

THE EFFECT OF AMARANTH SEEDS. SEA BUCKTHORN POMACE AND BLACK CHOKEBERRY POMACE IN FEED MIXTURES FOR **BROILER CHICKENS ON PRODUCTIVE PERFORMANCE, CARCASS** CHARACTERISTICS AND SELECTED INDICATORS **OF MEAT OUALITY***

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Abstract

The aim of the present study was to determine the effect of amaranth seeds, dried sea buckthorn pomace and dried chokeberry pomace on the growth performance of broiler chickens and on the fatty acid profile and oxidative stability of meat lipids. The study was conducted on 480 Ross 308 chickens assigned in the second production phase to 4 experimental groups. The birds from the control group received 3% flax oil, while the chickens from the experimental groups were fed with mixtures containing: 3% flax oil and 8% amaranth seeds (group II), 3% flax oil and 3% dried sea buckthorn pomace (group III), and 3% flax oil and 3% dried chokeberry pomace (group IV). Basic production parameters were evaluated for each feeding period. At 42 days of age, 8 birds with body weight close to the average from each group were slaughtered. A simplified analysis of the chicken carcasses was conducted and samples of the breast muscles were collected for further analysis. The addition of 8% amaranth seeds into the feed mixtures in the second feeding phase decreased body weight gains (P<0.05) and increased the feed conversion ratio compared with groups receiving sea buckthorn or chokeberry pomace. However, the addition of amaranth seeds into the feed mixtures increased breast muscle yield (P<0.05) and decreased fat content in comparison to the other experimental groups. Moreover, sensory analysis of the breast muscles from chickens fed the diet with amaranth seeds revealed that they were characterised by a better aroma (P<0.05) and flavour. The studied feed additives did not significantly affect the physicochemical properties of the breast muscles. Sea buckthorn pomace efficiently slowed down lipid oxidation in the breast muscles. The obtained results indicate that using tested plant additives in feed mixture may be an effective way to improved production parameters of broiler chicken and effectively enriched meat in *n-3* fatty acid and protect against excessive oxidation of lipids.

Key words: broiler chickens, meat quality, fatty acid profile, phytobiotics, tocopherols

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In recent years, consumer interest in meat quality, and especially fat content and fatty acid profile, has been on the rise. The n-3 polyunsaturated fatty acids are particularly in demand as components of human diet due to their beneficial effects on human health. These acids are indispensable for the normal development and functioning of the human and animal body. Also, a deficit of these acids or improper proportions of n-6/n-3 PUFAs in the diet of birds can negatively compromise their immunity to diseases, reduce body weight gain and increase fat content. Studies conducted to date have demonstrated high efficiency of dietary flax oil supplementation in the enrichment of meat in n-3 PUFA, especially in particularly desired ALA (Pietras et al., 2000; Cortinas et al., 2004; Kowalska et al., 2011). Flax oil additive in the diet of birds results in a significant increase in the concentration of ALA and its long-chain derivatives (LC-PUFA) in tissues with a general increase in the total content of polyunsaturated fatty acids (Poureslami et al., 2010; Konieczka et al., 2015). However, meat enriched in n-3 PUFA is more susceptible to lipid oxidation, which is associated with a deterioration of its stability (Cortinas et al., 2005; Bou et al., 2009; Jankowski et al., 2012), and also a reduction of its health-promoting and sensory attributes due to undesired smell and flavor caused by lipid peroxidation processes (López-Ferrer et al., 2001; Zouari et al., 2010).

Fatty acid oxidation products accumulate in meat and impair its texture, colour, nutritional value and food security (Gray et al., 1996; Grau et al., 2001), which can have a negative impact on human health. The harmful effects of lipid oxidation products are due to the fact that they are digested and absorbed in the small intestine; they are then incorporated into chylomicrons and appear in the blood, increasing the pool of oxidized lipids in the body (Staprans et al., 1994). It is also known that lipid oxidation products damage the native structure of digestive enzymes (pepsin, trypsin), thus changing their activity (Hęś and Korczak, 2007). The use of antioxidants slowing down oxidative processes is one of the simplest and most efficient methods of prolonging the stability of meat and its products (Gray et al., 1996; Betti et al., 2009). However, the most commonly used synthetic antioxidants raise many consumer doubts and concerns due to a possible carcinogenic effect and toxicity of some of them (Chen et al., 1992; Lanigan and Yamarik, 2002).

The highest antioxidant efficacy is presented by natural antioxidants which are derived from vegetable oils and components (Sklan et al., 2011; Loetscher et al., 2013). Antioxidants present in or added to the feed are absorbed into the intestines so that they can perform their functions at the body level (Fellenberg and Speisky, 2006). This is due to the fact that natural antioxidants are more effectively integrated into the lipids of tissues and thus have a beneficial effect on the meat's durability, as well as its sensory and dietary properties (Morrissey et al., 1997; Brenes et al., 2008). One of the most important natural antioxidants includes tocopherol (vitamin E) which is found in plant products (Sklan et al., 2011). Thanks to its lipophilic properties, this vitamin has a protective effect on membrane phospholipids, protecting them against oxidation, which is particularly important for cellular structures containing significant amounts of PUFA acids and those exposed to oxygen effects (Sklan et al., 2011). Tocopherols quench singlet oxygen and block formation of peroxide radicals which results in DNA damage level reduction (Wąsowicz et al., 2004). This vitamin

is mainly embedded in the hydrocarbon part of the lipid membrane bilayer, protecting phospholipids from oxidation (Wąsowicz et al., 2004).

Amaranth seeds have been shown to improve the sensory attributes of meat (Pisarikova, 2006), moreover they are rich in natural antioxidants such as squalene and tocopherols which endow these seeds with a high antioxidant potential (Paśko et al., 2009; Worobiej et al., 2009). Squalene, which occurs in the liver of deep-sea sharks and whales in the highest amounts, is the most valuable antioxidant. The oil contained in amaranth seeds has a squalene content 10 times higher than that of olive oil. Studies conducted on rats show that amaranth oil has a significant effect on the increase of squalene levels in blood (91.00 vs. 2.59 µg/mL of plasma), liver tissue (5,680.48 vs. 103.09 µg/mL of tissue homogenate) and subcutaneous fat (482.46 vs. $255.54 \mu g/mL$ of tissue homogenate) in experimental animals compared to those of animals with diet including soy oil. The enrichment of the rat diet with amaranth oil resulted in an increase of α -tocopherol in the brain tissue (Czaplicki et al., 2014). The oil is also rich in vitamin C, flavonoids, phenolic compounds and isothiocyanates which exhibit strong antioxidant properties (Grajeta, 1997). Quercetin is the most abundant among the flavonoids in amaranth seeds and has the strongest antioxidant effect (Hertog et al., 2002). In vivo studies have shown that this compound has the ability to chelate metals, "sweep" free peroxide radicals and prevent low-density lipoprotein oxidation (Khandaker et al., 2008). The oil contained in amaranth seeds supports detoxifying functions of the liver (Nikolaevsky et al., 2014). This is considered to be an important protective factor against myocardial infarction by the mechanism of blocking lipid peroxidation induction (Martirosyan et al., 2007). Moreover, it is worth emphasising that the nutritional value of amaranth seeds is higher than that of other cereals (Rywotycki, 2005). These seeds contain from 17.5% to 38.3% of total protein, of which 5% is lysine, a necessary amino acid, the deficiencies of which are found in cereal-based feed mixtures. The seeds of this plant also contain antinutritional ingredients such as phytic acid, oxalates, tannins and fibre that reduce the availability of nutrients (Geetha et al., 2009), which is why amaranth seeds can be used in a limited amount in bird's diet.

Sea buckthorn (*Hippophaea rhamnoides*) fruit pomace is also characterised by a high concentration of antioxidants: vitamin C, flavonoids, anthocyanins, tocopherols and carotenoids (Geetha et al., 2009; Christaki, 2012). It should be noted that vitamin C present in the fruits of this plant is resistant to decomposition by storage and temperature. This is because these fruits lack ascorbinase, an enzyme capable of degrading vitamin C. Vitamin C reduces the demand for vitamin E, as after oxidation it can be reduced again by vitamin C, which also prevents lipid oxidation processes (Kleszczewska, 2002). The supplementation of animal feed with vitamin C increases the concentration of vitamin E and C as well as the MDA decrease in plasma (Sahin et al., 2001). Sea buckthorn pomace is also a valuable source of micro- and macroelements such as iron and manganese. Due to the valuable medicinal properties of the fruits, sea buckthorn is grown in Poland on an increasing scale. Its fruits are used to extract juice, and the seeds are used for pressing oil, which is classified as a special oil due to the high content of bioactive substances used in medicine and cosmetics. The addition of sea buckthorn pomace extract to poultry meat as a source of natural antioxidants resulted in inhibition of fatty acid oxidation processes (Püssa et al., 2008). On the other hand, Biswas et al. (2010) report that pomace from sea buck-thorn can be a valuable feed component in mixtures for poultry.

Black chokeberry (Aronia melanocarpa) fruits are a valuable source of active substances such as anthocyanins, tannins, flavonoids and vitamins (Kokotkiewicz et al., 2010; Kim et al., 2013). Studies conducted by Faff and Frankiewicz-Jóźko (2003) and Kowalczyk et al. (2003) confirmed the antioxidant action of chokeberry in animals, which resulted from their ability to reduce lipid oxidation and increase the activity of antioxidant enzymes. When processing these fruits, a waste product is produced in the form of pomace which can also be a valuable source of active compounds (Oszmiański and Wojdylo, 2005; Nawirska et al., 2007; Wojdyło et al., 2008; Juśkiewicz et al., 2015). Especially the pomace formed after juice extraction contains a large amount of polyphenols and anthocyanins. The results of studies conducted by Oszmiański and Wojdylo (2005) showed that in chokeberry juice the average content of phenolic compounds is 3.73 g/100 g, while in the pomace the content of these compounds was as much as 10.58 g/100 g. Due to such a favourable active substance content, this pomace can be a promising feed additive in animal nutrition. There is an increasing number of reports in scientific literature, describing the effect of chokeberry pomace on production results and the quality of meat (Loetscher et al., 2013; Pieszka et al., 2010, 2017). The natural plant additives (phytobiotics) used in dietary doses enrich products of animal origin, including meat, with bioactive substances and at the same time help animals to maintain and improve health.

The aim of the present studies was to determine the effect of amaranth seeds, dried sea buckthorn pomace or dried chokeberry pomace on growth performance, fatty acid profile and oxidative stability of meat lipids in broiler chickens.

Material and methods

Animals, diets and treatment

The studies were conducted on 608 Ross 308 chickens (cockerels and cocks). The chickens from 1 to 42 days of age were raised in pens on litter under standard environmental conditions with feed and water available *ad libitum*. In the first period of rearing (1–21 days of age), the birds were fed a starter diet which did not contain the tested additives (Table 1). In the second period of rearing (22–42 days of age), the chickens were randomly assigned to 4 groups with 4 replicates of 38 birds each and fed a grower-finisher feed mixture. The birds from the control group I received a feed mixture containing 3% flax oil. The birds from the experimental groups were fed a feed mixture with 3% flax oil and 8% amaranth seeds (group II), 3% flax oil and 3% dried chokeberry pomace (group IV). The feed mixtures used in the experiment were prepared according to the Nutrition Requirements of Poultry (2005) and computed using WinPasze Pro (2006) software, taking into account the chemical composition of the experimental components used. The raw fibre in the experimental feed mixtures did not exceed

4%. The plant additives used in the feed mixtures were purchased from certified producers (HACCP: Quality Control; Food Safety Management System according to DIN ISO 22000:2005) and Certificates of Conformity (AGRO BIO TEST). The composition and nutritional value of the mixtures are presented in Table 1.

		Group						
Ingredient (%)	Ι	II	III	IV				
Maize	28.00	28.00	28.00	48.21				
Wheat	30.46	22.00	29.00	5.00				
Soybean meal (46%)	31.50	32.22	31.23	34.00				
Rapeseed oil	3.00	3.00	3.00	3.00				
Flax oil	3.00	3.00	3.00	3.00				
Amaranth seeds	-	8.00	-	-				
Dried sea buckthorn pomace	-	-	3.00	-				
Dried chokeberry pomace	-	-	-	3.00				
Limestone	1.15	1.10	1.10	1.20				
Dicalcium phosphate	1.70	1.50	1.50	1.40				
NaCl	0.35	0.35	0.35	0.35				
DL-Methionine	0.21	0.20	0.20	0.21				
L-Lysine HCL	0.13	0.12	0.12	0.13				
Vitamin-mineral premix (0.5%) *	0.50	0.50	0.50	0.5				
Content of nutrients in 1 kg of mixte	ure:							
metabolisable energy (MJ/kg)]	13.0					
total protein (%)	20.00							
lysine (%)	1.15							
methionine (%)	0.52							
Ca (%)	0.92							
available P (%)		(0.40					

Table 1. Composition and nutrient content of grower-finisher feed mixtures

* Vitamin-mineral premix supplied the following per kg of mixture: vitamin A – 10,000 IU; vitamin D₃ – 2,000 IU; vitamin E – 40 mg; vitamin K₃ – 2.0 mg; vitamin B₁ – 1.5 mg; vitamin B₂ – 5 mg; vitamin B₆ – 3 mg; vitamin B₁₂ – 0.02 mg; pantothenate Ca – 12 mg; folic acid – 1 mg; biotin – 1 mg; niacin – 25 mg; choline – 400 mg; Mn – 100 mg; I – 0.8 mg; Zn – 65 mg; Se – 0.2 mg; Co – 0.4 mg.

During the experiment, in all repetitions the following values were recorded: individual body weight of chickens at 1, 21 and 42 days of age and number of dead birds and feed intake per group in each pen. Based on the experimental results, the basic production parameters were calculated, i.e. body weight gain, feed conversion ratio (kg/kg BW) and mortality (%).

Collection of samples and chemical analysis

At 42 days of age, 8 birds (4 cockerels and 4 cocks) were randomly selected from each group and slaughtered. The birds were decapitated, bled for 2 min, scalded at 54°C for 120 seconds, mechanically defeathered and manually eviscerated, washed and hung to drip. The carcass characteristics were determined according to a simplified analysis as reported by Ziołecki and Doruchowski (1989). The organs (heart, liver, gizzard) as well as the abdominal fat pad were weighed. The other parts, such as breast and leg muscles, both without skin, were separated from the carcasses after cooling. Carcass yield was determined using live weight and carcass weight, and breast meat yield was determined using carcass and breast weight.

In the feed mixtures, the profile and amount of higher fatty acids, tocopherols and tocotrienols were measured. The content of the higher fatty acids was determined by the method of Loor and Herebain (2001) according to ISO 12966-2:2011. Fatty acids were separated and quantified as methyl esters using a gas chromatography system VARIAN 3400 equipped with a flame-ionisation detector (250° C, range = 11; carrier gas: helium, 3 ml/min; gas injection: 0.7 ml), and a capillary column Rtx 2330 ($105m \times 0.32 \text{ mm}$, 0.2 mm). Tocopherols and tocotrienols were determined with the use of liquid chromatography according to Manz and Philip (1981) on a HPLC system (Merck-Hitachi) equipped with LiChroCART® 250-4, Superspher® 100 RP-18 column 4 μ m and FL detector, Ex. 295 nm and Em. 350 nm.

The samples of the *musculus pectoralis major* were used to determine basic chemical composition, fatty acid content, sensory attributes and malonaldehyde (TBA) after 90 days of frozen storage at -20°C. Triglycerides, total cholesterol, glucose were measured in plasma with colorimetric and enzymatic methods using an Alpha-Diagnostic kit. Thyroxine and triiodothyronine were determined with radio-immunological method using a Diasource RIA kit.

In the breast muscle samples, the content of dry matter, total protein and crude fat was measured using the AOAC method (2000). The higher fatty acids in the meat lipids were determined with gas chromatography as methyl esters according to procedures validated in the Central Laboratory of the National Research Institute of Animal Production in Aleksandrowice (Zabierzów, Lesser Poland Voivodeship). Fat was extracted from the samples using a mixture of chloroform and methanol (2:1) according to the modified method of Folch et al. (1957), and the extract was evaporated at 65°C under nitrogen. The residue was saponified using 0.5 NaOH in methanol (20 min, 80°C), esterified with BF₃ in methanol (Morrison and Smith, 1964) for 10 min at 80°C, and then hexane was added. After salting out with saturated NaCl solution, a hexane layer was collected on a chromatographic vial and the determination was carried out on a gas chromatograph VARIAN 3400 (column Rtx2330, 105 m, 0.32 mm, 0.2 m, detector range = 11, 250°C; carrier gas: helium, 3 ml/min), using an autosampler 8200 CX and data processing software Varian Star 4.5.

The TBA value in the breast meat was measured with a colorimetric method in the presence of 2-thiobarbituric acid according to the modified method described by Pikul (1993).

The breast meat samples held for sensory analysis were frozen at -20° C until evaluation. The breast meat was analysed after cooking to determine the sensory impact of the used plant additives on flavour and aroma quality. The sensory evaluation of the meat samples was conducted by eight panelists. The day before the analysis, 200 g of each sample was thawed at 4°C, and cooked individually in a covered container in 400 ml of 0.6% saline until the temperature inside the meat reached 70°C (the temperature was measured using a special thermometer). After cooling, the meat samples were evaluated within 10 min. The aroma, juiciness, tenderness and flavour of the meat were evaluated based on a five-point scale, where 5 meant strong appreciation and 1 an extreme dislike according to the method reported by Baryłko-Pikielna (1975).

The physicochemical properties of the fresh breast meat were also determined. Acidity was measured with the use of a portable pH-meter CyberScan 10 with a glass electrode for studies of meat. The measurements were carried out at 15 min after slaughter and after 24-h refrigeration at a temperature of 4°C (pH_{24h}). Water holding capacity (WHC) was evaluated with the method described by Grau and Hamm (1953). Drip loss was determined after 24- and 48-h meat storage at a temperature of 4°C, and was calculated according to the following formula: Drip loss (%) = sample weight before refrigeration (g) – sample weight after refrigeration (g)/sample weight before refrigeration (g) × 100.

Cooking loss was assessed based on the loss of breast muscle weight during cooking. The samples weighing 80 g were placed individually in plastic bags and cooked in a water bath at 100°C for ca. 15 min until an inner temperature of 78°C was reached in the thickest part of the sample. When the cooking was finished, the samples were cooled at room temperature for 30 min and were then kept in a cold room at a temperature of 4°C. The cold samples were weighed and the cooking loss was calculated according to the formula:

Cooking loss (%) = sample weight before cooking (g) – sample weight after cooking (g)/sample weight before cooking (g) × 100.

Statistical analysis

Analyses of the treatment effect on parameter production, carcass yield, fatty acid profile, meat quality and TBA content were conducted by one-way analysis of variance ANOVA with comparison of means using Duncan's multiple range test and SAS software package v. 9.2.

Results

The supplementation of plant additives under study (amaranth seeds, sea buckthorn pomace, chokeberry pomace) to grower feed mixtures resulted in differences in the content of n-3 PUFA contents, especially ALA, between the feed mixtures used for feeding of different groups of chickens (Table 2).

Fatter and	Group				
Fatty acid	Ι	II	III	IV	
C14:0	0.077	0.072	0.070	0.065	
C16:0	7.842	8.282	8.032	7.678	
C16:1	0.103	0.076	0.531	0.171	
C18:0	2.518	2.813	2.710	2.687	
C18:1	42.186	36.770	35.820	36.040	
C18:2	26.519	27.313	26.855	28.260	
C20	0.746	0.758	0.707	0.751	
C18:3	19.538	23.610	25.098	23.540	
CLA c9-t11	0.000	0.000	0.000	0.536	
CLA t10-c12	0.045	0.028	0.032	0.028	
CLA t9-t11	0.118	0.057	0.036	0.024	
C20:4	0.115	0.150	0.000	0.111	
C22:1	0.000	0.006	0.011	0.001	
SFA	11.284	11.924	11.566	11.250	
UFA	88.716	88.076	88.434	88.750	
MUFA	42.289	36.853	36.362	36.210	
PUFA	46.427	51.223	52.072	52.540	
PUFA-6	26.634	27.463	26.855	28.370	
PUFA-3	19.631	23.675	25.150	23.591	
DFA (C18:0+UFA)	91.234	90.889	91.144	91.446	
OFA (SFA-C18:0)	8.766	9.111	8.856	8.560	
UFA/SFA	7.862	7.386	7.646	7.890	
DFA/OFA	10.407	9.976	10.292	10.683	
MUFA/SFA	3.748	3.091	3.144	3.220	
PUFA/SFA	4.114	4.296	4.502	4.672	
PUFA 6/3	1.357	1.160	1.068	1.203	
CLA	0.162	0.085	0.067	0.588	

Table 2. Fatty acid profile in feed mixtures used for broiler feeding (% of the sum of fatty acid contents)

Groups:

I control: 3% flax oil, 3% rapeseed oil.

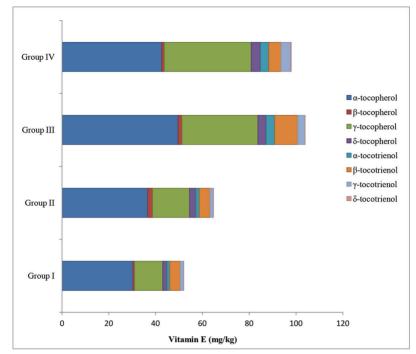
II: 3% flax oil, 3% rapeseed oil, 8% amaranth seeds.

III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.

In the control group I, ALA content amounted to 19.5%, while in the experimental groups it ranged from 23.5 to 25.1%. The total amount of saturated and unsaturated fatty acids remained unchanged. The ratio of *n*-6/*n*-3 PUFAs in different mixtures was 1.4, 1.2, 1.1 and 1.2, respectively.

The highest content of a-tocopherol exhibiting the strongest antioxidant action was observed in the feed mixtures containing sea buckthorn fruit pomace (49.56 mg/kg) (Figure 1).



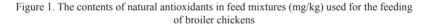
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III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.



In addition, the feed mixtures for the experimental group containing amaranth seeds and chokeberry fruit pomace showed higher levels of this form of tocopherol than the control group (36.62 and 42.46 vs. 30.03 mg/kg). The addition of amaranth seeds, dried sea buckthorn and chokeberry pomace to the feed also increased δ -tocopherol content by 31%, 170% and 209%, respectively, compared to the control (12.01 mg/kg). A higher content of d-tocopherol was observed in all the experimental groups (2.7, 3.48, 3.92 vs. 1.8 mg/kg).

Analysis of the data obtained in the second production period of the chickens revealed a significant (P<0.05) effect of amaranth seeds and also sea buckthorn and chokeberry pomace on feed intake by the birds (Table 3).

The addition of 8% amaranth seed to the feed mixture in the second production period reduced (P<0.05) broiler body weight gain and increased feed conversion ratio (FCR) compared to the groups fed sea buckthorn pomace and chokeberry pomace (Table 3). The FCR was significantly lower in the groups fed the feed containing 3% sea buckthorn and chokeberry fruit pomace. The percentage of dead chickens in the second production period amounted to 1.76% and 2.65% in groups I and II, respectively. Surprisingly, no dead chickens were noted in the groups receiving feed mixtures supplemented with sea buckthorn and chokeberry pomace.

Demander	Age		Group					
Parameter	(days)	Ι	II	III	IV	SEM		
Body weight gain (g)	1-21	799	775	788	805	4.62		
	22-42	2007 a	1905 b	2006 a	2007 a	34.03		
	1-42	2806	2680	2794	2812	29.56		
Feed intake (g/bird)	1-21	1137	1126	1200	1184	16.49		
	22-42	3879 a	3628 b	3595 b	3594 b	36.08		
	1–42	4821 a	4685 b	4778 a	4779 a	42.23		
Feed conversion ratio	1-21	1.42	1.45	1.52	1.47	0.02		
(kg/kg BW gain)	22-42	1.93 a	1.90 a	1.79 b	1.79 b	0.03		
	1-42	1.72	1.75	1.71	1.69	0.03		
Mortality (%)	1-21	0	0.88	1.28	0.56	-		
• • •	22-42	1.76	2.65	0	0	-		
	1-42	1.76	3.53	1.28	0.56	-		

Table 3	Production	parameters	of the	broiler	chickens

a, b, c – mean values marked with different letters differ statistically significantly at P<0.05. Groups:

I control: 3% flax oil, 3% rapeseed oil.

II: 3% flax oil, 3% rapeseed oil, 8% amaranth seeds.

III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.

Table 4. Carcass characteristics of broiler chick	cens
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Demonster		SEM				
Parameter	Ι	II	III	IV	SEM	
Body weight (g)	2828.3	2830.0	2831.7	2830.0	59.93	
Fresh carcass weight (g)	2120	2155	2130	2135	47.36	
Carcass weight after cooling (g)	2090.5	2123.3	2101.7	2096.7	44.81	
Carcass yield (%)	74.0	75.0	74.2	74.1	0.29	
Proportion (%) to carcass weight:						
breast muscle	27.64 b	29.22 a	26.96 b	26.61 b	0.30	
leg muscles	21.44	21.16	22.28	21.31	0.20	
liver (%)	1.84 a	2.17 b	1.83 a	2.42 b	0.16	
abdominal fat (%)	2.10 a	1.53 b	1.85 ab	1.78 ab	0.21	
skin with subcutaneous fat (%)	6.25 a	5.63 b	6.10 ab	6.0 ab	0.17	

a, b – mean values marked with different letters differ statistically significantly at P<0.05.

I control: 3% flax oil, 3% rapeseed oil.

II: 3% flax oil, 3% rapeseed oil, 8% amaranth seeds.

III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.

Groups:

The introduction of amaranth seeds and dried sea buckthorn or chokeberry pomace had no effect on fresh carcass weight compared to the control group (Table 4).

The greatest percentage of breast muscle was found in the carcasses of the chickens fed mixture with amaranth seed additive (P<0.05). The liver weight expressed as % of carcass weight in the groups fed amaranth seed or chokeberry pomace additives significantly increased (2.17–2.42%) compared to the control group I and group II fed with sea buckthorn pomace. A significant (P<0.05) reduction in the proportion of abdominal fat and skin with subcutaneous fat was observed in the chickens from group II, whereas a tendency towards a reduction in the proportion of abdominal fat content was observed in the chickens from groups III and IV.

The results of chemical analysis of the breast muscles (*pectoralis major*) of the broiler chickens are presented in Table 5. Crude fat content in group III (1.3%) was statistically significantly lower (P<0.05) compared with the other experimental groups (1.7%). Dry matter contents in the *pectoralis major* muscle of the chickens from the control group I and from the experimental groups fed mixtures supplemented with amaranth seeds or dried chokeberry pomace were similar, while the addition of sea buckthorn pomace to the feed mixture statistically significantly (P<0.05) reduced this parameter in comparison to the other groups.

Item		SEM			
Item	Ι	II	III	IV	SEM
Dry matter	25.61 a	25.35 a	24.83 b	25.60 a	0.13
Total protein	23.20	22.70	22.86	23.26	0.09
Crude fat	1.70 a	1.70 a	1.3 b	1.67 a	0.06

Table 5. Results of chemical analysis (%) of breast muscle (pectoralis major) in broiler chickens

a, b – mean values marked with different letters differ statistically significantly at P<0.05. Groups:

I control: 3% flax oil, 3% rapeseed oil.

II: 3% flax oil, 3% rapeseed oil, 8% amaranth seeds.

III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.

Component		SEM				
Component	Ι	II	III	IV	SEIVI	
Triglycerides (mg/dl)	56.75 a	53.83 b	51.17 b	58.33 a	1.33	
Cholesterol (mg/dl)	127.0 a	120.25 ab	110.33 b	126.08 a	1.66	
Glucose (mg/dl)	227.00 ab	232.58 a	204.92 b	237.00 a	2.51	
T3 (nmol/l)	2.45 a	1.71 b	1.22 c	1.83 b	0.18	
T4 (nmol/l)	27.43 a	26.32 a	20.17 b	17.68 b	1.10	

Table 6. Blood plasma components in broiler chicker	Tał	ble 6. Blood	plasma com	ponents in	broiler (chickens
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a, b, c – mean values marked with different letters differ statistically significantly at P < 0.05. Groups:

I control: 3% flax oil, 3% rapeseed oil.

II: 3% flax oil, 3% rapeseed oil, 8% amaranth seeds.

III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.

Analysis of blood plasma revealed a statistically significant (P < 0.05) reduction of triglyceride content in plasma from the chickens receiving amaranth seed or sea buckthorn pomace in the diet. Cholesterol and glucose contents were decreased in group III fed on the diet containing sea buckthorn pomace (Table 6).

In all the treatment groups, a statistically significant reduction (P<0.05) of triiodothyronine (T3) level by 25–50% was observed. The lowest T3 level (1.22 nmol/l) was noticed in the plasma of the birds from groups III and IV, and it was lower than in the control group by 26.5% and 35.5%, respectively.

No statistically significant impact of the studied plant additives on the physicochemical parameters of the broiler meat was found. In groups II, III and IV, the pH value in the breast muscle both after 12 h and 24 h was slightly higher than in the control group I. In the meat of the chickens from group II fed on the amaranth seed-supplemented diet, there was a tendency towards a reduction of drip losses (%) compared to the remaining groups (Table 7).

Demonster		SEM			
Parameter	Ι	II	III	IV	- SEM
pH _{15min}	6.32	6.47	6.50	6.54	0.04
pH_{24h}	5.99	6.04	6.08	6.02	0.03
Water holding capacity (%)	13.21	13.80	11.79	13.81	0.48
Drip loss 24h (%)	0.74	0.45	0.60	0.60	0.07
Drip loss 48h (%)	1.18	0.89	1.18	1.00	0.12
Cooking loss (%)	21.61	20.79	22.15	21.66	0.36
Total losses (%)	22.53	21.50	23.07	22.43	0.42

Table 7. Physicochemical properties of breast muscles (pectroralis major) of broiler chickens

Groups:

I control: 3% flax oil, 3% rapeseed oil.

II: 3% flax oil, 3% rapeseed oil, 8% amaranth seeds.

III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.

The breast muscle samples of chickens supplemented with dietary amaranth seeds and sea buckthorn pomace contained significantly lower contents of saturated fatty acids (P<0.05) by 2.65 and 2.2 percentage points, respectively, compared to the control group (Table 8). In these groups, a statistically significant (P<0.01) increase in palmitic acid (C16:0) was noticed. The proportion of monounsaturated fatty acid was lower in all the experimental groups, but the lowest (P<0.05) was observed in the chickens fed on a diet with sea buckthorn or chokeberry pomace in comparison to the control group I (by 1.68 and 3.73 percentage points, respectively). The meat of chickens from groups III and IV also contained a lower amount of oleic acid (C18:1) (P<0.01) and palmitoleic acid (C16:1). The addition of amaranth seeds, dried sea buckthorn and chokeberry pomace statistically significantly (P<0.01) increased the proportion of polyunsaturated fatty acids in the meat lipids. The proportion of these

acids increased (P<0.01) in all the treatment groups from 29.98% in the control group I to 35.91% in group III. The percentage proportion of *n*-3 PUFA significantly increased (P<0.01) in all the experimental groups. The ratio of *n*-6/*n*-3 PUFAs in all the groups receiving the tested dietary additives was significantly reduced (P<0.01) compared to the control group (1.7–1.9 vs. 2.28). In these groups, a tendency towards an increase in EPA and DHA contents was also observed.

	Group				
Fatty acid	Ι	II	III	IV	SEM
C16:0	18.65 Aa	16.60 Bb	16.70 Bb	17.28 Ab	1.183
C18:0	8.49	8.08	8.33	8.40	0.587
C16:1	1.88	1.63	1.62	1.50	0.150
C18:1	39.01 Aa	37.82 ABab	35.72 Bc	36.45 Bbc	2.250
C18:2	17.18 Bb	18.21 ABa	18.79 Aa	18.87 Aa	0.663
γC18:3	0.09	0.08	0.08	0.08	0.001
C20:0	0.10	0.09	0.08	0.08	0.001
C18:3	6.89 Bb	10.52 Aa	10.34 Aa	9.71 Aa	0.735
CLA T9-T11	0.05a	0.038 ab	0.034 b	0.035 b	0.001
C22:0	0.95	0.87	1.00	0.98	0.070
C20:4	3.53	3.07	4.00	3.62	0.582
C22:1	0.26	0.21	0.26	0.25	0.005
EPA	0.97	1.18	1.25	1.12	0.063
DHA	1.28	1.88	1.41	1.22	0.068
SFA	28.68 a	26.03 b	26.48 b	27.14 ab	2.972
UFA	71.32	73.97	73.52	72.87	2.973
MUFA	41.34 a	39.66 ab	37.61 b	38.21 b	4.882
PUFA	29.98 Bb	34.31 Aa	35.91 Aa	34.66 Aa	3.073
n-6 PUFA	20.80 Bc	21.37 ABbc	22.88 Aa	22.57 ABab	1.389
n-3 PUFA	9.14 Bb	12.90 Aa	13.00 Aa	12.05 Aa	0.699
DFA (C18:0 + UFA)	79.81 Bb	82.05 Aa	81.84 Aa	81.26 ABa	1.359
OFA (SFA-C18:0)	20.19 Aa	17.95 Bb	18.16 Bb	18.74 ABb	1.359
UFA/SFA	2.50 b	2.86 a	2.80 a	2.70 ab	0.058
DFA/OFA	3.96 Bb	4.59 Aa	4.55 ABa	4.35 ABab	0.125
MUFA/SFA	1.45	1.54	1.42	1.42	0.0251
PUFA/SFA	1.05 Bb	1.32 Aa	1.37 Aa	1.28 Aa	0.014
<i>n-6/n-3</i> PUFAs	2.28 Aa	1.66 Cc	1.76 BCbc	1.87 Bb	0.014
CLA	0.05a	0.04 ab	0.03b	0.04 ab	0.001

Table 8. Fatty acid profile of breast muscle lipids (*pectoralis major*) of broiler chickens (% of the sum of fatty acids)

a, b, c - mean values marked with different letters differ statistically significantly at P<0.05.

A, B, C – mean values marked with different letters differ statistically significantly at P<0.01. Groups:

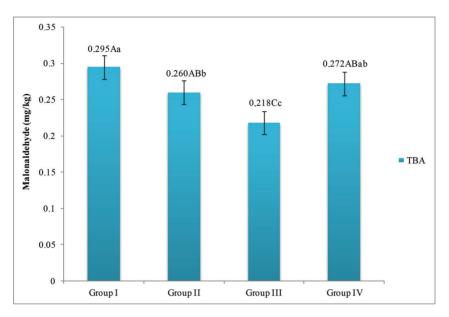
I control: 3% flax oil, 3% rapeseed oil.

II: 3% flax oil, 3% rapeseed oil, 8% amaranth seeds.

III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.

The results of evaluation of oxidant stability of the broiler meat are presented in Figure 2. TBA content in breast muscle samples of birds receiving sea buckthorn pomace measured after 3-month frozen storage at -20° C was significantly (P<0.01) lower compared with the control group I and the remaining treatment groups. This difference versus the control group amounted to 26.10% and versus groups supplemented with amaranth seeds and chokeberry pomace: 16.15% and 19.85%, respectively.



a, b, c – mean values marked with different letters differ statistically significantly at P<0.05. A, B, C – mean values marked with different letters differ statistically significantly at P<0.01. Groups:

I control: 3% flax oil, 3% rapeseed oil.

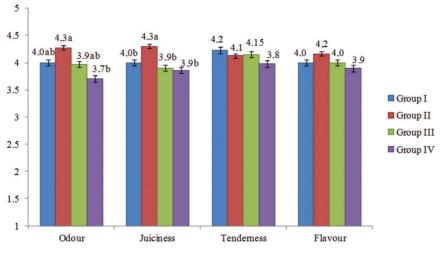
II: 3% flax oil, 3% rapeseed oil, 8% amaranth seeds.

III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.

Figure 2. Malonaldehyde content (mg/kg) in breast muscles (pectoralis major) of broiler chickens

Feed mixture supplementation with dried chokeberry pomace significantly (P<0.05) worsened meat aroma compared to the amaranth seed-fed group which had a beneficial effect on aroma, juiciness (P<0.05) and flavour (Figure 3). The addition of dried sea buckthorn pomace to the diet of the chickens (group III) had no effect on meat juiciness compared to the control group. However, chokeberry pomace as a feed component (group IV) negatively impaired meat tenderness and flavour versus the control group.



a, b – mean values marked with different letters differ statistically significantly at P<0.05. Groups:

I control: 3% flax oil, 3% rapeseed oil.

II: 3% flax oil, 3% rapeseed oil, 8% amaranth seeds.

III: 3% flax oil, 3% rapeseed oil, 3% sea buckthorn pomace.

IV: 3% flax oil, 3% rapeseed oil, 3% chokeberry pomace.

Figure 3. Results of sensory analysis of breast muscles *(musculus pectoralis major)* of broiler chickens (on a 5-point scale: 5 points – highest score, 1 point – lowest score)

Discussion

Studies on farm animals demonstrated a beneficial effect of plant additives on their health status (Arczewska-Włosek and Świątkiewicz, 2013), and on the taste and nutritional value of animal products (Rossi et al., 2013; Hanczakowska et al., 2015; Pieszka et al., 2017). The use of feed mixtures supplemented with flax oil, amaranth seeds, sea buckthorn pomace and chokeberry pomace significantly reduced feed intake, while amaranth seeds significantly reduced the body weight gain of chickens. The obtained results in our experiment are in contrast to the studies of Rouckova et al. (2004), who did not observe any impact of feeding broiler chickens with 8% amaranth seed feed mixture on the body weight and growth performance of birds. However, according to Pisarikova et al. (2006), the addition of 8% amaranth seeds to feed mixture did reduce the body weight of the chickens. The production data from the broiler feeding experiment with sea buckthorn pomace agreed with the results obtained by Stef et al. (2012), who also tested sea buckthorn pomace in poultry feeding. On the other hand, according to Kamel (2001) dried sea buckthorn showed appetite- and digestion-stimulating action which increased feed intake.

In our experiment, we observed the reduced feed intake by chickens fed rations with sea buckthorn pomace additive. However, Stef et al. (2012) indicated that

chicken diet supplementation with 2% buckthorn fruit pomace did not improve the production data of birds. These results are in line with studies by Ciurescu et al. (2007) who also applied sea buckthorn and chokeberry pomace as feed additives for broiler chickens and observed the best feed efficiency in the group of chickens receiving the mixture supplemented with 3% chokeberry pomace; these differences, however, were not supported by statistical analysis. Nevertheless, our experimental results confirmed a significant effect of chokeberry pomace on this parameter. According to Loetscher et al. (2013), the feed efficiency in the group of chickens fed a diet supplemented with 2.5% dried chokeberry was comparable with the control group. The observations of the above-mentioned authors demonstrated that chokeberry pomace as a component of feed mixture did not significantly affect the production results of broiler chickens. Moreover, studies conducted by Pieszka et al. (2010) aimed at assessing the efficiency of dried chokeberry pomace additive in feed mixture for fattening pigs did not confirm its impact on increased body weight gains and better feed efficiency.

In the present experiment, an interesting finding is the absence of mortality in group of the birds fed sea buckthorn pomace-supplemented feed mixture. Similar results were obtained by Patial et al. (2013) who investigated the effects of feeding Japanese quails with feed containing buckthorn fruit extract as an additive. It can be supposed that the active substances present in the fruits of this plant have a beneficial effect on the immune system function and show antibacterial and cytoprotective properties (Mishra et al., 2008; Stef et al., 2012; Dhanze et al., 2013). Ramasamy et al. (2010) in their studies on broiler chickens confirmed an immunomodulating activity of dried sea buckthorn fruits. They also observed zero mortality in chickens fed a feed mixture supplemented with 3% chokeberry pomace. On the other hand, Loetscher et al. (2013) reported that 2.5% of pomace in feed did not reduce the mortality of the broiler chickens. Presumably, the chokeberry pomace as well as sea buckthorn pomace, due to their antioxidant activity, can also exhibit a supportive function of the immune system and may prevent inflammatory processes in the intestines (Mishra et al., 2008).

The experiment did not show a hypocholesterolemic effect of amaranth seeds, but plasma triglyceride level was reduced. A reduction of this parameter was also observed in experiments with Japanese quails fed a mixture containing 7% amaranth seeds (Szczerbińska et al., 2015). In these birds, plasma glucose level was also decreased – both at 4% and 7% content of this component in the mixture. However, the results obtained in our experiment did not show a significant impact of 8% amaranth seeds in feed on plasma glucose level in the chickens. The above results are in agreement with those of Rouckova et al. (2004) who indicated that amaranth seeds in feed mixture for broiler chickens had no reducing effect on plasma cholesterol or triglyceride level. These data are in contrast to those reported by Qureshi et al. (1996). These researchers demonstrated that a high content of γ - and σ - tocotrienols and squalene in amaranth seeds blocked cholesterol biosynthesis in 6-day-old chicks, which resulted in the reduction of plasma cholesterol level in these birds. On the other hand, Mendonça et al. (2009) ascribed hypocholesterolemic properties to a protein component of amaranth seeds. However, despite that, the cause and the

mechanism of the inhibition of enzymes responsible in cholesterol synthesis have not been unequivocally explained. Grajeta (1999) indicated that the hypolipidemic effect of amaranth seeds was dependent on the type of dietary fat component. According to this author, sunflower oil rich in LA enhanced the hypolipidemic action of amaranth seeds. Studies on rats revealed that another amaranth variety, *Amaranthus hypochondriacus*, had a positive impact on plasma lipid profile (Czerwiński et al., 2004). This effect was closely dependent on the content of bioactive substances, including antioxidants in amaranth seeds (Czerwiński et al., 2004).

The results of our experiment showed a hypocholesterolemic and hypolipidemic effect of sea buckthorn pomace because its addition to feed significantly reduced plasma total cholesterol, triglyceride and glucose levels in the chickens. The ability of sea buckthorn pomace to reduce plasma cholesterol and triglyceride levels in animals has been attributed to flavonoids present in this additive. In contrast, the group fed a mixture supplemented with 3% chokeberry pomace showed a significant increase in the above plasma components. A reduction in triiodothyronine was observed with no changes in plasma thyroxin level in the chickens receiving feed with 8% amaranth seed additive compared to the control group. T3 reduction with unchanged T4 content suggests disturbances in T4 to T3 conversion. It is supposed that the observed changes result from anti-nutrients present in amaranth seeds which block the activity of deiodinases – the enzymes responsible for T4 to T3 conversion. On the other hand, when the chicken diet contained 3% sea buckthorn or chokeberry pomace, plasma analysis revealed diminished thyroid activity manifested by the reduced level of both T3 and T4.

In our experiment, feed mixture supplementation with amaranth seeds or sea buckthorn or chokeberry pomace did not have any significant effect on the dressing percentage of broiler chicken carcasses, which was similar in all groups. In addition, Nuernberg et al. (2015) did not show a negative impact of sea buckthorn pomace in pig feeding on production data and carcass quality. A significant increase was observed in the breast muscle percentage in carcasses of chickens fed 8% amaranth seed-supplemented feed mixture. These results are contrary to those reported by Rouckova et al. (2004) in which amaranth seeds did not influence breast muscle percentage yield. It appears that the rise in breast muscle proportion in our experiment could be caused by increased n-3 PUFA concentration in the feed due to supplementation of 3% flax oil which is rich in n-3 PUFA, whereas in the studies cited above, 2.2% soybean oil was used which is rich in *n*-6 PUFA. Energy released during β -oxidation of fatty acids can increase protein accumulation in the muscles and reduce fat deposition in the tissues. This was confirmed by analysis of the tissue composition of the carcasses of chickens receiving amaranth seeds in the feed, which were characterised by a significantly lower proportion of abdominal fat and skin with subcutaneous fat. On the other hand, Zhang et al. (2009) indicated that sea buckthorn supplementation to the feed mixtures for pigs reduced backfat thickness and decreased intramuscular fat content. In the presented experiment, the chokeberry pomace had no effect on carcass tissue composition. Also, Pieszka et al. (2010) noted no significant effect of this additive on the carcass characteristics in fattening pigs.

Jakubowska et al. (2013) did not find any impact of 4% or 7% amaranth seed in the feed mixtures for quails on the basic chemical composition of the breast muscles *(m. pectoralis major)*. Likewise in this experiment, the addition of 8% amaranth seeds and 3% chokeberry pomace to the feed mixture did not affect the chemical composition of the breast muscles of the broiler chickens *(pectoralis major)*. However, dry matter content and crude fat of the breast muscle *(pectoralis major)* in chickens receiving the feed mixture supplemented with sea buckthorn pomace were both significantly reduced. Similar results were also obtained by Zhang et al. (2009).

The technological value of meat – and thus indirectly the quality of its products – are dependent on pH, water holding capacity, cooking drip losses, among other things (Pietrzak et al., 2007). The muscles show the greatest water holding capacity immediately after slaughter, and as the pH decreases the water holding capacity is reduced as well. Muscle acidity determines such meat attributes as water holding capacity, colour, tenderness and juiciness (Le Bihan-Duval, 2004). An increase in pH is accompanied by the elevation of water holding capacity of meat proteins (Połtowicz, 2000) which reduces losses during technological processes. In our experiment, we did not observe an effect of the tested additives on the above-described parameters of breast meat quality (pectoralis major), although there was a tendency towards an improvement by decreasing drip and cooking losses in the breast muscles (pectoralis major) from the chickens receiving 8% amaranth seed in the diet. Similar results were obtained by Jakubowska et al. (2013), who demonstrated no negative effect of amaranth seeds on the physicochemical properties of the meat. Analogously, Nuernberg et al. (2015) did not note any disadvantageous effect of sea buckthorn pomace on the quality parameters of porcine meat.

The addition of amaranth seeds, sea buckthorn and chokeberry pomace in this study significantly increased n-3 PUFA level, especially ALA, and highly significantly reduced n-6/n-3 PUFA ratio. The obtained results are in agreement with the reports of other authors (Nuernberg et al., 2015; Jakubowska et al., 2013). Moreover, in our experiment, amaranth seeds and sea buckthorn pomace addition reduced the susceptibility of the breast muscles (pectoralis major) to oxidative changes. A considerable deceleration of lipid oxidation in the meat of chickens fed the diet with amaranth seeds and sea buckthorn pomace, as evidenced by a significantly reduced TBA value, was probably caused by a synergistic action of different antioxidant components of both feed additives. Squalene, isothiocyanates and phenolic compounds (mostly quercetin) and tocopherols present in amaranth seeds (Hertog et al., 1992; Geetha et al., 2009), and antioxidants found in sea buckthorn pomace - such as carotenoids, vitamin C, tocopherols and phytosterols - significantly preserve the health-promoting quality of meat without a worsening of food safety. On the other hand, the meat of the chickens receiving the feed mixture containing chokeberry pomace showed slightly lower TBA level compared with the meat of chickens from the control group; however, the difference was not statistically significant.

The studies of Loetscher et al. (2013) revealed that chokeberry pomace used in feed mixture for broiler chickens at 2.5% significantly delayed lipid oxidation in chicken meat, as manifested by a TBA-RS value lower by 32.18% than in the control group. The obtained result indicates a protective action of phenolic compounds, an-

thocyanins, flavonoids and tocochromanols present in chokeberry pomace (Pieszka et al., 2015). According to Strugała et al. (2015), the use of chokeberry fruit extracts in combination with flax oil delayed polyunsaturated fatty acid oxidation. The results of studies presented by Bou et al. (2009) demonstrated that these two antioxidants used in combination had a greater efficacy in slowing down lipid oxidation than either of them used separately. The results of the sensory analysis of the cooked meat carried out in the present study indicate that amaranth seed supplement significantly improves the aroma of the meat. However, the results of Jakubowska et al. (2013) did not confirm such an effect. These authors noted a significant influence of 4% amaranth seed additive on an improvement of meat juiciness, which was also observed in our own study.

Conclusions

In conclusion, it can be assumed that the addition of amaranth seeds and dried sea buckthorn or chokeberry pomace to the feed mixtures for broiler chicken has a beneficial influence on several meat quality and bird performance parameters. The most favourable chicken broiler production parameters were observed in the groups fed with sea buckthorn pomace and chokeberry pomace (groups III and IV). The amaranth seeds, as well as the sea buckthorn pomace and chokeberry pomace, can be an efficient method of modifying the fatty acid profile of the *pectoralis major* muscle by increasing the *n*-3 PUFA content and decreasing the ratio of n-6/n-3 PU-FAs which is desirable from the standpoint of human nutrition. The amaranth seed improved the taste attributes of the meat. The applied doses of the additives had no significant effect on the physiochemical characteristic of the meat. Both the sea buckthorn pomace and the amaranth seeds increased meat stability by delaying the lipid oxidation processes of the meat and improved some of its sensory properties. The obtained results indicate that using the tested plant additives in feed mixture may be an efficacious way of improving the production parameters of broiler chickens and effectively enriching the meat in n-3 fatty acid and protecting against excessive oxidation of lipids.

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