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EFFECT OF ADDING HERBAL EXTRACTS TO DRINKING WATER ON ACTIVITY OF ANTIOXIDANT ENZYMES, GSH AND MDA LEVELS, AND FATTY ACID PROFILE IN BROILER CHICKEN MUSCLES*

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Celem badań było określenie wpływu dodatku do wody pitnej ekstraktu z ziela melisy lekarskiej, szałwii lekarskiej oraz pokrzywy zwyczajnej na poziom enzymów antyoksydacyjnych (SOD, CAT, GPx), glutationu zredukowanego (GSH), dialdehydu malonowego (MDA) oraz profil kwasów tłuszczowych w mrożonych mięśniach piersiowych i nóg kurcząt brojlerów. Kurczęta brojlery Ross 308 przydzielono do czterech grup doświadczalnych: grupę I stanowiła kontrola, w grupie II, III i IV od 22. do 42. dnia odchowu ptaków dodawano do poideł z wodą odpowiednio: ekstrakt z ziela melisy lekarskiej, ekstrakt z szałwii lekarskiej oraz ekstrakt z pokrzywy zwyczajnej w ilości 2 ml/l wody. W 42. dniu 8 ptaków z każdej grupy ubito, a wypreparowane mięśnie zamrożono. Po 4 miesiącach w mięśniach oznaczono: SOD (aktywność dysmutazy ponadtlenkowej), GPx (aktywność peroksydazy glutationowej) oraz CAT (aktywność katalazy), poziom glutationu zredukowanego (GSH), dialdehydu malonowego (MDA) oraz profil kwasów tłuszczowych. Wyniki wskazują, że dodatek do wody pitnej ekstraktu z melisy lekarskiej oraz szałwi lekarskiej w ilości 2 ml/l ograniczył peroksydację lipidów w mięśniu piersiowym, jednakże nie stwierdzono pozytywnego wpływu ekstraktów z tych ziół na status antyoksydacyjny mięśni nóg kurcząt brojlerów. Suplementacja diety pokrzywą zwyczajną w zastosowanej formie i stężeniu nie przyniosła efektu antyoksydacyjnego w mięśniach kurcząt brojlerów, miała natomiast pozytywny wpływ na zmianę profilu kwasów tłuszczowych głównie w mięśniach nóg kurcząt brojlerów.

Słowa kluczowe: ekstrakty z ziół, antyoksydanty, peroksydacja lipidów, kwasy tłuszczowe, mięśnie kurcząt brojlerów

Poultry meat is characterized by high nutritional and dietetic value which often makes it a first choice food for increasingly more aware and demanding consumers. Health benefits of poultry meat undeniably include low fat content and relatively

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high level of polyunsaturated fatty acids (PUFA) (Kamboh & Zhu, 2013; Kirkpinar et al., 2014), which cannot by synthesized by the body and have to be delivered in the diet. Consumption of recommended amounts of PUFA, especially omega-3 acids, is essential for proper functioning of the body and is helpful in preventing and alleviation of a number of civilization diseases, like atherosclerosis, heart attack, autoimmune diseases or some cancers (Łoźna et al., 2012; Zdanowska-Sąsiadek et al., 2013). However, many authors reported that meat rich in PUFA was particularly susceptible to lipid oxidation (Zdanowska-Sąsiadek et al., 2013; Kasapidou et al., 2014) which is a serious problem for meat industry due to its negative effect on flavor, taste, texture and nutritional value (Ahn et al., 2007). Further, some compounds formed during lipid oxidation have negative health effects showing mutagenic, carcinogenic and cytotoxic actions (Jimènez-Colmenero et al., 2001).

Lipid oxidation can be reduced by different methods, for instance by the use of antioxidants either indirectly as dietary additives for chickens or directly as meat additives (Zdanowska-Sąsiadek et al., 2013). However, considering reluctance of consumers to buy food supplemented with synthetic additives, may studies aim to search for new antioxidants of natural origin able to efficiently suppress lipid peroxidation. Currently, a growing interest is focused on herbal plants containing biologically active substances, like phenols, polyphenols, carotenoids, flavonoids and essential oils, deemed to possess antioxidant properties (Kamboh & Zhu, 2013; Loetscher et al., 2013; Kasapidou et al., 2014).

Redox balance in animal cells is maintained by the antioxidant system, which comprises antioxidant enzymes, like superoxide dismutase, catalase, glutathione peroxidase, S-glutathione transferase, and other substances, e.g. glutathione or vitamins A, C, D and E, which act to remove reactive oxygen species (ROS) excess from the cell (Muchacka et al., 2016). According to some authors, diet supplementation with natural antioxidants rises the levels of antioxidant enzymes (Botsoglou et al., 2002; Hashemipour et al., 2013; Cong et al., 2017) and PUFA (Botsoglou et al., 2002; Kamboh & Zhu, 2013) in animal tissues.

Literature data have indicated that such plants as lemon balm (Marcinčáková et al., 2011; Kasapidou et al., 2014), sage (Lopez-Bote et al., 1998; Wereńska, 2013) and nettle (Bonetti et al., 2016) can elicit antioxidant actions.

Therefore, the aim of the present studies was to determine the effect of drinking water supplementation with herbal extracts from lemon balm (*Melissa officinalis* L.), common sage (*Salvia officinalis* L.) and common nettle (*Urtica dioica* L.*)* on the level of antioxidant enzymes (SOD, CAT, GPx), reduced glutathione (GSH), malondialdehyde (MDA) and on the fatty acid profile in frozen breast and leg muscles of broiler chickens.

Material and methods

The study was conducted on 640 1-d-old broiler chickens (Ross 308) purchased from Chicken Hatchery in Łężkowice. On the first day, chicks were weighed,

labeled and assigned to four groups, having four subgroups each. Group I chickens served as the control. Group II, III and IV chicks were treated with extracts of lemon balm (*Melissa officinalis* L.), common sage (*Salvia officinalis* L.) and common nettle (*Urtica dioica* L.*)* added to drinking water at 2 ml/l of water from day 22 to 42. Birds were housed on litter at stock density of 33 kg/m^2 (Regulation of the Minister of Agriculture and Rural Development, Dz. U. 2010.56.344) for 42 days. Indoor broiler house temperature was 30°C in the first days and then was gradually decreased to 20°C at 5 and 6 weeks. An L : D cycle was 23 :1 till day 7, 20 : 4 from day 8 to 38 and 23 : 1 from day 39 to 42. Chicks were fed *ad libitum* a starter diet till 3 weeks of age (ME 12.5 MJ; CP 22%), a grower diet from 4 to 5 weeks (ME 13MJ; CP 20.5%) and a finisher diet in week 6 (ME 13MJ; CP 20.5%), prepared from concentrates. Chicks had free access to feed and drinkers throughout the whole rearing period.

On day 42, 8 birds of approximately average weight were chosen from each group. These birds were slaughtered and after 24-hour cooling at 4°C, right breast muscle and right leg were excised. After removal of the skin and external fat, the muscles were packed into plastic bags and frozen at –18°C. After 4 months, meat samples were thawed and activities of the antioxidant enzymes: SOD (superoxide dismutase), GPx (glutathione peroxidase) and CAT (catalase) were determined. SOD activity was determined by spectrophotometric method of Rice-Evans et al. (1991). CAT activity was estimated by H_2O_2 degradation according to the method of Aebi (1984), GPx activity was assayed using Lück's method (1962). SOD, CAT and GPx activities in muscles were expressed as U/mg protein. Reduced glutathione (GSH) level in breast and leg muscles was determined by the method of Ellman (1959) and expressed as μmol/g protein and lipid peroxidation was estimated based on malondialdehyde concentration using thiobarbituric acid (TBA) (Ohkawa et al., 1979). MDA level was shown as nmol/mg protein. Further, fatty acid profile was estimated in muscle samples by gas chromatography on VARIAN 3400 CX, using helium as a carrier gas and column AGILENT J & W GC COLUMNS CP-WAX 58 FFAP (25 m).

Spirit extracts of herbs were obtained in a professional herbal manufacture certified for compliance with the standards (ZN-16/NX/900, ZN-07/NX/523, ZN-11/NX/546).

Statistical analysis of the data was performed by one-way analysis of variance and significance was estimated by Duncan test. Statgraphics plus 6.0 software package was used for statistical calculations.

Results

The lowest CAT level in breast muscles was observed in group IV broilers and the highest in group II ($P \leq 0.01$) (Tab. 1). CAT level in breast muscles of group I and II broilers also differed ($P \leq 0.05$). Chickens belonging to group I and IV showed lower GSH levels in breast muscles compared with birds of group II and III, respectively ($P \leq 0.01$). MDA levels in breast muscles in groups I and IV differed

from those in group II and III (P≤0.05) (Tab. 1). In leg muscles, GPx levels differed between group I and II while MDA level in groups I and III differed from that in group II (P≤0.05) (Tab. 2).

Table 1. Antioxidant enzymes activity and level of GSH and MDA in breast muscles of broiler chickens

Item	Group				SEM
	control	lemon balm	sage	nettle	
SOD (U/mg protein)	2,757	3,758	4,041	2,847	0,976
CAT (U/mg protein)	10,255 a	21,102 Bb	14,808	7,507 A	2,617
GPx (U/mg protein)	0,026	0,030	0,024	0,032	0,003
GSH (µmol/mg protein)	1,219 A	3,077 B	3,011 B	1,213 A	0,326
MDA (nmol/mg protein)	5,833 a	3,083 b	3,500 b	6,583 a	0,828

a, b – values in rows with different letters differ significantly (P≤0.05).
A, B – values in rows with different letters differ highly significantly (P≤0.01).

Table 2. Antioxidant enzymes activity and level of GSH and MDA in leg muscles of broiler chickens

Item	Group				SEM
	control	lemon balm	sage	nettle	
SOD (U/mg protein)	2,867	4,637	3,084	2,171	0,861
CAT (U/mg protein)	15,957	13,467	14,958	14,266	4,493
GPx (U/mg protein)	0,071 a	0,023 b	0,050	0,035	0,018
GSH (µmol/mg protein)	1,505	1,359	0,690	1,335	0,310
MDA (nmol/mg protein)	3,250 a	5,750 b	3,500 a	4,750	0,702

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

Table 3. Fatty acid profile (%) of breast muscles from broiler chickens

Item	Group				SEM
	control	lemon balm	sage	nettle	
C8	0,075	0,052	0,061	0,074	0,019
C10	0,100	0,080	0,083	0,094	0,020
C12	0,177 A	0,077 B	0,078 B	0,076 B	0,021
C14	0,449 A	0,381 B	0,371 B	0,355 B	0,014
C16	19,085	20,274	19,394	18,658	0,804
C16:1	0,689	0,894	0,802	0,568	0,110
C18	7,259	7,112	7,077	8,113	0,524
C18:1	40,458	42,084	41,386	39,534	1,500
C18:2	20,879	19,020	20,520	20,989	0,693
Gamma18:3	0,237	0,265	0,263	0,210	0,024
C20	0,087	0,082	0,078	0,100	0,007
C18:3	3,478	3,196	3,481	3,077	0,269
C22	0,621	0,582	0,584	0,588	0,088
C20:4	4,053	3,749	3,897	5,003	0,583
C22:1	0,064	0,063	0,063	0,059	0,003
EPA	1,008	0,972	0,834	0,881	0,183
DHA	1,249	1,087	0,994	1,597	0,177
CLA	0,031	0,031	0,033	0,023	0,004
SFA	27,853	28,637	27,727	28,057	1,300
UFA	72,147	71,363	72,273	71,943	1,300
MUFA	41,213	43,040	42,250	40,163	1,556
PUFA	30,933	28,317	30,020	31,780	0,865
PUFA-6	25,170	23,030	24,680	26,203	0,802
PUFA-3	5,733 a	5,253 b	5,307 b	5,553	0,106
DFA	79,403	78,473	79,350	80,057	0,887
UFA/SFA	2,613	2,500	2,623	2,577	0,168
MUFA/SFA	1,497	1,507	1,537	1,443	0,120
PUFA/SFA	1,120	0,993	1,087	1,133	0,059
PUFA6/3	4,390	4,383	4,653	4,717	0,122

a, b – values in rows with different letters differ significantly (P≤0.05).
A, B – values in rows with different letters differ highly significantly (P≤0.01).
SFA – saturated fatty acids.
UFA – unsaturated fatty acids.
PUFA – polyunsaturated fatty acids.
MUFA – monounsaturated fatty acids.
OFA – hypercholesterolemic fatty acids (C14:0 + C16:0).
DFA – neutral and hypocholesterolemic fatty acids (C18:0 + UFA).
n-6 acids (C18:2, C20:4, gamma 18:3).
n-3 acids (C18:3, EPA, DHA).

Table 4. Fatty acid profile (%) of leg muscles from broiler chickens

Item	Group				SEM
	I control	II lemon balm	III sage	IV nettle	
C8	0,009	0,009	0,009	0,009	8,165
C10	0,021 ^{A}	0,020 ^{A}	0,17	0,014 ^{B}	0,001
C12	0,050	0,038	0,051	0,039	0,007
C14	0,407	0,401	0,397	0,392	0,018
C16	18,179	19,580	18,720	17,600	0,693
C16-1	1,026	1,285	1,202	0,877	0,126
C18	5,392	5,171 ^{a}	5,148 ^{a}	5,957 ^{b}	0,194
C18-1	44,876	46,103 ^{a}	45,638 ^{a}	44,037 ^{b}	0,442
C18-2	22,636	20,629	21,715	23,075	0,683
Gamma18-3	0,158	0,141	0,170	0,137	0,018
C20	0,065	0,067	0,065	0,068	0,004
C18-3	4,488	4,072	4,249	4,111	0,115
C22	0,232	0,209 ^{a}	0,235	0,269 ^{b}	0,012
C20-4	1,684 ^{a}	1,554 ^{A}	1,657 ^{a}	2,403 ^{Bb}	0,159
C22-1	0,054	0,048	0,053	0,053	0,002
EPA	0,326	0,308	0,304	0,347	0,026
DHA	0,366 ^{a}	0,325 ^{a}	0,330 ^{a}	0,587 ^{b}	0,056
CLA	0,032 ^{a}	0,040 ^{Ab}	0,036 ^{A}	0,024 ^{Bb}	0,002
SFA	24,357	25,497	24,647	24,350	0,757
UFA	75,643	74,503	75,353	75,650	0,757
MUFA	45,957	47,433 ^{a}	46,893 ^{a}	44,967 ^{b}	0,511
PUFA	29,687	27,067	28,463	30,683	0,937
PUFA-6	24,477	22,323 ^{a}	23,543	25,613 ^{b}	0,793
PUFA-3	5,180	4,707	4,880	5,047	0,150
DFA	81,037	79,673	80,503	81,607	0,700
UFA/SFA	3,120	2,930	3,067	3,107	0,125
MUFA/SFA	1,893	1,863	1,907	1,847	0,061
PUFA/SFA	1,227	1,063	1,157	1,260	0,070
PUFA6/3	4,723 ^{A}	4,743 ^{A}	4,820 ^{A}	5,077 ^{B}	0,044

Explanations as above.

Tab. 3 presents fatty acid profile in breast muscles of broiler chickens. There was a difference in C12 and C14 acid contents in breast muscles between the control group and experimental groups at $P \leq 0.01$.

PUFA-3 level in breast muscles of group II and III chicks was reduced compared with the control group ($P \leq 0.05$). C10 acid level in group IV broilers was lower than in groups II and I ($P \leq 0.01$) (Tab. 4). It was also found that birds from this group had higher level of C18 acid and lower content of C18:1 acid and MUFA vs. group II and III birds ($P \leq 0.05$). In group IV chicken leg muscles, C22 acid and PUFA-6 levels were elevated vs. group II chicks ($P \leq 0.05$) whereas C20:4 acid and DHA contents were higher but CLA level was lower compared with the control group ($P \leq 0.05$). In leg muscles of group IV broilers, PUFA6/3 ratio was higher than in the remaining groups at $P \leq 0.01$.

Discussion

Antioxidant enzymes play an important role in protection of the cell against damage caused by reactive oxygen species (ROS). Yesilbag et al. (2011) reported that proper diet supplementation can increase activity of antioxidant enzymes in animal tissues. Hashemipour et al. (2013) evidenced a higher SOD activity in leg muscles of 42-d-old broiler chickens fed a diet supplemented with thymol and carvacrol. However, those authors did not note an effect of the antioxidant dietary supplements on the activity of antioxidant enzymes in chicken breast muscles. On the other hand, Cao et al. (2012) noted an increased T-SOD activity in breast muscles of broiler chickens fed antioxidant-supplemented diet but observed no effect of these additives on glutathione peroxidase activity. Our present studies revealed an increased CAT activity and GSH level in breast muscles of broiler chickens drinking the water supplemented with lemon balm extract and sage extract, respectively, compared with the control group. However, no beneficial effect of drinking water supplementation with herbal extracts on the activity of antioxidant enzymes was observed in leg muscles, even glutathione peroxidase (GPx) activity was reduced in lemon-balm extract-fed group vs. control.

Lipid peroxidation is one of the most important biological processes related to ROS action (Yagi, 1992). It is a cascade free-radical process of unsaturated fatty acid oxidation yielding their peroxides. Malondialdehyde (MDA) is one of many compounds formed during peroxidation of polyunsaturated fatty acids, which is often used to estimate oxidative damage (Hashemipour et al., 2013). MDA level is directly proportional to the degree of lipid peroxidation (Ismail et al., 2013). Many authors observed a positive effect of plant extracts on oxidative stability of chicken meat: rosemary and sage extracts added to feed at 500 $mg \cdot kg^{-1}$ (Lopez-Bote et al., 1998), oregano and rosemary essential oils added to feed at 150 and 300 $mg \cdot kg^{-1}$ (Basmacioglu et al., 2004), and oregano and rosemary essential oils supplemented at 100 and 200 $mg \cdot kg^{-1}$ (Papageorgiou et al., 2003).

The present studies demonstrated reduction of MDA level in breast muscles when drinking water was supplemented with lemon balm and sage extracts vs.

control group, which presumably could be linked to the higher CAT activity and GSH level in these muscles. On the other hand, in leg muscles, no positive effect of lemon balm, sage and nettle additives on reduction of lipid peroxidation was observed, and even lemon balm was shown to accelerate this process. However, Kasapidou et al. (2014) reported reduction of lipid peroxidation in breast and leg muscles of broiler chickens from organic farming in groups fed lemon balm-supplemented feed. Also Marcinčáková et al. (2011) noted a reduced MDA level in leg muscles stored at 4°C when chicken were fed a diet supplemented with lemon balm at 20 g/kg. Conversely, Koreleski & Świątkiewicz (2007) did not note reduction of lipid peroxidation in breast muscles frozen for 6 months at –20°C when broiler chickens were given feed with sage addition at 560 $mg \cdot kg^{-1}$. In the present studies, nettle extract did not show antioxidant properties which is in line with data reported by Loetscher et al. (2013).

Jung et al. (2010) demonstrated that diet supplementation with antioxidants was an excellent method to reduce saturated fatty acid (SFA) level and to elevate polyunsaturated acid (PUFA) content in broiler chicken muscles. Kamboh & Zhu (2013) obtained a positive effect of diet supplementation with different concentrations of bioflavonoids on fatty acid profile in breast muscles of broiler chicken. Dukić-Stojčić et al. (2016) reported a percentage increase in PUFA, linoleic acid and linolenic acid levels and narrowing of *n-6/n-3* ratio in breast muscles of Redbro chickens after addition of fresh nettle to birds' diet. In the present studies, drinking water supplementation with individual herbs reduced lauric acid (C12) and myristic acid (C14) levels vs. control group but had no significant effect on saturated fatty acids in breast muscles of birds from those experimental groups. In broiler chicken drinking the water supplemented with lemon balm and sage extracts, the level of omega-3 acids was also lower compared with the control group, however, PUFA level remained at similar level in all groups. On the other hand, Marcinčáková et al. (2011) obtained elevated levels of PUFA in breast muscles of broiler chicken fed lemon balm–supplemented feed, whereas studies of Koreleski & Świątkiewicz (2007) demonstrated an increase in percentage content of stearic acid and *n-3* levels and a lower percentage content of MUFA in breast muscles of chickens consuming sage-supplemented diet. In contrast, studies of Kasapidou et al. (2014) did not show the effect of diet supplementation with lemon balm on fatty acid profile in breast and leg muscles of broiler chicken from organic farming. However, when analyzing fatty acid profile in leg muscles, the present studies documented significant changes in fatty acid levels in chickens drinking the nettle-supplemented water compared with the control group. Birds of this group showed a lower content of capric acid (C10) and conjugated linoleic acid (CLA) and a higher level of arachidonic acid (C20:4) and docosahexaenoic acid (DHA) which is deficient in human diet and is essential for proper body functioning (Marcinčáková et al., 2011). In leg muscles of broiler chickens from this group, PUFA 6/3 ratio was wider compared with the remaining groups but still was in the range thought to be beneficial for human health (Gebauer et al., 2006).

In conclusion, addition of lemon balm and sage extracts at 2 ml/l to drinking water reduced lipid peroxidation in breast muscles of broiler chickens, however, no positive effect of these extracts on antioxidant status of leg muscles was observed.

Diet supplementation with nettle in the form and concentration used in this experiment did not produce an antioxidant effect in broiler chicken muscles but had a positive effect on fatty acid profile mostly in leg muscles of broiler chickens.

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Effect of adding herbal extracts to drinking water on activity of antioxidant enzymes, GSH and MDA levels, and fatty acid profile in broiler chicken muscles

SUMMARY

The aim of the study was to determine the effect of supplementing drinking water with extracts from lemon balm, sage and common nettle on the level of antioxidant enzymes (SOD, CAT, GPx), reduced glutathione (GSH), malondialdehyde (MDA) and fatty acid profile in frozen breast and leg muscles of broiler chickens. Ross 308 broiler chickens were assigned to four experimental groups: group I (control), and groups II, III and IV which received extracts from lemon balm, sage and common nettle, respectively, added to water in the drinking troughs (2 ml/l of water) from 22 to 42 days of rearing. At 42 days, 8 birds from each group were slaughtered, and the dissected muscles were frozen. After 4 months, the muscles were analysed for the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), the level of GSH and MDA, and the fatty aid profile. The results show that lemon balm and sage extracts added to drinking water at 2 ml/l reduced lipid peroxidation in breast muscle, but had no positive effect on the antioxidant status of leg muscle. Dietary inclusion of common nettle in the form and concentration used in the experiment did not show antioxidant effect in broiler muscles, but contributed to changes in the fatty acid profile, mainly in leg muscles of the chickens.

Key words: herbal extracts, antioxidants, lipid peroxidation, fatty acid, muscles of broiler chickens