 | 14.38 | 17.99 | 19.47 | 1.05 | 0.185 |
| Frozen / Smrznuto | 33.30 ^a | 30.68 ^a | 30.04 ^a | 24.34 ^b | 0.72 | 0.014 |

Treatments: P_1 control; P_2 0.5 β -alanine + 0.24 % MgO; P_3 0.25 % L-histidine + 0.24 % MgO; P_4 0.20 % β -alanine + 0.10 % L-histidine + 0.24 % MgO; a, b, $P < 0.05$; SEM – standard error of treatments

At the same time, the carnosine concentrations in the thighs muscle increased by 61.05%, 71.95%, and 89.52% in the experimental group. The feeding treatment was more efficient in the P_4 group when compared to the other treatments, although it included a lower amino acids concentration. However, MgO significantly affected the CIE a^* ($P = 0.07$) and drip loss ($P = 0.02$). The TBARS values (nmol/g tissue) were lower in the fresh than in the frozen breast muscle, and feeding treatments were not statistically significant ($P > 0.05$). A statistically significant difference in the TBARS values was found out in the frozen breast muscles. The P_4 group had a lower value (24.34 nmol/g tissue) when compared to the other groups (33.30, 30.68 and 30.04; $P < 0.05$). The results of the research by Kralik et al. (2018) circumstantiated that the combinations of β -alanine, L-histidine, and MgO effectively increased the carnosine deposition in the breast and thigh muscles, thus affecting the quality and maintenance of oxidative meat stability.

The most significant meat quality indicator is its pH, because it affects the color, water holding capacity, taste, and tenderness (Kralik et al., 2018). Oxidation affects the meat quality *ante mortem* or *postmortem*. Meat color is the criterium that either attracts or repels the consumers. A lower pH value is the result of biochemical processes, and it causes a higher degree of paleness and redness. In this research, the breast muscles had the lower CIE a^* values when compared to the control group, which was corroborated by the research conducted by Hu et al. (2009) and Kralik et al. (2015). The final pH₂ affects the myofibril structure and meat color. Hu et al. (2009) established a higher degree of CIE a^* values in the carnosine-enriched meat, but there were no changes in the CIE L^* and CIE b^* values. Drip loss ($P = 0.002$) in other experimental groups of meat was statistically significantly lower than in the control group, which was recorded in our previous research (Kralik et al., 2014).

The research conducted by Cong et al. (2017) has shown that adding carnosine to the broiler feed statistically significantly ($P < 0.05$) reduces the drip loss, cooking loss, and shear force (WBSF). The results are partially in line with our results in regard to the properties, such as the drip loss and shear force. The carnosine-enriched meat displays the higher WBSF values than the meat with a conventional composition. The results of research conducted by Everaert et al. (2013) show that the β aminotransferase is a limiting factor in the synthesis of carnosine in muscles. The β -alanine is ingested through food or is synthesized in the liver (Park et al., 2013). Apart from increasing the synthesis of carnosine in liver, β -alanine also increases both the CIE a^* and CIE L^* values (Kralik et al., 2014) and decreases the shear force values (Zhang, 2009; Qi et al., 2018). Qi et al. (2018) also state that an increase in the carnosine levels causes the increase of pH₁ and pH₂ in the breast muscles. Belviranl et al. (2015) did not detect any influence of carnosine on the oxidative stability of breast meat. However, Qi et al. (2018) established that carnosine reduces the MDA content ($P < 0.05$) in the breast muscles due to the carnosine's strong oxidative ability, which is also stated by Boldyrev et al. (2013) and Hu (2009).

CONCLUSION

Carnosine is a dipeptide synthesized from the β -alanine and L-histidine amino acids, with the presence of an enzyme called carnosine synthase. It can be found in the skeletal muscles in significant quantities. Carnosine has a polyfunctional biological role in metabolism, with a strong antioxidant effect. Adding β -alanine and L-histidine individually or in a combination increases the carnosine content in chickens' breast and thigh muscles. It improves the meat quality, maintains an oxidative stability, and decreases the TBARS values.

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KARNOZIN – POLIFUNKCIONALAN BIOLOŠKI AKTIVAN SASTOJAK

SAŽETAK

U radu se istražuje obogaćivanje mišića prsa i zabataka karnozinom, koji ima važnu funkciju u fiziološkim procesima. Istraživanja su pokazala da karnozin poboljšava kvalitetu pilećega mesa. Dodavanjem aminokiselina β -alanina i L-histidina u hranu pilića sintetizira se karnozin u skeletnim mišićima, mozgu, srčanome mišiću i stanicama čula mirisa. Ustanovljeno je da sadržaj karnozina ovisi o tipu mišića (bijelo ili tamno meso) te genotipu brojlera, kao i o spolu. Meso pilića osjetljivo je na oksidacijske procese, ali se lipidna oksidacija može efikasno prevenirati obogaćivanjem mesa karnozinom.

Ključne riječi: karnozin, β -alanin, L-histidin, mišići, brojleri

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