Animal Science Papers and Reports vol. 37 (2019) no. 4, 393-408
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Antioxidant effects of phytogenic herbal-vegetable mixtures additives used in chicken feed on breast meat quality

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(Accepted June 14, 2019)

Plants are important source of antioxidant substances. When fed to production animals they help to protect the characteristics of the final product. The aim of the current study was to demonstrate the influence of herbal and vegetable blends in the broiler chickens diet on the oxidative status and physiochemical properties of their breast muscles. Four hundred male broiler chickens (ROSS 308) were randomly divided into four feeding groups (100 birds each) and reared on litter at stocking density of 11 birds/m^2 until 42 days of age. Chickens were fed *ad libitum*. The control group (C) received the standard commercial diet, while experimental groups E1, E2 and E3 diet with 2% herbal-vegetables mixtures as an additive (70% onion, 25% thyme, 5% mint or 90% ginger, 7% rosemary, 3% chili or 30% onion, 20% garlic, 25% oregano, 10% fennel, 5% mint, 5% turmeric,

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5% ginger in group E1, E2 and E3 respectively). A generalised linear mixed model was applied on all measured parameters, including feeding group as fixed factor using PROC GLIMMIX of SAS v 9.3. Application of the vegetable and herbs mixtures affected meat physiochemical characteristics (pH, WHC, shear force, color) ($P<0.05$). Chickens receiving herbal and vegetable additives showed higher content of polyphenols, anthocyanins, retinol and α-tocopherol in the breast muscle ($P<0.05$). In all experimental groups, TBA was reduced.
Selected herbal-vegetable mixtures in broiler diet improved physiochemical properties of the meat, enriched it with health-promoting substances and protected it against the unfavorable oxidation process.

KEY WORDS: chicken / herbs / meat quality / oxidative process / vegetables

Antioxidants are widely and successfully applied in food because they protect lipids and proteins. The effectiveness of using direct antioxidants in meat and its products has been widely proven [Lara *et al*., 2011, Kim *et al*. 2013, Hoffman *et al*. 2014, Jasińska and Kurek 2017, Longato *et al.* 2017, Zdanowska-Sąsiadek 2018]. According to existing knowledge about the factors that shape the oxidative stability of meat, it is known that many factors influence the oxidation process, including ante-mortem processes. Future meat quality can be affected by protecting muscles functioning in a live animal body. Through animal diets, antioxidants are incorporated into the integral cellular structure of the membranes. As a consequence, they can fulfill the antioxidant function in meat and protect it more effectively [Descalzo and Sancho 2008]. In animal nutrition, synthetic antioxidants such as BHT, BHA or gallusans are used and their effectiveness in the later protection of meat against oxidation has been shown [Smet *et al*. 2005]. Nevertheless, there have been reports on the toxic and carcinogenic activity of these compounds on the body [Sarafian *et al.* 2002, Faine *et al*. 2006]. In addition, current trends in food production tend to eliminate synthetic feed additives in favor of their natural substitutes that, while fulfilling their antioxidant functions, do not have toxicological properties and are at the same time more safe for the health of consumers [Pokorny 2007, Czech *et al*. 2017, Attia *et al*. 2018]. Due to societal and consumer acceptance, the potential for using natural antioxidants directly into food or indirectly through farm animal diets has become high. The most commonly used additives to inhibit oxidation processes are herbs and vegetables and fruits, as they are natural sources of phenolic acids, flavonoids, anthocyanins and essential oils [Horbańczuk and Wierzbicka 2017, Carmona-Hernández *et al.* 2017, Islam *et al*. 2018, Pogorzelska-Nowicka *et al*. 2018, Horbańczuk *et al.* 2019]. Of course, not all herbs, vegetables and fruits can equally successfully be used in the protection of lipids and proteins from oxidation. Kong *et al.* [2010] divided herbs into two main groups: stronger antioxidant group, to which belong for instance rosemary, nutmeg, clove, cassia bark, round cardamom and to the weaker group represented by aniseed, oregano, prickly ash, pepper, angelica, dahurian angelica root, fennel.

The antioxidant effect of plants is mainly related to the presence of antioxidant compounds. However, their level depends primarily on the plant species [Wojdyło *et al.* 2007] as well as on growing conditions. Borguini *et al.* [2013] showed a higher antioxidant potential of plants grown in an organic system compared to conventional

ones, as the main functions of polyphenols in the plant organism are related to its response to adverse environmental conditions. Creating optimal growing conditions in a conventional system eliminates the need to create natural protection barriers such as polyphenols.

Consequently, conventional crops content less of these compounds. Using natural sources of antioxidants, such as herbs, vegetables and fruits in animal nutrition, it is important to choose not only species rich in these compounds but also consider their availability and price, which in different regions of the world may vary. The high price of organic farming products eliminates them from widespread use in animal nutrition.

The aim of the current study was to demonstrate the influence of herbal vegetable blends used in the broiler chickens diet on the oxidative status and physicochemical properties of their breast muscles. The mixtures used in this study were based on cheap, commonly available in Europe vegetables and herbs such as onions, garlic, thyme, rosemary or oregano.

Material and methods

Birds and diets

Four hundred broiler chickens (ROSS 308) (only males) were randomly divided into four feeding groups (100 birds each) in five replications with 20 chickens each, and reared until 42 days of age. Chickens were reared on litter at stock density in a pen reaching 11 birds per m^2 and fed *ad libitum* in a two-stage system. The control group (C) was receiving the basal diet and experimental groups E1, E2 and E3 diet with 2% herbal-vegetables mixtures as an additive. Herbal-vegetables mixtures were composed of 70% onion, 25% thyme, 5% mint or 90% ginger, 7% rosemary, 3% chili or 30% onion, 20% garlic, 25% oregano, 10% fennel, 5% mint, 5% turmeric, 5% ginger in group E1, E2 and E3 respectively (Tab. 1). On day 42 of birds life, 10 chickens from group (2 from each 5 replication) were randomly selected and fasted for 8 h. Then, the birds were transported to a slaughterhouse where they were slaughtered. The carcasses were chilled at 4°C for 12 h and transported to the laboratory where dissection was performed and breast muscle samples were collected for further analyses. According to Directive 2010/63/EU for the experimental procedures approved of the Ethical Commission was not required.

Physicochemical properties

The pH value of meat samples was measured 24 h after slaughter (pH_{24}). Samples were assayed according to the Polish Standard (PN-ISO 2917:2001) with a CP-411 pH-meter (Elmetron, Zabrze, Poland) using a combined glass-calomel electrode. Cooking loss was determined according to Iwańska and Jacórzyński [1973].Water holding capacity (WHC) was determined according to Grau and Hamm [1952]. Shear force was measured with the tensile tester ZWICK 1120 using a Warner-Bratzler shear blade. The maximum shear force (F_{max}) was measured at a cross-head speed of 50 mm/min. Colour parameters were analysed with a Minolta CR-410 chroma-meter in accordance with producer instruction.

Table 1. Composition and nutritional value of feed mixtures

Components[1]	Starter (1-21 day)	Grower (22-42 day)
Wheat	32.3	39.0
Maize	30.0	25.0
Soybean meal (46.5)	29.05	18.35
Sunflower meal[3]	3.00/1.00	5.0/3.00
Rapeseed meal	-	4.60
Wheat bran	-	2.00
Sunflower oil	2.00	2.00
Fat concentrate	-	0.86
Limestone Ca39	0.97	1.20
Sodium bicarbonate	0.14	0.10
NaCl (Salt)	0.26	0.28
Dicalcium phosphate	1.22	0.59
MHA methionine 84%	0.30	0.20
Lysine	0.26	0.32
Premix[4]	0.50	0.50
Herbal mixture[5]	0/2.0	0/2.0
Analysis[2]		
ME	12.68	12.73
Crude protein	21.37	19.58
Crude fiber	3.18	3.95
Crude fat	4.10	4.98
Crude ash	5.73	5.27

[1]Components were shown as a percentage.
[2]Analysis metabolic energy (ME) was shown in MJ, crude protein, fiber, fat and ash were shown as a percentage.
[3]Level of sunflower meal depend on feeding group. First value was taken to control group, second to group with herbal additives (E1, E2 and E3).
[4]The vitamin-mineral premix supplied the following per kilogram of complete feed: Ca. 1084.005 mg; Mg. 11 mg; K. 0.37 mg; Na. 63.775 mg; Cl. 76.285 mg; Se. 0.35 mg; Fe. 45 mg; Mn. 70 mg; Zn. 55 mg; Cu. 7.5 mg; J. 0.6 mg; vitamin A. 11000 I.U.; vitamin D_3. 2500 I.U.; vitamin E. 50 mg; vitamin K. 2.5 g; vitamin B_1. 2 mg; vitamin B_2. 7 mg; vitamin B_6. 4 mg; vitamin B_{12}. 0.02 mg; niacin. 40 mg; D-pantothenic acid. 12.5 mg; folic acid. 1 mg; choline chloride. 300 mg; biotin. 0.15 mg; 1.4-β-D-xylanase. 200 FX; 6-phytase. 1500 FT; ethoxyquin. 0.174 mg; citric acid (E330). 0.099 mg; gallate. 0.027 mg.
[5]Level of herbal mixture was 0 and 2.0 for control and experimental group respectively. Composition of herbs in herbal mixture: E1 – 70% onion, 25% thyme, 5% mint; E2 – 90% ginger, 7% rosemary, 3% chili; E3 – 30% onion, 20% garlic, 25% oregano, 10% fennel, 5% mint, 5% turmeric, 5% ginger.

Total anthocyanins content

2 g of a fragmented breast muscle sample was homogenized in 6 ml of 80% methanol acidified to pH = 2. The samples were then centrifuged at 4,000 x g for 15 minutes at 4°C. 1 ml of the obtained supernatant was transferred twice to tubes to which 4 ml of pH 4.5 buffer were added (450 cm^3 of 1M sodium acetate, 220 cm^3 of 1M hydrochloric acid and 330 cm^3 of ddH_2O) or pH = 1 (120 cm^3 of 0.2M sodium chloride, 390 cm^3 0.2M hydrochloric acid). The samples were thoroughly mixed and then extinction measurements were made using a Cary WinUV spectrophotometer (Varian Inc., Australia) at 526 nm using blank buffer as appropriate. Results were shown as a mg/100g tissue expressed as pelargonidin-3-glucoside equivalent.

Total phenols content

Muscle samples were perfused with PBS of pH 7.4, 1 g of tissue was homogenized in 10 ml of ultra-pure methanol with 1% acetic acid added chilled to 4°C. Samples were extracted at 40°C. The tubes were cooled and allowed to decant in the dark. Analysis of the total phenols in the result of the modified method by Skerget *et. al*. [2005]. The Folin - Ciocalteu reagent was used as the oxidizing reagent [AOCS 1990]. 0.5 ml of the reconstiled extract was transferred to a 6 ml test tube followed by 2.5 ml of the Folin - Ciocalteu reagent diluted 10-fold with demineralized water (Sigma-Aldrich, Switzerland). Samples were thoroughly mixed and after 8 minutes, 2 mL of saturated sodium carbonate solution was added. The next stage of the analysis was conducted at 40°C for 30 minutes (until a stable characteristic color was obtained). The absorbance was measured at 765 and 735 nm against a blank sample (experimental material replaced with 0.5 ml ddH_2O). Results were read using a calibration curve of 0 to 0.5 mg/ml. The results are expressed in mg of GAE / g tissue (GAE - gallic acid equivalent).

Total antioxidative capacity by DPPH radical reduction

Antioxidative activity of the tested samples was determined according to the modified method of Brand-Wiliams *et al*. [1995], using the synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl).Brest muscle samples were perfused with PBS pH 7.4. 1 g of tissue was homogenized in 10 ml of ultra-pure ethanol chilled to 4°C. Test-tubes with homogenates were aerated with nitrogen and sealed. The material prepared in this manner was extracted for 2 h in an ultrasonic bath at the temperature of 40° C. The tubes were next cooled and centrifuged the samples at 4,000 x g for 15 minutes at 4°C. To 0.5 ml of the obtained supernatant 0.5 ml of an ethanolic solution of 1,1-diphenyl-2-picrylhydrazyl (0.5 mM) was added, which was previously diluted so that its absorbance at wavelength $\lambda = 517$ nm was about 0.9. The samples were thoroughly mixed and set aside to a dark, cool place for 30 minutes to achieve color stabilization. Extinction measurements were made using a Cary WinUV spectrophotometer (Varian Inc., Australia) at a wavelength of 517 nm. The ability of the tested antioxidants to counter oxidation reactions was calculated as follows:

$$\% \text{ inhibition} = 100 (A0 - Aavg.) / A0$$

where:

Aavg. – average value of the absorbance of the test solution containing antioxidants;

A0 – absorbance of the DPPH radical solution.

Nonenzymatic antioxidant capacity

The analysis of breast muscles was carried out according to instructions of the Total Antioxidant Capacity Assay Kit by Abnova® (Taoyuan City, 320 Taiwan), strictly with the manufacturer's recommendations. Samples of breast muscle were homogenised in 1.96 ml of demineralized water (ddH_2O), chilled to 4°C, with the addition of 40 µl dimethyl sulphoxide (PubChem CID:679). The method is based

on the measurement of reduction of indicator ions, which as a result of reaction with the probe produces a coloured complex. The measurement of absorbance proceeded at λ_{570}. The total antioxidant capacity was expressed in mM/1g tissue expressed per single - electron Trolox equivalent.

TBA

To determine lipid oxidation of breast muscle, the thiobarbituric acid (TBA) value was defined with the extraction method according to SHAHIDI (1990). Thiobarbituric acid in breast muscle was determined twice after 1 and 5 days of storage in 4°C. The method was based on measuring the absorbance of colour solution, whereas the colour was a result of reaction between fat oxidation products (mainly malondialdehyde – AM) and 2-thiobarbituric acid (TBA). The absorbance was measured using a Hitachi U-1100 spectrophotometer at 532 nm in comparison to reagent blank. The TBA level was expressed in mg AM/kg.

Vitamin A and E concentration

Determination of vitamin A and E content in breast muscles was carried out with High Performance Liquid Chromatography (HPLC) (Perkin Elmer series 200, Waltham, USA). For the extraction of vitamins, the modified method Katsanidis and Addis (1999) was used. 4 ml absolute ethanol (99,8%) and 1 ml ddH_2O were added to 2 g of muscle tissue. The homogenate obtained was centrifuged at 1,500 x g for 10 minutes at 4°C. Placed in the vortex tubes, the supernatant was extracted with hexane (4ml). Samples were centrifuged at 1,500 x g for 10 minutes at 4°C. The resulting clear upper layer was transferred to new tubes. The operation was repeated twice. The hexane was evaporated under nitrogen in a heating block at 37°C. The obtained fat was dissolved in 1 ml of methanol. The chapter was carried out using a Thermo RP-18 column (250mm x 4.6mm, grain size 5 μm). The elution was of an isocratic nature. The mobile phase consisted of 90% MeOH and 10% of a mixture of C2H3N (60%) and HPLC water (40%). Spectrophotometric detection took place at a wavelength of 294 nm. The volume of the sample injected into the column was 20 μl, analysis time 50 min. Based on the reference samples, the retention time and areas of peak areas corresponding to vitamin A and E in the test samples were determined. The content of vitamins is expressed in mg / 100 g of meat.

Results and discussion

Physicochemical properties

The effects of dietary herbal-vegetable mixtures supplementation on physicochemical properties of breast muscles were detailed in Table 2. Cooking loss and redness (a*) were not affected by dietary herbal-vegetable mixtures ($P>0.05$), but difference in pH_{24}, WHC, shear force and color L* and b* parameter were observed ($P<0.05$).

Table 2. Means and their standard errors (SE) for physicochemical properties in chicken breast muscles with feeding herbal additives diet

Item	Feeding groups								P-value
	C		E1		E2		E3		
	mean	SE	mean	SE	mean	SE	mean	SE	
pH_{24}	5.86^{B}	0.02	5.98^{A}	0.03	5.83^{B}	0.02	6.00^{A}	0.04	0.001
Cooking loss (%)	19.9	1.05	19.6	0.81	20.2	0.63	19.8	0.82	0.960
WHC (cm^2/g)	11.1^{AB}	0.79	13.0^{A}	0.94	9.08^{B}	0.71	8.82^{B}	0.84	0.006
Shear force (N)	51.6^{A}	2.05	40.0^{BC}	1.98	39.4^{C}	2.02	45.7^{B}	1.93	0.001
Colour									
L*	55.1^{A}	0.47	51.7^{B}	1.04	53.6^{AB}	0.29	52.8^{B}	0.55	0.013
a*	-0.121	0.32	-0.378	0.07	-0.440	0.18	-0.077	0.15	0.472
b*	11.5^{B}	0.21	12.4^{A}	0.25	13.1^{A}	0.36	12.6^{A}	0.13	0.002

^{ABC}Within row means bearing the same superscript are not significantly different at $P<0.05$.
WHC – water holding capacity; C – contained no herbal extract; E1 – herbal extract composition: 70% onion, 25% thyme, 5% mint; E2 – herbal extract composition: 90% ginger, 7% rosemary, 3% chili; E3 – herbal extract composition: 30% onion, 20% garlic, 25% oregano, 10% fennel, 5% mint, 5% turmeric, 5% ginger.

Breast muscles of chickens receiving a mixture dominating in onions (E1) and herbal-vegetable mix (E3) were characterized by a higher pH than of control group and of chickens receiving a mixture dominating in ginger (E2). The highest WHC was found for chickens from the E1 group, higher than in the E2 and E3 groups. The breast muscles of the group C was characterized by a higher ($P<0.05$) shear force compared to the muscle of the other chicken groups. The lowest ($P<0.05$) shear force was found for breasts of chickens receiving the mixture with the majority of ginger. However, no significant differences were found between the E2 and E1 and E3 and E1 groups. The brightest color (L *) was demonstrated in the breast muscles of the group C chickens, which were lighter ($P<0.05$) compared to the muscle of chickens from groups E1 and E3. The remaining groups did not differ from each other ($P>0.05$) in the L * value of breast muscles. The influence of all herbal and vegetable mixtures used on saturation with yellow color was found (b *). The chicken breasts from the control group were characterized by a lower ($P>0.05$) value of the b *.

Genetic selection work on meat poultry, focused on the bird's body weight and other production characteristics, while not taking into account the health potential and immunity of the newly created hybrids [Siegel *et al*. 2008]. Many authors point to significant differences between the quality of meat including physicochemical features from slow and fast growing chickens [Fanatico *et al*. 2007, Mikulski *et al*. 2011]. As the progress in achieving higher body mass appeared undesirable characteristics of chickens such as excessive fatness, deterioration of sensory and technological quality of meat as well as greater susceptibility to stress of the birds. The increase of susceptibility of birds to stress is of particular importance in shaping physicochemical characteristics of meat. In meat of birds exposed to stress, there was a higher frequency of meat quality defects, including PSE and DFD [Zaboli *et al*. 2018]. Limited possibilities of adaptation to the prevailing conditions, which are one

of the main factors causing stress, increase the rate of glycolysis to form lactic acid in muscle tissue. If such conditions occur prior and during the birds slaughter, then increased and disturbed post-slaughter glycolysis will affect the dynamics of meat pH changes [Zaboli *et al*. 2018]. The results of many studies [Qiao *et al*. 2001, Le Bihan-Duval 2004] show a significant correlation between meat pH and some features such as WHC, color, cooking loss or meat tenderness.

Therefore, stress which birds may experience should be reduced, while improving production environment. Genetic and environmental factors should be included among the most important elements that shape resistance to stress. However, it is hard to imagine modern large-scale poultry production based on slow-growing genotypes, whose resistance to stress is much greater than rapidly growing hybrids [Nain *et al.* 2008]. Therefore, the greatest chances should be seen in improving environmental factors. The most important environmental factors that can significantly influence the bird's response to stress is nutrition. The research confirms that the proper composition of feed additives by shaping the stress response may improve the quality of meat. It was observed that in the groups of chickens fed with the addition of onions and herbs (groups E1 and E3), the pH of the meat was higher and the color darker in comparison to the control group. Onions are a very rich source of quercetin, whose strong antioxidant properties have been extensively studied and described [Harwood *et al*. 2007, Damaziak *et al*. 2017]. Higher pH of meat after using herbal supplements was also confirmed by other authors [Jang *et al*. 2008, Lee *et al*. 2013]. Akbarian *et al.* [2013] showed that during supplementation of chicken diets in orange peel extracts and *Curcuma xanthorrhiza*, essential oil increases the activity of antioxidant enzymes such as glutathione peroxidase or superoxide dismutase. Such interaction results in the reduction of oxidative stress in animals. This is particularly important at the time of the intensification of the stress stimulus that occurs during slaughter. Oxidative equilibrium allows to maintain adequate glycogen reserves after slaughter and the proper course of meat maturation.

In terms of color, saturation with yellow color of both whole carcasses and individual elements is very important for the consumer to [Sirri *et al*. 2010]. A greater willingness to purchase poultry products with a more yellow color is the basic criterion when searching for natural and synthetic supplements to strengthen saturation in the yellow color. When ochre was used in poultry diet, the observers noticed significantly higher yellow (b *) saturation of the breast muscles at the level of 2% of the supplement compared to the control group [Liu *et al*. 2008]. At the same time, the greater the amount of additive was used, the b * parameter was higher. Previously we confirmed the purposefulness of using a 2% addition of herbal and herbal vegetable mixtures in shaping the color of chicken broiler chickens (Tab. 2). Castaneda *et al*. [2005] observed, however, that using natural pigment to saturate poultry meat with yellow (b *) is much more effective than using synthetic pigments. Researchers obtained similar yellow saturation using a low level of natural pigment (25 ppm) and a high level of synthetic pigment (45 ppm). Based on the results of the above work and own research

it can be concluded that the natural additives used in poultry feed positively affect the color (in terms of parameter b *), and their use also has economic justification because with small amounts used in feed they give a positive effect. It is very important that these additives are fully accepted by consumers.

No differences in water holding capacity (WHC) between the control group and experimental groups was noted. Similarly, the lack of differences in the discussed parameter using the Medicinal Herb Extract Mix in broiler nutrition was confirmed by Jang *et al.* [2008]. Significant differences were noted between experimental groups. The highest WHC of meat observed in the group of chickens receiving a mixture dominating in onions, which may suggest that different vegetables and herbs may have different effects on the WHC.

One of the most important features of meat quality is its tenderness. The use of the addition of herbal mixtures significantly improved the tenderness of breast muscles of chickens. Holm and Fletcher [1997] observed that due to stress, muscle fibers are shrinking and this can have a direct effect on the increase in meat hardness. The use of herbs that have strong antioxidant properties may reduce the effects of stress and improve the meat tenderness [Shah *et al*. 2014]. An *et al*. [2015] feeding birds with the onion extract did not find any differences in the physicochemical properties of chicken meat. Similarly, the lack of differences was demonstrated by Jang *et al*. [2011] using the quercetin extract. The discrepancy between the results of our research and of the other authors may be influenced by the use of a mixture with high onion content (group E1) or as one of many components (group E3) but always with the addition of herbs, which are also a very rich source of antioxidant compounds . The synergistic effect of onions and herbs could have a more beneficial effect than using the onion itself or just one active compound in it, i.e., quercetin. In the E2 group, lower shear force was also observed compared to the control group. This results is consistent with other studies [Herawati 2011] and confirms that the addition of ginger in broiler nutrition can have a positive effect on the physico-chemical properties of meat.

Antioxidant activity

The effects of dietary herbal-vegetable mixtures supplementation on chosen antioxidants content and their activity as well as oxidation process were presented in Table 3.

The effect ($P<0.05$) of herbal and vegetable additives on the content of retinol, α-tocopherol, anthocyanins and polyphenols was found. Levels of γ-tocopherol and tocopherol acetate were not affected by dietary herbal-vegetable mixtures ($P>0.05$). The herb-vegetable mixes used in the bird diet ($P>0.05$) increased antioxidant activity including total antioxidative capacity (DPPH) and nonenzymatic antioxidant capacity (NEAC). TBA in fresh (TBA_1) as well as stored (TBA_5) meat was found in comparison with the control group. The highest level of retinol and α-tocopherol was found in breast muscles of chickens receiving a mixture dominating in onions. In the breasts of chickens receiving the herb-vegetable mix (E3), the highest level of anthocyanins and

polyphenols was found. As a result, the highest antioxidant activity measured by DPPH and NEAC were observed in breast muscles from the E3 group. In groups E1 and E3, in which the highest level of antioxidants (retinol, α-tocopherol, anthocyanins and polyphenols) were observed, lower (P>0.05) lipid oxidation was observed in both fresh (TBA_1) and stored meat (TBA_5).

In the animal feed, besides the basic nutrients, antioxidants support the body and prevent the harmful effects of free radicals and their products. The main antioxidants present in the feed include vitamins A, C and E, the properties of which have been widely known and repeatedly described [Karadas *et al*. 2016, Zangeneh *et al*. 2018]. In addition to the positive effects of antioxidants on animal organisms, they are also of great importance in shaping the quality of animal products. The use of natural or synthetic vitamins to limit changes in the quality of stored meat has been proven. Poultry nutrition confirmed the effectiveness of vitamin E in shaping physicochemical properties, as well as limiting the oxidation processes in meat [Zdanowska-Sąsiadek *et al*. 2016]. Vitamin E is considered the main chain-breaking antioxidant. However, recent studies show equally effective antioxidant effects of polyphenols, the source of which primarily are fruit, vegetables, herbs as well as green and black tea or red wine [Szliszka and Król 2011].

Table 3. Means and their standard errors (SE) for antioxidant activity characterization and oxidation process determination in chickens meat with feeding herbal additives diet

Item	Feeding groups								P-value
	C		E1		E2		E3		
	mean	SE	mean	SE	mean	SE	mean	SE	
Retinol (mg/100 g tissue)	0.119^{B}	0.01	0.242^{A}	0.02	0.169^{B}	0.01	0.149^{B}	0.03	0.001
Alfa Tocopherol (mg/100 g tissue)	1.11^{B}	0.12	1.53^{A}	0.09	1.14^{B}	0.10	1.28^{AB}	0.10	0.040
Gamma Tocopherol (mg/100g tissue)	0.070	0.01	0.111	0.01	0.093	0.01	0.092	0.01	0.059
Tocopherol Acetat (mg/100g tissue)	0.019	0.02	0.023	0.01	0.028	0.01	0.013	0.01	0.623
Anthocyanins (mg/100g tissue)	0.214^{C}	0.03	0.341^{B}	0.05	0.262^{BC}	0.03	0.592^{A}	0.03	<0.001
Polyphenols (mg GAE/g tissue)	0.817^{C}	0.03	1.06^{B}	0.04	1.04^{B}	0.01	1.20^{A}	0.06	<0.001
DPPH (%)	74.2^{C}	0.16	85.0^{B}	0.40	84.7^{B}	0.62	89.2^{A}	0.11	<0.001
NEAC (µmol/g tissue)	8.02^{C}	0.11	8.68^{BC}	0.07	8.75^{B}	0.34	9.77^{A}	0.27	<0.001
TBA_1 (mg AM/kg tissue)	0.463^{A}	0.03	0.328^{BC}	0.02	0.362^{B}	0.01	0.271^{C}	0.01	<0.001
TBA_5 (mg AM/kg tissue)	0.868^{A}	0.04	0.400^{C}	0.02	0.752^{B}	0.04	0.326^{C}	0.02	<0.001

^{ABC}Within row means bearing the same superscript are not significantly different at P<0.05.
C – contained no herbal extract, E1 – herbal extract composition: 70% onion, 25% thyme, 5% mint; E2 – herbal extract composition: 90% ginger, 7% rosemary, 3% chili; E3 – herbal extract composition: 30% onion, 20% garlic, 25% oregano, 10% fennel, 5% mint, 5% turmeric, 5% ginger. TBA_1 and TBA_5 were determined in 1st and 5th day of storage time, respectively.

It is difficult to assess the ability of the hyperoxidizing polyphenols due to the presence of vitamins A, C and E present in animal nutrition, which present antioxidant properties. Besides the mentioned polyphenols, fruit, vegetable, herbal supplements or their mixes bring into chicken diets the aforementioned vitamins. In chicken plasma there is a

constant level of vitamins (vitamin C 61-66 μM – McKee *et al.* [1997], vitamin E 13-15 μM – Surai [2002]), and the total antioxidant capacity in the plasma is very variable and can range from 337 μM [Brenes *et al.* 2008] up to 740-830 μM [Willemsen *et al.* 2011]. It is still unknown how strong antioxidant properties polyphenols have and whether the effect of their action is not overlapped by the effect of vitamins. Researchers observed an increase in polyphenol content in all experimental groups (E1, E2 and E3) but also higher content of vitamins A and E (group E1) in meat. Increased levels of potentially antioxidant polyphenols and antioxidant vitamins A and E (group E1) increased the higher binding capacity of free radical DPPH and nonenzymatic antioxidant capacity. All experimental groups were also characterized by lower TBA, both after 1 and after 5 days of meat storage compared to the control group. Brenes *et al.* [2008] demonstrated the effectiveness of grape pomace on the course of oxidative processes in stored meat. The authors emphasized that this may be related to the occurrence of polyphenols in the additive used. These studies contradict the results of Duthie and Morrice [2012] who showed a significant decrease of the polyphenol activity, also as antioxidants due to the very high restriction of their absorption from the gastrointestinal tract. Procházková *et al.* [2011] emphasized that polyphenols are characterized by strong antioxidant properties, however, acting mainly in the plants by which they have been synthetised. Polyphenols, as compounds produced by plants in response to stress, act as an antioxidants [Huminiecki and Horbańczuk 2017, Huminecki *et al*. 2017, Mozos *et al*. 2018, Wang *et al.* 2018, Yeung *et al*. 2018], but according to Duthie and Morrice [2012], this action is not equal in animal *in vivo* studies. Although strong antioxidant properties of polyphenols *in vitro* have been demonstrated by Andriantsitohaina *et al*. [2012], it is very difficult to determine their true *in vivo* activity. The study of Hashemipour *et al*. [2013] who used thymol and carvacrol which were reported to inhibit lipid peroxidation, confirmed the legitimacy of using the supplements due to the lower activity of antioxidant enzymes. Decreased antioxidant enzymes activity may indicate that thymol and carvacrol used in the experiment took over the antioxidative functions. Results of the experiment carried out by the authors of the above-mentioned work, stated that the addition of herbs and vegetables rich in polyphenols to the nutrition of chickens had a positive effect on the quality of the meat. The greater ability to bind free radical DPPH as well as the higher level of nonenzymatic antioxidant capacity indicated better protection of meat in experimental groups against oxidative changes progressing over time. The reduced TBA level in these groups indicated the possibility of longer storage of meat obtained from chickens that received a herbal or herbal-vegetable supplement in the diet.

Conclusion

The use of onions and various herbs used in mixtures in group E1 and E3 in chicken diets could have beneficial effects in reducing stress and, as a result, improving the physico-chemical properties of the meat.

Herbal and vegetable supplements in a good combination can give a measurable effect, however, further research should be carried out to determine whether it is the

vitamins between the polyphenols contained in these additives that give a beneficial effect.

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