Effects of dietary alfalfa protein concentrate on lipid metabolism and antioxidative status of serum and composition and fatty acid profile and antioxidative status and dietetic value of muscles in broilers

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ABSTRACT The aim of this study was to determine the effect of addition of alfalfa protein concentrate (**APC**) at the dose of 15 g or 30 g per 1 kg on the chemical composition, fatty acid profile, dietary value, and antioxidant status in muscles and serum of Ross 308 chickens. The experiment involved 150 1-day-old Ross 308 broiler chickens allocated into 3 groups in 5 replications (10 birds per pen). A 1-way system with 2 levels of APC, 15 g or 30 g per 1 kg of a complete diet, was used. Group C receiving a standard feed mixture without APC was the control. The addition of 15 and 30 g APC increased the CP content in the breast muscle and in the thigh muscle, and reduction in the cholesterol and fat level was noted. Higher content of polyunsaturated fatty acids from the n-6 group was noted in the breast muscles of the 30-g APC–supplemented chickens. The thigh muscles with APC were characterized by more favorable values of the atherogenic index, thrombotic index, and hypocholesterol-to-hypercholesterol ratio.The supplementation with 30 g APC reduced the cholesterol and triacylglycerol levels, increased the high-density lipoprotein level, and decreased the low-density lipoprotein level and improved the antioxidant parameters in plasma (increase in superoxide dismutase and in glutathione peroxidase and reduction of malondialdehyde), compared with group C. The results of this study indicate that the supplementation with 30 g APC improved the metabolic functions of the organism, meat resistance to oxidative processes, and the composition and profile of fatty acids. Therefore, APC can be a potential alternative to synthetic feed additives and soya protein in production of healthier poultry meat.

Key words: broiler chicken, antioxidant status, fatty acid, meat quality, alfalfa protein concentrate

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INTRODUCTION

Global poultry meat consumption exhibits an upward trend, which is associated with the availability, low price, and health benefits of this type of meat. It contains a large amount of digestible protein, low cholesterol and fat contents, and a high proportion of unsaturated fatty acids (**UFA**) (Dinh et al., 2008). Consumption of the latter is especially important for health and proper function of the organism and plays an important role in prevention and alleviation of many civilization diseases, that is coronary disease, myocardial infarction, cerebral stroke, autoimmune diseases, and some cancers (Bird et al., 2018). Through appropriate composition of animal feed, the quantity of fatty acids can be modified, which is particularly important in light of the low consumption of polyunsaturated fatty acids (**PUFA**) owing to the limited availability of n-3 acid–rich food products (Pisulewski, 2005).

There is a current trend of reducing the amounts of synthetic feed additives owing to consumer concerns about their toxicity (Falowo et al., 2014) in favor of inexpensive and effective natural antioxidants, which exert a positive effect on the texture of meat and meat products and on consumer health (Karre et al., 2013). These include for example plant extracts: when introduced into poultry feed, they inhibit lipid oxidation and degradation of meat pigments and influence individual organoleptic traits (Falowo et al., 2014). There are only few studies suggesting that alfalfa and its products can be used as a plant-derived antioxidant ingredient in

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poultry feed (Karwowska et al., 2010). One of the additives produced from alfalfa (*Medicago sativa* L.) is the alfalfa protein concentrate (**APC**); in the literature, it is referred to as protein–xanthophyll concentrate or l 'Extrait Foliaire de Luzerne. It contains more than 50% of total protein (EFSA, 2009) and 0.59% of crude fiber in DM (Grela and Pietrzak, 2014).

Previous studies analyzed the suitability of APC in the nutrition for laying hens (Grela et al., 2014, 2020) and turkeys (Krauze and Grela, 2010; Karwatowska et al., 2010). However, there is insufficient knowledge of the effects of APC concentrate used in feed mixes for broilers (Kwiatkowska et al., 2017).

To our knowledge, this is the first study on the effects of APC on the plasma antioxidative potential and lipid metabolism, fatty acid composition and profile, antioxidant status, and dietary value of broiler meat. Nevertheless, we believe that these limitations do not undermine the originality of our study.

It was assumed in the present study that the APC concentrate added to the broiler chicken diet would improve the metabolic functions in the organism, meat resistance to oxidative processes, and the composition and profile of fatty acids. Therefore, this study aimed to investigate the effect of APC applied at the doses of 15 and 30 g kg^{-1} on the chemical composition, fatty acid profile, dietary value, and antioxidant status of muscles and serum of Ross 308 chickens.

MATERIALS AND METHODS

All procedures used during the research were approved by the Local Ethics Committee for Animal Testing at the University of Life Sciences in Lublin, Poland (Resolution No. 4/2009 of 20 January 2009). The chickens were maintained in an animal house as per the guidelines of this committee. The experiment complied with the Guiding Principles for Research Involving Animals.

Experimental Design, Birds, and Diet

A total of 150 1-day-old Ross 308 male broiler chicks (purchased on Hatchery and Poultry Farm Sławomir Pastuszak, Janów Lubelski, Poland) were assigned randomly into 3 groups. Each group comprised 50 male broilers (10 birds in each replicate × 5 pen replicates) The experiment lasted for 6 wk. The birds in cages were placed in a room with an initial temperature of 32°C, which was gradually reduced while the chickens were growing to 22°C in the fourth week of the experiment (Aviagen, 2013). Grain (wheat and corn) meal and recommended postextraction soybean meal were used for the production of the basal diet. The control group (C) received a standard complete diet (NRC, 1994), while soya bean meal in the experimental diets was replaced with APC in an amount of 15 g•kg^{-1} or 30 g•kg^{-1} produced by Desialis, France Luzerne. The chemical composition of APC is shown in Table 1. A three-phase feeding scheme was used: starter (from 1–21 d, crumbles), grower (from 22–35 d, pellets), and finisher (from 36–42 d, pellets). The composition and nutritional value of the diets are presented in Table 2. From 1 to 42 d of age, all the chickens were provided with water and feed ad libitum.

Table 1. Chemical composition of APC.

Nutrients, g•kg^{-1}	APC	Microelements, mg•kg^{-1}	APC
CP	533.9	Fe	497.0
Crude fat	103.7	Cu	10.2
Crude fiber	5.9	Zn	19.4
Crude ash	102.3		
Amino acids, g•kg^{-1}	APC	Fatty acids, g•kg^{-1}	APC
Histidine	12.1	C 14:0	1.4
Isoleucine	23.3	C 16:0	16.8
Leucine	44.9	C 16:1	6.4
Lysine	30.5	C 18:0	2.7
Methionine	10.0	C 18:1	5.2
Phenylalanine	27.2	C 18:2n-6	18.8
Threonine	22.2	C 18:3n-3	41.7
Tryptophan	11.8	C 20:0	0.9
Valine	29.8	C 22:0	0.8
Tyrosine	20.4	C 24:0	0.7
Arginine	29.4	$\sum$ Saturated fatty acids	23.3
Vitamins, mg•kg^{-1}		$\sum$ Monounsaturated fatty acids	11.6
Vitamin E	428.2	$\sum$ Polyunsaturated fatty acids	60.5
Vitamin K	95.3	n-6/n-3	4.5
B-Carotene	303.7		
L-Canavanine	3.2		
Macroelements, g•kg^{-1}	APC	Saponins, g•kg^{-1}	APC
Ca	32.9	Zahnic acid tridesmoside	0.97
P	7.9	Medicagenic acid 3 GlcGlc. 28 AraRhaXyl	0.77
K	7.4	Medicagenic acid 3 GlcA. 28 AraRhaXyl	5.82
Na	0.13	Medicagenic acid 3 Glc. 28 AraRhaXyl	0.52
Mg	1.5	Soyasaponin I	2.30

Abbreviation: APC, alfalfa protein concentrate.

Table 2. Ingredients and nutritive values of the experimental diets.

	Diets[1]/Rearing periods								
	Starter (1–21 d)			Grower (22–35 d)			Finisher (36–42 d)		
		APC, g•kg^{-1}			APC, g•kg^{-1}			APC, g•kg^{-1}	
Item	C	15	30	C	15	30	C	15	30
Ingredients, g•kg^{-1}									
Maize	247.0	247.0	247.0	300.0	300.0	300.0	300.0	300.0	300.0
Wheat	427.0	427.0	427.0	359.0	359.0	359.0	368.0	368.0	368.0
Soybean meal, 46% CP	259.5	244.5	229.5	265.0	250.0	235.0	257.0	242.0	227.0
Soybean oil	25.5	25.5	25.5	40.0	40.0	40.0	45.0	45.0	45.0
Monocalcium phosphate	8.6	8.6	8.6	9.0	9.0	9.0	7.2	7.2	7.2
Limestone	14.0	14.0	14.0	11.0	11.0	11.0	9.3	9.3	9.3
Sodium bicarbonate	0.8	0.8	0.8	0.8	0.8	0.8	0.5	0.5	0.5
NaCl	2.9	2.9	2.9	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin–mineral premix[2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Choline chloride	0.8	0.8	0.8	0.7	0.7	0.7	0.4	0.4	0.4
DL-methionine 99%	3.0	3.0	3.0	2.2	2.2	2.2	2.0	2.0	2.0
L-lysine HCl	3.6	3.6	3.6	2.5	2.5	2.5	2.1	2.1	2.1
L-threonine 99%	1.8	1.8	1.8	0.6	0.6	0.6	0.5	0.5	0.5
Calprona PL (acidifier)	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4
APC	-	15.0	30.0	-	15.0	30.0	-	15.0	30.0
Calculated of nutrients in 1 kg:									
ME, MJ	12.66	12.51	12.34	13.02	13.05	13.00	13.15	13.17	13.13
Available phosphorus, g	4.2	4.1	4.1	4.9	4.8	4.1	3.7	3.8	3.8
Analyzed values (each value based on triplicate determinations)									
Total protein, g•kg^{-1}	201.0	197.7	193.4	190.3	190.7	190.4	182.9	183.1	178.6
Crude fiber, g•kg^{-1}	30.5	30.1	29.6	29.3	29.6	29.1	29.8	29.6	29.0
Lysine, g•kg^{-1}	12.9	12.7	12.5	11.0	11.1	10.9	10.5	10.6	10.4
Methionine, g•kg^{-1}	5.9	5.9	5.8	4.9	5.0	4.9	4.6	4.7	4.7
Methionine + cysteine, g•kg^{-1}	9.3	9.2	9.1	8.1	8.1	8.0	7.8	7.9	7.8
Total calcium, g•kg^{-1}	8.8	8.7	8.9	9.4	9.5	8.9	8.2	8.3	8.1
Total phosphorus, g•kg^{-1}	6.7	6.6	6.5	6.4	6.6	6.5	6.1	6.2	6.1
Sodium, g•kg^{-1}	1.5	1.6	1.6	1.3	1.4	1.4	1.3	1.4	1.4
Zinc, mg•kg^{-1}	491.0	492	494	495	496	493	498	495	497
Iron, mg•kg^{-1}	899.0	904	908	599	602	605	598	604	602
Copper, mg•kg^{-1}	188.0	192	194	188	182	181	189	181	183
Fatty acids (g·100 g^{-1} of total fatty acids)									
Myristic (14:0)	0.50	0.52	0.51	0.47	0.48	0.49	0.48	0.47	0.49
Palmitic (16:0)	22.8	23. 9	24.2	22.5	24.1	23.6	22.4	21.1	22.3
Stearic (18:0)	6.72	6.31	5.98	5.67	6.99	6.43	7.02	6.85	6.56
Linoleic (18:2 n-6)	0.26	0.29	0.25	0.32	0.30	0.29	0.31	0.32	0.30
Linolenic (18:3 n-3)	23.5	23.4	22.9	24.2	23.9	25.9	25.9	25.4	26.3

Abbreviation: APC, alfalfa protein concentrate.

[1]Diets: C = control diet without supplementation, 15 APC = diet supplemented with alfalfa protein concentrate at 15 g kg^{-1} feed, 30 APC = diet supplemented with alfalfa protein concentrate at 30 g kg^{-1} feed.

[2]Provided per kg feed (for feeding periods 1–21, 22–35 and 36–42 d, respectively):Mn, 100, 100, 100 mg; I, 1, 1, 1 mg; Zn, 100, 100, 100 mg; Fe, 40, 40, 40 mg; Cu, 16, 16, 16 mg; Se, 0.15, 0.15, 0.15 mg; vitamin A, 15,000, 12,000, 12,000 UI; vitamin D_3, 5,000, 5,000, 5,000 UI; vitamin, E 75, 50, 50 mg; vitamin K_3, 4, 3, 2 mg; vitamin B_1, 3, 2, 2 mg; vitamin B_2, 8, 6, 5 mg; vitamin, B_6 5, 4, 3 mg; vitamin B_{12}, 0.016, 0.016, 0.011 µg; biotin, 0.2, 0.2, 0.05 mg; folic acid, 2, 1.75, 1.5 mg; nicotinic acid, 60, 60, 60 mg; pantothenic acid, 18, 18, 18 mg; choline, 1,800, 1,600, 1,600 mg.

Experimental Measurements

Before slaughter, the birds were fasted for 8 h (with unlimited access to water). The slaughter and dissection of the cocks of all groups were carried out in the same technological conditions. On day 42, all cocks were weighed to determine their preslaughter weight, and then, 10 birds from each group (2 of 10 birds in each replicate × 5 pen replicates) with a BW representative for the group were selected for slaughter. Next, the chickens were weighed, and a simplified slaughter analysis was performed (Ziołecki and Doruchowski, 1989), that is breast and thigh muscles were collected and weighed. The skin was separated from the muscles, and the muscles were packed into individual sealed plastic bags, and kept frozen at −20°C until chemical analysis.

Chemical Analysis of the Muscles and Feed

The muscle samples (n = 30) were analyzed for the content of DM (procedure 945.15; AOAC, 2006), ether extract (procedure 945.16; AOAC, 2006), CP (procedure 984.13; AOAC, 2006), crude ash (procedure 942.05; AOAC, 2006), and moisture (Method 925.09; AOAC, 2019). The feed samples (n = 3) were analyzed for the content of CP (procedure 984.13; AOAC, 2006), and crude fiber was determined as per procedure 978.10 (AOAC, 2006) using an Ankom 220 Fiber Analyzer (Ankom Technology, Macedon, NY). The contents of

minerals (Ca, Zn, Cu, Fe, P) in the feed were determined, as described in detail by Kwiecień et al. (2016).

Before the determination of the amino acid composition in the feed, the samples were hydrolyzed in an aqueous solution (6N HCI + 0.5% phenol at 110°C for 24 h) and analyzed by ion-exchange chromatography in an AAA 400 amino acid analyzer (Ingos Ltd., Praha, Czech Republic) as described in detail by Kwiecień et al. (2016).

The β-carotene content was determined with the method proposed by Hager and Mayer (1996). The content of vitamins E and K was determined with the HPLC with fluorescence detection technique, using the Sigma-Aldrich standard. Quantification of the content of vitamin K and vitamin E was carried out at the excitation/emission wavelength of 330/400 nm and 295/330 nm, respectively, and their concentrations were calculated from the parameters of calibration curves for the individual vitamins. The content of L-kanavanin and saponins was analyzed by ultra-performance liquid chromatography and HPLC, respectively (Oleszek, 2002).

Fatty Acid and Cholesterol Analysis

The total cholesterol content in the muscles was determined with the colorimetric method using an EPOLL 20 colorimeter and C3045 standard (Sigma) (Winiarska-Mieczan and Kwiecień, 2015). The content of fatty acids in the feed and muscles was determined after extraction of fat with a mixture of chloroform and methanol using the method described by Folch et al. (1957). The content of fatty acids in the feed and muscles was analyzed using a Varian CP-3800 gas chromatograph with a GC-FID detector (Varian, The Netherlands) and a capillary column CP-Wax52CBWCOT Fused Silica with the following parameters:1) 60 m length, 0.25 mm inner diameter; 2) initial temperature of 120°C, increasing gradually by 2°C/min, and final temperature of 210°C; 3) temperature of the injector and detector as 260°C; 4) hydrogen flow of 30 ml/min, air flow of 300 mL/min, and helium flow of 1.4 mL/min; 5) volume of the injected sample is 1 mL.

The StarGC Workstation, version 6.30, was used for determination of the percentage content of fatty acids in the sample, and fatty acid methyl esters were identified by comparison with the retention time of 37 fatty acid methyl esters contained in the standard mixture (FAME Mix, C4-C24, No. 18919–1AMP; Sigma-Aldrich Poznań, Poland) analyzed in the same conditions.

The content of each fatty acid was expressed as a percentage of all the fatty acids detected in the analysis. The dietary value of meat, that is the atherogenic (**AI**) and thrombogenic (**TI**) indices and the hypocholesterolemic/hypercholesterolemic ratio (**h/H**), were calculated as follows:

$$AI = (C12:0 + 4 \times C14:0 + C16:0) \Big/ \left(\sum MUFA + \sum (n-6) + \sum (n-3)\right)$$

$$TI = (C14:0 + C16:0 + C18:0) \Big/ \left(\left(0.5 \times \sum MUFA + 0.5 \times \sum (n-6) + 3 \times \sum (n-3)\right)\left(\sum (n-3) \Big/ \sum (n-6)\right)\right)$$

based on the study by Ulbricht and Southgate (1991) and

$$h/H = (C18:In-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0)$$

based on the study by Fernández et al. (2007).

Lipid Profile in Serum and Antioxidant Status in Serum and Muscles

On day 42, blood was sampled from 10 cocks selected for the slaughter (2 broiler chickens from each repetition). Eight hours before blood collection, the cocks did not receive feed but were provided with water ad libitum. In the morning before slaughter, blood samples intended for determination of the lipid concentration and antioxidant status were collected from the vena cutanea ulnaris into 6-ml lithium heparin-containing Vacutest tubes (Vacutest Kima s.r.l.) (Kwiecień et al., 2017). Plasma, for analysis of biochemical parameters, was obtained by centrifugation of whole blood at 3,000 rpm (603 × *g*) for 15 min at 4°C in a laboratory centrifuge (MPW-350R centrifuge, MPW Medical Instruments, Warsaw, Poland) (Kwiecień et al., 2017). Cormay kits (PZ CORMAY SA, Łomianki, Poland) were used to determine the content of triglycerides (**TG**), total cholesterol (**TC**), high-density lipoprotein (**HDL**), and low-density lipoprotein (**LDL**).

Spectrophotometric methods were used to determine superoxide dismutase (**SOD**) activity in the muscles and serum (Misra and Fridowich, 1972) at a wavelength λ = 480 nm and glutathione peroxidase (**GPx**) activity at λ = 340 nm using ready Bioxytech GPx-340 assays (OxisResearch, Portland, OR). The catalase (**CAT**) activity was determined at λ = 570 nm with the colorimetric method developed by Sinha (1972). In addition, the biological material was analyzed for the levels of the lipid peroxidation product malondialdehyde (**MDA**) as the end product of tissue lipid oxidation with the method proposed by Salih et al. (1987). The MDA results were expressed in nmol per 1 mg protein for the meat and in nmol per 1 mL for the serum.

Statistical Analysis

The numerical data were analyzed with the 1-way ANOVA method, where the effect of the APC supplementation was evaluated (α = 95%; $P < 0.05$ and α = 99%; $P < 0.01$) with the following model:

$$yijk = \mu + \alpha i + eijk$$

where yijk = kth observation from the ith and jth groups, μ = mean value of the trait in the population, αi = effect of the ith group, eijk − error = effect related to individual variability and measurement error.

The significance of the differences between the mean values of the analyzed parameters was determined using the Duncan test (post test hoc) in the Statistica 10.0 program (Statsoft Inc., Tulsa).

RESULTS

Selected Indicators the Carcass

In the groups of cocks receiving the APC supplement, an average 10.6% increase ($P < 0.05$) was observed in the chilled carcass weight after slaughter and a significant ($P < 0.01$) increase in the weight of the breast (by 22.9% on average) and thigh (by 26.9% on average) muscles, compared with the control group (Table 3).

Chemical Composition of Raw Breast and Thigh Meat

The APC supplementation of the diets at the doses of 15 and 30 g kg^{-1} increased the CP content in the breast muscle (by 5.4 and 5.6%, respectively) and in the thigh muscle (by 5.4 and 6.5%, respectively), compared with group C (Table 4). The addition of APC at the levels of 15 and 30 g kg^{-1} contributed to a significant ($P < 0.01$) decrease in the fat content in the breast muscle (by 1.8 and 11.2%, respectively) and in the thigh muscle (on average by 6.5%), in comparison with group C. The DM and crude ash contents were similar in the groups. In the APC-supplemented groups, the cholesterol level decreased significantly ($P < 0.01$) by 17 and 14%, respectively, in the breast muscle and on average by 13.2% in the thigh muscle ($P < 0.05$).

Fatty Acid Contents and Indices of the Dietary Value of Meat

Compared with group C, chickens fed with the APC-supplemented diets had higher contents ($P < 0.05$) of eicosadienoic and arachidonic acids and a lower level of saturated margaric (C 17:0) and arachidic (C 20:0) acids in the breast muscle (Table 5). However, the PUFA/saturated fatty acids (**SFA**) ratio was unaffected ($P < 0.05$). The n-6/n-3 ratio was higher ($P < 0.05$) in the breast muscles of chickens receiving 15 g•kg^{-1} APC, in comparison with group C. The diet supplemented with 30 g•kg^{-1}APC was associated with higher content ($P < 0.05$) of n-6 fatty acids than in the control group, but there was no effect ($P > 0.05$) of the diet on the sum of PUFA and SFA.

The meat from chickens fed with the APC-supplemented diet had higher content ($P < 0.05$) of eicosadienoic acids and arachidonic acids with a lower level ($P < 0.05$) of saturated palmitic and margaric acids as well as significantly lower content ($P < 0.01$) of arachidic acid in the thigh muscle, compared with group C (Table 6). This was reflected in the PUFA/SFA ratio ($P < 0.05$). The lowest ($P < 0.05$) n-6/n-3 ratio in chicken thigh muscles was observed in the group

Table 3. Selected indicators the carcass analysis of male broilers on the 42 d of age.

	Treatments[1]				
		APC, g•kg^{-1}			
Item	C	15	30	SEM	*P* value
Chilled carcass weight (legs + head), g	1,499^{b}	1,638^{a}	1,678^{a}	31.940	0.049
Weight of carcass (g)					
Breast meat	344.5^{c,C}	406.7^{b,B}	439.9^{a,A}	7.230	0.001
Thigh meat	143.9^{c,C}	163.7^{b,B}	201.7^{a,A}	3.700	0.001

^{a,b}Means in the same rows with different letters differ significantly at $P < 0.05$.
$^{A-C}$Means in the same rows with different letters differ significantly at $P < 0.01$.
Abbreviation: APC, alfalfa protein concentrate.
[1]Treatments: C = control diet without supplementation, 15 APC = diet supplemented with alfalfa protein concentrate at 15 g kg^{-1} feed, 30 APC = diet supplemented with alfalfa protein concentrate at 30 g kg^{-1} feed.

Table 4. Moisture, and chemical composition of crude breast and thigh meat of male broilers.

	Treatments[1]				
		APC, g•kg^{-1}			
Item	C	15	30	SEM	*P* value
Breast meat (n = 30)					
Moisture, %	74.9	74.8	74.9	8.395	0.222
DM, %	25.1	25.2	25.1	0.652	0.062
Crude ash, %	1.28	1.24	1.30	1.986	0.054
CP, %	23.2^{b}	24.7^{a}	24.5^{a}	1.003	0.045
Crude fat, %	2.23^{a,A}	2.19^{a,B}	1.98^{b,C}	0.086	0.005
Cholesterol, mg•100 g^{-1}	54.2^{a,A}	45.0^{b,C}	46.6^{b,B}	2.188	0.004
Thigh meat (n = 30)					
Moisture, %	74.0	74.5	74.7	7.495	0.122
DM, %	26.0	25.5	25.3	0.706	0.061
Crude ash, %	1.21	1.23	1.20	2.554	0.054
CP, %	18.4^{b}	19.4^{a}	19.6^{a}	1.025	0.044
Crude fat, %	2.77^{a,A}	2.63^{a,b,B}	2.58^{b,B}	0.114	0.006
Cholesterol, mg•100 g^{-1}	47.4^{a}	40.2^{b}	42.1^{b}	0.025	0.042

^{a,b}Means in the same rows with different letters differ significantly at $P < 0.05$.
$^{A-C}$Means in the same rows with different letters differ significantly at $P < 0.01$.
Abbreviation: APC, alfalfa protein concentrate.
[1]Treatments: C = control diet without supplementation, 15 APC = diet supplemented with alfalfa protein concentrate at 15 g kg^{-1} feed, 30 APC = diet supplemented with alfalfa protein concentrate at 30 g kg^{-1} feed.

receiving 15 g•kg^{-1} APC compared with the other groups (C and 30 g•kg^{-1} APC). The thigh muscles of chickens receiving APC were characterized by higher dietary values, as demonstrated by the AI, TI, and h/H values (Table 6).

An effect ($P < 0.05$, $P < 0.01$) of the type of meat on the total fatty acid content was found (Table 7). Significantly higher amounts of these acids were determined in the thigh muscle, regardless of the experimental factor used. No significant differences were found only in the content of PUFA n-3 in the group receiving 15-g APC.

Antioxidant Status in Meat

It was found that the APC supplementation of the diets improved the oxidative stress markers in the chicken meat (Table 8). The SOD and CAT activities in the breast muscle of chickens receiving 30 g•kg^{-1}APC were higher than those in group C. Lipid peroxidation, determined based on the MDA concentration in the breast muscle, was significantly reduced in the group of birds receiving 30 g kg^{-1} of APC, compared with group C. In the thigh muscle, the addition of 30 g•kg^{-1} APC resulted in a 7.7% increase in CAT activity, in comparison with group C. The best effect in both muscles was obtained using APC at the dose of 30 g kg^{-1}.

Lipid Profile and Antioxidants in Serum

Compared with group C, the supplementation with 30 g•kg^{-1} APC reduced the TC and TG levels ($P < 0.05$) by 19.2 and 9.1%, respectively, increased the HDL content ($P < 0.05$) by 19.7%, and decreased ($P < 0.01$) the LDL level significantly in the APC-supplemented groups by 12.1 and 17.6%, respectively (Table 9).

It was found that APC improved the antioxidant parameters (Table 9). The SOD and GPx levels in the serum of 42-day-old chickens receiving diet supplemented with 30 g kg^{-1} of APC were higher ($P < 0.05$), that is by 8.3% for SOD and by 5.8% GPx, than in those of group C. As shown in Table 9, the addition of APC reduced the MDA serum levels. The level of MDA in chickens receiving 30 g•kg^{-1} APC was lower than that in group C ($P < 0.01$). This dose of APC proved to exert the best effect on the measured parameters.

DISCUSSION

Research on phytobiotics suggest that the biologically active compounds contained therein serve many functions in various biological systems, causing changes in their chemical structure and demonstrating anti-inflammatory, anticancer, antilipid activity, or affinity for estrogen receptors with an ability to activate them (Windisch et al., 2008). Because they can act similarly to hormones, phytobiotics may influence chicken growth (Chen et al., 2016; Ouyang et al., 2016; Kwiatkowska et al., 2017). This is associated with the fact that flavones can regulate the binding of growth hormone to the liver growth hormone receptor and cause an increase in the concentration of insulin-like growth factor-1. In addition, isoflavones can promote muscle protein synthesis and stimulate muscle growth (Kamboh and Zhu, 2013).

From the nutritional point of view, poultry meat has relatively low fat content (Barroeta, 2007). In the present study, APC (30 g kg^{-1}) reduced the fat content in both muscles and increased the amount of CP. Despite

Table 5. Fatty acid profile (g/100 g of total fatty acids) in male broilers breast meat.

	Treatments[1]				
		APC, g•kg^{-1}			
Fatty acids	C	15	30	SEM	*P* value
SFA					
14:0	0.381	0.391	0.394	0.021	0.120
15:0	0.120	0.122	0.125	0.012	0.235
16:0	22.91	23.53	23.84	0.846	0.114
17:0	0.101^{a,A}	0.090^{b,B}	0.089^{b,B}	0.026	0.010
18:0	8.352	8.871	8.461	0.183	0.201
20:0	0.150^{a}	0.125^{b}	0.120^{b}	0.023	0.032
MUFA					
16:1	0.461^{b}	0.513^{a}	0.481^{a}	0.028	0.013
18:1	31.21	30.24	30.62	0.833	0.084
20:1	0.230	0.222	0.221	0.033	0.261
PUFA					
18:2$_{n-6}$	22.25	23.21	23.34	0.012	0.086
20:2$_{n-6}$	0.224^{b}	0.277^{a}	0.270^{a}	0.290	0.021
20:4$_{n-6}$	0.254^{b}	0.311^{a}	0.312^{a}	0.017	0.011
18:3$_{n-3}$	2.660	2.562	2.690	0.026	0.078
20:3$_{n-3}$	0.140^{b,C}	0.161^{a,A}	0.152^{a,B}	0.006	0.008
∑ SFA	32.01	33.15	33.06	0.950	0.097
∑ MUFA	31.90	30.75	31.10	0.857	0.114
∑ PUFA	25.53	26.52	26.76	0.326	0.059
∑ UFA	57.43	57.27	57.87	1.082	0.068
∑ PUFA$_{n-6}$	22.73^{b}	23.80^{a,b}	23.92^{a}	0.292	0.014
∑ PUFA$_{n-3}$	2.800	2.723	2.842	0.028	0.087
∑ PUFA/SFA	0.797	0.800	0.809	0.026	0.331
Indices of the dietary value of meat					
n-6/n-3[2]	8.117^{b}	8.740^{a}	8.417^{a,b}	1.292	0.041
AI	0.425	0.440	0.442	0.011	0.185
TI	0.722	0.743	0.737	0.021	0.262
h/H	2.420	2.352	2.346	0.081	0.267

Data represent the mean of 10 broiler chickens per treatment.

^{a,b}Means in the same rows with different letters differ significantly at $P < 0.05$.

$^{A-C}$Means in the same rows with different letters differ significantly at $P < 0.01$.

Abbreviations: AI, atherogenic index; APC, alfalfa protein concentrate; h/H, hypocholesterolemic/hypercholesterolemic ratio; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TI, thrombogenic index; UFA, unsaturated fatty acids.

[1]Treatments: C = control diet without supplementation, 15 APC = diet supplemented with alfalfa protein concentrate at 15 g kg^{-1} feed, 30 APC = diet supplemented with alfalfa protein concentrate at 30 g kg^{-1} feed.

[2]The calculated n-6/n-3 ratio was a sum of [(C18:2 n-6, C20:2 n-6, C20:4 n-6)/(C18:3 n-3, 20:3n-3)].

Table 6. Fatty acid profile (g/100 g of total fatty acids) in male broilers thigh meat.

	Treatments[1]				
		APC, g•kg^{-1}			
Fatty acids	C	15	30	SEM	*P* value
SFA					
14:0	0.503	0.481	0.483	0.013	0.757
15:0	0.100	0.103	0.104	0.003	0.905
16:0	24.01^{a}	22.41^{b}	22.43^{b}	0.044	0.029
17:0	0.153^{a}	0.143^{b}	0.145^{b}	0.006	0.036
18:0	6.86^{b}	7.22^{a}	6.93^{a,b}	0.174	0.048
20:0	0.126^{a}	0.115^{b}	0.106^{c}	0.005	0.043
MUFA					
16:1	0.250^{b}	0.310^{a}	0.320^{a}	0.013	0.044
18:1	33.86	34.57	34.58	0.230	0.351
20:1	0.280^{b,B}	0.320^{a,A}	0.320^{a,A}	0.006	0.003
PUFA					
18:2$_{n-6}$	23.86	24.57	24.58	0.010	0.096
20:2$_{n-6}$	0.250^{b}	0.310^{a}	0.320^{a}	0.402	0.046
20:4$_{n-6}$	0.280^{b}	0.320^{a}	0.320^{a}	0.001	0.015
18:3$_{n-3}$	2.390^{b}	2.600^{a}	2.440^{b}	0.047	0.032
20:3$_{n-3}$	0.150^{b}	0.170^{a}	0.161^{a}	0.004	0.011
∑ SFA	31.73	30.47	30.22	0.760	0.646
∑ MUFA	34.39	35.2	35.22	0.255	0.189
∑ PUFA	26.93	27.96	27.82	0.421	0.074
∑ UFA	61.32	63.17	63.04	0.595	0.052
∑ PUFA$_{n-6}$	24.39	25.20	25.22	0.048	0.208
∑ PUFA$_{n-3}$	2.540^{b}	2.770^{a}	2.601^{b}	0.397	0.031
∑ PUFA/SFA	0.849^{b}	0.918^{a}	0.921^{a}	0.028	0.041
Indices of dietary value of meat					
n-6/n-3[2]	9.60^{a}	9.10^{b}	9.70^{a}	0.171	0.033
AI	0.424^{a}	0.385^{b}	0.386^{b}	0.011	0.025
TI	0.673^{a}	0.632^{b}	0.625^{b}	0.021	0.042
h/H	2.464^{b}	2.711^{a}	2.702^{a}	0.081	0.017

Data represent the mean of 10 broiler chickens per treatment.

^{a,b}Means in the same rows with different letters differ significantly at $P < 0.05$.

$^{A-C}$Means in the same rows with different letters differ significantly at $P < 0.01$.

Abbreviations: AI, atherogenic index; APC, alfalfa protein concentrate; h/H, hypocholesterolemic/hypercholesterolemic ratio; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TI, thrombogenic index; UFA, unsaturated fatty acids.

[1]Treatments: C = control diet without supplementation, 15 APC = diet supplemented with alfalfa protein concentrate at 15 g kg^{-1} feed, 30 APC = diet supplemented with alfalfa protein concentrate at 30 g kg^{-1} feed.

[2]The calculated n-6/n-3 ratio was a sum of [(C18:2 n-6, C20:2 n-6, C20:4 n-6)/(C18:3 n-3, 20:3n-3)].

its many positive properties, APC also contains antinutritional compounds such as saponins, which are involved in reduction of total cholesterol levels through formation of insoluble complexes with cholesterol, thereby inhibiting absorption thereof. Moreover, they increase secretion of bile, gastric, pancreatic, and intestinal juices (Khaleel et al., 2005). In the present study, the addition of APC (15 and 30 g kg^{-1}) contributed to reduction of muscle cholesterol levels. This may prove the inhibitory dietary effects of flavonoids on the key enzyme in cholesterol biosynthesis, that is HMG-CoA reductase (Lien et al., 2008), or may be associated with reduced absorption of cholesterol and bile acids from the intestinal lumen (Cavallini et al., 2009). Reduction of muscle cholesterol content is valuable to consumers, as it lowers the risk of cardiovascular disease. As indicated by research results, even a low level of flavonoids may enhance cholesterol metabolism and reduce its accumulation in the organism, thus improving meat quality (Chen et al., 2016).

The main reason for the introduction of phytobiotic additives is that, besides improvement of production yields (Kwiatkowska et al., 2017), they can influence the quality of animal products. This in turn can be valuable for prevention of some human diseases (Bird et al., 2018). Production and consumption of such chicken meat can be safe even at prolonged supplementation: APC practically does not contain *Aspergillus flavus* toxins, pesticides, or heavy metals, and the number of antinutritional compounds does not exceed permissible values, which seems to be safe for human and animal health (EFSA, 2009). Furthermore, the APC is dominated by PUFA, with the largest amount of omega-3 fatty acids and has an impact on the chemical composition of broiler meat (Grela and Pietrzak, 2014).

Table 7. Impact of the type of meat on the total fatty acid content.

Item	CBM × CTB	15BM × 15 TM	30 BM × 30 TM
	P value		
SFA	0.121	0.031	0.043
MUFA	0.008	0.009	0.003
PUFA	0.010	0.006	0.031
UFA	0.001	0.004	0.010
$PUFA_{n-6}$	0.001	0.007	0.006
$PUFA_{n-3}$	0.010	NS	0.021
PUFA/SFA	0.003	0.007	0.010

Abbreviations: CBM, control diet, breast meat; CTM, control diet, thigh meat; MUFA, monounsaturated fatty acids; NS, statistically insignificant; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; 15 BM, diet supplemented with alfalfa protein concentrate at 15 g kg^{-1} feed, breast meat; 15 TM, diet supplemented with alfalfa protein concentrate at 15 g kg^{-1} feed, thigh meat; 30 BM, diet supplemented with alfalfa protein concentrate at 30 g kg^{-1} feed, breast meat; 30 TM, diet supplemented with alfalfa protein concentrate at 30 g kg^{-1} feed, thigh meat.

In recent years, there has been clearly increasing interest in PUFA, especially from the n-3 group, in human and animal nutrition. Consumption of n-3 PUFA, in particular those with >18 C atoms, is low owing to the limited availability of food products (e.g., fish). On the one hand, there is interest in enrichment of poultry meat with these fatty acids, especially among consumers that lead a healthy lifestyle (Sirri et al., 2011). On the other hand, these acids are more susceptible to oxidation (Al-Khalifa, 2015).

Oxidation stability in poultry meat is important owing to the high content of UFA from the n-3 and n-6 groups (Attia et al., 2019). It has been shown that alfalfa preparations added to the diets for birds can increase the content of fatty acids in muscles and improve their profile (Jiang et al., 2012). In the present study, the most favorable n-6/n-3 ratio in the breast muscle was found in the group supplemented with 15 g•kg^{-1} APC, whereas the 30-g APC dose contributed to the most optimal value of the ratio in the thigh muscle. The experimental factor did not cause changes in the total fatty acid content. The n-3 fatty acids ($P < 0.05$) had the greatest effect on the n-3 PUFA proportions in the thigh muscle at the lower APC level. Based on the present results, it can be concluded that APC (30 g kg^{-1}) can sufficiently inhibit lipid oxidation (MDA production) in the breast meat of broiler chickens, thereby preventing oxidation of oxidatively labile PUFA rather than the more stable SFA in meat. In poultry, increased PUFA concentrations can reduce monounsaturated fatty acid (**MUFA**) synthesis through inhibition of the activity of the 9-desaturase complex, that is a key enzyme that converts SFA into MUFA, and supplementation with antioxidants is the basic strategy for reducing SFA and increasing the proportion of PUFA in poultry meat (Kamboh and Zhu, 2013). Moreover, the increased ratios of PUFA: SFA (15 and 30 g APC) and n-6:n-3 (30 g APC) in the thigh muscle observed in our study may be related to the protective role of antioxidants, which act as electron donors, providing them with electrons required for reduction of certain UFA, and are metabolized by microorganisms to these donors (Chikunya et al., 2004). To the best of our knowledge, the additional effect of APC on the fatty acid profile in broiler chicken meat is largely unknown, as there is no research in this field.

The fatty acid composition depends mainly on the poultry species and may change depending on the feed composition. Other factors, for example the genotype, type, and age of the animals, may also influence the fatty acid composition of poultry meat (Woods and Fearon, 2009). Changes in the proportions between saturated and unsaturated acids are an unfavorable phenomenon from the dietary point of view. In the present study, there was no significant effect of APC on the proportions between SFA and UFA (SFA, MUFA, PUFA, UFA), despite the differences between the groups in the content of some acids. There were also no differences in the fatty acid content of the breast and thigh muscles, despite the higher fat content in the thigh muscle. Because these were not directional modifications, it is not possible based on the present

Table 8. Antioxidant enzyme activity in crude male broilers breast and thigh meat.

Item	Treatments[1]			SEM	*P* Value
		APC, g•kg^{-17}			
	C	15	30		
Breast meat					
SOD, U/g protein	42.33[b]	43.82[a,b]	46.13[a]	0.214	0.036
CAT, U/mg protein	0.278[b]	0.290[a,b]	0.303[a]	0.012	0.034
GPx, U/mg protein	2,541.0	2,624.0	2,621.0	31.47	0.219
MDA, nmol/mg protein	1.515[a]	1.510[a,b]	1.439[b]	0.023	0.021
Thigh meat					
SOD, U/g protein	40.53	41.47	40.12	0.351	0.056
CAT, U/mg protein	0.273[b]	0.285[a,b]	0.294[a]	0.035	0.041
GPx, U/mg protein	2,531.0	2,598.0	2,601.0	30.41	0.232
MDA, nmol/mg protein	1.435	1.399	1.389	0.045	0.111

[a,b]Means in the same rows with different letters differ significantly at $P < 0.05$.

Data represent the mean of 10 broiler chickens per treatment.

Abbreviations: APC, alfalfa protein concentrate; CAT, catalase; GPx, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

[1]Treatments: C = control diet without supplementation, 15 APC = diet supplemented with alfalfa protein concentrate at 15 g kg^{-1} feed, 30 APC = diet supplemented with alfalfa protein concentrate at 30 g kg^{-1} feed.

Table 9. Lipid concentration and antioxidant enzyme in male broilers plasma.

	Treatments[1]				
		APC, g•kg^{-1}			
Item	C	15	30	SEM	*P* Value
TC, mg•dL^{-1}	2.76^{a}	2.38^{a,b}	2.23^{b}	0.228	0.031
TG, mg•dL^{-1}	0.44^{a}	0.42^{a,b}	0.40^{b}	0.021	0.025
HDL, mg•dL^{-1}	1.83^{b}	2.00^{a,b}	2.19^{a}	0.006	0.016
LDL, mg•dL^{-1}	1.99^{a,A}	1.75^{a,b,B}	1.64^{b,C}	0.025	0.009
SOD, U•mg^{-1} of protein	43.52^{b}	45.18^{a,b}	47.15^{a}	2.388	0.031
CAT, U•mg^{-1} of protein	56.30^{b}	58.19^{a,b}	59.54^{a}	0.782	0.024
GPx, U•mg^{-1} of protein	5.42	5.39	5.48	0.024	0.068
MDA, nmol•mL^{-1} of serum	40.51^{a,A}	38.51^{a,b,B}	37.28^{b,C}	0.311	0.003

^{a,b}Means in the same rows with different letters differ significantly at $P < 0.05$.
$^{A-C}$Means in the same rows with different letters differ significantly at $P < 0.01$.
Data represent the mean of 10 broiler chickens per treatment.
Abbreviations: APC, alfalfa protein concentrate; CAT, catalase; GPx, glutathione peroxidase; HDL, high density lipoprotein; LDL, low density lipoprotein; MDA, malondialdehyde; SOD, superoxide dismutase; TC, total cholesterol; TG, total triglyceride.
[1]Treatments: C = control diet without supplementation, 15 APC = diet supplemented with alfalfa protein concentrate at 15 g kg^{-1} feed, 30 APC = diet supplemented with alfalfa protein concentrate at 30 g kg^{-1} feed.

results to determine unequivocally the effect of the APC application on the fatty acid profile in the muscles of the studied broiler chickens. Similarly, no significant impact of the experimental factor on the proportion of acids in both muscles was found in a study conducted by Winiarska and Kwiecień (2015). In other investigations (Kiczorowska et al., 2015), only a tendency toward a reduction in the amount of SFA sum in favor of the MUFA sum was observed in breast and thigh muscles.

Owing to the substantial amounts of UFA, poultry meat is more susceptible to oxidation processes and changes in the dietary value (Attia et al., 2019). Atherogenic index and TI values indicate to what extent the fatty acid–containing components of the human diet may contribute to an increase in the incidence of coronary artery disease and atherosclerosis. The lower value is associated with the lower probability of atherosclerosis and blood clot formation (Simitzis et al., 2011). The present study showed a clearly positive effect of the experimental factor on the AI and TI observed only in the thigh meat of the chickens receiving APC, compared with group C, whereas no such effect on the examined parameters in the breast muscle was found. Similarly, the value of the h/H ratio in the thigh muscle of chickens receiving APC, regardless of the dose, was more favorable (higher) than in group C. There was no such effect on the breast muscle.

In the present study, the supplementation with APC at a dose of 30 g•kg^{-1} yielded a significant increase in the SOD activity in the breast muscle and a decrease in the MDA levels, compared with group C. In addition, higher CAT activity than the value in group C was observed in both muscles. This suggests that administration of 15 g•kg^{-1} APC does not produce differences in the activity of antioxidant enzymes; therefore, a higher amount of the compound (30 g kg^{-1}) is required to protect tissues against lipid oxidation products. This amount increased the antioxidant capacity primarily in the chicken breast muscles, probably via improvement of the ability to scavenge free radicals and inhibit oxidative processes.

Appropriate nutritional strategies can modify the quantitative composition of fatty acids, mainly by changing the cholesterol content via the complex mechanism of cholesterol biosynthesis, regulation, and metabolism (Ting et al., 2011). The present study showed that the supplementation of 30 g•kg^{-1} APC caused a decrease in the content of TC, TG, and LDL and an increase in the level of HDL. This may indicate that the bioactive substances contained in APC play an important role in modulation of lipid metabolism. The present results are in agreement with those reported by Ouyang et al. (2016), where a decline in serum TC and LDL levels was observed with a simultaneous increase in HDL levels. Similarly, Chen et al. (2016) demonstrated that different amounts of alfalfa flavonoids added to the diet reduced the levels of TG, TC (300 mg/kg), and LDL (150, 300, and 450 mg/kg) and improved the HDL content in goose serum (150, 300, and 450 mg/kg).

Investigations of antioxidant substances of plant origin indicate that they exert antioxidant effects through free radical scavenging, reduce the levels of LDL and DNA susceptibility to oxidative stress, and increase the activity and expression of antioxidant enzymes (Erba et al., 2012). The addition of 15 g•kg^{-1} APC did not change the SOD, CAT, MDA, and GPx activity, compared with group C, suggesting that this dose may not be sufficient to increase the antioxidant potential in chicken serum. In contrast, the supplementation with 30 g•kg^{-1} APC caused a significant increase in SOD and CAT activity and reduced the concentration of MDA in the serum, compared with group C. This improvement in the antioxidant capacity may be related to the fact that the increase in the dose of APC (up to 30 g kg^{-1}), which has strong antioxidant molecules, can exert a protective effect by activating

antioxidant enzymes or increasing the concentration of low-molecular-weight antioxidants (Brenes et al., 2016). In turn, Ouyang et al. (2016) demonstrated that application of alfalfa flavonoids increased the levels of CAT, SOD, and glutathione peroxidase and reduced MDA content in serum in a dose-dependent manner. The best effect was achieved at a dose of 15 mg/kg. Similarly, Jiang et al. (2007) reported a dose-dependent (10–80 mg/kg) effect of soy flavonoids on the increase in total antioxidant activity in the serum of broiler chickens. It can therefore be assumed that the effect of polyphenolic compounds on the antioxidant status and plasma lipid oxidation in chickens may be dependent on various factors, for example the composition, concentration, or bioavailability of these molecules as well as the species and sex of chickens (Gessner et al., 2017).

CONCLUSION

The scientific novelty of this study is the first-time description of the effects of APC concentrate on plasma antioxidant potential and lipid metabolism as well as the composition and profile of fatty acids, antioxidant status, and dietary value of muscle in broiler chickens. It was found that the inclusion of APC in the diet, regardless of the amount, increased carcass weight and crude protein content in both muscles (on average by 6%) and reduced the cholesterol level (meat: breast by 16%, thigh by 13%) and the content of crude fat (meat: breast by 6.5%, thigh by 13%).

The higher dose of APC increased the content of n-6 PUFA in the pectoral muscle, but a more favorable n-6/n-3 ratio was observed at the dose of 15 g kg^{-1}. The highest level of n-3 PUFA in the thigh muscle was found in the group supplemented with the lower dose of APC; however, an unfavorable ratio of n-6/n-3 acids was detected in this group. The addition of APC, regardless of the amount, contributed to the improvement of atherogenic parameters in thigh muscles expressed by lower values of AI and TI and a higher h/H value.

The addition of APC at the dose of 30 g kg^{-1} increased the activity of antioxidant enzymes in the muscles breast muscles – SOD by 8.9%, CAT by 9% – thigh muscles – CAT by 7.7% – and simultaneously reduced MDA activity (by 6%) in the breast muscle.

The study has demonstrated that biologically active substances contained in APC can play an important role in modulation of lipid metabolism, which was reflected in the lower plasma lipid levels (TC by 19%, TG by 9%, LDL by 18%) and the higher HDL (by 19.6%) content in plasma. The higher APC dose was found to be sufficient to increase the antioxidant potential in chicken plasma (increased SOD by 8.3% and CAT by 5.8% activity and reduced MDA by 7.9% concentration).

Therefore, APC can be a potential alternative to synthetic feed additives and soya protein in production of healthier poultry meat.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

Al-Khalifa, H. 2015. Production of added-value poultry meat: enrichment with n-3 polyunsaturated fatty acids. World Poult. Sci. J. 71:319–326.

AOAC. 2006. Offcial Methods of Analysis. 18th ed. Association of Offcial Analytical Chemists, AOAC International, Gaithersburg, MD.

AOAC. 2019. Association of Offcial Analytical Chemists. Offcial Methods of Analysis. 21st ed. AOAC, Gaithersburg, MD.

Attia, Y. A., N. F. Addeo, A. A. E. Abd Al-Hamid, and F. Bovera. 2019. Effects of phytase supplementation to diets with or without zinc addition on growth performance and zinc utilization of white pekin ducks. Animals 9:280.

Aviagen. 2013. Ross 308 parent stock nutrition specifications. Accessed Nov. 2020. www.en.aviagen.com.

Barroeta, A. C. 2007. Nutritive value of poultry meat: relationship between vitamin and PUFA. World Poult. Sci. J. 63:277–284.

Bird, J. K., P. C. Calder, and M. Eggersdorfer. 2018. The role of n-3 long chain polyunsaturated fatty acids in cardiovascular disease prevention, and interactions with statins. Nutrients 10:775.

Brenes, A., A. Viveros, S. Chamorro, and I. Arija. 2016. Use of polyphenol-rich grape by-products in monogastric nutrition. A review. Anim. Feed Sci. Tech. 211:1–17.

Cavallini, D., R. Bedani, L. Bomdespacho, R. Vendramini, and E. Rossi. 2009. Effects of probiotic bacteria, isofiavones and simvastatin on lipid profile and atherosclerosis in cholesterol-fed rabbits: a randomized double-blind study. Lipids Health Dis. 8:1.

Chen, Y., X. Gong, G. Li, M. Lin, Y. Huo, S. Li, and G. Zhao. 2016. Effects of dietary alfalfa flavonoids extraction on growth performance, organ development and blood biochemical indexes of Yangzhou geese aged from 28 to 70 days. Anim. Nutr. 2:318–322.

Chikunya, S., G. Demirel, M. Enser, J. D. Wood, R. G. Wilkinson, and L. A. Sinclair. 2004. Biohydrogenation of dietary n-3 PUFA and stability of ingested vitamin E in the rumen, and their effects on microbial activity in sheep. Br. J. Nutr. 91:539–550.

Dinh, T. T. N., J. R. Blanton, Jr, J. C. Brooks, M. F. Miller, and L. D. Thompson. 2008. A simplified method for cholesterol determination in meat and meat products. J. Food Compos. Anal. 21:306–314.

EFSA. 2009. Opinion on the safety of 'Alfalfa protein concentrate' as food. EFSA. J. 997:1–19.

Erba, D., M. C. Casiraghi, C. Martinez-Conesa, G. Goi, and L. Massaccesi. 2012. Isoflavone supplementation reduces DNA oxidative damage and increases O-β-N-acetyl-D-glucosaminidase activity in healthy women. Nutr. Res. 32:233–240.

Falowo, A. B., P. O. Fayemi, and V. Muchenje. 2014. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: a review. Food Res. Int. 64:171–181.

Fernández, M., J. A. Ordóñez, I. Cambero, C. Santos, C. Pin, and L. De la Hoz. 2007. Fatty acid compositions of selected varieties of Spanish dry ham related to their nutritional implications. Food Chem. 9:107–112.

Folch, J., M. Less, and G. H. S Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Gessner, D. K., R. Ringseis, and K. Eder. 2017. Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. J. Anim. Physiol. Anim. Nutr. 101:605–628.

Grela, E. R., and K. Pietrzak. 2014. Production technology, chemical composition and use of alfalfa protein-xanthophyll concentrate as dietary supplement. J. Food Process. Technol. 5:373–377.

Grela, E. R., S. Knaga, A. Winiarska-Mieczan, and G. Zięba. 2020. Effects of dietary alfalfa protein concentrate supplementation on performance, egg quality, and fatty acid composition of raw, freeze-dried, and hard-boiled eggs from Polbar laying hens. Poult. Sci. 99:2256–2265.

Grela, E. R., K. Ognik, A. Czech, and J. Matras. 2014. Quality assessment of eggs from laying hens fed a mixture with lucerne protein concentrate. J. Anim. Feed Sci. 23:236–243.

Hager, A., and T. Mayer-Berthenrath. 1966. Die Isolierung und quanttative Bestimung der Carotenoide und Chlorophyll von Blatern, Algen und isolierten Chloroplasten mit Hilfe Dunnschicht-chromatographischer Methoden. Planta 69:198–217.

Jiang, Z. Y., S. Q. Jiang, Y. C. Lin, P. B. Xi, D. Q. Yu, and T. X. Wu. 2007. Effects of soybean isoflavone on growth performance, meat quality, and antioxidation in male broilers. Poult. Sci. 86:1356–1362.

Jiang, J. F., X. M. Song, X. Huang, J. L. Wu, W. D. Zhou, H. C. Zheng, and Y. Q. Jiang. 2012. Effects of alfalfa meal on carcass quality and fat metabolism of Muscovy ducks. Br. Poult. Sci. 53:681–688.

Kamboh, A. A., and W. Y. Zhu. 2013. Effect of increasing levels of bioflavonoids in broiler feed on plasma anti-oxidative potential, lipid metabolites, and fatty acid composition of meat. Poult. Sci. 92:454–461.

Karre, L., K. Lopez, and J. K. Getty. 2013. Natural antioxidants in meat and poultry products. Meat Sci. 94:220–227.

Karwowska, M., J. Stadnik, Z. J. Dolatowski, and E. R. Grela. 2010. Effect of protein-xanthophylls (PX) concentrate of alfalfa supplementation on physico-chemical properties of Turkey breast and thigh muscles during ageing. Meat Sci. 86:486–490.

Khaleel, A. E., M. Z. Gad, S. A. El-Maraghy, M. S. Hifnawy, and E. Abdel-Sattar. 2005. Study of hypocholesterolemic and antiatheroscierotic properties of Medicago sativa cultivated in Egypt. J. Food Drug Anal. 13:212–218.

Kiczorowska, B., W. Samolińska, A. R. M. Al-Yasiry, A. Winiarska-Mieczan, and M. Kwiecień. 2015. Nutritional value of poultry meat produced in conventional and organic systems. Probl. Hig. Epidemiol. 96:598–600.

Krauze, M., and E. R. Grela. 2010. Influence of protein-xanthophyll (APC) cocncentrate of alfalfa additive in Turkey diet on performance and some blood indice. Archiv Geflügelk 74:226–232.

Kwiatkowska, K., M. Kwiecień, and A. Winiarska-Mieczan. 2017. Fast-growing chickens fed with lucerne protein-xanthophyll concentrate: growth performance, slaughter yield and bone quality. J. Anim. Feed Sci. 26:131–140.

Kwiecień, M., A. Winiarska-Mieczan, A. Milczarek, and R. Klebaniuk. 2017. Biological response of broiler chickens to decreasing dietary inclusion levels of zinc glycine chelate. Biol. Trace Elem. Res. 175:204–213.

Kwiecień, M., A. Winiarska-Mieczan, A. Milczarek, E. Tomaszewska, and J. Matras. 2016. Effects of zinc glycine chelate on growth performance, carcass traits and bone quality of broilers chicken. Livest. Sci. 191:43–50.

Lien, T. F., H. S. Yeh, and W. T. Su. 2008. Effect of adding extracted hesperetin, naringenin and pectin on egg cholesterol, serum traits and antioxidant activity in laying hens. Arch. Anim. Nutr. 62:33–43.

Misra, H. P., and I. Fridowich. 1972. The role of superoxide anion in the autoxidation of Epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247:3170–3175.

NRC, National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Oleszek, W. A. 2002. Chromatographic determination of plant saponins. J. Chromatogr. A. 967:147–162.

Ouyang, K., M. Xu, Y. Jiang, and W. Wang. 2016. Effects of alfalfa flavonoids on broiler performance, meat quality, and gene expression. Can. J. Anim. Sci. 96:331–340.

Pisulewski, P. M. 2005. Nutritional potential for improving meat quality in poultry. Anim. Sci. Pap. Rep. 23:303–315.

Salih, M., D. M. Smith, J. F. Price, and L. E. Dawson. 1987. Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. Poult. Sci. 66:1483–1488.

Simitzis, P. E., G. K. Symeon, M. A. Charismiadou, A. G. Ayoutanti, and S. G. Deligeorgis. 2011. The effects of dietary hesperidin supplementation on broiler performance and chicken meat characteristics. Can. J. Anim. Sci. 91:275–282.

Sinha, A. K. 1972. Colorimetric assay of catalase. Anal. Biochem. 47:389–394.

Sirri, F., G. Minelli, N. Iaffaldano, N. Tallarico, and A. Franchini. 2011. Oxidative stability and quality traits of n-3 PUFA enriched chicken meat. Ital. J. Anim. Sci. 2:450–452.

Ting, S., H. S. Yeh, and T. F. Lien. 2011. Effects of supplemental levels of hesperetin and naringenin on egg quality, serum traits and antioxidant activity of laying hens. Anim. Feed Sci. Technol. 163:59–66.

Ulbricht, T. L., and D. A. T. Southgate. 1991. Coronary heart disease: seven dietary factors. Lancet 338:985–992.

Windisch, W. M., K. Schedle, C. Plitzner, and A. Kroismayr. 2008. Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci. 86:E140–E148.

Winiarska-Mieczan, A., and M. Kwiecień. 2015. The effects of copper-glycine complexes on chemical composition and sensory attributes of raw, cooked and grilled chicken meat. J. Food Sci. Technol. 52:4226–4235.

Woods, V. B., and A. M. Fearon. 2009. Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: a review. Livestock Sci. 126:1–20.

Ziołecki, J., and W. Doruchowski. 1989. The Method of Assessment of Slaughter Poultry (In Polish). COBRD Publishing, Poznań, Poland.