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The effect of *Amaranthus caudatus* supplementation to diets containing linseed oil on oxidative status, blood serum metabolites, growth performance and meat quality characteristics in broilers*

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The study evaluated the effect of amaranth (*Amaranthus caudatus*) grain (AMG) supplementation to diets containing linseed oil on the oxidative status, blood serum metabolites, growth performance and meat quality. A total of 132 90-d-old female Big Ray broilers were randomly divided into 3 groups of 44 broilers each (11 broilers per cage, 4 cages per treatment) and fed on a diet containing 50 g/kg linseed oil supplemented with 0, 50 or 100 g/kg AMG, respectively, for 32 d. At the end of the experiment 30 broilers (10 per treatment) were sacrificed and breast muscle samples were prepared for analysis. Growth performance was significantly lower ($P<0.05$) in the broilers fed on the diets supplemented with AMG. Serum antioxidant power was significantly higher (250 and 219 *vs* 177 µEq/l; $P<0.05$) and serum lipid peroxidation levels were lower (262 and 419 *vs* 700 µmoles/l) in the broilers fed on a diet containing 100 or 50 g/kg AMG, respectively, as compared with the broilers given a diet without AMG supplementation. Cholesterol and triglyceride levels were significantly lower ($P<0.05$) in the broilers fed on AMG diets than in those given a diet without supplementation. No differences in alanine aminotransferase or albumin levels were found. Broilers fed on diets rich in linseed oil, which contains a high content of polyunsaturated fatty acids and either supplemented or not with AMG, showed a good meat fatty acid profile. No differences in other meat quality characteristics were found between broilers fed on a diet containing AMG and those not administered this supplementation.

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Nutrition plays a pivotal role in health promotion and disease prevention. Recognizing the adverse health effects potentially associated with a high dietary intake of saturated fatty acids (SFAs) [Szostak-Węgierek *et al*. 2013], nutritionists have recommended partly replacing SFAs with polyunsaturated fatty acids (PUFAs) to improve consumer health [Sanders 2014]. In response to new dietary recommendations and changes in consumer preferences, food manufacturers have looked for ways to produce meat and meat products with a higher PUFAs content in the fat through the addition of various different lipid sources to animal feed [Peiretti 2012, Raj *et al*. 2010, Ribeiro *et al*. 2013, Wood *et al.* 2008, Poławska *et al*. 2011, 2013]. However, because in comparison to SFA-enriched meat PUFA-enriched meat is more subject to lipid peroxidation, flavour deterioration, and the formation of unhealthy compounds, animals fed on diets supplemented with PUFAs require an adequate intake of dietary antioxidants [Jacobsen *et al*. 2008]. The inclusion of vegetable oils in broiler feed has been shown to increase the level of unsaturated fatty acids in poultry meat. Rezar *et al*. [2003] showed that a high PUFA intake with an unbalanced diet can cause lipid peroxidation in the body. Moreover, dietary modification with other natural substances in poultry diets has been found to enhance antioxidant activity, thus protecting broilers and their feed from lipid peroxidation [Nain *et al*. 2015, Anjum *et al*. 2013].

Amaranth (*Amaranthus caudatus*), a member of the *Amaranthaceae* family native to Central America, was cultivated for its grain by the ancient Incas, Mayas and Aztecs [Caselato-Sousa and Amaya-Farfŕn 2012]. One reason amaranth grain (AMG) is again gaining popularity among grains for human and animal nutrition is because it is an optimal source of essential amino acids, unsaturated fatty acids, antioxidants and active compounds [Ulbricht *et al*. 2009]. AMG contains variable amounts of crude protein and lipid (125 to 176 g/kg and 52 to 77 g/kg, respectively) depending on environmental conditions and genotype [Budin *et al*. 1996]. The lipid fraction is rich in linoleic acid (LA; C18:2n-6; 367 to 559 g/kg total fatty acid methyl esters (FAME)), oleic acid (OA; C18:1n-9; 187 to 389 g/kg total FAME), palmitic acid (PA; C16:0; 191 to 234 g/kg total FAME) [He *et al*. 2002], and small quantities of α-linolenic acid (ALA; C18:3n-3; 14 g/kg total FAME) [Berger *et al*. 2003]. Amaranth is considered a functional food thanks to its many beneficial effects on consumer health. Its hypocholesterolemic and antioxidant properties derive from several active compounds. For example, its ability to lower serum cholesterol levels has been mainly attributed to the high content of squalene [Caselato-Sousa and Amaya-Farfŕn 2012] and lunasin [Huerta-Ocampo and de la Rosa 2011] in the grain. Paśko *et al*. [2009] demonstrated that AMG contains significant amounts of phenolic compounds, particularly gallic, *p* hydroxybenzoic, and vanillic acid, as well as flavonoids such as rutin that confer AMG its antioxidant activity.

The aim of this study was to evaluate the effect of AMG supplementation on the oxidative status, blood serum metabolites, growth performance and meat quality in

broilers fed on diets rich in linseed oil, which contains a high proportion of PUFAs. Indeed, poultry meat is destined to become the most widely consumed meat in the world due to its low production costs and the absence of religious restrictions [Landoni and Albarellos 2015].

Material and methods

Experimental design and animal management

The study was carried out in accordance with regulations of the Institutional Review Board for animal use and ethics of the Department of Veterinary Sciences, University of Torino.

A total of 132 90-d-old female Big Ray broilers were randomly distributed into 3 groups of 44 broilers each (11 broilers per cage, 4 cages per treatment) under controlled environmental conditions. Each cage measured 1.50 x 1.50 m and was equipped with a feeder and a watering place. The treatments consisting of 3 isoproteic and isoenergetic diets containing 50 g/kg linseed oil were formulated as follows: no AMG supplementation, supplementation with 50 g/kg and with 100 g/kg AMG, respectively (Tab. 1). The diets were formulated according to requirements specific for meat chickens [National Research Council 1994]. Each mixed feed was stored in silos sheltered from light to avoid self-oxidation of lipid components and was offered in the powdered form. Diets and clean drinking water were offered *ad libitum*. Feed consumption per replicate was measured every 3 days and each replicate was weighed weekly to evaluate growth performance. Mortality rate was recorded daily. All broilers were slaughtered after 32 d in a poultry slaughterhouse according to current standards [Regulation E.C. 2009].

Proximate analysis

Diet samples were analysed for dry matter (DM; cod. 930.13), ash by ignition to 550°C, crude protein (CP; cod. 954.01) and ether extract (EE; cod. 945.16) according to AOAC procedures [AOAC 1990], neutral detergent fibre without sodium sulphite or α-amylase, and acid detergent fibre, as described in Van Soest *et al*. [1991], expressed exclusive of residual ash, and gross energy determined using an adiabatic bomb calorimeter (C7000, IKA, Staufen, Germany).

Biochemical parameters

Blood samples from 30 tagged broilers (10 per treatment) were collected via brachial puncture into 5-mL heparin vacuum tubes at 0 and 32 days. Blood was allowed to clot and it was centrifuged at 3000 rpm for 10 min at room temperature. Serum was then separated and stored at -70°C until analysis.

Biochemical analysis was carried out on serum samples and levels of cholesterol, triglycerides, liver enzyme alanine aminotransferase (ALT), and albumin were determined using an automated clinical biochemistry analyzer (ILab Aries

analyzer, Instrumentation Laboratory, Milan, Italy) using dedicated commercial kits (Instrumentation Laboratory).

Analysis of oxidative status

Reactive oxygen metabolites and the biological antioxidant activity in serum were determined using the LP-Cholox test, anti-ROMs test 1 (state of rapid antioxidant capacity) and the anti-ROMs test 2 (state of slow anti-oxidant capacity) run on an automated analyser (Free Carpe Diem, Diacron International, Grosseto, Italy) and using commercial kits (Diacron International) according to the manufacturer's instructions. The LP-Cholox test evaluates lipid peroxidation, an important marker of oxidative stress. The test is based on the ability of peroxides to promote the oxidation of ferrous iron to ferric iron. The ferric iron bound to thiocyanate develops a coloured complex that can be measured spectrophotometrically. The increase in absorbance is directly proportional to the concentration of lipoperoxides in the sample, and the values are related to specific standard solutions [Macri *et al*. 2015].

The anti-ROMs test is based on the ability of antioxidants to reduce ferric iron to ferrous iron which, on reacting with αα-dipyridyl, produces a red-purple colouration. The colour intensity increases proportionally to the quantity of iron reduced by the antioxidants in the plasma. The test allows to discriminate between the state of rapid antioxidant capacity (the first concentration determined by the instrument), i.e. fast-acting antioxidants such as vitamin C or vitamin E, and the state of slow antioxidant capacity (the second concentration determined by the instrument), i.e. slow-acting antioxidants such as thiol-SH groups, uric acid, polyphenols and anthocyanins. The results are expressed in μEq of reduced iron/L using ascorbic acid as a standard according to Meineri *et al*. [2015]. In a study conducted with human subjects Maruoka *et al.* [2013] classified anti-ROMs test 1 results into four oxidative stress levels: normal (optimal value >200 μEq/l), slightly low (200-150 μEq/l), moderately low (150-100 μEq/l), and very low (<100 μEq/l), and anti-ROMs test 2 results into five stress levels: normal (optimal value >1,000 μEq/l), borderline (1,000-900 μEq/l), slightly low (900-700 μEq/l), moderately low (700-500 μEq/l), and very low (<500 μEq/l).

Preparation of meat samples

Thirty tagged broilers (10 from each treatment group) were slaughtered after 32 d and eviscerated. Broiler breast muscle was extracted from carcasses and divided into two parts: one part was used to measure pH and colour, and the other was frozen at -20 C°, freeze-dried and used to determine the FA profile.

pH and colour measurement

The pH of the breast muscle was measured in duplicate using a Crison portable pH meter (Crison Instruments, S.A., Alella, Spain) fitted with a spear-type electrode and an automatic temperature compensation probe. Meat colour was measured on a freshly cut surface of the breast muscle at room temperature (20°C) using a Minolta

Colorimeter CR-331C (Konica Minolta, Tokyo, Japan) (25 mm measuring area, 45° circumferential illumination/0° viewing angle geometry) with the D65 illuminant and a 2° standard observed angle. Colour measurements are reported as lightness (L*), redness (a*), and yellowness (b*) in the CIELAB colour space model [CIE 1976]. Chroma (C*), which is a measure of colour intensity, and hue angle (H*), which describes the fundamental colour of a substance, were calculated as (a*2+b*2) 0.5 and $\tan^{-1}$ (b*/a*), respectively. The hue angle was converted from radians to degrees for data analysis. The colour values were obtained from the average of three readings per meat sample.

Fatty acid composition

Lipid extraction was performed on AMG, diets, and meat samples according to Hara and Radin [1978]. The FA were analysed as their methyl esters. Gas chromatography was applied as described in Peiretti *et al*. [2007]. The analysis was carried out using a Dani GC 1000 DPC (Dani Instruments S.P.A., Cologno Monzese, Italy), equipped with a fused silica capillary column – Supelcowax-10 (60 m x 0.32 mm (i.d.), 0.25 μm). The PTV injection and flame ionization detector (FID) ports were set at 245°C and 270°C, respectively. The oven temperature program was initially set at 50°C for the first min, and then increased at a rate of 15°C/min to 200°C, where it was maintained for 20 min and then increased at a rate of 5°C/min to 230°C, where it was maintained for the last 3 min. The carrier gas was hydrogen. One microlitre was injected using a Dani ALS 1000 auto sampler with a 1:50 split ratio. The peak area was measured using a Dani Data Station DDS 1000, with each peak identified and quantified by pure methyl ester standards (Restek Corporation, Bellefonte, PA, USA).

The saturation (S/P), atherogenic (AI), and thrombogenic (TI) indexes were calculated according to Ulbricht and Southgate [1991] as follows:

$$S/P = (C14:0 + C16:0 + C18:0) / \Sigma \text{ MUFAs} + \Sigma \text{ PUFAs};$$

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / [\Sigma \text{ MUFAs} + \Sigma (n\text{-}6) + \Sigma (n\text{–}3)],$$

$$TI = (C14:0 + C16:0 + C18:0) / [0.5 \times \Sigma \text{ MUFAs} + 0.5 \times \Sigma (n\text{-}6) + 3 \times \Sigma (n\text{-}3) + \Sigma (n\text{-}3) / \Sigma (n\text{-}6)],$$

where: Σ MUFAs and Σ PUFAs denote the sum of monounsaturated fatty acids and polyunsaturated fatty acids, respectively and Σ (n-6) and Σ (n-3) denote the sum of PUFA n-6 and PUFA n-3, respectively.

Statistical analyses

The experimental unit for growth performance was a cage of 11 broilers, while one bird was an experimental unit for the other parameters. The effect of diet on growth performance, oxidative status, blood serum metabolites, meat chemical composition, and the FA profile was evaluated using one-way analysis of variance (ANOVA). Treatment means were compared using Duncan's new multiple range test. Statistical analyses were performed using SPSS version 11.5.1 for Windows (SPSS Inc., Chicago, IL, USA).

Results and discussion

AMG and diet composition

The chemical composition of the 3 diets was similar in terms of their contents of dry matter, acid detergent fibre, gross energy, crude protein and ether extract, while the diets differ only in ash and neutral detergent fibre contents (Tab. 1).

The most abundant FAs in the diets were ALA, LA, and OA, accounting for 828 to 830 g/kg of total FAME. A significant decrease in the percentage of LA and an increase in ALA and stearidonic acid (STA, C18:4n-3) was observed with an increase in AMG supplementation (Tab. 1).

Table 1. Ingredients (g/kg), chemical composition and fatty acid profile (LS mean and standard deviation) of the experimental diets

Item	Diet/group[1]		
	C (n=4)	AMG50 (n=4)	AMG100 (n=4)
Components			
corn	485	240	0
wheat	0	180	360
soy	335	300	280
wheat bran	100	150	180
amaranth grain	0	50	100
linseed oil	50	50	50
dicalcium phosphate	30	30	30
Chemical composition			
dry matter (g/kg fresh matter)	894.6 (6.8)	891.1 (1.9)	896.7 (2.9)
organic matter (g/kg DM)	936.9 (2.8)	944.5 (2.5)	940.6 (0.6)
crude ash (g/kg DM)	63.1^{A} (2.8)	55.5^{C} (2.5)	59.4^{B} (0.6)
neutral detergent fibre (g/kg DM)	137.6^{A} (8.7)	150.1^{A} (2.1)	163.4^{B} (10.4)
acid detergent fibre (g/kg DM)	59.2 (7.8)	55.1 (10.1)	68.6 (3.8)
ether extract (g/kg DM)	51.9 (8.6)	57.2 (4.1)	54.9 (3.0)
crude protein (g/kg DM)	225.6 (6.7)	233.1 (8.0)	234.5 (3.3)
gross energy (MJ/kg DM)	19.1 (0.2)	19.4 (0.1)	19.2 (0.1)
Fatty acid profile			
C16:0 (g/kg total FAME)	81.7 (2.7)	82.2 (3.9)	82.7 (2.8)
C17:0 (g/kg total FAME)	18.8 (10.9)	18.0 (6.0)	14.1 (7.8)
C18:0 (g/kg total FAME)	36.5 (1.2)	37.5 (3.1)	36.9 (1.3)
C18:1n-9 (g/kg total FAME)	201.0 (12.9)	194.4 (5.1)	185.0 (2.9)
C18:1n-7 (g/kg total FAME)	5.5 (4.7)	6.9 (6.3)	9.7 (0.9)
C18:2n-6 (g/kg total FAME)	315.1^{A} (3.9)	300.3^{B} (6.3)	286.9^{C} (1.6)
C18:3n-4 (g/kg total FAME)	13.6 (1.3)	10.2 (8.8)	9.2 (8.4)
C18:3n-3 (g/kg total FAME)	311.4^{A} (10.0)	333.3^{A} (4.8)	358.1^{B} (16.6)
C18:4n-3 (g/kg total FAME)	16.3^{a} (0.7)	17.3^{b} (0.2)	17.6^{b} (0.2)

[1]C – control group; AMG50 – amaranth grain group (50 g/kg of diet); AMG100 – amaranth grain group (100 g/kg of diet).

[aA...]Within rows means bearing different superscripts differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

Growth performance

Table 2 presents the data on productive efficiency. Final weight decreased ($P<0.05$) with an increasing AMG inclusion in the diet. Weight gain and daily weight gain ($P<0.001$) were lower in the broilers fed on diets containing AMG. Acar *et al*. [1988] and Roučkova *et al*. [2004] found no differences in live weights of broilers fed with heat-treated or raw AMG. In contrast, Ravindran *et al*. [1996] reported that weight gain, feed intake, and feed gain are lower with increasing levels of raw AMG, whereas autoclaved AMG does not affect growth performance. Popiela *et al*. [2013] reported that 50 and 100 g/kg of extruded AMG supplementation resulted in a greater final body weight in laying hens as compared to layers fed on a diet without AMG. Takeda and Kiriyama [1991] showed that intestinal absorption of nutrients and growth are inhibited in rats fed on a diet containing 50 g/kg amaranth. The reason is that AMG contains anti-nutritional factors (e.g. saponins and trypsin inhibitors) that can interfere with the digestive process or the metabolic utilization of feed [Martens *et al*. 2012]. Heat treatment can inactivate anti-nutritional factors, but adversely affect the nutritional properties of AMG by reducing antioxidant activity and the amount of FAs in the seeds [Repo-Carrasco-Valencia *et al*. 2009, Queiroz *et al*. 2009, Venskutonis and Kraujalis 2013]. Furthermore, it seems that the content of anti-nutritional factors in AMG is not always such as to inhibit the absorption of proteins and minerals in animals [Ologunde *et al*. 1992]. We supplemented the diets with raw AMG in order to maintain its antioxidant activity. Nonetheless, the anti-nutritional factors contained in the AMG may have influenced nutrient uptake, thus inhibiting normal growth despite the fact that the diets were nutritionally balanced.

Table 2. Block assignment, mortality and growth performance (LS mean and standard deviation) of broilers

Item	Diet/group[1]		
	C	AMG50	AMG100
Number of cages	4	4	4
Number of broilers/cage	11	11	11
Mortality	3/44	3/44	4/44
Initial live weight (g)	2156 (73)	2167 (56)	2135 (30)
Final live weight (g)	2687^{a} (111)	2408^{b} (143)	2400^{b} (42)
Feed intake (g)	4535 (413)	3916 (261)	4139 (236)
Daily feed intake (g)	141.7 (12.9)	122.4 (8.2)	129.3 (7.4)
Weight gain (g)	531.5^{A} (40.6)	241.0^{B} (123.5)	265.0^{B} (68.9)
Daily weight gain (g)	16.6^{A} (1.3)	7.5^{B} (3.9)	8.3^{B} (2.2)

[1]C – control group; AMG50 – amaranth grain group (50 g/kg of diet); AMG100 – amaranth grain group (100 g/kg of diet).
[aA...]Within rows means bearing different superscripts differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

Blood serum metabolites

Cholesterol levels were significantly lower ($P<0.01$) in the serum of broilers fed on the AMG-supplemented diets than in those fed on the diet without AMG supplementation, while triglyceride levels were significantly lower ($P<0.05$) in the serum of broilers fed on the diet containing 100 g AMG/kg in comparison to those fed on the diet containing 50 g AMG/kg or without AMG. No differences in ALT or albumin levels were found (Tab. 3). The nutritional state was normal in relation to the protein fraction, inasmuch as the serum albumin levels were within the standard limits. Our observation of a lipid-lowering effect of AMG on broilers is consistent with findings from previous studies involving humans and animals.

Table 3. Blood serum metabolites (LS mean and standard deviation) in blood samples taken after 32 days

Item	Diet/group[1]		
	C (n=10)	AMG50 (n=10)	AMG100 (n=10)
Cholesterol (mg/dl)	93.4^{A} (12.2)	83.1^{B} (9.4)	72.0^{C} (8.4)
Triglycerides (mg/dl)	84.4^{a} (25.3)	68.8^{a} (28.9)	42.4^{b} (12.8)
Albumin (g/dl)	1.49 (0.13)	1.53 (0.16)	1.60 (0.08)
ALT[2] (U/l)	2.70 (0.67)	3.20 (1.23)	2.44 (0.88)

[1]C – control group; AMG50 – amaranth grain group (50 g/kg of diet); AMG100 – amaranth grain group (100 g/kg of diet).
[2]ALT – alanine aminotransferase.
$^{aA,\ldots}$Within rows means bearing different superscripts differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

Martirosyan *et al*. [2007] reported that the inclusion of amaranth oil in the diet significantly reduces total cholesterol and triglyceride levels in human patients with hypertension and coronary heart disease. Mendonça *et al*. [2009] found that amaranth protein has a hypocholesterolemic effect in hamsters. Plate and Areąs [2002] showed that extruded amaranth has a cholesterol-lowering effect in hypercholesterolemic rabbits. Finally, Qureshi *et al*. [1996] reported that dietary supplementation with amaranth inhibits cholesterol biosynthesis in broilers.

Our results were not consistent with those presented in a study of Popiela *et al.* [2013] on Lohmann Brown laying hens fed on diets containing 0, 50 or 100 g/kg of extruded AMG for 5 and 10 weeks. In this study no significant changes in cholesterol or triglyceride concentrations were observed during the whole experiment period. Furthermore, the levels of ALT were also investigated; the level of ALT in all dietary treatments was lower after 10 weeks of the experiment in comparison with results obtained after 5 weeks of feeding. In poultry the ALT activity in blood serum depends on age; in fact, the values decrease with the increasing age of the broilers [Krasnodębska-Depta 2005].

Oxidative status

The LP-Cholox test showed that lipoperoxides were decreased ($P<0.01$) with a higher AMG inclusion in the feed (Tab. 4). Lipoperoxides comprise a group of oxygenated compounds produced during the oxidation of cholesterol and PUFA [Rahman 2007]. The tissue damage they cause results from the inhibition of protein synthesis, blood macrophage activity, chemotactic signalling and enzyme activity. Accordingly, the serum lipoperoxide level is used as an indicator of systemic oxidative stress in humans and experimental animals [Repetto *et al*. 2012]. Pasko *et al*. [2011] studied the relationship between lipid peroxidation and antioxidant activity of AMG by measuring plasma malondialdehyde and evaluating changes in antioxidant enzyme activity. They demonstrated that AMG supplementation has a dose-dependent effect on reducing lipid peroxidation in rats under oxidative stress induced by dietary fructose.

Table 4. Lipoperoxides (LS mean and standard deviation) in blood samples taken after 32 days

Item	Diet/group[1]		
	C (n=10)	AMG50 (n=10)	AMG100 (n=10)
Lipoperoxides (μmoles/l)	700.6^{A} (62.6)	419.0^{B} (80.5)	262.5^{C} (67.8)
Anti-ROMs test 1 (μEq of Fe3+/l)[2]	176.9^{A} (24.3)	218.9^{B} (24.7)	249.6^{C} (23.3)
Anti-ROMs test 2 (μEq of Fe3+/l)[3]	1449^{A} (73)	1651^{B} (80)	1787^{C} (76)

[1]C – control group; AMG50 – amaranth grain group (50 g/kg of diet); AMG100 – amaranth grain group (100 g/kg of diet).
[2]State of rapid antioxidant capacity.
[3]State of slow antioxidant capacity.
[ABC]Within rows means bearing different superscripts differ significantly at P<0.01.

We noted elevated oxidative stress levels in the serum of broilers fed on diets containing 50 g/kg linseed oil. Analysis of the total serum lipoperoxide level indicated that AMG can prevent the generation of free radicals in the serum of broilers fed diets rich in PUFAs. Furthermore, the anti-ROMs test results suggest that AMG possesses good antioxidant activity, as demonstrated by the significant increase ($P<0.01$) with the inclusion of AMG in the diet (Tab. 4). The antioxidant power of the serum of broilers fed on the AMG-supplemented diets was significantly higher than that of the broilers fed on the diet containing only linseed oil.

Dietary AMG supplementation can enhance the body's antioxidant defences. In a randomized, double-blind placebo-controlled study Maruoka *et al.* [2013] found no significant changes in the antioxidant potential with rapid and slow reactivity in two groups of overweight humans: one group received liquorice flavonoid oil capsules and L-carnitine capsules (with antioxidant capacity), while the other group received placebo capsules only. The results of anti-ROMs test 1 and anti-ROMs test 2 showed a moderately low stress level (150-100 μEq/l) and a slightly low stress level (900-700 μEq/l), respectively, in the group that received dietary supplementation with liquorice

flavonoid oil and L-carnitine. In our study the results of anti-ROMs tests 1 and 2 measuring serum antioxidant potential showed optimal values (>200 μEq/l and >1,000 μEq/l, respectively) in the serum of broilers fed on the AMG-supplemented diets as compared with those fed on a diet containing linseed oil only, which had lower test values, demonstrating that AMG has an antioxidant potential sufficient to normalize reactive oxygen species (ROS) levels in broilers.

Meat quality characteristics

Table 5 presents the data on lipid content, pH and colour measurements of the breast meat. Lightness (L*) was increased ($P<0.01$), whereas yellowness (b*) and chroma (C*) decreased ($P<0.005$) with a higher AMG inclusion in the diet. This may have been due to the different composition of the 3 diets (see Tab. 1), particularly owing to the presence of corn, which contains pigments and may have affected meat colour [Kean *et al*. 2008].

Table 5. Breast meat characteristics (LS mean and standard deviation)

Item	Diet/group[1]		
	C (n=10)	AMG50 (n=10)	AMG100 (n=10)
Lipid (g/kg DM)	41.6 (11.9)	45.0 (10.8)	42.5 (12.9)
pH	5.73 (0.28)	5.82 (0.26)	5.83 (0.22)
Lightness (L*)	48.74^{a} (3.82)	48.78a (3.16)	51.25^{b} (3.01)
Redness (a*)	-1.87 (0.89)	-2.16 (0.56)	-1.78 (0.61)
Yellowness (b*)	5.42^{A} (1.38)	5.01^{A} (1.23)	3.22^{B} (0.84)
Chroma (C*)	5.79^{A} (1.39)	5.48^{A} (1.25)	3.74^{B} (0.76)
Hue (H*)	-63.9 (30.0)	-66.5 (5.5)	-60.4 (11.1)

[1]C – control group; AMG50 – amaranth grain group (50 g/kg of diet); AMG100 – amaranth grain group (100 g/kg of diet).
$^{aA...}$Within rows means bearing different superscripts differ significantly at: small letters – $P<0.05$; capitals – $P<0.01$.

Few studies to date have evaluated meat quality characteristics of animals fed on diets containing AMG. In a study by Sokňl *et al*. [2001] conducted on pigs dietary supplementation with 250 g/kg AMG in the flour or extruded form had no significant effect on meat quality. When animal protein was replaced with amaranth in a pig diet, Zraly *et al*. [2006] found no significant differences in meat colour between the controls and the experimental animals.

As regards the FA composition, no statistically significant differences in the saturated fatty acid/unsaturated fatty acid ratio (S/P), atherogenic index (AI) or thrombogenic index (TI) were found in the meat of the broilers fed on diets with or without AMG (Tab. 6). The n-6/n-3 PUFA ratio, AI and TI were in line with dietary recommendations for human nutrition [Simopoulos 2002]. The AI and TI were lower (0.58 and 0.56, respectively) than those reported by Laudadio and Tufarelli [2010] in an experiment investigating the effect of 40 g/kg of pea seed in diets for broilers.

Table 6. Fatty acid (FA) composition (g/kg of total FAME; LS mean and standard deviation) in the breast muscle and indexes related to human health

Item	Diet/group[1]		
	C (n=10)	AMG50 (n=10)	AMG100 (n=10)
C14:0	3.3 (1.3)	2.5 (0.7)	3.3 (1.0)
C15:0	12.7 (9.1)	28.1 (27.1)	17.4 (9.8)
C16:0	219.9 (16.9)	215.1 (16.0)	210.1 (18.9)
C16:1n-7	26.2 (7.6)	24.3 (5.4)	24.8 (8.3)
C17:0	16.6 (8.5)	18.8 (13.0)	15.3 (13.4)
C17:1	5.9 (4.9)	9.3 (4.7)	7.2 (3.4)
C18:0	83.2 (7.4)	88.2 (8.7)	90.8 (17.6)
C18:1n-9	285.1 (30.9)	276.1 (34.0)	271.0 (27.3)
C18:1n-7	19.6 (2.4)	19.9 (1.3)	19.8 (2.2)
C18:2n-6	180.6 (24.4)	167.3 (15.4)	180.3 (25.1)
C18:3n-3	90.6 (36.1)	91.2 (29.2)	104.3 (30.8)
CLA	14.7 (33.5)	4.3 (1.3)	4.8 (1.3)
C20:1n-9	2.4 (1.0)	2.1 (0.2)	2.3 (0.4)
C20:2n-6	1.7 (0.4)	1.6 (0.3)	1.9 (1.2)
C20:3n-6	3.7 (1.5)	3.8 (1.0)	3.5 (1.4)
C20:4n-6	25.4 (11.9)	37.2 (13.8)	36.2 (26.4)
C21:0	3.5 (6.3)	1.5 (0.9)	1.5 (0.6)
C20:5n-3	5.7 (1.4)	5.8 (1.4)	5.7 (1.9)
SFA	339.4 (31.4)	354.2 (38.3)	338.2 (35.9)
MUFA	338.1(36.7)	331.7 (34.0)	325.1 (31.9)
PUFAs	322.5 (41.2)	311.3 (35.3)	336.7 (36.2)
PUFAs n-3	96.3 (35.5)	97.0 (29.1)	110.0 (29.8)
PUFAs n-6	217.1 (28.1)	215.8 (17.8)	227.6 (24.2)
n-6/n-3	3.48 (4.38)	2.40 (0.71)	2.27 (0.96)
S/P	0.47 (0.05)	0.48 (0.04)	0.46 (0.05)
Atherogenic Index	0.36 (0.04)	0.35 (0.03)	0.34 (0.04)
Thrombogenic Index	0.57 (0.17)	0.55 (0.09)	0.51 (0.11)

[1]C – control group, AMG50 – amaranth grain group (50 g/kg of diet), AMG100 – amaranth grain group (100 g/kg of diet).
SFA – saturated fatty acid; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids; PUFAs n-3 – polyunsaturated fatty acid series n-3; PUFAs n-6 – polyunsaturated fatty acid series n-6; n-6/n-3 – PUFAs n-6/ PUFAs n-3 ratio; S/P – saturated fatty acid/unsaturated fatty acid.

The most abundant FAs in the broiler meat were OA, PA and LA. ALA content ranged from 91 to 104 g/kg of total FAME. The proportions of SFA, MUFA, and PUFA contents were one third of each FA group. Moretti and Corino [2009] reported that the SFA and MUFA contents of chicken meat are approximately 300 to 400 g/kg of total FAME, while the PUFA content is around 300 g/kg of total FAME. Our analysis of breast meat showed a well-balanced FA composition, similar to that reported by Jakubowska *et al*. [2013], who evaluated the effect of AMG supplementation in quail diet on meat quality, sensory characteristics and the FA profile. They found no differences in FA

contents of breast meat from quails fed on diets supplemented with 0, 40, and 70 g/kg AMG for 14 weeks. In a study on pigs fed on diets containing 0, 100, and 200 g/kg AMG, Bobiel and Sokol [1999] found no significant differences in the FA profile of pig meat between the control and the experimental groups. Popiel *et al*. [2013] evaluated the effect on the egg yolk FA composition of eggs from laying hens fed on diets containing 0, 50 or 100 g/kg extruded AMG. Extruded AMG supplementation did not affect the FA profile of the egg yolk; however, the PUFA level was slightly higher and the n-6/n-3 PUFA ratio lower in the hens fed on a diet with 50 g/kg AMG.

Raw AMG supplementation in broiler diets rich in PUFAs, which are susceptible to lipid peroxidation, can help prevent the generation of ROS, enhance the body's antioxidant defences, as well as reduce triglyceride and cholesterol levels in serum of broilers. Broilers fed on diets rich in linseed oil and either supplemented or not with AMG showed a good meat PUFAs profile. However, raw AMG supplementation inhibited growth performance. Further studies are needed to evaluate the use of lower AMG doses or the inclusion of flaked or extruded AMG, which can partially inactivate anti-nutritional factors, in order to overcome the problem with growth performance while preserving the positive effects of AMG supplementation.

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