EFFECTS OF SUPPLEMENTING DRINKING WATER WITH MIXED HERB EXTRACT OR OUTDOOR ACCESS ON MEAT QUALITY CHARACTERISTICS IN BROILER CHICKENS*

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Abstract

The aim of the present study was to determine the effects of supplementing drinking water with an extract of mixed herbs or housing with outdoor access on carcass traits, levels of antioxidant enzymes (SOD, CAT, GPx), reduced glutathione (GSH), malondialdehyde (MDA), and selected quality parameters of meat from broiler chickens. One-day-old Ross 308 broiler chickens were allocated to three groups: group I (control), in which birds were kept in compartments on litter and had no outdoor access; group II, in which birds were kept in compartments on litter without outdoor access and were supplemented with an extract of mixed herbs (50% *Melissa officinalis* L. and 50% *Urtica dioica* L.) at 2 ml/l of drinking water; and group III, in which birds were raised in compartments on litter and had outdoor access from day 1 of rearing. Throughout the rearing period, the broilers had free access to feed and water. On day 42, 20 birds were selected from each group, slaughtered and subjected to simplified slaughter analysis. Their breast and leg muscles were measured for pH, colour, water holding capacity (WHC) and drip loss, and analysed for the content of antioxidant enzymes (SOD, CAT, GPx), reduced glutathione (GSH), malondialdehyde (MDA) and fatty acids. The outdoor access reduced dressing percentage, both with ($P \leq 0.01$) and without giblets ($P \leq 0.05$). The supplementation of drinking water with the mixed herb extract (2 ml/l) improved the muscle antioxidant status (higher SOD, CAT and GSH content) and reduced lipid peroxidation in the leg muscles of the broilers (lower MDA level). In general, the supplementation of the diet with the mixture of herbs in the applied form and concentration, as well as the outdoor access had no effect on the other examined quality parameters of broiler meat.

Key words: rearing system, herb extract, meat quality, muscle antioxidant status, broiler chickens

In recent years, avian welfare and food safety have become a priority for poultry producers. Under pressure from public opinion, there is a move away from artifi-

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cial feed additives and extreme conditions of intensive production towards animal welfare and environmental conservation. More and more consumers prefer products from animals kept in free-range or organic systems, which they believe provide more comfort and guarantee safe and wholesome foods.

After the European Union imposed a ban on in-feed antibiotics as growth stimulants in 2006, scientists started to actively search for alternatives. In addition to prebiotics, probiotics and organic acids, there is increasing interest in all kinds of plant-derived preparations (phytobiotics), which are considered “natural” and “safe” additives characterized by wide-ranging effects on animals, including antibacterial, antiviral, antifungal, stimulating digestive enzymes secretion, immunostimulating, and antioxidant.

In view of the above, we undertook a study to test the hypothesis whether dietary herbs or outdoor access has a positive effect on the quality of broiler chicken meat.

Poultry meat is characterised by high nutritional and dietetic value, which makes it attractive for an increasingly health conscious and demanding consumer. The benefits of poultry meat, from the viewpoint of human health, include low fat content and relatively high level of polyunsaturated fatty acids (PUFA) (Kamboh and Zhu, 2013; Kirkpinar et al., 2014), the composition (*n-6* to *n-3* ratio) of which, especially in the breast muscle, is closest to the optimum for humans (Sierżant et al., 2018). The consumption of recommended amounts of PUFA, in particular omega-3 fatty acids, is essential for normal body function and at the same time contributes to preventing and moderating the course of the diseases of modern civilization, such as coronary artery disease, heart failure, autoimmune diseases, and certain forms of cancer (Ipçak and Alçiçek, 2018; Saleh et al., 2018). However, meat rich in polyunsaturated fatty acids is particularly susceptible to lipid oxidation (Kasapidou et al., 2014; Giannenas et al., 2018; Sierżant et al., 2018), which presents a serious problem for the meat industry due to the negative effect on its aroma, taste, texture and nutritive value (Ahn et al., 2007). In turn, Zhang et al. (2017) report that muscle antioxidant capacity is associated with qualitative parameters such as pH, drip loss, and colour. Furthermore, some compounds formed during lipid oxidation are detrimental to health due to their mutagenic, carcinogenic and cytotoxic properties (Jiménez-Colmenero et al., 2001; Sierżant et al., 2018).

A way of limiting lipid oxidation is the use of synthetic or natural antioxidants (Skomorucha et al., 2017; Zhang et al., 2017; Bakhshalinejad et al., 2018; Sierżant et al., 2018), either indirectly when added to animal diets or directly when added to meat post-mortem. Because many consumers are reluctant to consume synthetic food additives, many studies have been conducted to find new antioxidants of natural origin that effectively prevent lipid peroxidation. Current interest is focused on herbal plants containing biologically active substances such as phenols, polyphenols, carotenoids, flavonoids and essential oils that possess antioxidant properties (Kamboh and Zhu, 2013; Kasapidou et al., 2014; Giannenas et al., 2018; Ipçak and Alçiçek, 2018; Saleh et al., 2018).

The literature indicates that lemon balm (Kasapidou et al., 2014) and nettle (Bonetti et al., 2016) are included in the herbal plants with antioxidant properties. Some researchers suggest that improved effects are obtained when active ingredi-

ents are added to the ration in the proper combination as an effect of their synergism rather than when they are individually incorporated (Giannenas et al., 2018; Ipçak and Alçiçek, 2018).

In animal organisms, cellular balance is maintained by the antioxidant system, which includes antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase and other substances, including glutathione and vitamins A, C, D and E, which help to remove excessive reactive oxygen species from cells (Muchacka et al., 2016; Skomorucha et al., 2017; Bakhshalinejad et al., 2018). According to some authors, dietary inclusion of natural antioxidants increases the level of antioxidant enzymes (Cong et al., 2017; Zhang et al., 2017; Bakhshalinejad et al., 2018; Yu et al., 2018) and PUFA (Kamboh and Zhu, 2013; Saleh et al., 2018) in animal tissues.

Nowadays consumers feel that alternative rearing systems provide more comfort to animals and thus result in healthier products compared to those derived from conventional rearing systems (Połtowicz and Doktor, 2011; Funaro et al., 2014; Sales, 2014). Numerous studies on the alternative rearing systems have shown that they may improve the yield of breast and leg muscles, enhance the sensory quality of meat, reduce the fat content of bird carcasses (Castellini et al., 2002; Fanatico et al., 2005; Dou et al., 2009), affect meat colour (Chen et al., 2013; Sales, 2014) and pH (Castellini et al., 2002; Połtowicz and Doktor, 2011), which further affects physico-chemical characteristics of the meat (Funaro et al., 2014).

The aim of the present study was therefore to determine the effects of supplementing drinking water with an extract of antioxidant herbs or housing with outdoor access on carcass traits, levels of antioxidant enzymes (SOD, CAT, GPx), reduced glutathione (GSH), malondialdehyde (MDA), and selected meat quality parameters of broiler chickens.

Material and methods

Experimental site

The experiment was carried out at the poultry farm of the National Research Institute of Animal Production in Aleksandrowice, Poland.

Birds and experimental design

Four hundred and eighty sexed one-day-old Ross 308 broiler chickens (240 ♂ and 240 ♀) originating from the Poultry Hatchery in Łęzkowice were used in the present study. At one day of age, the chicks were weighed, identified with tags and assigned to 3 treatment groups, each of which consisted of 4 subgroups. In group I (control), birds were kept in compartments on litter and had no outdoor access; in group II, broilers were kept in compartments on litter without outdoor access and from 1 to 42 days of rearing supplemented with an extract of mixed herbs (50% *Melissa officinalis* L. and 50% *Urtica dioica* L.) at 2 ml/l of drinking water; in group III, birds were raised in compartments on litter and had outdoor access from day 1 of

rearing. The size of the compartments in each subgroup was 3 m². Birds from group III had outdoor access between 07:00 and 20:00 h. The outdoor paddock provided an area of 1 m² per bird. The outdoor area was equipped with drinkers and shelters to protect birds from excessive sunshine. Birds were kept for 42 days and the stocking density did not exceed 33 kg/m² (Regulation of the Minister of Agriculture and Rural Development, Journal of Laws 2010 no. 56 item 344). The temperature in the broiler house was 32°C on the first day and it was gradually decreased to 20°C during week 5 and 6 of rearing. The lighting schedule consisted of 23 h of light/d until day 7, 20 h of light/d from day 8 to 38, and 23 h of light/d from days 39 to 42. The chickens were fed on an *ad libitum* basis with a starter diet until 3 weeks, a grower diet from 4 to 5 weeks, and a finisher diet at 6 weeks; all the diets were based on concentrates (Table 1), fed as pellets. Drinking water with the herb extract was given in bell drinkers. The water was manually replenished twice a day. Throughout rearing, birds had free access to the water drinkers.

Table 1. Ingredient composition (%) and nutritive value of starter, grower and finisher diets

Item	Diet	
	Starter ¹ 1–21 days	Grower ² -Finisher ³ 22–42 days
Maize	55.35	47.21
Soybean meal	37.5	33.75
Wheat	–	10.00
Rapeseed oil	2.90	4.80
Ground limestone	1.10	1.15
Dicalcium phosphate	2.10	2.00
Sodium chloride	0.30	0.30
Vitamin-mineral premix ^{1,2,3}	0.50	0.50
L-Lysine	0.03	0.09
DL-Methionine	0.22	0.20
Nutritive value per kg:		
crude protein (%)	22.00	20.5
metabolisable energy (MJ)	12.5	13.0
lysine (g)	12.0	11.5
methionine (g)	5.50	5.20
calcium (g)	9.50	9.30
phosphorus (g)	4.30	4.10

¹Supplements per kg starter diet: vit. A 13 500 IU; vit. D₃ 3 600 IU; vit. E 45 mg; vit. B₁ 3.25 mg; vit. B₂ 7.5 mg; vit. B₆ 5 mg; vit. B₁₂ 32.5 mcg; vit. K₃ 3 mg; biotin 150 mcg; nicotinic acid 45 mg; calcium pantothenate 15 mg; folic acid 1.5 mg; choline chloride 447.6 mg; Mn 100 mg; Cu 1.75 mg; Fe 76.5 mg; Se 0.275 mg; I 1 mg; Zn 75 mg; Co 0.4 mg; Seldox HM (antioxidant) 25 mg; Clinacox 0.5% 1 mg.

²Supplements per kg grower diet: vit. A 12 000 IU; vit. D₃ 3 250 IU; vit. E 40 mg; vit. B₁ 2 mg; vit. B₂ 7.3 mg; vit. B₆ 4.25 mg; vit. B₁₂ 30 mcg; vit. K₃ 2.25 mg; biotin 100 mcg; nicotinic acid 40 mg; calcium pantothenate 12 mg; folic acid 1.0 mg; choline chloride 335.7 mg; Mn 100 mg; Cu 1.5 mg; Fe 65 mg; Se 0.25 mg; I 0.9 mg; Zn 65 mg; Co 0.4 mg; Seldox HM (antioxidant) 25 mg; Coxidin 200 100 mg.

³Supplements per kg finisher diet: vit. A 10 500 IU; vit. D₃ 2 500 IU; vit. E 40 mg; vit. B₁ 1.5 mg; vit. B₂ 5.5 mg; vit. B₆ 3.25 mg; vit. B₁₂ 25 mcg; vit. K₃ 2 mg; biotin 100 mcg; nicotinic acid 35 mg; calcium pantothenate 12 mg; folic acid 1.0 mg; choline chloride 298.4 mg; Mn 80 mg; Cu 8 mg; Fe 60 mg; Se 0.2 mg; I 0.8 mg; Zn 50 mg; Co 0.4 mg; Seldox HM (antioxidant) 25 mg.

The experiment was conducted with approval (no. 1205/2015) of the Local Ethics Committee for Animal Experimentation.

Data, sample collection and laboratory analyses

On day 42, 10 pullets and 10 cockerels whose body weights were similar to the group average, were selected from each group and slaughtered. Fifteen minutes post-mortem and 24 h after chilling the carcasses to 4°C, the pH of the breast and leg muscles (pH₁₅ and pH₂₄) was measured using a CyberScan 10 pH meter Cole-Parmer (USA), and EC-FG 73905 electrode. The chilled carcasses were then subjected to simplified slaughter analysis and the colour of the dissected breast and leg muscles was determined with a Minolta CR 310 (Konica Minolta, Japan) reflectance chroma meter set on the CIE L*a*b* system, where L* represents lightness, a* redness, and b* yellowness. Water holding capacity (WHC) was determined by the Grau and Hamm method (1953) and drip loss of the meat was measured after 24 h chilling at 4°C. To this end, samples weighing ~80 g (e = 0.001 g) were collected from the left breast (*M. pectoralis maior*) and left thigh muscles, placed in airtight containers and cold stored for 24 h. The samples were then weighed again, and drip loss was determined as a percentage loss of initial weight (Sosnowka-Czajka et al., 2017). Meat samples were collected from the dissected breast and leg muscles and were placed in plastic bags and frozen at -18°C. After 6 months the samples were removed and thawed to determine antioxidant enzymes: SOD (superoxide dismutase activity), GPx (glutathione peroxidase activity) and CAT (catalase activity). SOD activity was determined spectrophotometrically according to the method of Rice-Evans et al. (1991). CAT activity was assayed by measuring H₂O₂ breakdown using Aebi's (1984) method. GPx activity was estimated by the method of Lück (1962). SOD, CAT and GPx activity in the muscles was expressed as U/mg of protein. The breast and leg muscles were also analysed for the level of reduced glutathione (GSH) by the method of Ellman (1959) and expressed in µmol/g of protein. Lipid peroxidation was assayed by measuring the concentration of malondialdehyde (MDA) using thiobarbituric acid (TBA) (Ohkawa et al., 1979). The concentration of MDA was expressed as nmol/mg of protein. Additionally, the muscle samples were analysed for fatty acid profile by gas chromatography (VARIAN 3400 CX) using helium as carrier gas and column AGILENT J & W GC COLUMNS CP-WAX 58 FFAP (25 m). Injector temperature was 200°C and detector temperature 240°C. The samples were prepared by the method of Folch et al. (1957) using BF3/methanol methylation.

An ethanol herb extract was produced by a professional herbal company and was certified to conform with in-house quality standard (ZN-16/NX/900).

Statistical analyses

The results were statistically analysed with one-way analysis of variance and significant differences were detected with Duncan's test. The data were expressed as means ± SEM. Effects were considered significant at a probability of P ≤ 0.05 and P ≤ 0.01. The statistical calculations were performed using Statgraphics plus 6.0.

Results

Broiler chickens from group I were characterized by a greater dressing by 1.35%, both with giblets ($P \leq 0.01$) and without giblets ($P \leq 0.05$), as well as by lower by 0.07% heart ($P \leq 0.01$) compared to chickens from group III (Table 2).

Table 2. Carcass traits (%) of broilers as affected by water supplementation with a herb extract or outdoor access

Item (%)	Group			SEM
	I	II	III	
Dressing percentage with giblets	79.93 A	79.25 AB	78.58 B	0.31
Dressing percentage without giblets	77.05 a	76.37 ab	75.70 b	0.32
Breast muscles	29.73	29.91	30.50	0.38
Leg muscles	21.23	21.19	20.93	0.35
Leg bones	4.87	4.76	4.73	0.09
Abdominal fat	1.67	1.56	1.41	0.11
Giblets	3.48	3.45	3.62	0.08
Liver	1.94	1.95	2.04	0.04
Gizzard	1.09	1.20	1.03	0.06
Heart	0.44 A	0.48 AB	0.51 B	0.01

Group I – litter without outdoor access.

Group II – litter without outdoor access and supplemented with an extract of mixed herbs to drinking water.

Group III – litter with outdoor access.

a, b – values in rows with different letters differ significantly ($P \leq 0.05$).

A, B – values in rows with different letters differ highly significantly ($P \leq 0.01$).

Table 3. Quality parameters of breast and leg muscles from broiler chickens as affected by water supplementation with a herb extract or outdoor access

Item		Group			SEM
		I	II	III	
Breast muscles					
pH	15 min	6.69	6.72	6.68	0.04
	24 h	5.71	5.75	5.72	0.02
Drip loss (%)		0.50 a	0.88 b	0.83 ab	0.12
WHC (%)		18.42	20.21	19.45	0.86
Colour	L*	57.76	58.07	57.60	0.57
	a*	8.63	8.20	8.97	0.25
	b*	10.48	10.92	10.66	0.26
Leg muscles					
pH	15 min	6.58	6.63	6.62	0.04
	24 h	6.09	6.14	6.10	0.03
Drip loss (%)		0.21	0.35	0.21	0.04
WHC (%)		13.61	15.38	14.62	0.72
Colour	L*	47.18	47.22	46.53	0.34
	a*	15.29	15.15	15.67	0.33
	b*	8.24	8.53	8.17	0.15

Group I – litter without outdoor access.

Group II – litter without outdoor access and supplemented with an extract of mixed herbs to drinking water.

Group III – litter with outdoor access.

a,b – values in rows with different letters differ significantly ($P \leq 0.05$).

Table 4. Activity of antioxidant enzymes and levels of GSH and MDA in the muscles of broiler chickens as affected by water supplementation with a herb extract or outdoor access

Item	Group			SEM
	I	II	III	
Breast muscles				
SOD (U/mg protein)	3.541 a	4.728 b	4.657 ab	0.476
CAT (U/mg protein)	11.194 a	19.052 b	15.388 ab	2.617
GPx (U/mg protein)	0.027	0.029	0.024	0.003
GSH (μmol/mg protein)	1.215 a	2.207 b	1.218 a	0.326
MDA (nmol/mg protein)	3.501 ab	3.082 a	3.835 b	0.228
Leg muscles				
SOD (U/mg protein)	2.656 a	4.672 b	3.834 ab	0.359
CAT (U/mg protein)	14.745	15.432	14.568	3.122
GPx (U/mg protein)	0.048	0.053	0.0490	0.019
GSH (μmol/mg protein)	1.115	1.363	1.024	0.305
MDA (nmol/mg protein)	5.370 b	3.250 a	5.750 b	0.678

Group I – litter without outdoor access.

Group II – litter without outdoor access and supplemented with an extract of mixed herbs to drinking water.

Group III – litter with outdoor access.

a, b – values in rows with different letters differ significantly ($P \leq 0.05$).

Table 5. Fatty acid profile of breast muscles from broiler chickens as affected by water supplementation with a herb extract or outdoor access

Item	Group			
	I	II	III	SEM
1	2	3	4	5
C8	0.00	0.00	0.00	0.00
C10	0.009	0.007	0.004	0.001
C12	0.019	0.010	0.05	0.008
C14	0.27	0.24	0.25	0.01
C16	14.38	14.24	14.71	0.27
C16-1	1.34	2.02	1.60	0.17
C18	5.88	5.34	5.77	0.21
C18-1	34.99	36.42	35.26	0.49
C18-2	28.86	28.37	27.75	0.52
Gamma18-3	0.15	0.12	0.13	0.02
C20	0.07	0.08	0.07	0.01
C18-3	5.14	5.14	4.67	0.19
C22	0.28	0.27	0.28	0.02
C20-4	7.64	6.91	8.46	0.69
C22-1	0.034 a	0.031 b	0.029 b	9.23
EPA	0.29	0.30	0.32	0.04
DHA	0.49	0.50	0.68	0.09

Table 5 – contd.

1	2	3	4	5
SFA	20.91	20.18	21.08	0.29
UFA	79.09	79.82	78.92	0.29
MUFA	36.36	38.47	36.89	0.54
PUFA	42.73	41.35	42.03	0.43
PUFA-6	36.64	35.40	36.35	0.50
PUFA-3	6.07	5.94	5.67	0.14
DFA	84.96	85.16	84.69	0.27
UFA/SFA	3.79	3.96	3.75	0.07
MUFA/SFA	1.74	1.91	1.75	0.05
PUFA/SFA	2.04	2.05	2.00	0.03
PUFA6/3	6.05	5.97	6.43	0.22

Group I – litter without outdoor access.

Group II – litter without outdoor access and supplemented with an extract of mixed herbs to drinking water.

Group III – litter with outdoor access.

a, b – values in rows with different letters differ significantly ($P \leq 0.05$).

SFA – saturated fatty acids.

UFA – unsaturated fatty acids.

PUFA – polyunsaturated fatty acids.

MUFA – monounsaturated fatty acids.

DFA – neutral and hypocholesterolemic acids (C18:0 + UFA).

n-6 – fatty acids (C18-2, C20-4, gamma 18-3).

n-3 – fatty acids (C18-3, EPA, DHA).

Table 6. Fatty acid profile of leg muscles from broiler chickens as affected by water supplementation with a herb extract or outdoor access

Item	Group			
	I	II	III	SEM
C8	3.33	6.67	6.67	4.71
C10	0.01	0.01	0.01	0.002
C12	0.02	0.02	0.02	0.002
C14	0.29	0.27	0.31	0.02
C16	14.00	14.24	14.24	0.25
C16-1	2.01	2.85	2.42	0.24
C18	4.74	4.31	4.64	0.23
C18-1	37.44	38.06	37.98	0.38
C18-2	30.87	29.83	29.79	0.36
Gamma18-3	1.19	0.17	0.19	0.03
C20	0.07	0.06	0.06	0.01
C18-3	5.83	5.65	5.52	0.11
C22	0.18	0.18	0.16	0.01
C20-4	3.92	3.92	4.12	0.27
C22-1	0.03	0.02	0.02	0.002
EPA	0.16	0.16	0.14	0.02
DHA	0.24	0.23	0.28	0.03

Table 6 – contd.

1	2	3	4	5
SFA	19.31	19.08	19.53	0.25
UFA	80.69	80.92	80.47	0.25
MUFA	39.48	40.94	40.42	0.50
PUFA	41.22	39.98	40.05	0.54
PUFA-6	34.98	33.93	34.10	0.49
PUFA-3	6.23	6.04	5.94	0.13
DFA	85.43	85.23	85.10	0.26
UFA/SFA	4.18	4.24	4.12	0.07
MUFA/SFA	2.05	2.14	2.07	0.04
PUFA/SFA	2.14	2.10	2.05	0.05
PUFA6/3	5.62	5.62	5.75	0.12

Group I – litter without outdoor access.

Group II – litter without outdoor access and supplemented with an extract of mixed herbs to drinking water.

Group III – litter with outdoor access.

SFA – saturated fatty acids.

UFA – unsaturated fatty acids.

PUFA – polyunsaturated fatty acids.

MUFA – monounsaturated fatty acids.

DFA – neutral and hypocholesterolemic acids (C18:0 + UFA).

n-6 – fatty acids (C18-2, C20-4, gamma 18-3).

n-3 – fatty acids (C18-3, EPA, DHA).

The breast muscles of the birds from the group that received the herb extract had a 0.38% higher ($P \leq 0.05$) drip loss compared to that of group I (Table 3). No other significant differences were observed for the other quality parameters in the breast and leg muscles among broiler groups. The breast muscles of birds from group II also contained increased SOD (by 1.19 U/mg protein), CAT (by 7.86 U/mg protein) and GSH (by 0.99 $\mu\text{mol/mg}$ protein) levels in relation to birds from group I ($P \leq 0.05$) and had an 0.99 $\mu\text{mol/mg}$ protein increased GSH level and an 0.75 nmol/mg protein decreased MDA levels compared to group III ($P \leq 0.05$) (Table 4). For leg muscles, group II had a 2.02 U/mg protein increased level of SOD compared to group I, as well as a decreased MDA level compared to the other two groups, by 2.12 and 2.50 nmol/mg protein ($P \leq 0.05$) (Table 4) respectively. A lower percentage of erucic acid (C22-1) was noted in the breast muscles of broilers from the experimental groups (by 0.003 and 0.005) compared to those from the control group ($P \leq 0.05$) (Table 5). There were no differences in the fatty acids content of leg muscles among the different groups of broilers (Table 6).

Discussion

Many authors have reported that the rearing system has an effect on carcass traits of birds (Wang et al., 2008; Sun et al., 2013; Sales, 2014; Cömert et al., 2016). This finding is in agreement with our research, which showed decreased dressing

percentage, both with and without giblets, for broiler chickens raised with outdoor access compared to the group housed without outdoor access. This could have been influenced by the fact that birds in outdoor runs show increased motor activity and are also exposed to various environmental stimuli such as lower temperature compared to a controlled facility, which may result in reduced production results, including dressing percentage. Similar results were obtained by Fanatico et al. (2008) and Skomorucha et al. (2008). On the other hand, no effect of housing system on dressing percentage in chickens was observed by Połtowicz and Doktor (2011) and Puchala et al. (2015).

Koreleski and Świątkiewicz (2007) did not observe any effect of supplementing poultry diet with sage and thyme extract ($560 \text{ mg} \cdot \text{kg}^{-1}$) on dressing percentage, weights of breast muscles and abdominal fat, and percentage of breast. Similarly, Kirkpınar et al. (2014) demonstrated no positive effect of the dietary inclusion of oregano and garlic essential oils ($300 \text{ mg} \cdot \text{kg}^{-1}$) on carcass characteristics of broiler chickens. As also indicated in the present study, mixed herb extract added to drinking water failed to improve the examined carcass characteristics. Maślanko and Pisarski (2009) noted a lower proportion of abdominal fat in the group of chickens fed a diet supplemented with 2% dried nettle, however, no significant effects were observed in birds supplemented with 2% dried lemon balm.

One of the most important meat quality indicators is the pH value, the level of which depends on glycogen repositories in muscles (Tong et al., 2014). These authors report that foraging activity may influence muscle metabolism in birds. Changes in the pH of chickens with outdoor access were observed by Chen et al. (2013) and Funaro et al. (2014). Birds raised with outdoor access have more yellow meat, which is connected with the ingestion of carotenoid-rich plants (Chen et al., 2013; Sales, 2014). However, our study failed to confirm any effect of outdoor access on changes in the pH and colour of the broiler muscles. Likewise, Tong et al. (2014) found no differences in meat colour between chickens raised with and without outdoor access. Połtowicz and Doktor (2011) and Sarica et al. (2011) showed no effect of the housing system on water holding capacity of bird muscles, which is consistent with our study.

The herbs and plant components used as natural antioxidants generally affect the colour of poultry meat (Karre et al., 2013). Eleroğlu et al. (2013) observed lighter colour of breast muscles in broiler chickens fed a diet supplemented with lemon balm at 10 g/kg feed. Kirkpınar et al. (2014) found that supplementing the diet with the strong antioxidant oregano caused a reduction in the redness of breast muscles when compared to the control group. Aksu et al. (2014) noted an effect of dietary inclusion of thyme (200, 400 and $600 \text{ mg} \cdot \text{kg}^{-1}$) on the a^* value and no effect on the L^* and b^* values for breast muscles of quail. Ipçak and Alçiçek (2018) did not show any effect of the dietary inclusion of bioactive substances ($150 \text{ mg} \cdot \text{kg}^{-1}$) on the L^* , a^* and b^* values of chicken breast and leg muscles in relation to the control group. Similarly in our study, no effect of the mixed herb extract (2 ml/l) on broiler muscle colour was observed.

In our study, higher percentage chilling loss from the breast muscles was observed for broilers that received water with the mixed herb extract, which may be indicative of poorer water holding capacity of the meat.

Our study found that outdoor access has no effect on the antioxidant status of broiler muscles, which is consistent with the findings of Muchacka et al. (2017). On the other hand, Yesilbag et al. (2011) report that appropriate dietary supplementation may increase the activity of antioxidant enzymes in animal tissues. In our study, dietary inclusion of the mixed herb extract increased SOD, CAT and GSH activity in the breast muscles and SOD activity in the leg muscles of broiler chickens. This may indicate that the antioxidants found in the herbs making up the experimental diet, had a positive effect on the antioxidant status of broiler muscles. Hashemipour et al. (2013) also observed higher SOD activity in the leg muscles of 42-day-old broiler chickens, which were fed a diet supplemented with thymol and carvacrol at 60, 100 and 200 mg·kg⁻¹ feed. However, the authors did not observe any effect of the dietary antioxidants on the activity of antioxidant enzymes in chicken breast muscles. In turn, Cao et al. (2012) noted an increase in T-SOD activity in the breast muscles of broiler chickens fed a diet supplemented with antioxidants (0.2, 0.35, and 0.5% *Aspergillus niger*-fermented *Ginkgo biloba* leaves in the starter and 0.4, 0.7, and 1.0% *Aspergillus niger*-fermented *Ginkgo biloba* leaves in the grower phase), but the same supplements had no effect on the activity of glutathione peroxidase. Zhang et al. (2017) showed that supplementation of the grower diet of broiler chickens with 400 mg/kg resveratrol increased T-SOD and GSH-Px activity and reduced lipid peroxidation in muscles. Ismail et al. (2013) reported that the level of MDA is directly proportional to the degree of lipid peroxidation. Also in our study, addition of the herb extract into the drinking water reduced MDA level in the leg muscles of broiler chickens, a finding that is possibly related with the increased SOD activity in the leg muscles of these broilers. Reduced MDA levels in chicken muscles, following the application of a diet enriched with antioxidants were also obtained by Kasapidou et al. (2014), Rostami et al. (2017), Giannenas et al. (2018) and Sierżant et al. (2018).

Several studies have shown a different fatty acid profile of meat in birds with outdoor access (Chen et al., 2013; Puchała et al., 2015; Cömert et al., 2016). At the same time, Jung et al. (2010) reported that enrichment of the diet with antioxidants is an excellent method to reduce the level of saturated fatty acids (SFA) and to increase the level of (PUFA) in broiler chicken muscles. Similarly, Ipçak and Alçiçek (2018) and Saleh et al. (2018) achieved a positive change in the fatty acid profile of meat after feeding broiler chickens a diet rich in antioxidants. However, in our study outdoor access and supplementation of the water with an extract of antioxidant herbs had no effect on the percentage of fatty acids in leg muscles. In the case of breast muscles, only a lower level of erucic acid was noted in the experimental groups, which is beneficial to human health.

In summary, the outdoor access reduced dressing percentage in broiler chickens. The supplementation of drinking water with the mixed herb extract (2 ml/l) improved the muscle antioxidant status and reduced lipid peroxidation in the leg muscles of broilers. In general, dietary inclusion of the mixture of herbs in the applied form and concentration, and the outdoor access had no effect on the other meat quality parameters of broiler chickens.

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