Influence of dietary replacement of sunflower oil with milk thistle (*Silybum marianum*) oil on chicken meat quality and antioxidant status of liver

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Abstract

The research focused on the effects of dietary replacement of 3% sunflower oil (SO group) with 3% milk thistle oil (MTO group) on the technological quality of meat, such as pH value, colour (CIE L*, CIE a*, CIE b*), drip loss (%), shear force (N), and cooking loss (%), as well as on the content of fatty acid lipids in broiler breast and thigh muscles. Significant difference ($P < 0.05$) was determined for pH_i pH_u, CIE a*, CIE b* values between groups, although the values for the stated indicators were within the standard range. Lipids of breast meat of the MTO group contained more arachidic acid ($P < 0.001$), octadecenoic acid isomer B ($P = 0.047$) and eicosatrienoic acid ($P = 0.041$), and less α-linolenic acid ($P < 0.001$) and Σn-3PUFA. Lipids of thigh meat of the MTO group contained more ΣSFA, myristoleic acid, eicosatrienoic acid ($P < 0.05$) and eicosenoic acid ($P < 0.001$), and less α-linolenic acid, and had narrower Σn-3/n-6 PUFA ratio than the SO group. According to the antioxidant status of broiler liver, there was significantly higher catalase activity determined in the MTO group.

Breast meat, technological quality, fatty acids, catalase, glutathione-S-transferase

Broiler meat and its products are consumed worldwide. The quality of meat highly depends on the content and composition of fat in meat. Oils in diets are required as a source of energy. This research focused on the usage of milk thistle (*Silybum marianum*) oil as a replacement for sunflower oil. Milk thistle is a plant used for production of silymarin – an ingredient which acts as antioxidant, absorbs free radicals and shows anti-inflammatory activity (Fiebrich and Koch 1979; Bosisio et. al. 1992; De La Puerta et al. 1996). Positive effects of this medicinal plant referring to improved health conditions and better performances of broilers were reported by Yakhkeshi et al. (2012) and Elmakki et al. (2013). Many plants, including also *Silybum marianum,* have been examined for their influences in treatments of some liver disorders (Luper 1998). Several studies demonstrated its anti-inflammatory effects (Polyak et al. 2007), including mast cell stabilization, inhibition of neutrophil migration, Kuppfer cell inhibition, strong inhibition of leukotriene synthesis, and prostaglandin formation (Kang et al. 2004). Scott (1998) pointed out the significant activity of milk thistle in medical applications, since it was one of the oldest known plants containing active components, such as silybin (50–60%), isosilybin (5%), silydianin (10%), silychristin (20%), all referred to under the common name of silymarin. Silymarin acts as a hepatoprotective agent, and is used in treatments of liver regeneration, fatty liver disease, cirrhosis, and toxic radiation. Immunomodulating

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effects are also attributed to silymarin. Cold pressed oil of milk thistle is an excellent source of linoleic acid (C18: 2, n-6), which is important in synthesis of biologically active substances, such as prostaglandin, prostacyclin, tromboselicines and other substances that regulate metabolism. For its high content of linoleic acid, milk thistle oil is similar to sunflower oil. Several authors (Mekala et al. 2006; Schiavone et al. 2007; Mojahedtalab et al. 2013) investigated supplementation of silymarin in broiler diets and its effects on growth, consumption, and conversion of feed. However, there is still lack of knowledge on influences of milk thistle oil on the technological properties of broiler meat, fatty acid profile in muscles, as well as liver enzymes that regulate metabolic processes, all of which were the objectives of our research. Kralik et al. (2015) reported on the influences of milk thistle oil on the fattening characteristics of broilers and on carcass quality. This paper presents a continuation of that research.

Materials and Methods

The research was carried out on a total of 60 Cobb 500 broilers, of which 30 broilers (3 × 10) were included in the SO group fed 3% sunflower oil (SO), and 30 broilers (3 × 10) were included in the MTO group fed 3% milk thistle oil (MTO). During the first three weeks of fattening, broilers consumed powdered starter diets of the same composition. At the beginning of the 4th fattening week (since the 22nd day), broilers were randomly divided into two groups and fed pelleted finisher diets of modified composition. The feeding and watering of broilers was *ad libitum* and automatically regulated.

The starter diet contained 24.3% crude protein and 13.5 MJ·kg^{-1} ME, and the finisher diets were balanced at the level of 20.8% crude protein and 13.2 MJ·kg^{-1} ME. Diets were prepared to meet the physiological needs of the Cobb 500 broilers. Broilers were fattened in groups and kept on deep litter. The ambient temperature was gradually reduced from 32 to 20 °C during the experiment. Relative humidity inside the room varied from 65% to 75%. The lighting programme was 24 h/per day during the first eight days of the experiment, and afterwards it included 3 h darkness. After 42 days, the broilers were weighed, slaughtered and defeathered. Carcasses were processed according to the EC regulations 543/2008 and cut into main parts: breasts, drumsticks with thighs, backs and wings. Breast and thigh muscles were used in the research.

Meat quality indicators referred to pH_i (45 min after the slaughtering of broilers) and pH_u (24 h after the slaughtering of broilers), determined on the left side of m. pectoralis major by a probe of the digital pH meter Mettler MP 120-B. Meat colour was determined 24 h after the cooling of carcasses by a chromatograph Minolta Camera CR-300 and presented as CIE L*, a* and b* (Commission Internationale de l'Eclairage 1976), referring to lightness (black-white axis), degree of redness (red-green spectrum), and degree of yellow (yellow-blue spectrum). Calibration of the device was performed by using the standard white plate (Y = 93.0, x = 0.3195; y = 0.3324). The optical lens diameter was 8 mm, illumination D65, and standard observation 10°. Prior to the measuring of colour, there was a fresh section made on the breast muscle and then left for 10 min to develop colour. The colour of each breast muscle resulted from three consecutive measurements, and was presented as a mean value. Resistance of muscles to cutting was determined by TA.XT*plus* Texture Analyser device with Warner-Bratzler blade according to the modified method of Liu et al. (2004). After storing the samples at -20 °C for 14 days, the meat was defrosted, closed in plastic bags, cooked in a water bath at 85 °C for 25 min, and then cooled to room temperature. There were three subsamples (3 cm × 1.9 cm × 1.9 cm) taken from each sample. Maximum force (N) required for the cutting of a subsample with Warner-Bratzler shear force blade was calculated by Texture Exponent 4.0 software (Stable Microsystems, UK). Drip loss from the breast muscle was determined according to the method of Honikel (1998), as follows: a sample of breast muscle was cut in the diameter of 2 cm and the height of 3 cm, then it was weighed and kept in a plastic bag in the refrigerator for 48 h at +4 °C.

Drip loss was calculated as: drip loss (%) = {initial tissue mass (g) – final tissue mass (g) / initial tissue mass (g)} × 100.

Cooking loss was determined in samples of breast muscle tissue, calculated as follows: cooking loss (%) = {(sample mass before cooking (g) – sample mass after cooking (g) / sample mass before cooking (g)} × 100.

The content of fatty acids in diets and meat were determined by the Chrompack CP-9000 chromatograph equipped with flame ionization detector. Quantitative evaluation was obtained on the basis of percentage relations of chromatographic peaks of pure methyl ester and heights of chromatographic peaks of samples. Percent proportion of fatty acids in a sample was calculated as a function of comparative weight percentage of fatty acid methyl ester (Csapo et al. 1986). Individual fatty acids were presented as % of total fatty acids.

In order to perform homogenization and extraction of the tissue, broiler liver was cut to thin slices, slices diced, and 150 mg of diced liver was mixed with 1.5 of 50 mM phosphate buffer pH 7.0 containing 5 mM EDTA-2Na. After vortexing for 15 s, ultrasonic homogenization of suspension was performed in ice-cold water bath (+4 °C),

during four 30-s intervals at full cycle and 100% amplitude. Extracts were clarified by centrifugation (15 000 *g*, 4 °C, 10 min) and used for protein content and antioxidant enzyme activity determination. The protein content in extracts was determined by Bradford method (Bradford 1976) with bovine serum albumin as a standard.

Free sulfhydryl group determination was performed according to the method of Sedlak and Lindsay (1968). Prior to free sulfhydryl content determination, proteins from extracts were removed by 15 min precipitation with 10% trichloroacetic acid, followed by supernatant clarification (15 000 *g*, 4 °C, 10 min). Glutathione reductase (GR), glutathione-S-transferase (GST) and catalase (CAT) were measured in liver extracts diluted as follows: (i) 10-fold dilution was used for GR and GST activity determination, and 100-fold CAT. Catalase activity was determined by the method of Aebi (1986) using hydrogen peroxide as a substrate. One unit (U) of catalase activity was defined as the amount of enzyme decomposing 1 µmol of hydrogen peroxide to oxygen and water per minute at pH 7.0, 25 °C and 10 mM hydrogen peroxide concentration. Glutathione reductase activity was determined by UV assay using glutathione reductase assay kit (Sigma, USA). One unit (U) of glutathione reductase activity was defined as amount of enzyme causing oxidation of 1 µmol of NADPH at 25 °C and pH 7.5. Glutathione-S-transferase activity was measured by modified method of Habig et al. (1974) as described by Habdous et al. (2002). The reaction mixture (1.1 ml) contained 1 mM EDTA, 2 mM 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate and 5 mM reduced glutathione (GSH) as co-substrate in 100 mM potassium phosphate buffer at pH 5.5. One unit (U) of GST activity was defined as the amount of enzyme producing 1 µmol of GS-DNB conjugate at pH 5.5 and 25 °C.

Analysis of variance (ANOVA) was performed by using the GLM procedure for two treatments (SO and MTO). Obtained differences between groups were tested by the Fisher's LSD test. Significant differences between experimental groups were assigned with different subscripts (^{a,b} - $P < 0.05$, ^{A,B} - $P < 0.01$, ^{X,Y} - $P < 0.001$).

Results

Table 1 presents concentrations of fatty acids in plant oils and in broiler diets. The experiment proved that milk thistle oil was richer in n-6 PUFA (63.11 vs 58.85%) and poorer in n-3 PUFA (0.30% vs 0.41%) compared to sunflower oil. The finisher diet for the MTO broiler group contained more ΣSFA (16.92% vs 13.73%), and less ΣMUFA (23.89% vs 25.89%) compared to the finisher diet given to the SO broiler group. Palmitic acid (C16:0) was the most represented in ΣSFA of both oils (13.46% vs 12.79%). In ΣMUFA, oleic acid (C18:1n9c) was represented at a higher concentration in the finisher diet of SO compared to MTO group (23.70% vs 21.25%). No differences were determined in the content of n-6 PUFA between SO and MTO finisher diets. The finisher diet of the SO group contained more Σn-3 PUFA (referring to α-linolenic acid, C18:3n3) than the finisher diet of MTO group (2.33% vs 1.13%). The ratio of n-6/n-3 PUFA was more favourable in the SO finisher diet compared to the MTO finisher diet (24.91:1, i.e. 51.38).

Research into the technological and sensory quality of breast muscles indicated higher pH_i values ($P = 0.026$), and lower pH_u values ($P = 0.003$) in the SO group compared to the MTO group (Table 2). The values of pH_i and pH_u obtained in our research were within the standard range defined for meat of normal technological traits. Results of breast muscle tissue colour showed a higher CIE a* value ($P < 0.030$), and lower CIE b* value ($P < 0.001$) in the SO group compared to the MTO group.

Breast muscles of the SO group had greater values of drip loss (%), shear force (N) and cooking loss (%) than the MTO group, however, the differences were not significant ($P > 0.05$).

White meat of the MTO group contained more octadecenoic acid isomer (2.47% vs 2.08%; $P = 0.047$), eicosenoic acid (0.42% vs 0.30%; $P < 0.001$), as well as eicosatrienoic acid (0.96% vs 0.74%; $P = 0.041$) compared to white meat of the SO group (Table 3). Breast muscle lipids of the SO group exhibited significantly higher concentration of α-linolenic acid (1.03% vs 0.50%; $P < 0.001$), slightly higher concentration of eicosatrienoic acid (0.06% vs 0.04%, $P = 0.021$), and significantly lower concentration of eicosapentaenoic acid (0.22% vs 0.32%, $P = 0.040$) compared to breast muscle lipids of the MTO group. The sum of n-3 PUFA in breast muscle lipids in the SO group was higher compared to the MTO group (2.48% vs 1.91%, $P > 0.05$). Both broiler groups had a high and unfavourable n-6/n-3 PUFA ratio (15.48 and 17.76) in breast meat.

Table 1. Concentrations of fatty acids in plant oils and chickens' diet.

Fatty acid	Sunflower oil	Milk thistle oil	Starter diet	Finisher diets	
				SO group	MTO group
Lauric acid (C 12:0)	0.20	-	0.03	0.01	0.02
Myristic acid (C 14:0)	0.20	0.09	0.19	0.10	0.21
Pentadecanoic acid (C 15:0)	6.30	0.02	0.06	0.03	0.06
Palmitic acid (C 16:0)	8.30	8.01	17.46	12.71	13.96
Heptadecanoic acid (C 17:0)	-	0.07	0.35	0.12	0.10
Stearic acid (C 18:0)	3.10	0.06	0.10	0.07	0.09
Arachidic acid (C 20:0)	0.15	2.32	0.51	0.28	1.38
Behenic acid (C 22:0)	0.25	1.86	0.24	0.41	1.10
∑ SFA	18.50	12.43	18.94	13.73	16.92
Myristoleic acid (C 14:1)	-	-	-	-	0.01
Palmitoleic acid isomer (C 16:1)	-	0.02	0.09	0.08	0.04
Palmitoleic acid (C 16:1n7)	-	0.07	0.35	0.12	0.10
Elaidic acid (C 18:1n9t)	-	-	0.27	0.12	0.11
Oleic acid (C 18:1n9c)	21.94	22.27	21.10	23.70	21.25
Octadecenoic acid isomer A (C 18:1)	0.10	0.46	1.05	0.75	0.56
Octadecenoic acid isomer B (C 18:1)	-	0.55	1.65	0.94	1.32
Eicosenoic acid (C 20:1n9)	0.20	0.79	0.23	0.18	0.50
∑ MUFA	22.24	24.16	24.74	25.89	23.89
Linoleic (C 18:2n6)	58.85	63.05	47.37	55.48	54.78
Eicosadienoic acid (C 20:2n6)	-	0.03	0.08	0.03	0.04
Eicosatrienoic acid (C 20:3n6)	-	-	0.16	0.09	0.14
Arachidonic acid (C 18:4n6)	-	-	0.38	0.03	0.01
Octadecadienoic acid isomer A (C 18:2)	-	-	1.78	0.82	1.05
Octadecadienoic acid isomer B (C 18:2)	-	-	1.21	0.59	0.73
Octadecadienoic acid isomer C (C 18:2)	-	0.03	1.16	0.52	0.68
Octadecadienoic acid isomer D (C 18:2)	-	-	1.09	0.49	0.63
∑n-6 PUFA	58.85	63.11	53.23	58.05	58.06
α-linolenic acid (C 18:3n3)	0.41	0.24	3.09	2.33	1.13
Eicosatrienoic (C 20:3n3)	-	0.06	-	-	
∑n-3 PUFA	0.41	0.30	3.09	2.33	1.13
n-6/n-3 PUFA	143.54	210.37	17.23	24.91	51.38

SO 3% sunflower oil; MTO 3% milk thistle oil

The fatty acid profile in thigh muscle lipids is presented in Table 4. The MTO group was richer in myristic acid (0.43% vs 0.36%, $P = 0.032$), palmitic acid (21.58% vs 19.90%; $P = 0.014$) arachidic acid (0.20% vs 0.11%; $P < 0.001$), myristoleic acid (0.08% vs 0.05%; $P = 0.044$), palmitoleic acid isomer (0.43% vs 0.34%; $P = 0.028$) and eicosenoic acid (0.36% vs 0.27%; $P < 0.001$). Lipids in thighs of the SO group contained more linoleic acid (34.80% vs 29.10%; $P = 0.024$), and less eicosatrienoic acid (0.40% vs 0.48%; $P = 0.022$) than those of the MTO group. Lipids in thighs of the SO group had more α-linolenic acid (1.57% vs 0.87%; $P < 0.001$), and more eicosatrienoic acid (0.03% vs 0.01%; $P = 0.003$) compared to the MTO group. Higher concentration of Σn-3 PUFA was determined in lipids

Table 2. Breast muscle quality traits.

Indicator	SO group ($\bar{x}$ ± SD)	MTO group ($\bar{x}$ ± SD)	*P* value
pH_i (n = 30)	6.10^{a} ± 0.19	6.09^{b} ± 0.14	0.026
pH_u (n = 30)	5.75^{B} ± 0.17	5.88^{A} ± 0.15	0.003
CIE L* (n = 30)	49.40 ± 3.91	49.51 ± 2.42	0.893
CIE a* (n = 30)	2.03^{a} ± 0.62	1.66^{b} ± 0.29	0.030
CIE b* (n = 30)	9.81^{Y} ± 2.16	12.01^{X} ± 1.70	< 0.001
Drip loss, % (n = 10)	2.18 ± 0.90	1.71 ± 0.34	0.069
Shear force WBSF, N (n = 10)	36.34 ± 7.75	35.83 ± 3.31	0.865
Cooking loss, % (n = 10)	19.72 ± 2.13	19.42 ± 3.23	0.816

SO 3% sunflower oil, MTO 3% milk thistle oil; $\bar{x}$ = arithmetic means; SD = standard deviation; $^{a,b}P < 0.05$; $^{A,B}P < 0.01$; $^{X,Y}P < 0.001$

of thighs of the SO group (2.29% vs 1.57%; $P < 0.001$), as well as a narrower n-6/n-3 PUFA ratio (17.43 : 22.36; $P < 0.001$) if compared to MTO group.

Silybum marianum oil dietary supplementation did not exhibit a significant effect on the free sulfhydryl group content in broiler liver. However, increased ($P = 0.020$) catalase activity was determined in liver extracts of broilers fed *Silybum marianum* oil (Table 5). A decrease of glutathione-S-transferase ($P = 0.005$) and glutathione reductase ($P < 0.001$) activity was determined in this research.

Discussion

In this research, it was important to compare concentrations of α-linolenic acid in the feed and in meat because α-linolenic acid is a precursor of EPA and DHA. During the processes of desaturation and elongation with specific enzymes, α-linolenic acid is converted to n-3 PUFA of a long carbon chain. Finisher diets used in this research contained 2.33% α-linolenic acid in lipids of the SO group, and 1.13% in lipids of the MTO group. Analysis of breast muscle samples proved significantly higher concentration of EPA ($P = 0.40$) in the MTO group compared to the SO group. These results were in accordance with the findings of Youdim et al. (2000) and Cortinas et al. (2004). Relations between the content of PUFA in the feed and in broiler muscle tissue were explained by Cortinas et al. (2004) as a combination of FA deposition from the feed and endogenous synthesis, which was also confirmed by Kralik et al. (2012). Endogenous synthesis of EPA was not determined in lipids of thigh muscles. This research proved two aspects of the technological quality of meat. It was determined that 3% sunflower oil can be replaced with 3% milk thistle oil from the 22nd to 42nd day of broiler fattening. The fatty acid profile and the ratio of n-6/n-3 PUFA were more favourable in muscle lipids of broilers fed sunflower oil. Since both types of oil contain high concentrations of n-6 PUFA, it is advisable to combine them with oils that are rich in n-3 PUFA to reach a balance of n-6/n-3 PUFA in diets, and consequently, in the lipids of broiler meat.

The value of pH_i in breast muscles was higher in the SO group than in the MTO group. Slower glycolytic processes increased pH_u value in the MTO group compared to the SO group (Table 2). Le Bihan-Duval et al. (2008) pointed out that the rate of decrease of pH value *post mortem,* as well as the final pH value were important for the technological quality of meat. A rapid decline in pH results in pale, soft, and exudative breast muscles (PSE), and a high pH_u value indicates DFD meat (Dry-Firm-Dark). The authors determined that the pH value 15 min *p.m.* ranged 6.02–6.79, with a mean of 6.45. The pH_u value ranged 5.35–6.04, with a mean of 5.64. Raach-Moujahed and Haddad (2013) determined that

Table 3. Content of fatty acids in lipids of broiler breast muscle (% of total fatty acids, n = 5).

Fatty acid	SO group $\bar{x}$ ± SD	MTO group $\bar{x}$ ± SD	*P* value
Lauric acid (C12:0)	0.03 ± 0.01	0.02 ± 0.0005	0.180
Myristic acid (C14:0)	0.38 ± 0.04	0.37 ± 0.01	0.794
Pentadecanoic acid (C 15:0)	0.08^{a} ± 0.01	0.06^{b} ± 0.01	0.031
Palmitic acid C (16:0)	23.01 ± 1.95	24.26 ± 1.75	0.315
Heptadecanoic acid (C 17:0)	2.08 ± 0.61	2.34 ± 0.69	0.543
Stearic acid (C 18:0)	0.15 ± 0.01	0.12 ± 0.03	0.073
Arachidic acid (C 20:0)	0.14^{Y} ± 0.01	0.26^{X} ± 0.04	< 0.001
Behenic acid (C 22:0)	0.13 ± 0.03	0.17 ± 0.03	0.096
∑ SFA	25.99 ± 2.22	27.61 ± 1.58	0.222
Myristoleic acid (C 14:1)	0.05 ± 0.02	0.05 ± 0.02	0.978
Palmitoleic acid isomer (C 16:1)	0.29 ± 0.02	0.34 ± 0.06	0.163
Palmitoleic acid (C 16:1n7)	2.07 ± 0.61	2.34 ± 0.69	0.543
Octadecenoic acid isomer A (C 18:1)	1.54 ± 0.34	2.02 ± 0.93	0.302
Octadecenoic acid isomer B (C18:1)	2.08^{b} ± 0.23	2.47^{a} ± 0.30	0.047
Elaidic acid (C18:1n9t)	0.34 ± 0.04	0.46 ± 0.14	0.104
Oleic acid (C18:1n9c)	27.08 ± 2.72	28.96 ± 2.49	0.286
Eicosenoic acid (C20:1n9)	0.30^{Y} ± 0.02	0.42^{X} ± 0.03	< 0.001
∑ MUFA	33.75 ± 3.16	37.05 ± 2.83	0.119
Octadecadienoic acid isomer A (C 18:2)	0.51 ± 0.10	0.52 ± 0.20	0.933
Octadecadienoic acid isomer B (C 18:2)	0.35 ± 0.07	0.33 ± 0.13	0.864
Octadecadienoic acid isomer C (C 18:2)	0.30 ± 0.06	0.29 ± 0.12	0.874
Octadecadienoic acid isomer D (C 18:2)	1.53 ± 0.50	1.91 ± 0.57	0.291
Linoleic (C 18:2n6)	27.90 ± 2.39	22.01 ± 2.52	0.005
γ-linolenic (C 18:3n6)	0.17 ± 0.04	0.16 ± 0.04	0.500
Eicosadienoic (C 20:2n6)	0.83 ± 0.19	0.77 ± 0.16	0.604
Eicosatrienoic acid (C:20:3n6)	0.74^{b} ± 0.09	0.96^{a} ± 0.18	0.041
Arachidonic acid (C 20:4n6)	5.06 ± 1.70	5.91 ± 1.27	0.400
Docosatetraenoic acid (C2 2:4n6)	0.39 ± 0.16	0.58 ± 0.23	0.180
∑n-6 PUFA	37.78 ± 3.72	33.43 ± 3.28	0.085
α-linolenic acid (C 18:3n3)	1.03^{X} ± 0.14	0.50^{Y} ± 0.08	< 0.001
Eicosatrienoic (C 20:3n3)	0.06^{a} ± 0.01	0.04^{b} ± 0.01	0.021
Eicosapentaenoic acid (C 20:5n3)	0.22^{b} ± 0.04	0.32^{a} ± 0.08	0.040
Docosapentaenoic acid (C22:5n3)	0.69 ± 0.26	0.54 ± 0.13	0.300
Docosahexaenoic acid (C22:6n3)	0.48 ± 0.22	0.51 ± 0.18	0.817
∑n-3 PUFA	2.48 ± 0.46	1.91 ± 0.32	0.051
n-6/n-3 PUFA	15.48 ± 1.63	17.76 ± 2.14	0.094

SO 3% sunflower oil, MTO 3% milk thistle oil; $\bar{x}$ = arithmetic means; SD = standard deviation; $^{a,b}P < 0.05$; $^{X,Y}P < 0.001$

Table 4. Content of fatty acids in lipids of broiler thigh muscle (% of total fatty acids, n = 5).

Fatty acid	SO group $\bar{x}$ ± SD	MTO group $\bar{x}$ ± SD	*P* value
Lauric acid (C12:0)	0.02 ± 0.01	0.03 ± 0.01	0.103
Myristic acid (C14:0)	0.36^{b} ± 0.02	0.43^{a} ± 0.05	0.032
Pentadecanoic acid (C 15:0)	0.08 ± 0.01	0.07 ± 0.01	0.109
Palmitic acid C (16:0)	19.90^{b} ± 0.92	21.58^{a} ± 0.76	0.014
Heptadecanoic acid (C 17:0)	2.60 ± 2.62	3.56 ± 0.84	0.073
Stearic acid (C 18:0)	0.14 ± 0.05	0.11 ± 0.02	0.349
Arachidic acid (C 20:0)	0.11^{Y} ± 0.01	0.20^{X} ± 0.02	< 0.001
Behenic acid (C 22:0)	0.09 ± 0.02	0.11 ± 0.01	0.150
∑ SFA	23.30 ± 1.43	26.08 ± 1.59	0.019
Myristoleic acid (C 14:1)	0.05^{b} ± 0.01	0.08^{a} ± 0.02	0.044
Palmitoleic acid isomer (C 16:1)	0.34^{b} ± 0.03	0.43^{a} ± 0.07	0.028
Palmitoleic acid (C 16:1n7)	2.59 ± 0.62	3.55 ± 0.44	0.073
Octadecenoic acid isomer A (C 18:1)	0.26 ± 0.06	0.29 ± 0.01	0.557
Octadecenoic acid isomer B (C18:1)	1.39 ± 0.18	1.80 ± 0.37	0.060
Elaidic acid (C18:1n9t)	0.15 ± 0.02	0.15 ± 0.03	0.967
Oleic acid (C18:1n9c)	29.47 ± 2.10	30.64 ± 1.50	0.340
Eicosenoic acid (C20:1n9)	0.27^{Y} ± 0.02	0.36^{X} ± 0.03	< 0.001
∑ MUFA	34.52 ± 0.02	37.30 ± 2.43	0.127
Octadecadienoic acid isomer A (C 18:2)	0.11 ± 0.02	0.12 ± 0.04	0.446
Octadecadienoic acid isomer B (C 18:2)	0.06 ± 0.01	0.08 ± 0.03	0.191
Octadecadienoic acid isomer C (C 18:2)	0.07 ± 0.01	0.07 ± 0.02	0.762
Octadecadienoic acid isomer D (C 18:2)	0.75 ± 0.24	0.87 ± 0.17	0.392
Linoleic (C 18:2n6)	34.80^{a} ± 2.79	29.10^{b} ± 3.66	0.024
γ-linolenic (C 18:3n6)	0.20 ± 0.03	0.20 ± 0.04	0.811
Eicosadienoic (C 20:2n6)	0.44 ± 0.09	0.37 ± 0.09	0.213
Eicosatrienoic acid (C:20:3n6)	0.40^{b} ± 0.04	0.48^{a} ± 0.05	0.022
Arachidonic acid (C 20:4n6)	2.83 ± 0.86	3.48 ± 0.64	0.216
Docosatetraenoic acid (C2 2:4n6)	0.23 ± 0.13	0.28 ± 0.07	0.503
∑n-6 PUFA	39.89 ± 3.88	35.05 ± 3.74	0.079
α-linolenic acid (C 18:3n3)	1.57^{X} ± 0.10	0.87^{Y} ± 0.11	< 0.001
Eicosatrienoic (C 20:3n3)	0.03^{A} ± 0.01	0.01^{B} ± 0.01	0.003
Eicosapentaenoic acid (C 20:5n3)	0.13 ± 0.02	0.15 ± 0.02	0.175
Docosapentaenoic acid (C22:5n3)	0.33 ± 0.10	0.30 ± 0.06	0.578
Docosahexaenoic acid (C22:6n3)	0.24 ± 0.10	0.23 ± 0.04	0.928
∑n-3 PUFA	2.29^{X} ± 0.25	1.57^{Y} ± 0.15	< 0.001
n-6/n-3 PUFA	17.43^{Y} ± 0.72	22.36^{X} ± 0.79	< 0.001

SO 3% sunflower oil, MTO 3% milk thistle oil; $\bar{x}$ = arithmetic means; SD = standard deviation; $^{a,b}P < 0.05$; $^{A,B}P < 0.01$; $^{X,Y}P < 0.001$

Table 5. Influence of *Silybum marianum* oil feed supplementation on antioxidative status of chicken liver (n = 27).

Indicator	SO group $\bar{x}$ ± SD	MTO group $\bar{x}$ ± SD	*P* value
Free sulfhydryl groups (μmol/g $_{liver}$)	0.71 ± 0.09	0.73 ± 0.14	0.558
Catalase activity (U/mg)	67.62^{b} ± 33.07	83.09^{a} ± 9.21	0.020
Glutathione-S-transferase activity (mU/mg)	118.10^{A} ± 13.48	101.55^{B} ± 18.83	0.005
Glutathione-reductase activity (mU/mg)	13.22^{X} ± 3.65	9.36^{Y} ± 2.03	< 0.001

SO 3% sunflower oil, MTO 3% milk thistle oil; $\bar{x}$ = arithmetic means; SD = standard deviation; $^{a,b}P < 0.05$; $^{A,B}P < 0.01$; $^{X,Y}P < 0.001$

pH_u 24 h post-slaughter was 6.1 in domestic broilers, and 5.79 in Arbor Acres broilers. Kralik et al. (2014a) reported pH values in breast muscles of Cobb 500 broilers as follows: pH_1 5.95–6.2, and pH_u 5.73–5.84, which is in line with the results of the present research.

Broiler meat colour depends on the genotype (Fletcher 1995), age (Fanatico et al. 2005), animal technology conditions, and feeding regime (Ponte et al. 2008). Husak et al. (2008) stated that the colour of breast muscles of Arbor Acres broilers was: CIE L* value 62.57, CIE a* value 11.87, and CIE b* value 11.37. Lonergan et al. (2003) reported the following breast colour values in 8-week-old broilers: CIE L* 40.31; CIE a* 6.08 and CIE b* 12.52. Fraqueza et al. (2006) classified breast meat as PSE if CIE L* value > 50, and pH_u value < 5.8. According to Petracci et al. (2004), the colour of breast meat (CIE L* value) should be 41–68, Lesiow et al. (2007) set that value range at 43–56, and Woelfel et al. (2002) at 42–71. Fletcher (1999) studied the correlation between breast muscle colour and pH_2 values. On the basis of obtained indicators, muscle tissue was classified to lighter than normal (CIE L* > 48.8 and pH_2 5.63), normal (48.8 ≥ CIE L* ≤ 43.1, mean values were 45.6 and pH_2 5.70), and darker than normal (L* < 43.1 and pH_2 5.81). Accordingly, there is a clear correlation between CIE L* values and pH_2 values in broiler breast muscles. The colour of muscle tissue is important for the consumers' perception; therefore, produced meat should have optimal values for colour. Van Laack et al. (2000) classified breast muscle tissue as lighter than normal (CIE L* > 60.0) and normal (CIE L* ≤ 55.0). Kralik et al. (2014a) investigated the breast muscle colour of Cobb 500 broilers and determined the variability for the following indicators: CIE L* 53.34–55.29, CIE a* 1.78–2.88 and CIE b* 7.34–8.32. Kralik et al. (2014b) also investigated the indicators of breast meat quality with respect to meat colour. On the basis of paleness (CIE L*), breast meat was classified as DFD (CIE L* < 44), "normal" (CIE L* = 44–53) and PSE (CIE L* > 53). Seventy five percent of breast meat from Cobb 500 and 70% from Hubbard Classic were classified as "normal". Breast meat classified as PSE had higher CIE L*, lower pH_u and higher drip loss (%) than meat of normal characteristics. The opposite was found for DFD meat. Negative correlation between CIE L* and pH_u and positive correlations between CIE L* and CIE b* values, as well as between CIE L* and drip loss (%) in both hybrid lines were determined. Abdullah et al. (2010) investigated meat quality indicators of commercial broiler hybrids and determined that cooking loss was between 28.45–28.95%, W.H.C. 15.10–19.12%, and shear force 1.49–2.74 kg/cm^2. Kralik et al. (2014a) reported that drip loss value in breast muscles of 42-day-old Cobb 500 broilers varied from 3.09–4.14%, cooking loss was 21.49–33.29%, and shear force was 35.38–39.93 N.

According to the antioxidant status of broiler liver, authors of the present paper found significantly higher catalase activity in the MTO group. Silymarin proved to have strong antioxidant and hepatoprotective effects on the liver after induced hepatocellular damage in rats (Pradeep et al. 2007). Recent investigation showed that silymarin functioned as a

free radical scavenger, increasing reduced available glutathione (GSH) which functions as detoxicant of intermediary oxygen reactive products of lipoperoxidation (Soto et al. 2003). In a situation with increased reactive oxygen species (ROS) production, significant increase in the activities of SOD, GSHPX, and CAT in mice organs such as liver were measured (Sonnenbichler et al. 1976). They reported that transcription in the livers of rats and mice was accelerated *in vivo* under the influence of silymarin. Therefore, it can be supposed that *Silybum marianum* oil used in this study led to induced liver catalase biosynthesis, enhancing hepatocellular antioxidant defence. This is not in accordance with Blevins et al. (2010), who reported no influence of silymarin on the glutathione S transferase activity in broiler liver. Kiruthiga et al. (2007) found that silymarin prevented reduction in GSH activity, which was not the case in our research. The increase in hydrogen peroxide removal by liver catalase, and the subsequent decrease in H_2O_2-derived-ROS probably lead to a decrease of other antioxidative liver enzymes activities, such as glutathione-S-transferase, and glutathione reductase.

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