Research Article

Oxidative Stability of the Meat of Broilers Fed Diets Supplemented with Various Levels of Blackcurrant Extract (*Ribes nigrum* L.) during Different Time Period

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In the 20 d experiment, the influence of different concentration and supplementation period of commercial blackcurrant extract (BC) in the broiler diets on the oxidative stability of breast and thigh meat, as well as selected performance indices, was investigated. A number of 120 fifteen-d-old Hubbard Flex male chicks (initial BW 363.5 ± 22.9 g) were randomly allocated to five groups: the control and four treatments (6 replicates, 4 birds per cage in each group). The BC extract was administered to treatment groups at two concentrations (1.25 and 2.5 g/kg) and in different periods within the trial (i.e., from 15 to 35 d and from 25 to 35 d of life). Body weight gain (BWG) and feed conversion ratio (FCR) were determined during the 20-d experiment. At d 35, two randomly selected birds from each cage were decapitated, and pectoral and thigh muscles were collected. Extent of lipid oxidation after storage at chilling (2–3°C, 1, and 5 d) and freezing conditions (after 90 d, –18°C) was determined. The chickens' growth performance and FCR were not affected by the concentrations and periods of BC supplementation. The enrichment of grower diet with 1.25 g/kg of BC extract reduced the malondialdehyde (MDA) formation in frozen thigh muscles ($P < 0.05$), and this effect tended to appear ($P < 0.089$) irrespective of duration of the supplementation period. Significant extent of lipid oxidation process was found in 1-d-chilled pectoral muscles of chickens receiving BC diet for 20 d or diet containing 2.5 g/kg of the extract. The results showed that BC extract may be an efficient source of antioxidants in chicken diet, which may increase oxidative stability of frozen dark meat. However, the conditions and ability of some polyphenols to initiate oxidation processes have not been fully understood and further studies are required.

1. Introduction

Over the last decades, significant increase in global production and consumption of poultry meat has been observed, mainly due to its dietary and sensory value, relatively low production costs, low price, and short time of thermal processing [1]. Poultry meat is a source of a high biological value of animal protein and due to low fat content, the energetic value of poultry meat is lower than other types of meat, but at the same time, poultry meat is a valuable source

of polyunsaturated fatty acids (PUFAs). It is stated that poultry meat lipids contain the best composition of fatty acids, corresponding with human requirements. The average $n-6$ to $n-3$ ratio in broiler chicken meat ranges from 7:1 to 12.5:1, while the $n-6$ to $n-3$ ratio in pectoral muscles is the most optimal among all poultry meat (approx. 3.7:1) [2, 3]. This value is close to the optimal $n-6$ to $n-3$ ratio, which is exactly 4:1 and should not exceed 10:1 [4].

However, increased content of essential PUFAs, as well as $n-3$ fatty acids in poultry meat, is beneficial to human

health, it decreases an oxidative stability of the meat. This is due to a high susceptibility of unsaturated lipids to oxidation processes, which increases in a geometric progression along with number of unsaturated bonds. As a result, poultry meat is particularly prone to oxidative rancidity, that might be arranged, according to sequence of lipid susceptibility to oxidation in the following order: fish > poultry (turkey) > poultry (broilers) > pork > beef > mutton [5]. During peroxidation, decomposition of lipids proceeds simultaneously generating a wide range of secondary products, like aldehydes, ketones, alcohols, peroxides, or hydrocarbons. These compounds are responsible for an incidence of so called unpleasant, acute, rancid odour, and flavour of meat known as warmed-over flavour, WOF [6]. Moreover, some products of lipid peroxidation, such as malondialdehyde and oxysterols, demonstrate cancerogenic and atherogenic activity [7, 8].

One of the methods of the meat rancidity limitation is an application of feed antioxidants to animal diets. It has been demonstrated that the addition of synthetic antioxidants like butylated hydroxytoluene (BHT), etoxiquin (EQ), or α -tocopherol to concentrated mixtures for broiler chickens decreases the rate of lipid oxidation during storage, both chilled and frozen poultry meat [9]. However, the use of effective, synthetic antioxidants to food might be controversial, mainly due to potential carcinogenic properties [10]. As an alternative to the synthetic antioxidants, natural polyphenols from various plant species may be used. These compounds have an ideal chemical structure to “scavenge” free radicals, demonstrating, at the same time, higher antioxidant capacity (e.g., cyanidin and malvidin) than vitamins C and E [11]. Previous investigations performed on meat of broilers fed diets supplemented with tea catechins and grape seed extract have shown that oxidative stability of the meat was improved and development of rancid deterioration was minimized [12, 13]. Similar inhibitory effect of white mulberry (*Morus alba* L.), honeysuckle (*Lonicera flos*), and the Chinese goldthreath (*Coptis chinensis*) herb mixtures applied to broiler chicken diet on muscle lipid oxidation was reported by Jang et al. [14]. The limited lipid oxidation was also confirmed in chilled meat of broilers receiving diets supplemented with 5 or 10 g/kg of rosemary powder (*Rosmarinus officinalis* L.) and vitamin E [15].

Blackcurrant (*Ribes nigrum* L.) is a plant species belonging to the *Grossulariaceae* family, originated from Europe and temperate Asia. The blackcurrant juice is a rich source of polyphenols (1.91 g/L), and it has been shown to exhibit more than three times higher antioxidant activity comparing to apple or orange juices [16, 17]. Polyphenolic extracts derived from blackcurrant fruit contain a considerably higher amount of these compounds than juice, that is, approx. 245 mg/g, where about 100 mg/g are anthocyanins [18]. The preliminary studies of this paper (using 1,1-diphenyl-2-picrylhydrazyl, DPPH free radical assay) have shown [19] that blackcurrant extracts demonstrated very high ability (expressed as 50% inhibition concentration) to neutralize free radicals ($IC_{50} = 10.68 \pm 0.83$ mg/L), which was approx. 2-3 times higher than rosemary extracts ($IC_{50} = 26.36\text{--}37.88 \pm 0.85\text{--}2.54$ mg/L) and comparable to

pure ferulic acid ($IC_{50} = 9.69 \pm 1.37$ mg/L). Although, the high efficacy of blackcurrant extract to limit development of peroxidation process in raw pork patties during 9-d-storage was previously given by Jia et al. [20], there is still lack of data regarding the use of condensed polyphenol sources, such as blackcurrant extract, to livestock diet. Taking above into consideration, the present study was carried out to determine the influence of various concentrations and administration periods of blackcurrant extract to grower diet on the oxidative stability of chicken meat. Evaluation of selected performance indices of chickens was determined only as an additional part of the experiment.

2. Materials and Methods

2.1. Chemicals, Reagents, and Blackcurrant Extract. Neochlorogenic acid was purchased from TRANS MIT GmbH (Giessen, Germany). Delphinidin-3-O-glucoside and 3-O-rutinoside, cyanidin-3-O-glucoside and 3-O-rutinoside, quercetin-3-O-glucoside and 3-O-rutinoside, myricetin-3-O-rutinoside, and 3-O-galactoside and kaempferol-3-O-rutinoside, (+)-catechin, and (–)-epicatechin were purchased from Extrasynthese (Lyon, France). Acetonitrile was purchased from Merck (Darmstadt, Germany). Commercial extracts of blackcurrant (BC) was obtained from PK Components (Warsaw, Poland).

2.2. Identification and Quantification of Polyphenols by UPLC-PDA-MS Method. The solvent for identification (LC/MS Q-TOF) and quantitative (UPLC-PDA) analysis of polyphenols (anthocyanin, flavan-3-ol, flavonol, and phenolic acid) were performed as detailed described previously by Wojdyło et al. [21]. Briefly, polyphenols of the tested BC extract were identified using an ACQUITY Ultra Performance LC™ system (UPLC™) with binary solvent manager and PDA detector (Waters Corporation, Milford, MA, USA) and a Micromass Q-TOF spectrometer (Waters, Manchester, UK) with an electrospray ionisation (ESI) source operating in negative and positive mode. Separation of individual polyphenols was realized using a UPLC BEH C18 column (1.7 μ m, 2.1 x 50 mm; Waters Corporation) at 30°C. The obtained results were expressed as mg per 100 g of the BC extract.

2.3. Other Chemical Analytical Methods Used for Diets and Blackcurrant Extract. The chemical composition of feed components and blackcurrant extract was analyzed according to Association of Official Analytical Chemists [22]: dry matter (DM)—using Zalmed SML 32/250 dryer (AOAC, 930.15); crude ash (CA)—muffle furnace (AOAC, 942.05); the nitrogen content—by Kjeldahl method using Kjeltac 2300 Foss Tecator (Sweden; AOAC, 984.13); crude protein (CP) by multiplying the nitrogen content by 6.25; crude fat (CFA) by ether extraction (Büchi Extraction System B-811; AOAC, 920.39); crude fiber (CF) content was determined according to the Henneberg-Stohmann method, with the use a Foss Fibertec 1020 Tecator (Sweden; AOAC, 978.10).

2.4. Animals and Diets. The experiment was performed at the Wrocław University of Environmental and Life Sciences experimental facilities (RZD Swojec, Wrocław, Poland) in compliance with the 2nd Local Ethics Commission for Animal Experiments in Wrocław. The chickens were maintained according to European Union and Ethical Commission regulations [23]. The lighting program consisted of 18 h of light and 6 h of darkness. Birds had unlimited access to feed (in mashed form) and drinking water (nipples).

In the 20 d trial, 120 fifteen-d-old Hubbard Flex broiler males (initial BW 363.5 ± 22.9 g) were randomly allocated to 5 groups (control group + 4 treatments), each in 6 replicates (4 birds per cage). Throughout the experiment, an ambient temperature was maintained at 28°C (at d 15th) followed by the gradual reduction to 23°C (at the d 35th) according to breeding recommendations [24].

The isoenergetic and isoprotein grower diets based on maize, wheat, and soy bean meal (Table 1) were formulated according to the recommendations for Hubbard broilers [24]. The basal grower diet contained about 210 g/kg crude protein (CP) and 13.10 MJ/kg metabolizable energy [25] and was offered to control birds (I–C) in period of 15–35 d (20 d) and from 15 to 25 d (10 d) to birds in treatment groups II and IV (Table 1). The treatment grower diets were based on the same control concentrate mixture and supplemented with 1.25 (groups II and III) and 2.50 g/kg (groups IV and V) of blackcurrant extract mixed with rapeseed oil used for greasing of the tested feed mixtures and administered to birds for 10 and 20 d periods of the experiment.

2.5. Experimental Measurements. The feed intake and body weight gain (BWG, kg) were measured once a week and feed conversion ratios (FCR, kg of feed per kg of BWG) were calculated for each cage (kg of feed intake per 1 kg BW gain). Mortality and body weight of dead birds were recorded daily in each cage.

At 35 d of life, all birds were weighed to assess the average body weight in each group. Following, two birds from each cage with a body weight reflecting the average within the group were slaughtered, defeathered, and eviscerated. The pectoral and thigh muscles samples were then collected in order to determine the development of lipid oxidation during storage at chilling and freezing conditions.

2.6. Determination of MDA Content in Raw Chicken Muscles. Analyses of the muscles were performed after 1 d and 7 d of storage under chilling conditions (2–3°C) and after 90 d of frozen storage (–18°C). The extent of lipid peroxidation in breast (*pectoralis superficialis*) and thigh (*biceps femoris*) muscles was estimated by TBA-assay according to the modified procedure by McDonald and Hultin [26]. The raw pectoral and thigh muscles were grounded in a meat grinder, homogenized, and 0.5 g of the material was taken and mixed with 2 ml of 10% TCA (trichloroacetic acid) and the suspension was centrifuged for 10 min at 4000 g (Sigma 3K30 Polygen). Next, 2 ml of 0.02 M TBA (thiobarbituric acid) was added to 2 ml of the collected supernatant and stirred vigorously. The samples were incubated at 95–100°C in

a water bath (Julabo EcoTemp TW 12) for 40 min. After 20 min. of cooling under tap water, the absorbance was read against the blank (distilled water) at $\lambda = 530$ nm (Evolution 160 UV-VIS Thermo Scientific spectrophotometer). Results were calculated using the standard calibration curve based on the concentration of malonic dialdehyde (1,1,3,3-tetra-methoxypropane) and expressed as mg MDA per kg of meat. The procedure was carried out in triplicate.

2.7. Statistical Analyses. The data obtained in this study were evaluated by two-way ANOVA (factorial designs with a separate control), and differences between groups were determined according to Tukey's Multiple Comparison Test [27]. The differences between treatments for all parameters were performed using the statistical model: $y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$, where y_{ijk} is the variance associated with parameter; μ is the overall mean; α_i is the treatment effect; β_j is the period of extract administration effect; $(\alpha\beta)_{ij}$ is the interaction effect; and e_{ijk} is the impact of specific factors. For the TBA results of meat samples, the statistical analysis was performed with selection of repeated measures in the two-way ANOVA using General Linear Model (GML) section.

3. Results and Discussion

3.1. Polyphenolic Compounds Determined in the Blackcurrant Extract. The main LC/MS quantification of polyphenolic groups of blackcurrant extract revealed that berries used for extraction contained anthocyanidins >> flavonols > flavan-3-ols and >> phenolic acid. Anthocyanins were clearly the major group of phenolics in blackcurrant fruits. These compounds contributed to 83% of total polyphenols in the BC extract. Delphinidin and cyanidin of 3-O-glucoside and 3-O-rutinoside were also previously reported to be the main anthocyanins in blackcurrant berries contributing to more than 80% of the total content of polyphenol compounds [21, 28, 29].

The major flavonols found in BC extract were derivatives of myricetin and quercetin (Table 2). In addition, small amounts of kaempferol derivatives were quantified. The major compounds were glucosides and rutinosides of myricetin and quercetin, which constituted about 95% of total content of these compounds. Furthermore, flavonols are effective antioxidants because these compounds with 3',4'-dihydroxy substitution in the B-ring and conjugation between the A- and B-rings have high antioxidant potential. Flavones, in general, have higher antioxidant activity as compared with anthocyanins with the same hydroxylation patterns measured with the ORAC assay [30]. The content of flavan-3-ols was only 6% of total phenolic compounds and represented by monomeric form as catechin.

The phenolic acids are minor group of substances found in the studied extract. The dominant phenolic acids were 3-*p*-caffeoylglucoside acid followed by neochlorogenic and *p*-coumaroylquinic acids. The contents of phenolic derivatives were consistent with the data obtained by Maatta et al. [31] and Anttonen and Karjalainen [29].

TABLE 1: Composition of the experimental diets fed to broilers.

Diets	Grower (g/kg)		
Groups and period of BC extract supplementation	Control (I-C)	II/III 25–35 d/15–35 d	IV/V 25–35 d/15–35 d
Duration of BC extract suppl.	0 d	10 d/20 d	10 d/20 d
<i>Components (g/kg)</i>			
Maize	250.00	249.38	248.75
Wheat	285.50	284.79	284.07
Soy bean meal	364.80	363.89	362.98
Rapeseed oil	59.20	59.05	58.90
Chalk	12.60	12.57	12.54
Dicalcium phosphate	14.30	14.26	14.23
Sodium chloride	3.60	3.59	3.58
Supermix DS broiler	10.00	9.98	9.95
Blackcurrant extract (BC)	0.00	1.25	2.50
Total BC polyphenols (mg/kg)	0.00	288.40	576.68
Total BC anthocyanins (mg/kg)	0.00	225.00	449.90
<i>Energy value (MJ) and essential nutrients (g/kg)</i>			
ME, MJ/kg	13.10	13.10	13.09
Dry matter	898.70	898.65	898.81
Crude protein ($N \times 6.25$)	210.00	209.84	209.68
Crude fiber	40.00	39.99	39.97
Crude fat g/kg	88.10	88.00	87.91
Crude ash g/kg	55.60	55.54	55.49
Nitrogen-free extractives	505.00	505.35	505.70
Ca	10.00	9.98	9.95
P available	4.70	4.69	4.68
Na	1.70	1.70	1.69

Chemical composition of premix per 1 kg of diet: CaCO_3 , 0.9 g; P, 0.8 g; S, 250 μg ; Mn, 80 mg; J, 1 mg; Zn, 80 mg; Fe, 70 mg; Co, 400 μg ; Se, 300 μg ; retinol, 7.8 mg; cholecalciferol, 75 μg ; α -tocopherol, 60 mg; menadione, 3.25 mg; thiamin, 3.03 mg; riboflavin, 8 mg; pyridoxine hydrochloride, 5.53 mg; cyanocobalamin, 30 μg ; pantothenic acid, 15 mg; biotin, 200 μg ; nicotinic acid, 60 mg; folic acid, 2 mg; choline, 500 mg; Lys, 1.85 g; Met, 2.25 g; Tre, 0.6 g; phytase; coccidiostat-salinomycin.

TABLE 2: Identification and quantification of phenolic compounds in blackcurrant extract by LC/MS Q-TOF.

Compounds	MS/MS ($M+H$) ⁺ (m/z)	Polyphenolic compounds (mg/100 g)
<i>Anthocyanins</i>		
Delphinidin-3- <i>O</i> -glucoside	465/303 ⁺	233.45 $\pm$ 13.23
Delphinidin-3- <i>O</i> -rutinoside	611/303 ⁺	712.60 $\pm$ 32.54
Cyanidin-3- <i>O</i> -glucoside	449/287 ⁺	155.16 $\pm$ 16.44
Cyanidin-3- <i>O</i> -rutinoside	595/449/287 ⁺	707.27 $\pm$ 36.11
<i>Hydroxycinnamic acid</i>		
3- <i>p</i> -caffeoylglucoside acid	341/191	61.43 $\pm$ 4.33
Neochlorogenic acid	353/191	31.14 $\pm$ 3.43
<i>p</i> -Coumaroylquinic acid	337/325	26.55 $\pm$ 2.11
<i>Flavonols</i>		
Myricetin-3- <i>O</i> -rutinoside	625/316	106.06 $\pm$ 9.43
Myricetin-3- <i>O</i> -galactoside	479/316	97.77 $\pm$ 3.66
Quercetin-3- <i>O</i> -rutinoside	609/301	68.15 $\pm$ 2.17
Quercetin-3- <i>O</i> -glucoside	463/301	11.55 $\pm$ 1.22
Quercetin-3- <i>O</i> -malonylglucoside	549/301	49.55 $\pm$ 2.54
Kaempferol-3- <i>O</i> -rutinoside	593/285	6.73 $\pm$ 0.98
<i>Flavan-3-ols</i>		
(+)-catechin	289	67.45 $\pm$ 3.52
(-)-epicatechin	289	80.41 $\pm$ 5.81
Total of phenolic compounds		2307.27

($M+H$)⁺ (m/z) anthocyanins were obtained in the positive ion mode.

3.2. Growth Performances. The present study has demonstrated that the enrichment of grower diet with BC extract

for the last 10 d (from 25 to 35 d) and 20 d (from 15 to 35 d) of broilers rearing did not change significantly bird's growth

performance and FCR. In most cases, the use of natural phytogetic additives (*Syzygium aromaticum*, *Thymus vulgaris*) in broiler nutrition has a positive impact on chickens performance [32, 33], or at least does not affected these parameters significantly (*Zingiber officinale*, *Thymus vulgaris*, *Origanum vulgare*, *Armoracia rusticana*, *Trigonella foenum-graceum* L., and *Matricaria chamomilla* L.) [32, 34, 35]. On the other hand, significantly lower BWG and higher FCR were found in broilers treated with 1% of green tea powder [36].

During the experiment, no significant differences were found in regards to mortality of chickens fed with diets containing BC extract (Table 3). Slightly higher mortality rate was noted only for birds receiving BC extract for the last 10 d of rearing. However, it appears to be an accidental finding, since no similar mortality incidences were found when the BC supplementation period was extended.

3.3. MDA Concentration in the Muscles of Chickens. The lipid oxidation in breast and thigh muscles after 1 d of chilled storage was not affected by dietary BC extract used 1.25 and 2.5 g/kg and supplemented for 10 or 20 d, in relation to control group (Table 4). Significantly ($P < 0.05$) higher concentration of MDA was found in pectoral muscles of chicken fed diets supplemented with 2.50 g/kg BC extract or supplemented with the BC diet for 20 d. The two-way ANOVA revealed significant interaction of BC concentration and period of supplementation on the MDA levels in the breast meat samples.

On d 7, no differences were found in MDA concentration in the analyzed pectoral and thigh muscles between treatments. Although the MDA content in 7-d-chilled pectoral muscles of BC supplemented chickens was noticeably lower in relation to control, these observed values were statistically insignificant. The increase of MDA content during the 7-d-chilled storage of the meat was observed and was more visible in the case of pectoral ($P < 0.05$) than the thigh ($P > 0.05$) muscles (except. 1.25 g/kg BC) and mostly insignificant in the muscles of chicken receiving control diet (Table 4). The delayed oxidation in thigh meat was confirmed in samples collected from chickens fed 2.5 g/kg BC diet.

After 90 d of frozen storage, thigh muscles of broilers fed diets containing 1.25 g/kg BC extract demonstrated significantly ($P < 0.05$) lower MDA concentration (0.258 mg/kg) compared to the control group (0.302 mg/kg). A noticeable tendency ($P = 0.089$) to inhibition of lipid oxidation in thigh muscles of broilers receiving BC grower diets for 10 d (0.264 mg/kg) and 20 d (0.264 mg/kg), related to control. The significant interaction of BC concentration and period of supplementation on MDA values in thigh meat was also noted. At the same time, no significant differences were found in the intensity of lipid oxidation in chicken breast muscles. The increase of MDA content during frozen storage was significant in all samples of meat when compared to the 1-d-chilled fresh muscles ($P < 0.01$). However, this process tended to be less visible in thigh muscles, where significant differences were obtained in most cases only after 90 d of freezing.

The collected results showed, that the supplementation of grower diet with 1.25 g/kg BC extract had protective effect against MDA formation in frozen thigh muscles and also tended ($P = 0.089$) to decrease MDA concentration in the frozen thigh muscles irrespective to period of supplementation. Although relatively limited, this demonstrated antioxidant effect of dietary BC is in accordance with numerous literature reports, where protective effects of dietary phytogetic additives (grape pomace concentrate, tea catechins, rosemary, citrus waste) against lipid oxidation in broiler meat were observed [12, 15, 37, 38]. Despite, no data were found to compare the effect of pure blackcurrant extracts supplementation on susceptibility of chicken or other livestock meat to oxidation. However, the diet supplemented with 50 g/kg of blackcurrant pomace was proven to lower the concentration of thiobarbituric acid reactive substances (TBARS) both in raw and cooked meat of chilled and frozen breast and thigh of turkeys [39]. Moreover, Szachowicz-Petelska et al. [40] reported a protective effect ($P < 0.05$) of blackcurrant juice polyphenols on oxidative stress in liver cell membranes during simultaneous intoxication of rats with ethanol. Stabilizing effect on vascular oxidative stress was also observed when polyphenol rich blackcurrant juice was supplemented to cirrhotic rats with portal hypertension [41]. Lower TBARS values in serum of rabbits fed with high-fat diet supplemented with 15 g/kg blackcurrant polyphenolic extract was also confirmed by Jurgoński et al. [42].

The hypothesis regarding the use of herbal and other phytogetic supplements in poultry or other livestock nutrition assumes the transfer of active compounds to meat that exerts an antioxidant property [43]. This suggestion is consistent with findings of Jang et al. [14], who demonstrated significant increase of phenolic constituents ($P < 0.05$) in breast muscles of chickens fed diet enriched with *Morus alba* L., *Lonicera flos*, *Coptis chinensis* extract mixture. This hypothesis may be partly confirmed by the outcomes of this study, since the substantial effect of BC on oxidation intensity in 1-d-chilled breast muscles and frozen thigh muscles was stated. The absorption of anthocyanins of blackcurrant from gastrointestinal tract has been also proved, as evidenced by the presence of these compounds in the blood and tissues of the eye of rabbits [44] and the urine of rabbits and human [45]. Faiz et al. [38] indicated lower TBARS values and higher activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, connected with the dose-dependent improvement in total phenolic compound identified in meat of chickens receiving various levels of citrus waste.

In the present study, a significant increase in lipid oxidation process was found in 1-d-chilled breast muscles of chickens fed diets supplemented BC extract for 20 d and or receiving diet containing a higher concentration of BC (2.5 g/kg). These differences were insignificant in relation to control group and occurred only in comparison with breast meat samples of chickens fed a lower concentration or the shorter period of supplementation. Similar discrepancies were reported by Liu et al. [46], who noted a significantly higher MDA content in muscles of rabbits fed with diet enriched with 10 g/kg of a tannin-rich chestnut extract

TABLE 3: Effect of experimental treatments on body performance indices of broiler chickens.

Group	Treatment	BWG (g) d 15–35	FCR (kg feed/kg BW gain) d 15–35	Mortality rate (%) d 15–35
<i>Concentration (C) (g/kg)</i>				
0.00		1364	1.67	0.00
1.25		1321	1.80	4.17
2.50		1249	1.86	4.17
<i>Period of supplementation (PS)</i>				
Control		1364	1.67	0.00
25–35 d (10 d)		1284	1.86	8.33
15–35 d (20 d)		1286	1.80	0.00
SEM		18.842	0.034	1.982
<i>P value</i>				
Concentration (C)		0.085	0.382	1.000
Period (PS)		0.957	0.390	0.070
Interaction C x PS		0.627	0.034	1.000

^{a,b}Means in the column with different superscripts differ significantly at $P < 0.05$. ^{A,B}Means in the column with different superscripts differ significantly at $P < 0.01$.

TABLE 4: TBA values presented as MDA content (mg/kg) in breast (*pectoralis superficialis*) and thigh (*biceps femoris*) muscles stored at 2–3°C for 1 and 7 d or stored at –18°C for 90 d.

Item	Muscles					
	<i>Pectoralis superficialis</i> (day of storage)			<i>Biceps femoris</i> (day of storage)		
	1	7	90	1	7	90
<i>Concentration (C) (g/kg)</i>						
0.00	0.180 _(X)	0.243	0.262 _(Y)	0.175 _(XX)	0.215 _(YY)	0.302 ^a _(Y)
1.25	0.169 ^A _(X)	0.236 _(Y)	0.234 _(Y)	0.176 _(X)	0.214 _(Y)	0.258 ^b _(Y)
2.50	0.184 ^B _(X)	0.213	0.246 _(Y)	0.185 _(X)	0.214 _(X)	0.270 _(Y)
<i>Period of supplementation (PS)</i>						
Control	0.180 _(X)	0.243	0.262 _(Y)	0.175 _(X)	0.215 _(X)	0.302 _(Y)
15–35 d (10 d)	0.166 ^A _(XX)	0.214 _(Y)	0.236 _(YY)	0.178 _(X)	0.214 _(X)	0.264 _(Y)
25–35 d (20 d)	0.187 ^B _(XX)	0.235 _(Y)	0.244 _(YY)	0.183 _(X)	0.214 _(X)	0.264 _(Y)
SEM	0.005	0.009	0.007	0.004	0.006	0.008
<i>P value</i>						
Concentration (C)	0.039	0.302	0.499	0.335	0.972	0.040
Period (PS)	0.005	0.343	0.601	0.557	0.960	0.089
Interaction C x PS	0.000	0.273	0.740	0.196	0.132	0.001
Conc. (Rep. measures)	0.001	0.001	0.001	0.001	0.001	0.001
Period (Rep. measures)	0.001	0.001	0.001	0.001	0.001	0.001

_(x,y)Means in the rows with different under scripts differ significantly at $P < 0.05$. _(x,y)Means in the rows with different under scripts differ significantly at $P < 0.01$. ^{a,b}Means in the column with different superscripts differ significantly at $P < 0.05$. ^{A,B}Means in the column with different superscripts differ significantly at $P < 0.01$.

compared to rabbits fed with only 5 g/kg of the extract in feed. Although the obtained results seem to be controversial and difficult to explain, however, they can be a result of a “dual nature” of some antioxidants that, in some specific conditions, may act as prooxidants. This phenomenon has been well documented for numerous natural antioxidants, such as ascorbic acid [47], β -carotene [48], whether α -tocopherol [49], and also some flavonoids. Prooxidant properties have been also observed for some blackcurrant polyphenols present in the tested BC extract, such as cyanidin and delphinidin, as well as malvidin, pelargonidin or peonidin, and their glucosides [50]. A suggestion that polyphenols used in animal nutrition might initiate the oxidation process of meat lipids is supported by Du et al. [51], who reported a significant increase in thiobarbituric

acid reactive substances in thigh muscles of broilers fed with diet supplemented with sorghum tannins. The authors have suggested that polyphenols deposited in muscle tissue may maintain the nonheme iron in reduced form, which lead to initiation of Fenton’s reaction increasing the intensity of muscle lipids oxidation. Also, a possible explanation of the dietary polyphenols “prooxidant” paradox was given by Liu et al. [46]. According to the cited paper, the muscles of rabbits treated with 10 g/kg chestnut extract had higher iron content in comparison to control group. This could cause stronger interactions between the nonheme iron ions (due to their chelating properties) and chestnut tannins, deposited in meat. The effect of such interactions could be a depletion of available amount of phenolic compounds (or other antioxidants) in muscles and

paradoxically contributed to a significant increase of lipid oxidation compared to rabbits fed with a lower dose of the extract in feed (5 g/kg). Moreover, phenolic compounds are transformed to less active forms during the absorption in digestive system (i.e., glucuronide, sulfate and methylated derivatives) [52, 53] and further metabolized to other by-products in various tissues [54]. These further endogenous biotransformations are still not fully understood, but probably affect further antioxidant properties *in vivo*, which in a certain way could explain the limited and even prooxidative effect of the tested BC in the muscles of chickens.

4. Conclusions

It should be emphasized, that blackcurrant extract, rich in anthocyanins, can be an efficient source of antioxidants in chicken diet, which may increase an oxidative stability of frozen thigh meat. However, the conditions and ability of some polyphenols to initiate oxidation processes have not been fully understood.

Data Availability

The [radical scavenging ability of the blackcurrant extract, performance indices of broiler chickens, MDA concentration] data used to support the findings of this study have been deposited in the "Additives of natural polyphenolic extracts to the broiler chickens diet and their effect on oxidative stability of meat and selected production parameters" repository, Ph.D. thesis (in Polish): http://www.dbc.wroc.pl/dlibra/docmetadata?id=23332&from=&dirids=1&ver_id=&lp=1&QI=50D8F03C7DE90720D104FEA4B43C70EC-1. The [quantification of polyphenols of blackcurrant extract] data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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