# Antioxidant status of turkey breast meat and blood after feeding a diet enriched with histidine

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**ABSTRACT** The objective of this study was to investigate the effects of 1) spray dried blood cells rich in histidine and 2) pure histidine added to feed on the antioxidant status and concentration of carnosine related components in the blood and breast meat of female turkeys. The experiment was performed on 168 Big7 turkey females randomly assigned to 3 dietary treatments: control; control with the addition of 0.18% L-histidine (His); and control with the addition of spray dried blood cells (SDBC). Birds were raised for 103 d on a floor with sawdust litter, with drinking water and feed ad libitum. The antioxidant status of blood plasma and breast muscle was analyzed by ferric reducing ability (FRAP) and by 2,2-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals scavenging ability. The activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) was analyzed in the blood and breast meat, with the content of carnosine and anserine quantified by HPLC. Proximate analysis as well as amino acid profiling were carried out for the feed and breast muscles. Growth performance parameters also were calculated. Histidine supplementation of the turkey diet resulted in increased DPPH radical scavenging capacity in the breast muscles and blood, but did not result in higher histidine dipeptide concentrations. The enzymatic antioxidant system of turkey blood was affected by the diet with SDBC. In the plasma, the SDBC addition increased both SOD and GPx activity, and decreased GPx activity in the erythrocytes. Feeding turkeys with an SDBC containing diet increased BW and the content of isoleucine and valine in breast muscles.

**Key words:** turkey meat, histidine, antioxidative potential, carnosine

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## INTRODUCTION

Turkey meat is more susceptible to oxidation than chicken meat (Peiretti et al., 2011), but the antioxidant status of turkey muscle has been less extensively studied than that of chickens. A few reports on the supplementation of turkey diets with soy oil and tocopherol show the effect of modified feed on the oxidation processes in muscle microsomal membranes (Mercier et al., 2001; Batifoulier et al., 2002). Daun and Akesson (2004) compared glutathione peroxidase (**GPx**) activity in the muscles of various animals and found higher GPx activity in thigh muscles, with dominating oxidative metabolism, than in breast muscles. Among the studied poultry species, the highest activity of GPx in both breast and thigh muscles was found in ducks. Turkey breast muscles were characterized by slightly lower GPx activity than that in chickens. Sarraga et al. (2006) studied the effect of the addition of tocopherol acetate and $\beta$-carotene to turkey feed on antioxidant enzyme activity. The cited authors found that the supplementation increased catalase (**CAT**) activity only in turkey breast. Superoxide dismutase (**SOD**) and GPx activities were not affected, which is consistent with the findings of Renerre et al. (1999) who did not observe any effect of vitamin E on GPx in turkey muscle.

Histidine heterodipeptides, such as carnosine and anserine, play many important roles in poultry tissues, including their effect on antioxidant status (Furuse et al., 2007). Turkey meat is characterized by the highest content of carnosine and anserine among poultry species, with the breast muscle abundant in dipeptides (predominantly anserine) (Tinbergen and Slump, 1976; Gil-Agust et al., 2008; Peiretti et al., 2011). In a study conducted on broiler chickens fed a diet supplemented with histidine-rich materials, including spray dried blood cells (**SDBC**), an increased concentration of carnosine was found in breast muscle, including


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**Table 1.** Composition of experimental diets.

| | Diets*** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 7 to 9 wk | | | 10 to 12 wk | | | 13 to 15 wk | | |
| Components | Control | His | SDBC | Control | His | SDBC | Control | His | SDBC |
| Maize, % | 30.9 | 36.5 | 34.8 | 31.1 | 30.4 | 30.5 | 33.7 | 33 | 35 |
| Wheat | 20 | 12.7 | 15 | 20 | 20 | 20 | 20 | 20 | 20 |
| Spray dried blood cells SDBC | - | - | 4 | - | - | 4 | - | - | 4 |
| Soya bean oil | 3.0 | 3.0 | 2.7 | 4.7 | 4.9 | 4.6 | 5.8 | 5.9 | 5.4 |
| Wheat bran | 5.7 | 6.8 | 11.8 | 8.2 | 8.4 | 13.7 | 11.3 | 11.5 | 15 |
| Soya bean meal | 35.5 | 35.9 | 26.9 | 31.3 | 31.4 | 22.5 | 24.6 | 24.7 | 16 |
| Dicalcium phosphate | 0.52 | 0.53 | 0.50 | 0.45 | 0.43 | 0.45 | 0.24 | 0.32 | 0.27 |
| Limestone | 0.21 | 0.20 | 0.30 | 0.16 | 0.16 | 0.25 | 0.25 | 0.25 | 0.33 |
| NaCl | 0.09 | 0.08 | - | 0.09 | 0.09 | - | 0.09 | 0.09 | - |
| Premix IB* | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| L-Histidine (83%) | - | 0.22 | - | - | 0.22 | - | - | 0.22 | - |
| L-Lysine (98%) | 0.08 | 0.07 | - | - | - | - | 0.02 | 0.02 | - |
| Metabolisable energy, MJ/kg** | 12.10 | 12.22 | 12.09 | 12.70 | 12.82 | 12.62 | 13.16 | 13.22 | 13.17 |
| Crude protein, g/kg | 232.2 | 227.7 | 231.4 | 214.3 | 215.1 | 210.2 | 189.5 | 188.0 | 186.3 |
| Crude fibre | 30.9 | 28.8 | 29.2 | 29.2 | 29.8 | 28.9 | 30.1 | 30.5 | 29.5 |
| Ca | 11.1 | 11.0 | 12.0 | 11.3 | 10.8 | 11.4 | 10.4 | 11.0 | 11.0 |
| P - available | 6.2 | 6.0 | 5.9 | 6.3 | 6.0 | 5.9 | 5.7 | 5.7 | 5.5 |
| Na | 1.8 | 1.7 | 2.1 | 1.7 | 1.7 | 2.1 | 1.7 | 1.8 | 2.3 |

*In 1 kg of premix: Ca 198 g (chelated calcium); P 90 g (as Ca3(PO4)2); Mg 10 g (as MgCl); Na 28 g (as NaCl); Se 8 mg (as Na2SeO3 · 5 H2O); Fe 1700 mg (as FeSO4 · H2O); Mn 2950 mg (as MnO2); Zn 2800 mg (as ZnO); Cu 625 mg (as CuSO4 · 5 H2O); J 45 mg (as Ca(IO3)2); L-Lysine 50 g; DL-Methionine 60 g; Threonine 5 g; vitamin A (retinyl acetate) 337,500 I.E.; vitamin $D_3$ (cholecalciferol) 95,000 I.E.; vitamin E (DL-$\alpha$-tocopheryl acetate) 1,250 mg; vitamin K (hetrazeen vit. $K_3$ free from menadione) 75 mg; vitamin $B_1$ (thiamine mononitrate) 80 mg; vitamin $B_2$ 250 mg; vitamin $B_6$ (pyridoxine HCl) 140 mg; vitamin $B_{12}$ (cyanocobalamin) 1 mg; nicotinic acid 2,150 mg; pantothenic acid 500 mg; folic acid 60 mg; choline chloride 15,500 mg.

**Energy value of diets was calculated with data of European Tables of Energy Values of Feed for Poultry, 1989.

***control - the non-supplemented diet; His - control diet with 0.18% L-histidine; SDBC - control diet with 4% spray dried porcine blood cells rich in histidine.

improvements in antioxidative enzymes activity (SOD, GPx, CAT) (Kopec et al., 2013a).

The aim of this study was to investigate the effect of diet supplementation with SDBC rich in histidine and pure histidine on antioxidant status, as well as the concentrations of carnosine and anserine in the blood and muscles of female turkeys.

## MATERIAL AND METHODS

### *Turkeys, Diets, Performance Indices*

The experiment was conducted on 42-day-old Big7 female turkeys (n = 168) with an average initial BW of 1.61 (±0.04) kg. The birds had previously been kept together for the first 6 wk and fed 2 types of diets: starters 1 and 2 (27.5% and 26.0% crude protein and 11.7 MJ/kg and 11.9 MJ/kg metabolizable energy, respectively). The 168 selected female birds were then randomly assigned to the 3 feed treatments: control; control with added L-histidine (His); and control containing SDBC, with 7 replications, each of them consisting of 8 birds. The turkeys were raised on a floor covered with sawdust litter. The ambient temperature was reduced by 1°C per wk from 24 to 21°C over the first 4 wk then stabilized at 21°C, with an environmental humidity of 64 to 70%. The lighting program comprised 18 h of light and 6 h of darkness.

Turkeys were fed ad libitum (free access to feeders and drinking water) with a core diet in mashed form in which the crude protein concentration was reduced from 22.8 (7 to 9 wk) to 21.2 (10 to 12 wk) to 18.7% (13 to 15 wk), while the metabolizable energy increased from 12.1 to 13.0 MJ/kg (7 to 13 wk) (Table 1). Treatment "control" was a diet of the non-supplemented core feed; treatment His control diet was supplemented with 0.18% L-histidine (Table 2); and treatment SDBC control diet contained 4% spray dried porcine blood cells rich in histidine (Table 3). The composition of all diets was calculated using a simple linear optimization on the basis of analytical data collected in the initial study for feed components and complete diets. Robenidyne® (30 mg/kg feed) was used to protect the flock against coccidiosis.

The individual BW, feed consumption, and conversion were measured for 12 randomly selected birds from each treatment at d 42 and 103. The mortality also was monitored during the whole experiment; none of the birds was found dead during the entire study.

All procedures carried out were approved by the Local Ethics Commission for experiments on animals.

### *Blood Collection and Muscle Preparation for Analyses*

Blood samples were collected during slaughtering, then immediately heparinized, chilled on ice, and centrifuged (5,000 G) to separate plasma from blood

**Table 2.** Amino acid concentration in experimental diets [g/kg].

| | Diets* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 7 to 9 wk | | | 10 to 12 wk | | | 13 to 15 wk | | |
| Aminoacids | Control | His | SDBC | Control | His | SDBC | Control | His | SDBC |
| Asp | 21.73 | 21.12 | 22.96 | 19.97 | 19.42 | 20.00 | 17.62 | 16.82 | 18.48 |
| Thr | 8.55 | 8.57 | 8.49 | 7.92 | 7.82 | 7.38 | 6.95 | 7.03 | 6.86 |
| Ser | 11.13 | 11.26 | 11.26 | 10.40 | 10.55 | 9.98 | 8.91 | 8.92 | 8.81 |
| Glu | 47.88 | 48.33 | 47.09 | 43.82 | 44.40 | 41.68 | 37.63 | 38.11 | 37.94 |
| Pro | 14.58 | 15.31 | 16.07 | 13.40 | 14.36 | 14.31 | 11.94 | 12.62 | 12.82 |
| Gly | 9.06 | 9.37 | 9.08 | 8.45 | 8.61 | 8.02 | 7.41 | 7.57 | 7.12 |
| Ala | 9.92 | 9.98 | 10.94 | 9.13 | 9.35 | 9.80 | 8.06 | 7.99 | 8.43 |
| Val | 10.31 | 9.89 | 10.91 | 9.53 | 8.96 | 9.42 | 8.20 | 8.07 | 8.49 |
| Ile | 8.95 | 8.80 | 8.08 | 8.33 | 8.25 | 7.18 | 7.10 | 7.13 | 6.46 |
| Leu | 17.14 | 16.59 | 19.32 | 15.80 | 15.31 | 17.00 | 13.56 | 13.22 | 15.48 |
| Tyr | 5.63 | 5.86 | 5.88 | 5.13 | 5.34 | 5.14 | 4.55 | 4.70 | 4.74 |
| Phe | 10.65 | 11.25 | 11.83 | 9.97 | 10.31 | 10.44 | 8.75 | 9.28 | 9.42 |
| **His** | **5.84** | **7.83** | **7.21** | **5.35** | **7.34** | **6.35** | **4.88** | **6.28** | **5.62** |
| Lys | 13.72 | 12.79 | 13.00 | 12.61 | 11.86 | 11.64 | 10.86 | 10.33 | 10.07 |
| Arg | 16.97 | 16.69 | 16.06 | 15.48 | 15.69 | 13.93 | 13.64 | 13.13 | 12.84 |
| Cys | 3.45 | 3.39 | 3.50 | 3.19 | 3.09 | 3.11 | 2.76 | 2.74 | 2.74 |
| Met | 5.70 | 5.84 | 6.11 | 5.22 | 5.29 | 5.29 | 4.58 | 4.78 | 4.94 |
| Try | 2.49 | 2.39 | 2.45 | 2.30 | 2.19 | 2.17 | 2.03 | 1.93 | 1.94 |
| Total | 223.73 | 225.24 | 225.70 | 206.01 | 208.14 | 198.83 | 179.43 | 180.68 | 179.64 |

*control - the non-supplemented diet; His - control diet with 0.18% L-histidine; SDBC - control diet with 4% spray dried porcine blood cells rich in histidine.

**Table 3.** Chemical composition of spray dried porcine blood cells.

| Item | SDBC |
|---|---|
| Crude protein, g/kg | 871.1 |
| Crude ash | 44.0 |
| Ca | 1.03 |
| P – total | 2.50 |
| Mg | 0.32 |
| Zn, mg/kg | 19.7 |
| Fe | 2378 |
| Mn | 4.13 |
| Cu | 3.81 |
| Amino acid, g/kg | |
| Asp | 103.6 |
| Thr | 32.3 |
| Ser | 42.4 |
| Glu | 75.4 |
| Pro | 34.8 |
| Gly | 41.4 |
| Ala | 73.3 |
| Val | 72.0 |
| Ile | 6.7 |
| Leu | 112.2 |
| Tyr | 18.5 |
| Phe | 59.0 |
| His | 63.5 |
| Lys | 76.3 |
| Arg | 45.7 |
| Cys | 8.6 |
| Met | 7.0 |
| Try | 9.2 |

cells. The activity of GPx, as well as total antioxidant capacity—2,2-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (**ABTS**); 1,1-Diphenyl-2-picrylhydrazyl (**DPPH**); ferric reducing ability (**FRAP**)—were determined in the plasma. The sediment of blood cells was used for CAT activity assay after being washed 3 times with cold 0.15 M NaCl solution and centrifuged at 5,000 G.

Within 10 min after slaughter, the *Pectoralis superficialis* and *profundus* muscles were excised from the carcass, chilled on ice in polyethylene bags, then kept in a freezer at –20°C. All samples used for amino acid profiling remained stored at –20°C, while the meat directed to chemical evaluation of antioxidant status was stored for 30 min at –20°C then transferred to a deep freezer (–80°C) and kept until analysis. Prior to analyses, meat samples were placed in a freezer at –20°C, followed by a refrigerator until a temperature of –2°C was reached.

The hydrophilic fraction of breast muscles used for the total antioxidant capacity assays (ABTS, DPPH, FRAP), was prepared by homogenization (Mixer B-400 Buchi, Flawil, Switzerland) of the muscles with redistilled water as described by Sacchetti et al. (2008).

## Radical Scavenging Ability

The total antioxidant capacity was analyzed in hydrophilic fractions of turkey breast muscles and blood plasma using the following methods:

The activity of reducing ABTS radicals was determined using Trolox-equivalent antioxidant capacity assays according to Re et al. (1999). Studied samples were combined with $ABTS^{+}$ radical solution diluted with phosphate buffered saline, pH 7.4 (PBS) and vortexed for 1 min. After 10 min incubation at room temperature, samples were measured at 734 nm. The inhibition of $ABTS^{+}$ radical by the samples was compared to the Trolox standard and expressed as $\mu$mol Trolox per g wet tissue or per mL plasma.

Scavenging activity towards DPPH radicals was conducted according to Jang et al. (2008) with modifications by Milardović et al. (2006). Analyzed samples were reacted with DPPH in an ethanol solution for one h in a dark room and measured at 517 nm. The ability to scavenge DPPH radicals was expressed as $\mu$mol Trolox per g wet muscles or per mL plasma.

FRAP of plasma was analyzed according to Benzie and Strain (1996). The assay is based on the reduction of tripyridyltriazine $Fe^{3+}$ by the formation of tripyridyltriazine (TPTZ)-$Fe^{2+}$ with an intensive blue color measured at 593 nm. FRAP was expressed as $\mu$mol $Fe^{2+}$ per g wet muscles or per mL plasma.

All chemicals used in this study were purchased from Sigma-Aldrich (Poznan, Poland).

## Activity of Antioxidant Enzymes

CAT (EC 1.11.1.6) activity was analyzed by the method of Aebi (1983). The principle of the method was based on enzymatic hydrolysis of $H_2O_2$. The loss of $H_2O_2$ during the initial 30 s of the reaction was monitored at 240 nm. Erythrocytes were homogenized with 1% Triton X-100 solution and the obtained hemolyzate was further diluted with 0.05 M phosphate buffer (pH 7.0). Protein concentration was determined by biuret assay (Gornall et al., 1949). Breast muscles were homogenized with 0.05 M phosphate buffer (pH 7.0) and centrifuged (7,000 G) according to Hernandez et al. (2002). Following that, the supernatant was reacted with 10 mM $H_2O_2$. One unit (**U**) of CAT activity was defined as the amount of erythrocyte protein (hemolyzate) or amount of muscle needed to decompose one mmol of $H_2O_2$ per min.

Superoxide dismutase (**SOD**, EC 1.15.1.1) activity was analyzed using a Cayman Superoxide Dismutase Assay Kit No. 7060002 (Biokom, Janki, Poland). Breast muscle tissue was homogenized with 5 vol. of cold 20 mM HEPES containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose buffer (pH 7.2). After centrifugation (1,500 G) the supernatant was collected and analyzed. Detection of superoxide radicals generated by xanthine oxidase was monitored for 20 min at room temperature at 450 nm. One U of SOD activity was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical per g wet muscle or per mL plasma.

The activity of GPx (EC 1.11.1.9) was determined by measuring the oxidation of nicotinamide adenine dinucleotide phosphate (**NADPH**) in the presence of reduced glutathione and hydrogen peroxide (Cayman Chemicals, Kit No. 703102, Biokom, Janki, Poland). The breast muscle tissue was homogenized with 5 vol. of cold 50 mMTris-HCl, 5 mM EDTA, and 1mM DTT (pH 7.5) buffer and centrifuged (10,000 G). The reaction was monitored for 5 min at 340 nm. One unit of GPx activity was defined as the amount of enzyme to oxidize 1 mmol of NADPH to NADP+ per min at 25°C per g wet muscles or per mL plasma.

## Histidine Dipeptides Content

Preparation of the samples for dipeptide (carnosine, anserine) determination was carried out according to Aristoy et al. (2004), with modification of the ratio of muscle to redistilled water to 1:1, using a Mixer B-400 homogenizer (Buchi, Switzerland). After centrifugation (10,000 G) the collected supernatant was deproteinized by 3 vol. of methanol (HPLC grade) and centrifuged (12,000 G). Plasma was analyzed after deproteinization by solid phase extraction (**SPE**) system and with methanol. The samples were reacted with O-phthalaldehyde (**OPA** pre-column derivation method) and analyzed on an 1100 Series HPLC system (Agilent Technologies) equipped with a binary pump, an autosampler, and a vacuum degasser. The chromatographic separation was developed using a ZORBAX Eclipse-AAA column (4.6 × 150 mm, 3.5 $\mu$m, Agilent Technologies) with variable gradient at a flow rate of 2.0 mL/min. Phosphate buffer (40 mM $Na_2HPO_4$, pH 7.8) (A), and acetonitrile : methanol : water (45 : 45 : 10) (B), were used as mobile phases. Fluorescent detection was monitored by a G1321A FLD detector at 340/450 nm and at 266/305 nm. Standard curves were prepared using L-carnosine and L-anserine (as L-anserine nitrate salt) (Sigma-Aldrich, Poznań, Poland).

## Chemical Analytical Methods

The proximate analysis of feed components and complete diets was determined according to the standard AOAC methods (2005): nitrogen by Kjeldahl-method (984.13) using Kjeltec 2300 Foss Tecator apparatus (Hillerod, Denmark); crude protein by multiplying the nitrogen content by 6.25; crude fat by ether extraction (920.39); and crude fiber by the method of Hennenberg-Stohmann (978.10) using a Fibertec Tecator (Hillerod, Denmark). The mineralization of samples with $HNO_3$ was performed using a MarsX (CEM Corp., Kamp-Linfort, Germany). Phosphorus content was analyzed post mineralization with nitric acid and perchloric acid by the ammonium vanadomolybdate method (995.11) using a Specol 11 (Carl Zeiss, Jena, Germany) spectrophotometer at 470 nm. Calcium and other mineral (Mg, Zn, Fe, Mn, Cu) concentrations were determined by atomic absorption spectrophotometry using an AA 240 FS with SIPS 20 (Varian, Santa Clara, CA) (968.08).

Amino acid determination (994.12) was performed on feed components and breast muscles after hydrolysis with 6 M hydrochloric acid for 24 h at 110°C. The amino acids were separated according to Moore (1963) and Moore and Stein (1963). For sulfur amino acid determinations, feed samples were oxidized (0°C, 16 h)

**Table 4.** Average BW, feed conversion, and mortality of turkeys.

| Item | Control* | His* | SDBC* | SEM | *P*-value |
|---|---|---|---|---|---|
| | Body weight, kg** | | | | |
| at d 42 | 1.605 | 1.623 | 1.614 | 0.009 | 0.735 |
| at d 103 | 7.014[b] | 7.456[a] | 7.330[a] | 0.074 | 0.031*** |
| Feed conversion, kg feed/kg BW gain | 2.944 | 2.790 | 2.787 | 0.043 | 0.238 |
| Daily feed intake, g | 291 | 295 | 292 | 3.124 | 0.810 |
| Mortality, % | 5.2 | 5.2 | 1.8 | 1.278 | 0.485 |
| Skinless breast muscles, kg | 1.320 | 1.520 | 1.360 | 0.040 | 0.091 |
| Breast muscles as a percentage of BW (%) | 19.5 | 21.2 | 19.5 | 0.455 | 0.227 |

*control - the non-supplemented diet; His - control diet with 0.18% L-histidine; SDBC - control diet with 4% spray dried porcine blood cells rich in histidine.

**Values are presented as means.

***Differences in rows between treatments signed a, b, c significant at $P < 0.05$.

with formic acid and hydrogen peroxide (9:1 v:v) prior to HCl hydrolysis, then separated using an Analysator AAA 400 Ingos (Prague, Czech Republic) (985.25). For tryptophan content, samples after alkaline hydrolysis with lithium hydroxide (110°C, 16 h) and 4 – dimethylaminobenzaldehyde were analyzed at 590 nm (Landry and Delhaye, 1992) (988.15).

The energy values of the diets were calculated on the basis of the European Tables of Energy Values of Feeds for Poultry (1989) and Polish Recommendations of Poultry Feeding (2005).

All analyses of the indices of antioxidant characteristics and amino acids content in muscles were repeated twice.

### Statistical Analysis

The collected data were statistically analyzed by one-factorial ANOVA using StatSoft Statistica® Software (2009). The differences among treatments for all parameters were tested according to the following statistical model:

$$Y_{ij} = \mu + a_i + e_{ij}$$

where $Y_{ij}$ is the variance associated with parameter a; $\mu$ is the overall mean; $a_i$ is the treatment effect; and $e_{ij}$ the error term. For individual measurements (BW, blood, and meat parameters) or averages for pens: replications of feed consumption and feed conversion were treated as the experimental units, and differences among treatment means were analyzed for significance ($P < 0.01$ or $P < 0.05$) using Tukey's tests. The data are presented as mean accompanied by the standard error of the mean (**SEM**).

## RESULTS

### Performance Indices of Turkeys

The diets used in this study significantly ($P < 0.05$) influenced the BW of the turkeys (Table 4). Supplementation of the turkey diets with L-histidine or SDBC, as a source of histidine, increased the BW of the birds by 6.3% and 4.5%, respectively. No significant ($P < 0.05$) differences were observed among the treatments for feed conversion ratio and breast muscle weight and yield. A tendency to increase breast muscle weight and yield was observed when turkeys were fed the diet with pure L-histidine (Table 4).

Carnosine and anserine concentrations in turkey breast muscles were not affected by the diet ($P < 0.05$). No relationship between the sum of dipeptides and diet was found (Table 5). Supplementation of the diets with histidine or SDBC did not influence carnosine and anserine contents or the sum of dipeptides in blood plasma (Table 6).

Superoxide dismutase, GPx, and CAT activities in turkey muscle were not affected by the diet (Table 5). In blood plasma and erythrocytes, significant differences ($P < 0.05$) in SOD activity were observed (Table 6). Turkeys fed with the diet containing pure L-histidine exhibited lower SOD activity in blood plasma and erythrocytes ($P < 0.05$), while the diet with SDBC increased SOD activity in blood plasma ($P < 0.01$). The turkey diets with increased histidine content (treatments 2 and 3) had no effect on CAT activity in erythrocytes, but they increased GPx activity in blood plasma. Opposite results, i.e. decreased GPx activity in erythrocytes, were found for turkey diet supplemented with spray dried porcine blood cells. GPx activity in erythrocytes from the control treatment and the diet with L-histidine were the same (3.22 U/mg) (Table 6).

The FRAP of plasma in turkey breast muscles was not affected by the diet ($P < 0.05$). The antioxidant potential measured by ABTS in the hydrophilic breast muscle extract was not affected by the diet (average 21 $\mu$M Trolox/g) (Table 5). However, diet supplemented with pure L-histidine (treatment 2) increased the total antioxidant capacity measured by the ability to scavenge DPPH radicals ($P = 0.03$). No differences in DPPH scavenging ability were observed for the control (treatment 1) or SDBC (treatment 3) diets. The highest ability to scavenge DPPH radical was measured in blood plasma of turkeys fed the diet with L-histidine (treatment 2) (Table 6). The total antioxidant capacity of blood plasma analyzed by ABTS and FRAP was not affected by diet ($P < 0.05$).

**Table 5.** Antioxidant characteristics of turkey breast muscles.

| Treatments* | Carnosine mg/g | Anserine mg/g | Sum of dipeptides mg/g | SOD U/g | GPx U/g | CAT U/g | ABTS [$\mu$mol Trolox/g] | DPPH [$\mu$mol Trolox/g] | FRAP [$\mu$mol Fe/g] |
|---|---|---|---|---|---|---|---|---|---|
| Control | 2.35 | 3.98 | 6.33 | 1.904 | 0.972 | 200.1 | 2.181 | $15.20^{b}$ | 0.144 |
| His | 2.15 | 3.87 | 6.02 | 1.844 | 0.867 | 203.0 | 2.193 | $22.2^{a}$ | 0.145 |
| SDBC | 2.30 | 3.89 | 6.19 | 1.894 | 0.935 | 197.2 | 2.085 | $16.4^{b}$ | 0.136 |
| SEM | 0.097 | 0.043 | 0.051 | 0.160 | 0.066 | 3.232 | 0.052 | 1.88 | 0.031 |
| *P*-value | 0.338 | 0.197 | 0.053 | 0.965 | 0.564 | 0.213 | 0.297 | $0.031^{**}$ | 0.168 |

*control - the non-supplemented diet; His - control diet with 0.18% L-histidine; SDBC - control diet with 4% spray dried porcine blood cells rich in histidine.

**Values are presented as means, Differences in columns signed with A, B, C significant at $P < 0.01$; a, b, c significant at $P < 0.05$.

**Table 6.** Antioxidant enzymes activities in erythrocytes as well as in blood plasma and selected antioxidant content in blood plasma.

| | Blood plasma | | | | | | | | Erythrocytes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments* | Carnosine [$\mu$g/ml] | Anserine [$\mu$g/ml] | Sum of dipeptdes [$\mu$g/ml] | SOD [U/ml] | GPx [U/ml] | ABTS [$\mu$mol Trolox/ml] | DPPH [$\mu$mol Trolox/ml] | FRAP [$\mu$mol Fe/ml] | SOD U/mg | GPx U/mg | CAT U/mg |
| Control | 62 | 153 | 215 | $2.015^{B}$ | $2.568^{b}$ | 2.818 | $8.142^{C}$ | 1.164 | $0.379^{a}$ | $0.326^{a}$ | 399 |
| His | 61 | 169 | 230 | $1.870^{B}$ | $3.412^{a}$ | 2.762 | $9.278^{A}$ | 1.129 | $0.241^{b}$ | $0.322^{a}$ | 398 |
| SDBC | 59 | 172 | 231 | $2.672^{A}$ | $3.733^{a}$ | 2.757 | $8.659^{B}$ | 1.171 | $0.362^{a,b}$ | $0.284^{b}$ | 394 |
| SEM | 5.23 | 25.68 | 11.5 | 0.179 | 0.332 | 0.075 | 0.237 | 0.054 | 0.045 | 0.012 | 5.134 |
| *P*-value | 0.823 | 0.771 | 0.846 | $0.001^{**}$ | $0.035^{**}$ | 0.830 | $0.001^{**}$ | 0.761 | 0.040 | 0.030 | 0.213 |

*control - the non-supplemented diet; His - control diet with 0.18% L-histidine; SDBC - control diet with 4% spray dried porcine blood cells rich in histidine.

**Values are presented as means, Differences in columns signed with A, B, C significant at $P < 0.01$; a, b, c significant at $P < 0.05$.

**Table 7.** Concentration of amino acids in turkey breast muscles (g/kg wet tissue).

| | Control* | His* | SDBC* | SEM | *P* value |
|---|---|---|---|---|---|
| **Asp** | 22.75 | 22.34 | 22.66 | 0.208 | 0.364 |
| **Thr** | 10.94 | 10.71 | 10.72 | 0.097 | 0.160 |
| **Ser** | 9.77 | 9.51 | 9.59 | 0.092 | 0.130 |
| **Glu** | 33.62 | 33.00 | 33.10 | 0.268 | 0.221 |
| **Pro** | 9.53 | 9.65 | 9.78 | 0.163 | 0.587 |
| **Gly** | 9.97 | 9.86 | 9.99 | 0.116 | 0.699 |
| **Ala** | 13.85 | 13.64 | 13.86 | 0.126 | 0.398 |
| **Val** | $11.67^{B}$ | $11.85^{B}$ | $12.24^{A}$ | 0.131 | 0.003** |
| **Ile** | $10.89^{b}$ | $11.00^{a,b}$ | $11.36^{a}$ | 0.122 | 0.010** |
| **Leu** | 19.33 | 19.20 | 19.33 | 0.191 | 0.863 |
| **Tyr** | 8.02 | 7.92 | 7.88 | 0.086 | 0.482 |
| **Phe** | 9.50 | 9.48 | 9.45 | 0.095 | 0.926 |
| **His** | 14.65 | 14.64 | 14.89 | 0.156 | 0.457 |
| **Lys** | 23.97 | 23.70 | 23.75 | 0.223 | 0.738 |
| **Arg** | 18.29 | 17.97 | 17.93 | 0.191 | 0.344 |
| **Cys** | 2.57 | 2.51 | 2.55 | 0.024 | 0.210 |
| **Met** | 6.99 | 6.80 | 6.91 | 0.059 | 0.085 |
| **Try** | 2.33 | 2.35 | 2.30 | 0.025 | 0.695 |

*control - the non-supplemented diet; His - control diet with 0.18% L-histidine; SDBC - control diet with 4% spray dried porcine blood cells rich in histidine.

Values are presented as means.

**Differences in rows signed with A, B, C significant at $P < 0.01$; a, b, c significant at $P < 0.05$.

Diet supplemented with SDBC (treatment 3) significantly increased isoleucine ($P < 0.05$) and valine ($P < 0.01$) concentrations in turkey breast meat (Table 7).

## DISCUSSION

In the present study the enzymatic system in turkey breast muscles was not affected by the diets with histidine rich materials (SDBC) or pure histidine, the precursor of carnosine-related compounds. For chickens fed a diet containing SDBC, Kopec et al. (2013a) found an increase in the activity of antioxidant enzymes in breast muscle, especially SOD and GPx, while the blood plasma enzymes were not affected by that diet (Kopec et. al., 2013a,b). The above-mentioned relations indicate that applied feeds stimulate the antioxidative mechanism differently in turkey and chicken tissues. This also can be confirmed by the relationship found between chicken and turkey erythrocytes. The addition of histidine and SDBC to the chicken diet increased the activity of enzymatic triad or at least SOD activity (Kopec et al., 2013b), whereas SOD and GPx activities in turkey erythrocytes decreased after diet supplementation with L-histidine and SDBC.

There is still not enough data concerning antioxidant enzyme activity comparisons among animal species. Daun and Akesson (2004) found similar GPx activity in turkey, chicken, and ostrich breast muscles, while in duck muscles the activity was several times higher due to relatively high selenium content. Previous studies on chicken (Kopec et al., 2013b) and the current study on turkey muscle and blood showed similar antioxidant enzyme activity, but different relations after feeding the birds a diet supplemented with histidine.

The effect of histidine on the antioxidant mechanism increased the concentration of cytosol antioxidants, i.e. histidine dipeptides (carnosine, anserine) in chicken muscles (Haug et al., 2008). In previous studies on diet supplementation with histidine and SDBC, Kopec et al. (2013a) observed an increased carnosine content in chicken breast muscles. Higher histidine dipeptide (carnosine, anserine) concentrations were not confirmed in breast muscles after 9 wk of feeding turkeys with diets supplemented with pure L-histidine and SDBC.

Peiretti et al. (2012) reported 2.73% anserine concentration in freeze-dried muscle, which was equivalent to about 6 to 7 mg anserine and about 2 to 3 mg carnosine per g wet tissue. In the present study, the content of anserine and carnosine in turkey breast muscle was about 4 mg/g and 2 mg/g, respectively. Present data agreed with Gil-Agust et al. (2008), who found 4.2 mg/g anserine, but only 0.7 mg/g of carnosine in muscle. The differences in the reported levels of dipeptides can be explained by insufficient separation of homocarnosine, which, according to Peiretti et al. (2012), is present in turkey breast muscles in concentrations about 3 times lower than carnosine.

The concentration of histidine dipeptides in turkey blood plasma (Table 6) was as low as in chickens and was not affected by the diet (Kopec et al., 2013a). However, higher amounts of anserine compared to carnosine were observed in turkey erythrocytes.

It seems that the increased ability to scavenge DPPH radicals observed in this study after turkey diet supplementation with L-histidine and SDBC was not related to histidine dipeptide content. Intarapichet and Maikhunthod (2005) showed that hydrophilic extracts of chicken breast meat expressed a higher antioxidant capacity than carnosine solutions, which can be related to protein content as well as free amino acid composition. This also was shown by Wu et al. (2005) where high antioxidant ability was associated with specific molecular peptides (1400 Da) present in chicken meat homogenates. Superior DPPH scavenging ability also can be explained by the higher concentration of available histidine in the diet. Wu et al. (2003) showed that histidine scavenged DPPH radicals at about 20% of the ability of carnosine or anserine. Such a hypothesis seems to be confirmed in the present study by the increased DPPH in plasma, but not in breast muscles after feeding turkeys the diet with SDBC. Robbins et al. (1977) found a linear increase of histidine content in chicken blood plasma when dietary histidine was higher than 0.4%. In the present study histidine concentrations in the turkey diets varied between 0.56 and 0.78%.

Important discrepancies in growth performance were observed as the effect of feeding chickens and turkeys a diet with a high content of spray dried porcine blood cells. Blood or blood cell application in chicken diets (from 4 to 15%) decreased growth performance indices (BW decreased by 10% or more) (Makinde et al., 2008; Auh et al., 2010; Kopec et al., 2013b). Such an effect was not observed in the current experiment conducted

on turkeys. Higher BW of turkeys was observed when the diet was supplemented with 4% SDBC and with pure L-histidine. This agrees with the previous results of Kopec et al. (2013b) for chickens fed a diet supplemented with histidine.

The increase of isoleucine content in the breast muscle of turkeys fed the diet with SDBC was not expected because the amount of isoleucine in the porcine blood cells was low. In addition to that, valine and isoleucine are used for glutamine and alanine synthesis (Wu, 2009). The amount of Ile (more than 0.65%) and the ratio of Val to Leu (0.65 to 0.90) were quite high in the experimental diets, which, according to Ishibashi and Yonemochi (2002), leads to optimal poultry growth. Moreover, the dietary requirements of birds towards branched chain amino acids are affected by the concentration of these amino acids in feed (Ishibashi and Yonemochi, 2002). It is worth underlining that alanine content in the diet supplemented with spray dried porcine blood cells was higher than in the control diet, so probably valine and isoleucine were not utilized in alanine synthesis. This might be the reason for higher valine and isoleucine concentrations in breast muscles of turkeys fed the diet supplemented with SDBC.

It can be concluded that the enzymatic antioxidant system of turkey blood plasma was activated by the diets supplemented with L-histidine and SDBC. However, GPx activity in erythrocytes was lower than in control birds. In contrast, the antioxidant enzymatic system and histidine dipeptide (carnosine, anserine) concentration in turkey breast muscle were not affected by diet supplementation with histidine and SDBC. Feeding turkeys a diet supplemented with histidine increased DPPH radical scavenging capacity in both blood plasma and muscle, whereas SDBC affected DPPH only in blood plasma. Diet supplementation with histidine and SDBC improved the production yield, especially the BW of the turkeys.

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