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## Influence of dietary combinations of *Amphora coffeaeformis* with linseed oil or sunflower oil on performance, fatty and amino acid profiles, oxidative stability and meat quality of broiler chickens

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### ABSTRACT

This study aimed to evaluate the effect of dietary linseed oil, sunflower oil, and their supplementation with *Amphora coffeaeformis* (AC) on growth performance, carcass characteristics, fatty and amino acid profiles, oxidative stability, and meat quality of broiler chickens. One hundred and eighty day-old broiler chicks were divided into four groups (five replicates per group, 9 birds per each replicate pen). Birds in groups 1, 2, 3, and 4 fed diets containing 3% sunflower oil without AC (SFO 3%), 3% SFO with 1 g/kg diet of AC (SFAC), 3% linseed oil without AC (LSO 3%) and 3% LSO oil with 1 g/kg diet of AC (LSAC), respectively for 32 days. Final weight and weight gain were increased whereas fat pads were decreased in birds fed diets containing SFAC or LSAC compared to other groups. The values of omega-3 fatty acids, essential amino acids, and antioxidants were increased while saturated fatty acids (SFA) declined in the meat of birds fed diets supplemented with AC (SFAC & LSAC) compared to other groups. Lysine levels in the meat of birds fed AC and/or linseed diets were inversely related to hepatic L-carnitine levels. Breast meats of different broiler groups did not differ significantly in quality traits. However, broilers fed AC-containing diets had relatively lower yellowness, redness, and Chroma values. Conclusively, dietary combinations of AC with either SFO or LSO improved the performance, fatty and amino acid profiles, oxidative stability, and meat quality of broiler chickens.

### HIGHLIGHTS

- Supplying *Amphora coffeaeformis* (AC) to broiler sunflower (SFAC) or linseed oil (LSAC) diets improved performance.
- Dietary AC, 1 g/kg, improved the meat nutritional value of broilers fed SFAC or LSAC diets.
- Dietary AC, 1 g/kg, improved the meat oxidative stability of broilers fed SFAC or LSAC diets.

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## Introduction

Human requirements for essential fatty and amino acids are derived mainly from animal and poultry meat (Long et al. 2020). From a health perspective, omega-3 polyunsaturated fatty acids (PUFA) are specifically or indirectly incorporated into the remediation protocol of cardiovascular disease (CVD) (Sokoła-

Wyszczńska et al. 2018). As a result, nutritionists still seek to provide healthier PUFA-enriched meat to improve human health. Besides, they are also concerned to keep the prescribed n-6: n-3 ratio (n-3 PUFA derived from  $\alpha$ -linolenic acid (18:3) and n-6 PUFA derived from linoleic acid) below 4 otherwise a risk factor for human coronary heart disease (Wood et al. 2004) and oxidative meat quality (Alagawany et al.

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2019) will be considered. In addition to being less expensive, feeding functional additives to poultry could generate meat rich in long-chain polyunsaturated fatty acids (LC-PUFA), making it more desirable and marketable than red meat in the modern diet (Alagawany et al. 2019). Fortunately, increasing dietary supplementation of poultry with omega-3 fatty acid sources always results in meat with a high LC-PUFA content (Bou et al. 2005). Dietary fish oil is used as a source of n-3 PUFA in broiler chickens. However, this oil is also used for aquaculture. This makes it more costly and not easy to purchase. In addition, the use of fish oil in poultry diets showed low oxidative stability and unfavourable sensory changes (Wood et al. 2004). Therefore, the development of suitable alternatives is essentially required. Plant-derived oils have therefore emerged as alternative sources to fish oil with a high content of n-3 and n-6 PUFA. Sunflower oil (SFO) is indeed one of the foods that provide high levels of LC-PUFA, especially omega-6 fatty acids. SFO contains 69% linoleic acid (18:2n-6), with a PUFA/SFA ratio of 6.4. However, it contains <0.1% alpha-linolenic acid (ALA, C18:3n-3) (Meydani et al. 1991). Linseed oil (LSO) is one of the best sources of plant-based n-3 fatty acids. It contains more than 50% of ALA. Though inferior mechanism, adequate amounts of linoleic acid and alpha-linolenic acid could function in the liver via a series of competitive desaturation and elongation reactions to produce long-chain fatty acids such as arachidonic acid (ARA, 20:4 n-6), eicosapentaenoic acid (EPA, 20:5 n-3), DPA, and docosahexaenoic acid (DHA, 22:6 n-3) (Yalçın and Ünal 2010; Calder 2012). Therefore, it perceives an alternative to marine foods (Rodriguez-Leyva et al. 2010). High vegetable oil diets including SFO and LSO reduced the concentration of low-density lipoprotein cholesterol (LDL-c) and the risk of blood pressure and CVD accordingly (Rodriguez-Leyva et al. 2010). Similarly, the dietary SFO and LSO had been correlated with the meat fortification with different LC-PUFA (Kishawy et al. 2019) and other health benefits for poultry (Lee et al. 2019). However, a high concentration of PUFA in meat particularly omega-3 fatty acids increased the susceptibility of meat to lipid oxidation (Lee et al. 2019). Microalgae used extensively in human, animal and aquaculture as a source of LC-PUFAs (Lee et al. 2019). Algae had the ability to produce potent and beneficial natural components. For this cause, in the last few years, Use of algae in the pharmaceutical industry has drawn world attention (Enwereuzoh and Onyeagoro 2014). *Amphora coffeaeformis* (Agardh) is one of the most commonly observed species in alkaline freshwater and

brackish water places (Bhosle et al. 1993). *Amphora coffeaeformis* (AC) evaluation showed high contents of PUFAs, in particular, linolenic acid, EPA, and DHA in addition to its antioxidant capacity (El-Bahr et al. 2020). More recently, dietary AC seemed to have superior positive effects on the performance, nutritional values, antioxidant status, and meat quality of chicken broilers particularly in comparison to *Chlorella vulgaris* (CV) and *Spirulina platensis* (SP) (El-Bahr et al. 2020). Earlier studies focusing on the effects of individual dietary incorporation of plant-derived oils (Crespo and Esteve-Garcia 2001; López-Ferrer et al. 2001; Panda et al. 2015; Kalakuntla et al. 2017; Long S et al. 2020) or algae (Abudabos et al. 2013; Long et al. 2018; Alwaleed et al. 2020; El-Bahr et al. 2020), but to our knowledge, no studies investigated the effects of their dietary combinations on broiler performance, oxidative stability, and meat quality. Here, we hypothesised that supplementation of AC to diets containing sunflower or linseed oil would increase the PUFA content of the meat and improve its oxidative stability. Therefore, the objective of this study was to determine the influence of supplementing *Amphora coffeaeformis* (AC) to broiler sunflower (SFAC) or linseed oil (LSAC) diets on performance, fatty and amino acids profiles, oxidative stress biomarkers and meat quality parameters.

## Materials and methods

### *Bird's management and experimental design*

Institutional Animal Care and Use Committee Research Ethics of Benha University, Faculty of Veterinary Medicine, Egypt reviewed and approved all experimental protocols (BUFVTM, 07032020) conducted in this study. The current nutritional study was carried out on 180 one-day mixed-sex broiler chicks (Ross 308) purchased from an approved local hatchery (Benha, Egypt) and transported to Poultry Research Farm, Faculty of Agriculture, Benha University, Egypt. After three days of adaptation, birds were randomly assigned to 20-floor pens (1.25 m<sup>2</sup>) divided into four groups with five replicates of 9 birds per replicate pen (4 × 5 × 9), each fitted with fresh wood shavings for bedding and supplied with feeder and waterer until the age of 35 days. Birds were randomly allocated into four groups. The first to fourth broiler groups were fed one of four dietary treatments for 32 days: sunflower oil basal diet (SFO; 3%), SFO diet supplemented with 1 g/kg *Amphora coffeaeformis* (SFAC), linseed oil basal diet (LSO; 3%), and LSO diet supplemented with 1 g/kg *Amphora coffeaeformis* (LSAC), respectively. Based on good performance results from our earlier

study (El-Bahr et al. 2020), *Amphora coffeaeformis* was applied at a 1 g/kg diet, as well as to avoid unfavourable concerns associated with sensory attributes of meat when algae fed at higher concentrations of 2.8%, 5.5% and 7.4% (Ribeiro et al. 2014; Lee et al. 2019), as opposed to low concentrations of 0.3%, 0.6% or 1.2% (Vossen et al. 2017). All birds during the experiment were fed ad libitum with mash feed and clean water. All diets tested were isonitrogenous and isocaloric, as shown in Tables 1–3. The proximate

chemical composition of various broiler growth phases diets (g/kg) was also determined according to Horwitz and AOAC (2000) and illustrated in the same Tables 1–3. The dried powder of *Amphora coffeaeformis* (AC) was obtained from the Algae Production Unit (APU), National Research Institute, Cairo, Egypt. The experiment's ambient temperature was kept at 33° C for the first week, and then gradually decreased to 25° C until the chicks were 21 days old, while the relative humidity was kept at 50–60% throughout the trial

**Table 1.** Ingredient composition, calculated nutrient analysis, and proximate chemical composition of starter linseed oil and sunflower oil diets and their supplementation with *Amphora coffeaeformis* (AC).

Ingredients (g/kg, as fed basis)	Starter diets			
	Groups			
	1	2	3	4
	SFO diet	SFAC diet	LSO diet	LSAC diet
<i>Amphora coffeaeformis</i>	0	1	0	1
Yellow corn	561	561	561	561
Soybean meal	270.3	270.3	270.3	270.3
Wheat bran	30	30	30	30
Corn gluten meal	15.6	15.6	15.6	15.6
Poultry by-product meal	60	60	60	60
Linseed oil	0	0	30	30
Sunflower oil	30	30	0	0
Lime stone	10.8	10.8	10.8	10.8
Mono calcium phosphate	10	10	10	10
Common salt	3.3	3.3	3.3	3.3
L-lysine	2	2	2	2
DL-methionine	3	3	3	3
<sup>a</sup> Vitamin and mineral premixes	3	3	3	3
Choline chloride	1	1	1	1
Nutrient specifications (g/kg)				
ME (MJ/kg)	12.76	12.76	12.76	12.76
DM	876.2	876.2	876.2	876.2
Crude protein	215	215	215	215
calcium	9	9	9	9
Available phosphorus	4.5	4.5	4.5	4.5
Methionine + cysteine	9.8	9.8	9.8	9.8
methionine	6.8	6.8	6.8	6.8
lysine	13.2	13.2	13.2	13.2
ADF	44.3	44.3	44.3	44.3
NDF	106.7	106.7	106.7	106.7
Ash	62.5	62.5	62.5	62.5
<sup>b</sup> Proximate chemical composition of diets (g/kg)				
Dry matter	874.6		874.4	
Crude protein	214.3		214.1	
Crude fat	60.2		60.6	
Crude fibre	34.5		34.3	
Ash	62.0		62.2	
Metabolizable energy (MJ/kg)	12.76		12.77	

<sup>a</sup>Vitamin and mineral premix supplied each kg of feeds with: Vitamin A 12,000 U; vitamin D3 2000 U; vitamin E 10 mg; vitamin K3 2 mg; vitamin B1 1 mg; vitamin B2 5 mg; vitamin B6 1.5 mg; vitamin B12 0.01 mg; Biotin 0.05 mg; pantothenic acid 10 mg; Nicotinic acid 30 mg; Folic acid 1 mg; zinc 50 mg; Manganese 60 mg; Iron 30 mg; Copper 10 mg; Iodine 1 mg; Selenium 0.01 mg; Cobalt 0.01 mg.

<sup>b</sup>Proximate chemical composition of diets was conducted according to Horwitz and AOAC (2000). Group 1: birds fed basal diets mixed with sunflower oil 3% (SFO). Group 2: birds fed basal diets mixed with a combination of SFO oil 3% and *Amphora coffeaeformis* (1 g/kg; SFAC). Group 3: birds fed basal diets mixed with linseed oil 3% (LSO). Group 4: birds fed basal diets mixed with a combination of LSO oil 3% and *Amphora coffeaeformis* (1 g/kg; LSAC).

**Table 2.** Ingredient composition, calculated nutrient analysis, and proximate chemical composition of grower linseed oil and sunflower oil diets and their supplementation with *Amphora coffeaeformis* (AC).

Ingredients (g/kg, as fed basis)	Grower diets			
	Groups			
	1	2	3	4
	SFO diet	SFAC diet	LSO diet	LSAC diet
<i>Amphora coffeaeformis</i>	0	1	0	1
Yellow corn	630	630	630	630
Soy bean meal	224.1	224.1	224.1	224.1
Wheat bran	23.4	23.4	23.4	23.4
Corn gluten meal	10	10	10	10
Poultry by-product meal	50	50	50	50
Linseed oil	0	0	30	30
Sunflower oil	30	30	0	0
Lime stone	10.6	10.6	10.6	10.6
Mono calcium phosphate	9.8	9.8	9.8	9.8
Common salt	3.3	3.3	3.3	3.3
L-lysine	2	2	2	2
DL-methionine	2.8	2.8	2.8	2.8
<sup>a</sup> Vitamin and mineral premixes	3	3	3	3
Choline chloride	1	1	1	1
Nutrient specifications (g/kg)				
ME (MJ/kg)	13.05	13.05	13.05	13.05
DM	874	874	874	874
Crude protein	190	190	190	190
Calcium	8.8	8.8	8.8	8.8
Available phosphorus	4.3	4.3	4.3	4.3
Methionine + cysteine	8.9	8.9	8.9	8.9
Methionine	6.2	6.2	6.2	6.2
Lysine	11.9	11.9	11.9	11.9
ADF	40.1	40.1	40.1	40.1
NDF	102.3	102.3	102.3	102.3
Ash	58.1	58.1	58.1	58.1
<sup>b</sup> Proximate chemical composition of diets (g/kg)				
Dry matter	872.9		873.4	
Crude protein	189.6		189.4	
Crude fat	61.1		61.5	
Crude fibre	28.0		27.8	
Ash	58.2		58.4	
Metabolizable energy (MJ/kg)	13.14		13.15	

<sup>a</sup>Vitamin and mineral premix supplied each kg of feeds with: Vitamin A 12000 U; vitamin D3 2000 U; vitamin E 10 mg; vitamin K3 2 mg; vitamin B1 1 mg; vitamin B2 5 mg; vitamin B6 1.5 mg; vitamin B12 0.01 mg; Biotin 0.05 mg; pantothenic acid 10 mg; Nicotinic acid 30 mg; Folic acid 1 mg; zinc 50 mg; Manganese 60 mg; Iron 30 mg; Copper 10 mg; Iodine 1 mg; Selenium 0.01 mg; Cobalt 0.01 mg. Group 1: birds fed basal diets mixed with sunflower oil 3% (SFO).

<sup>b</sup>Proximate chemical composition of diets was conducted according to Horwitz and AOAC (2000). Group 2: birds fed basal diets mixed with a combination of SFO oil 3% and *Amphora coffeaeformis* (1 g/kg; SFAC). Group 3: birds fed basal diets mixed with linseed oil 3% (LSO). Group 4: birds fed basal diets mixed with a combination of LSO oil 3% and *Amphora coffeaeformis* (1 g/kg; LSAC).

**Table 3.** Ingredient composition, calculated nutrient analysis, and proximate chemical composition of finisher linseed oil and sunflower oil diets, and their supplementation with *Amphora coffeaeformis* (AC).

Ingredients (g/kg)	Finisher diets			
	Groups			
	1	2	3	4
	SFO diet	SFAC diet	LSO diet	LSAC diet
<i>Amphora coffeaeformis</i>	0	1	0	1
Yellow corn	670	670	670	670
Soy bean meal	176.2	176.2	176.2	176.2
Wheat bran	20	20	20	20
Corn gluten meal	24.8	24.8	24.8	24.8
Poultry by-product meal	50	50	50	50
Linseed oil	0	0	30	30
Sunflower oil	30	30	0	0
Lime stone	9.4	9.4	9.4	9.4
Mono calcium phosphate	8.2	8.2	8.2	8.2
Common salt	3.3	3.3	3.3	3.3
L-lysine	1.9	1.9	1.9	1.9
DL-methionine	2.2	2.2	2.2	2.2
<sup>a</sup> Vitamin and mineral premixes	3	3	3	3
Choline chloride	1	1	1	1
Nutrient specifications (g/kg)				
ME (MJ/kg)	13.35	13.35	13.35	13.35
DM	873	873	873	873
Crude protein	180	180	180	180
Calcium	7.9	7.9	7.9	7.9
Available phosphorus	4.1	4.1	4.1	4.1
Methionine + cysteine	8.2	8.2	8.2	8.2
Methionine	5.6	5.6	5.6	5.6
Lysine	10.5	10.5	10.5	10.5
ADF	36.3	36.3	36.3	36.3
NDF	99.1	99.1	99.1	99.1
Ash	52.9	52.9	52.9	52.9
<sup>b</sup> Proximate chemical composition of diets (g/kg)				
Dry matter	871.3		871.2	
Crude protein	179.4		179.2	
Crude fat	62.1		62.4	
Crude fibre	28.0		28.2	
Ash	52.4		52.2	
Metabolizable energy (MJ/kg)	13.35		13.36	

<sup>a</sup>Vitamin and mineral premix supplied each kg of feeds with: Vitamin A 12000 U; vitamin D3 2000 U; vitamin E 10 mg; vitamin K3 2 mg; vitamin B1 1 mg; vitamin B2 5 mg; vitamin B6 1.5 mg; vitamin B12 0.01 mg; Biotin 0.05 mg; pantothenic acid 10 mg; Nicotinic acid 30 mg; Folic acid 1 mg; zinc 50 mg; Manganese 60 mg; Iron 30 mg; Copper 10 mg; Iodine 1 mg; Selenium 0.01 mg; Cobalt 0.01 mg. Group 1: birds fed basal diets mixed with sunflower oil 3% (SFO).

<sup>b</sup>Proximate chemical composition of diets was conducted according to Horwitz and AOAC (2000). Group 2: birds fed basal diets mixed with a combination of SFO oil 3% and *Amphora coffeaeformis* (1 g/kg; SFAC). Group 3: birds fed basal diets mixed with linseed oil 3% (LSO). Group 4: birds fed basal diets mixed with a combination of LSO oil 3% and *Amphora coffeaeformis* (1 g/kg; LSAC).

period. During the feeding trial, a lighting schedule of 23 hours per day and 1 hour of darkness was established.

### Growth performance, carcass characteristics and sample collection

At the end of the experiment, birds were weighed individually to determine the average weight gain

(WG), whereas feed intake (FI) was calculated by subtracting the amount of rejected feed from offered feed for each replicate. The feed conversion ratio (FCR) was determined as the ratio between the real feed consumed (FI) and the weight gained. At the end of the experiment, 15 birds of each group (3 birds per replicate) were selected randomly, weighed, fastened for 12 hours before euthanization to reduce the contamination of the meat during processing (El-Bahr et al. 2020). The weights of carcasses, liver, heart, spleen, gizzard, fat pad and breasts were taken immediately after deskinning and evisceration for individual calculation of different yields and percentages. Carcass yields were calculated as a percentage of total weight, and carcass parts and organs were calculated as a percentage of carcass weight (Wiley 2007). In addition, liver and breast muscle sections (Pectoralis major) were dissected and frozen in a sealed polythene bag at  $-80^{\circ}\text{C}$  for further laboratory assessment of amino and fatty acids profiles and enzymatic antioxidant status. The remaining portions of the breast meat were immediately used for the estimation of physicochemical attributes.

### Determination of profiles of fatty and amino acids in breast muscles

Extraction of total breast muscle lipids was performed in a mixture of chloroform and methanol (2:1; v/v) solution after 2 minutes of vortexing and 10 minutes of centrifugation at 1.792 g. The fatty acid methyl ester (FAME) was then prepared from the derived supernatant by esterification using a mixture of methanol/sulphuric acid (95:5) and hexane (Salah et al. 2019). Free fatty acids were quantified from hexane extract obtained from FAME using gas chromatography (GC; Agilent Technologies 7890 A) using column SP2330 (30 mm  $\times$  0.32 mm  $\times$  0.2  $\mu\text{m}$  film thickness; Supelco Analytical, Bellefonte, PA, USA) and flame ionisation detector (Radwan and Ahmed 2016). As regards the amino acid profiles, breast muscle tissues were subjected to homogenisation, centrifugation, and purification (Salah et al. 2019) before derivatization (Ali and Elgoly 2013). HPLC (Agilent HP 1200 Series Apparatus, USA) mounted with Nova-Pak<sup>TM</sup> column C18 (4  $\mu\text{m}$ , 3.9  $\mu\text{m}$ , 4.6 mm) was injected with extracted samples as well as standards for amino acids (Sigma-Aldrich, St. Louis, MO, USA) to differentiate and quantify free amino acids (nmol/g meat) using Hughes et al. (2002) technique, with some modifications (Salah et al. 2019).

### **Analysis of oxidative stress biomarkers and antioxidants in the liver and breast muscles tissues**

HPLC (Agilent HP 1200 Series Apparatus, USA) was used to determine the levels of malondialdehyde (MDA), reduced glutathione (GSH), oxidised glutathione (GSSG), and 8-hydroxy-deoxyguanosine (8-OHdG) in the liver and breast muscles in this experiment. Earlier protocols described for the determination of MDA were applied (Ahmed-Farid et al. 2017; Abd-Elrazek and Ahmed-Farid 2018). The same HPLC protocol was used for the determination of thiol compounds of oxidised and reduced glutathione in the liver and breast muscles tissues. However, in this case, HPLC was equipped with a Bondapak column (30 cm × 3.9 mm C18 $\mu$ ) and loaded with a mobile phase consisting of 0.0025 M sodium phosphate buffer, pH 3.5, 0.005 M tetrabutylammonium phosphate and 13% methanol. The hepatic and muscular levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were separated using C18 reversed-phase columns in series (Supelco, 5 p.m., I.D. 0.46 × 25 cm) at a flow rate of 0.68 mL/min and a wavelength of 245 nm. H<sub>2</sub>O/methanol (85:15 v/v) was implemented as an eluting solution, with 50 mM of KH<sub>3</sub>PO<sub>4</sub>, pH 5.5. Superoxide dismutase (SOD) activity was calculated by a spectrophotometric method. Briefly, five millilitres of cold PBS were used to homogenise one gram of tissue (1:5 dilution). All of these samples were centrifuged for 15 minutes at a speed of 1,968 g $\times$  and a temperature of 4°C. Supernatants were collected and stored at -20°C until biochemical analyses of superoxide dismutase (SOD) activity was carried out. The detection is based on estimating the activity of the SOD enzyme inhibiting the autoxidation of pyrogallol for 2 minutes intervals (Marklund and Marklund 1974).

In hepatic tissue, non-enzymatic antioxidants and energy marker which including vitamin E, Coenzyme Q10 and L-carnitine were measured. For vitamin E determination, liver samples were deproteinized by homogenisation with an equal volume of methanol and n-hexane at temperatures below 8°C, vortexed for 5 minutes, and centrifuged at 5000 rpm for 15 minutes. The upper hexane layer extraction was evaporated and reconstructed in the mobile phase and then injected into the HPLC, where the Waters Symmetry C18 column (3.9 mm × 150 mm) guarded with the Waters Symmetry C18 column (3.9 mm × 10 mm) was used to differentiate the  $\alpha$ -tocopherol. Methanol - n-hexane 72:28 (v/v) was a mobile injected phase that worked at a 2 ml/min flow rate, while UV detection was performed at 292 nm wavelength

(Karpińska et al. 2006). For the determination of hepatic Co Q10, the extraction of liver samples was performed by centrifuging 1-propanol at 2000 × g for 10 min at 4°C. The separation was run in the reverse phase Microsorb-MV column (4.6 mm × 15 cm) at a flow rate of 1 mL/min, while the detection was performed at a wavelength of 275 nm (Tang et al. 2001). In the case of L-carnitine, the liver sample prepared by Sonication in 100 ml of water and filtration in 0.45  $\mu$ m was used for chromatographic, HPLC (Agilent HP 1200 Series Apparatus, USA), determination of L-carnitine by comparing the sample area to the external standard. The Mobile phase (Isocratic mixture of Acetonitrile: Buffer (65:35)) was loaded and programmed to run at a flow rate of 1 ml/min in the column (Waters, Spherosorb, NH<sub>2</sub>, (150 × 4.6 mm)) and was then spectrophotometrically detected at 205 nm (Li and Sun 2010).

### **Determination of meat quality parameters**

The collected breast meat was tested for physico-chemical characteristics as described earlier (Elokil et al. 2019; El-Bahr et al. 2020). In detail, the ultimate pH (pHu) was recorded directly from breast meat by inserting a pH meter 24 hours post-mortem. The drip loss (48 h) was measured to reflect the water holding capacity of the breast muscle. When compared to a 24-hour drip-loss measurement, a longer drip-loss period of 2 days or more reduces variability and increases reliability (Honikel and Hamm 1994). Breast meat cuts of the same size (50 ± 5 gm) and shapes were suspended over a plastic net in an airtight plastic box, held at 5°C for 48 h, and then re-weighted to assess the drip loss (48 h). Drip loss is calculated to be the percentage of weight loss relative to the original weight of the meat sample (Honikel 1998). To assess the thawing loss, the breast fillet was sliced, wiped dry, weighed and frozen at -18°C for one week, followed by thawing at 5°C for 24 hours to obtain final weight. The proportion of the difference between the starting and ending weights was the value of the thawing loss (Honikel 1998). Cooking loss percentages were also measured as reported earlier by Honikel (1998), where muscle cuts were placed separately in thin-walled plastic thermo-tolerant bags in the water bath until the core temperature was 75°C, then cooled to 5°C in crushed ice, and re-weighted to quantify the cooking loss. The cores extracted from the cooked breast fillet samples were then used to analyse the Warner-Bratzler Shear Force (WBSF) using the 3343 Universal Test System Mono column (Instron,

USA) by shearing perpendicular to the direction of the fibre (AMSA 2015). Lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) of the raw breast fillet colour values were calculated by Chromametre CR-410 (Konica Minolta Sensing INC., Osaka, Japan), which were then used to predict colour Chroma (saturation index) and Hue angle (HA). Chroma can be calculated using the equation  $C = (a^{*2} + b^{*2})^{1/2}$ , with higher chroma values indicating greater saturation of the sample's primary hue. While the hue angle (or colour intensity) can be estimated using the equation  $HA = [\arctangent(b^*/a^*)]$ , with larger values indicating less red meat (AMSA 2012).

### Statistical analyses

The obtained data were normally distributed and exposed to two-way Analysis of Variance (ANOVA), as  $2 \times 2$  factorial arrangements (2 sources of oil  $\times$  2 levels of AC) Using SPSS (version 20; IBM, Chicago, IL, USA) followed by Tukey's post hoc tests to compare the differences between dietary treatments, where significant differences were observed ( $P < .05$ ).

## Results

### Growth performance and carcass characteristics

Effects of dietary linseed oil, sunflower oil, and their combinations with *Amphora coffeaeformis* on growth performance and carcass characteristics of broiler chickens are shown in Table 4. Final body weight and WG were significantly ( $P < .05$ ) increased in birds fed the basal diet supplemented with either LSAC or SFAC compared to those of birds fed the basal diet

supplemented with SFO or LSO which remained comparable (Table 4). Fat pads weights were significantly ( $P < .05$ ) decreased in birds fed the basal diet supplemented with either LSAC or SFAC compared to those of birds fed the basal diet supplemented with SFO or LSO, which remained comparable (Table 4). Significant ( $P < .05$ ) low feed conversion ratio and higher spleen and gizzard percentages were observed in birds fed diets containing LSO either alone or in a combination with AC. However, feed intake, carcass yield, breast yield, liver percentage, and heart percentage remained unchanged significantly ( $P > .05$ ) among all experimental groups (Table 4).

### Profiles of fatty and amino acids in breast muscles

Effect of dietary linseed oil, sunflower oil, and their combinations with *Amphora coffeaeformis* on profiles of fatty acids in breast muscles of broiler chickens are shown in Table 5. Significant differences were noted for palmitoleic acid (PtA), oleic acid (OA), eicosatetraenoic acid (ETA), and eicosapentaenoic acid (EPA) resulting from the interaction between LSO and AC ( $P < .05$ ). Diets containing linseed and/or AC enriched broiler muscles with ETA and EPA. Broiler muscles' total MUFA (PtA and OA) reached the highest when birds fed a diet containing LSO but decreased in birds fed a diet containing LSAC ( $P < .05$ ). All other major changes were recorded based on a direct comparison between groups attributed primarily to the oil source or the inclusion of AC. In detail, obvious low muscles' myristic acid values were mainly seen in birds fed a diet containing linseed oil (LSO, LSAC) as well as an

**Table 4.** Effect of dietary linseed oil, sunflower oil, and their supplementation with *Amphora coffeaeformis* on growth performance and carcass characteristics of broiler chickens.

Parameters	Groups				SEM	p values		
	1	2	3	4		Oils	AC	Oils*AC
Initial weight (g/bird)	84.44	83.42	83.84	84.77	0.32	.56	.95	.15
Final weight (g/bird)	2124.42 <sup>b</sup>	2168.68 <sup>a</sup>	2127.02 <sup>b</sup>	2210.60 <sup>a</sup>	14.81	.46	.046	.52
WG (g/bird)	2039.98 <sup>b</sup>	2085.26 <sup>a</sup>	2043.18 <sup>b</sup>	2125.83 <sup>a</sup>	14.73	.47	.045	.53
FI (g/bird)	3196.00	3207.00	3085.20	3135.00	34.83	.21	.67	.78
FCR	1.57	1.54	1.51	1.47	0.01	.04	.25	.91
Carcass Yield (%)	58.23	59.24	57.43	57.46	0.31	.05	.41	.43
Breast Yield (%)	31.18	32.18	32.93	31.86	0.54	.52	.97	.35
Liver (%)	4.57	4.44	4.58	4.64	0.08	.52	.84	.57
Spleen (%)	0.29 <sup>b</sup>	0.31 <sup>b</sup>	0.40 <sup>a</sup>	0.35 <sup>a</sup>	0.02	.04	.60	.29
Heart (%)	0.87	0.87	0.86	0.87	0.03	.97	.94	.93
Gizzard (%)	2.21	2.09	2.54	2.34	0.05	.01	.17	.71
Fat Pad (%)	3.78 <sup>a</sup>	2.93 <sup>b</sup>	3.41 <sup>a</sup>	3.07 <sup>b</sup>	0.11	.58	.010	.24

<sup>a-c</sup>Means within a row not sharing a common superscript differ significantly with the corresponding p value. Group 1: birds fed basal diets mixed with sunflower oil 3% (SFO). Group 2: birds fed basal diets mixed with a combination of SFO oil 3% and *Amphora coffeaeformis* (1 g/kg; SFAC). Group 3: birds fed basal diets mixed with linseed oil 3% (LSO). Group 4: birds fed basal diets mixed with a combination of LSO oil 3% and *Amphora coffeaeformis* (1 g/kg; LSAC). WG: weight gain; FI: feed intake; FCR: feed conversion ratio; AC: *Amphora coffeaeformis*; SEM: Standard error of the mean.

**Table 5.** Effect of dietary linseed oil, sunflower oil, and their supplementation with *Amphora coffeaeformis* on profiles of fatty acids in breast muscles of broiler chickens mg/g tissue.

Parameters	Groups				SEM	<i>p</i> values		
	1	2	3	4		Oils	AC	Oils*AC
	SFO	SFAC	LSO	LSAC				
<b>SFA</b>								
MA (C14:0)	0.35 <sup>a</sup>	0.31 <sup>b</sup>	0.27 <sup>b</sup>	0.29 <sup>b</sup>	0.008	.014	.548	.137
PA (C16:0)	8.14 <sup>a</sup>	6.58 <sup>abc</sup>	6.91 <sup>ab</sup>	5.42 <sup>c</sup>	0.313	.081	.031	.956
SA (C18:0)	3.88 <sup>a</sup>	3.19 <sup>b</sup>	3.40	3.53	0.126	.796	.289	.127
Arachidic acid (C20:0)	0.64	0.52	0.54	0.56	0.024	.628	.321	.173
<b>MUFA</b>								
PtA (C16:1)	0.93 <sup>b</sup>	1.24 <sup>a</sup>	1.41 <sup>a</sup>	1.11 <sup>b</sup>	0.029	.011	.861	.000
OA (C18:1)	5.93 <sup>b</sup>	7.35	9.21 <sup>a</sup>	7.03 <sup>b</sup>	0.291	.026	.524	.009
<b>PUFA</b>								
LA (C18:2)	5.40 <sup>c</sup>	6.84 <sup>b</sup>	7.45 <sup>b</sup>	8.43 <sup>a</sup>	0.239	.002	.026	.637
ALA (C18:3)	0.73 <sup>b</sup>	0.96 <sup>a</sup>	0.99 <sup>a</sup>	1.15 <sup>a</sup>	0.034	.007	.014	.629
ETA (C20:4n-3)	0.23 <sup>b</sup>	0.30 <sup>a</sup>	0.32 <sup>a</sup>	0.29 <sup>a</sup>	0.011	.055	.347	.036
EPA (C20:5 n-3)	0.21 <sup>b</sup>	0.29 <sup>a</sup>	0.27 <sup>a</sup>	0.26 <sup>a</sup>	0.009	.535	.072	.023
AA (C20:4n-6)	0.38 <sup>b</sup>	0.43 <sup>b</sup>	0.47 <sup>ab</sup>	0.49 <sup>a</sup>	0.012	.008	.220	.494
DHA(C22:6n-3)	0.32 <sup>b</sup>	0.38 <sup>a</sup>	0.40 <sup>a</sup>	0.43 <sup>a</sup>	0.008	.004	.019	.450
Total SFA	13.00 <sup>a</sup>	10.59 <sup>b</sup>	11.12 <sup>b</sup>	9.80 <sup>c</sup>	0.244	.018	.002	.287
Total MUFA	6.85 <sup>b</sup>	8.59 <sup>b</sup>	10.62 <sup>a</sup>	8.14 <sup>b</sup>	0.299	.017	.546	.004
Total PUFA	7.26 <sup>c</sup>	9.19 <sup>abc</sup>	9.90 <sup>ab</sup>	11.05 <sup>a</sup>	0.279	.002	.017	.497
Total n-3	1.49 <sup>c</sup>	1.93 <sup>b</sup>	1.97 <sup>b</sup>	2.13 <sup>a</sup>	0.043	.002	.004	.122
PUFA/SFA	0.57 <sup>c</sup>	0.89 <sup>ab</sup>	0.90 <sup>ab</sup>	1.13 <sup>a</sup>	0.037	.002	.003	.550

<sup>a-c</sup>Means within a row not sharing a common superscript differ significantly with the corresponding *p* value. Group 1: birds fed basal diets mixed with sunflower oil 3% (SFO). Group 2: birds fed basal diets mixed with a combination of SFO oil 3% and *Amphora coffeaeformis* (1 g/kg; SFAC). Group 3: birds fed basal diets mixed with linseed oil 3% (LSO). Group 4: birds fed basal diets mixed with a combination of LSO oil 3% and *Amphora coffeaeformis* (1 g/kg; LSAC). AC: *Amphora coffeaeformis*; SEM: Standard error of mean; SFA: Saturated fatty acids; MA: (C14:0) Myristic acid; PA: (C16:0) Palmitic acid; SA: (C18:0) Stearic acid; MUFA: monounsaturated fatty acids; PtA: (C16:1) Palmitoleic acid; OA: (C18:1) Oleic acid; PUFA: polyunsaturated fatty acid; LA: (C18:2) Linoleic acid; ALA: (C18:3)  $\alpha$ -Linolenic acid; ETA: Eicosatetraenoic acid (20:4n-3); EPA: (C20:5n-3) Eicosapentaenoic acid; AA: (C20:4n-6) Arachidonic acid; DHA: (C22:6n-3) Docosahexaenoic acid.

*Amphora coffeaeformis* compared to birds fed a diet containing SFO ( $P < .05$ ). While AC supplementation was largely associated with similar changes in muscle concentrations of palmitic acid in the same groups compared to birds fed a diet containing SFO ( $P < .05$ ). In addition, the dietary inclusion of linseed oil or supplementation of *Amphora coffeaeformis* (SFAC, LSO, and LSAC) increased the muscle levels of Linoleic acid, alpha-linolenic acid, arachidonic acid, docosahexaenoic acid, total PUFA, total n-3, and PUFA/SFA ratio compared to birds fed with SFO ( $P < .05$ ). Effect of dietary linseed oil, sunflower oil, and their combinations with *Amphora coffeaeformis* on profiles of amino acids in breast muscles of broiler chickens are shown in Table 6. The prevalent theme was the increased broiler muscle content for essential amino acids such as arginine, histidine, isoleucine, methionine, phenylalanine, threonine, and valine in the muscle of birds fed diets containing either AC (SFAC and LSAC) or linseed only (LSO) ( $P < .05$ ). Leucine showed a comparable elevated pattern, but when AC was fed with linseed (LSAC) it developed lower broiler muscle content than SFAC and LSO diets. Completely contrary to the prevalent pattern, lysine content decreased in muscles of birds fed a diet containing AC and/or linseed diets ( $P < .05$ ). The most changes noticed in non-essential amino acids including alanine, asparagine,

glycine, serine, and tyrosine were attributed to the interaction between oil source and AC ( $P < .05$ ). Compared with the SFO group, a small increase in alanine and proline and a major improvement in glutamine, glycine, and serine muscle composition were correlated with the addition of AC and/or linseed to the diet. Interestingly, aspartic acid content decreased and increased when AC was included in broiler diets in combination with SFO and linseed, respectively ( $P < .05$ ). Tyrosine pattern was shown to be the opposite of aspartic acid.

### **Oxidative stress biomarkers and antioxidants in liver and muscle tissues**

Effect of dietary linseed oil, sunflower oil, and their combinations with *Amphora coffeaeformis* on oxidative stress biomarkers and antioxidants in liver and muscle tissues of broiler chickens are shown in Table 7. The supplementation of AC to SFO and LSO diets increased hepatic GSH and decreased GSSG and 8-OHdG levels in comparison to broilers fed SFO or LSO ( $P < .05$ ). In addition, SOD activity in the liver was slightly increased and MDA was slightly reduced in broiler groups fed AC diets in comparison to broiler groups fed SFO or LSO diets ( $P > .05$ ). Although the LSO diet showed comparable hepatic oxidative



**Table 6.** Effect of dietary linseed oil, sunflower oil, and their supplementation with *Amphora coffeaeformis* on profiles of amino acids in breast muscles of broiler chickens.

Parameters	Groups				SEM	p values		
	1	2	3	4		Oils	AC	Oils*AC
	SFO	SFAC	LSO	LSAC				
<b>Essential amino acids</b>								
Arginine	14.25 <sup>c</sup>	18.77 <sup>b</sup>	20.25 <sup>b</sup>	23.67 <sup>a</sup>	0.828	.006	.033	.744
Histidine	11.05 <sup>c</sup>	14.73 <sup>ab</sup>	15.72 <sup>ab</sup>	16.88 <sup>a</sup>	0.380	.001	.008	.122
Isoleucine	9.19 <sup>b</sup>	12.43 <sup>a</sup>	12.54 <sup>a</sup>	12.03 <sup>a</sup>	0.437	.117	.145	.053
Leucine	13.34 <sup>c</sup>	17.87 <sup>a</sup>	18.48 <sup>a</sup>	15.56 <sup>b</sup>	0.725	.347	.589	.025
Lysine	23.37 <sup>a</sup>	16.92 <sup>b</sup>	17.50 <sup>b</sup>	14.11 <sup>c</sup>	0.417	.000	.000	.092
Methionine	3.92 <sup>c</sup>	4.76 <sup>b</sup>	5.45 <sup>a</sup>	4.76 <sup>b</sup>	0.103	.003	.727	.003
Phenylalanine	6.59 <sup>c</sup>	8.30 <sup>b</sup>	8.82 <sup>ab</sup>	9.77 <sup>a</sup>	0.199	.001	.006	.363
Threonine	8.08 <sup>b</sup>	10.23 <sup>a</sup>	11.39 <sup>a</sup>	10.44 <sup>a</sup>	0.315	.016	.361	.030
Valine	10.76 <sup>c</sup>	15.35 <sup>ab</sup>	15.53 <sup>ab</sup>	17.40 <sup>a</sup>	0.551	.009	.013	.240
<b>Non-essential amino acids</b>								
Alanine	11.64	12.92	15.00	12.74	0.409	.08	.56	.05
Asparagine	28.30 <sup>a</sup>	22.21 <sup>b</sup>	23.61 <sup>ab</sup>	26.67 <sup>a</sup>	0.771	.94	.34	.01
Glutamine	25.87	32.44	34.52	31.92	1.373	.16	.48	.12
Glycine	10.25 <sup>b</sup>	13.40 <sup>a</sup>	11.99 <sup>ab</sup>	11.12 <sup>ab</sup>	0.406	.75	.19	.03
Proline	4.53	5.87	6.01	5.95	0.250	.14	.22	.19
Serine	6.56 <sup>b</sup>	8.70 <sup>a</sup>	9.14 <sup>a</sup>	7.35 <sup>ab</sup>	0.354	.40	.81	.02
Tyrosine	7.80 <sup>b</sup>	9.57 <sup>b</sup>	11.10 <sup>a</sup>	9.06 <sup>b</sup>	0.316	.05	.84	.01

<sup>a-c</sup>Means within a row not sharing a common superscript differ significantly with the corresponding *p* value. Group 1: birds fed basal diets mixed with sunflower oil 3% (SFO). Group 2: birds fed basal diets mixed with a combination of SFO oil 3% and *Amphora coffeaeformis* (1 g/kg; SFAC). Group 3: birds fed basal diets mixed with linseed oil 3% (LSO). Group 4: birds fed basal diets mixed with a combination of LSO oil 3% and *Amphora coffeaeformis* (1 g/kg; LSAC). AC: *Amphora coffeaeformis*; SEM: standard error of the mean.

**Table 7.** Effect of dietary linseed oil, sunflower oil, and their supplementation with *Amphora coffeaeformis* on oxidative stress biomarkers and antioxidants in liver and muscle tissues of broiler chickens.

Parameters	Groups				SME	p values		
	1	2	3	4		Oils	AC	Oils*AC
	SFO	SFAC	LSO	LSAC				
<b>Liver</b>								
SOD (nM/min/g)	31.95	39.54	46.26	55.52	4.66	.13	.38	.93
MDA (nM/g)	36.17	31.43	25.59	33.27	3.72	.57	.85	.42
8-OHdG (nM/g)	267.00 <sup>a</sup>	225.00 <sup>b</sup>	194.0 <sup>b</sup>	186.00 <sup>b</sup>	3.92	<.001	.01	.05
GSH (nM/g)	2.65 <sup>b</sup>	3.56 <sup>a</sup>	3.57 <sup>a</sup>	3.88 <sup>a</sup>	0.12	.02	.02	.23
GSSG (nM/g)	0.34 <sup>a</sup>	0.26 <sup>b</sup>	0.30 <sup>a</sup>	0.26 <sup>b</sup>	0.01	.12	<.001	.13
CoQ10(nmol/gm)	17.90 <sup>b</sup>	21.93 <sup>ab</sup>	21.17 <sup>ab</sup>	21.20 <sup>a</sup>	0.80	.45	.23	.24
Vit. E (µg/g)	0.24 <sup>bc</sup>	0.27 <sup>b</sup>	0.30 <sup>ab</sup>	0.35 <sup>a</sup>	0.01	.02	.14	.80
L-Carnitine ( nmol/gm)	251.25 <sup>c</sup>	311.75 <sup>bc</sup>	331.25 <sup>ab</sup>	385.75 <sup>a</sup>	8.58	.001	.01	.86
<b>Muscle</b>								
MDA (nM/g)	26.47 <sup>b</sup>	29.93 <sup>b</sup>	41.24 <sup>a</sup>	28.73 <sup>b</sup>	0.89	.003	.03	.001
8-OHdG (nM/g)	162.40 <sup>b</sup>	149.80 <sup>bc</sup>	226.17 <sup>a</sup>	175.06 <sup>b</sup>	3.27	<.001	<.001	.01
GSH (nM/g)	3.24 <sup>ab</sup>	3.09 <sup>a</sup>	2.30 <sup>b</sup>	3.06 <sup>a</sup>	0.12	.07	.23	.09
GSSG (nM/g)	0.26 <sup>ab</sup>	0.22 <sup>b</sup>	0.28 <sup>a</sup>	0.21 <sup>b</sup>	0.01	.68	.005	.23

<sup>a-c</sup>Means within a row not sharing a common superscript differ significantly with the corresponding *p* value. Group 1: birds fed basal diets mixed with sunflower oil 3% (SFO). Group 2: birds fed basal diets mixed with a combination of SFO oil 3% and *Amphora coffeaeformis* (1 g/kg; SFAC). Group 3: birds fed basal diets mixed with linseed oil 3% (LSO). Group 4: birds fed basal diets mixed with a combination of LSO oil 3% and *Amphora coffeaeformis* (1 g/kg; LSAC). AC: *Amphora coffeaeformis*; SOD: superoxide dismutase; MDA: malonaldehyde; 8-OHdG: 8-hydroxy-2'-deoxyguanosines; GSH: reduced glutathione; GSSG: oxidised glutathione; CoQ10: coenzyme Q10; Vit. E: vitamin E; AC: *Amphora coffeaeformis*; SEM: Standard error of the mean; nM: nanomole.

stability to broilers fed AC diets (SFAC and LSAC), it provided the worst muscular oxidative indices compared with all other groups ( $P < .05$ ), but the addition of AC enhanced the antioxidant potential of LSAC and SFAC groups.

Effect of dietary oil source, linseed oil or sunflower, and their combinations with *Amphora coffeaeformis* on non-enzymatic antioxidants and energy markers of broiler chickens are shown in Table 7. Non-enzymatic

antioxidants, namely Coenzyme Q10 and vitamin E increased slightly in the liver of birds fed diets containing AC and/or linseed oil ( $P > .05$ ). The concentration of vitamin E was elevated significantly in the livers of birds fed diets containing LSAC than that of birds fed diets containing sunflower oil, SFO, and SFAC ( $P < .05$ ). Higher L-Carnitine contents were noticed in the livers of birds fed AC and/or linseed diets (SFAC, LSO, LSAC), with a more pronounced high

**Table 8.** Effect of dietary linseed oil, sunflower oil, and their supplementation with *Amphora coffeaeformis* on meat quality parameters of broiler chickens.

Parameters	Groups				SEM	<i>p</i> values		
	1	2	3	4		Oils	AC	Oils*AC
pH	5.61	5.59	5.53	5.74	0.03	.48	.08	.05
Drip loss (%)	1.01	1.71	1.67	1.67	0.18	.40	.35	.35
Thawing loss (%)	4.06	3.27	4.69	3.27	0.29	.59	.07	.60
Cooking loss (%)	17.09	17.48	15.33	15.17	0.49	.06	.91	.78
L*	54.39	53.14	54.56	55.53	0.47	.20	.89	.26
a*	12.67 <sup>a</sup>	10.48 <sup>ab</sup>	12.29 <sup>a</sup>	11.85 <sup>ab</sup>	0.31	.44	.05	.18
b*	13.36 <sup>ab</sup>	11.96 <sup>b</sup>	13.78 <sup>a</sup>	12.31 <sup>b</sup>	0.22	.40	.01	.94
Chroma (c*)	18.41 <sup>a</sup>	15.95 <sup>b</sup>	18.47 <sup>a</sup>	17.10 <sup>ab</sup>	0.30	.33	.01	.38
Hue angle (HA)	46.49	48.97	48.36	46.09	0.80	.76	.95	.16
WBSF (kgf)	3.22	2.96	2.79	2.13	0.16	.06	.17	.53

<sup>a-c</sup>Means within a row not sharing a common superscript differ significantly with corresponding *P* value. Group 1: birds fed basal diets mixed with sunflower oil 3% (SFO). Group 2: birds fed basal diets mixed with a combination of SFO oil 3% and *Amphora coffeaeformis* (1 g/kg; SFAC). Group 3: birds fed basal diets mixed with linseed oil 3% (LSO). Group 4: birds fed basal diets mixed with a combination of LSO oil 3% and *Amphora coffeaeformis* (1 g/kg; LSAC). pH: hydrogen ion concentration; L\*: lightness; a\*: redness; b\*: yellowness; c\*: chroma; Hue angle (HA): colour saturation; WBSF: Warner-Bratzler Shear Force; AC: *Amphora coffeaeformis*; SEM: standard error of the mean.

content detected in the livers of LSO and LSAC-fed birds ( $P < .05$ ) (Table 7).

### Meat quality parameters

Effects of dietary linseed oil, sunflower oil, and their combinations with *Amphora coffeaeformis* on meat quality parameters of broiler chickens are shown in Table 8. Marked modifications in meat quality parameters due to either dietary inclusion of certain plant oils or interactions between AC and the oil source were not observed in the present study ( $P > .05$ ). Dietary AC significantly affected both the yellowness (b\*) and the chroma (c\*) of breast meat ( $P < .05$ ), but no other changes were observed. Breast meat b\* values were lower in birds fed *Amphora coffeaeformis*, SFAC, and LSAC than in birds fed diets without *Amphora coffeaeformis*, SFO, and LSO ( $P < .05$ ). Smaller chroma (c\*) values were associated with breast meat of birds fed diets containing *Amphora coffeaeformis*, particularly the SFAC diet ( $P < .05$ ) when compared to other diets, SFO and LSO.

## Discussion

### Growth performance and carcass characteristics

Except for final body weight and WG, the overall growth performance of the broiler chickens of all experimental groups was comparable. Similarly, higher final body weight, WG, and lower FCR values were observed in broiler chickens fed a diet mixed with AC (El-Bahr et al. 2020) or 1% of microalgae oil, linseed oil, and soybean oil as a replacement mixture (Long et al. 2020). However, FCR was not changed significantly

in broilers fed diet mixed with microalgae as a replacement of dietary oil (Yan and Kim 2013). Despite the fact that linseed oil has a high nutritional value (El-Beltagi et al. 2007) and that earlier dietary inclusion (Ibrahim et al. 2018) was associated with better broiler performance than a sunflower oil-based diet, the results of this study revealed that the final body weight and WG of birds fed either LSO or SFO diets were not significantly different. Improved broiler performance previously observed was due to increased dietary n-3 PUFA, which improves fat digestion by activating bile in the intestine, thus increasing feed digestion and absorption efficiency. These parameters increased significantly in birds fed LSO or SFO diets supplemented with AC. The promotional effect of AC on broiler performance may be attributed to the presence of biologically active components such as polyphenolic compounds, gallic acid, catechin, and p-coumaric acid, all of which are antioxidants (Lee et al. 2009), antivirals, and antibacterial (El-Bahr et al. 2020). These components protected the integrity of the intestines, improved the overall beneficial microbiota, counteract the effects of harmful dietary ingredients (El-Sayed et al. 2018) and maximising nutrient absorbability (Alwaleed et al. 2020). Meanwhile, the slow feed flow rate in the broiler small intestine, which may be correlated with dietary microalgae and linseed oil (Long et al. 2020), would further increase nutrient digestibility and contribute to improved performance. This fact was confirmed in the current study by observing higher relative gizzard weights in birds fed diet mixed with linseed oil either alone (LSO) or in a combination with AC (LSAC). The unchanged relative weight of the liver and spleen in birds fed diets supplemented with AC in the form of combinations with

either SFO or LSO are consistent with the earlier study in broiler chicken fed microalgae supplemented diets (Yan and Kim 2013). Broilers-fed diets contained *Amphora coffeaeformis* and microalgae/linseed oil mixture yielded lower liver and spleen relative weights (Alwaleed et al. 2020; Long et al. 2020). Dietary 1% or 2% DHA-rich microalgae induced a higher percentage of liver of broiler chickens, and this attributed to the high DHA content of microalgae (Long et al. 2018). In the present study, the relative weight of fat pad decreased significantly in birds fed AC supplemented diets. Previous studies (Abudabos et al. 2013; Long et al. 2018; Alwaleed et al. 2020) have linked this decline to MA's high n-3 PUFA content, which regulates lipid utilisation in serum and liver, presumably by promoting HDL-C synthesis and accelerating TC, TG, and LDL-C metabolism.

### **Profiles of fatty and amino acids in breast muscles**

The concentrations of PUFA (LA, ALA, AA, and DHA) increased significantly in the muscle of birds fed diets containing AC mixed with either LSO or SFO compared to those of other groups. These findings may be attributable to the direct integration from the high natural content of these FAs within AC (Yan and Kim 2013; El-Bahr et al. 2020) and linseed (Gonzalez et al. 2014; Panda et al. 2015; Kalakuntla et al. 2017; Kralik et al. 2018). Although it has received much less attention than the preceding attribution, the potential of AC microalgae (Long et al. 2018) and linseed (López-Ferrer et al. 2001) to enhance the de novo synthesis of these fatty acids from their precursors (LA and ALA) should be considered. These suggestions are supported by the determination of higher levels of ETA, EPA, and DHA in the muscle of broilers fed diets containing a combination of AC and SFO (SFAC) compared to those of birds fed diets containing SFO alone. In line with current findings, improved muscle concentrations of ALA, EPA, DHA were reported from broiler chickens fed dietary linseed oil (Mirshekar et al. 2015) or mixtures of microalgae and linseed oil (Long et al. 2020). These birds had lower concentrations of MUFA compared to those of broilers fed fish oil diets (Long et al. 2020). In the current study, the concentrations of MUFA were increased significantly in the muscle of birds fed the combination of AC and SFO. Incorporation of AC and/or linseed oil in the diets of the current study induced a significant reduction of MUFA and SFA particularly myristic acid (MA) and palmitic acid (PA) in the muscle of investigated birds. The

concentration of DHA was increased and the concentration of SFA was decreased in muscle of broilers fed nearly similar dose of AC (El-Bahr et al. 2020) and marine microalgae powder (Yan and Kim 2013). Previously, the concentrations of SFA (Ibrahim et al. 2018) and MUFA (Kishawy et al. 2019) were reduced significantly in muscles of broilers fed diet containing LSO. These data are compatible with earlier evidence that higher concentrations of palmitic and stearic acids increase the deposition of SFA, MUFA, and n-6 PUFA in broiler meat (El-Katcha et al. 2014). Overall, the present amino acid study, excluding lysine, leucine, and tyrosine, showed that the implementation of AC-containing diets (SFAC and LSAC) and linseed-only (LSO) marginally and/or substantially enriched broiler muscles with essential and non-essential amino acids. Referring to the high concentrations of amino acids in AC (El-Bahr et al. 2020), current findings seem rational because AC improved the nutritional intensity of the diets provided with these amino acids, which is why microalgae species were used as protein sources to substitute traditional proteins or supplements in animal feed (El-Bahr et al. 2020). Another contributing factor is the high DHA and PUFA levels of AC and linseed, which were shown to increase the efficiency of feed digestion and absorption in the intestine (Jameel and Sahib 2014). Also, the detoxification, antioxidants, anti-inflammatory, immunomodulatory functions of AC biological agents (El-Sayed et al. 2018) and DHA (Calder 2010) should indeed be considered. The exact reasons behind the lower muscular content of leucine and tyrosine in LSAC-fed broilers, in the meantime slightly higher than the SFO diet, are uncertain. Nonetheless, the substantial low muscular lysine content, as well as higher body weight gain and final body weight provided by this group compared to other groups, suggested that the metabolic activity of this group (LSAC) is positively modulated by dietary AC. It should be highlighted that lysine was previously enriched in muscle when broiler fed diets containing AC and soya bean oil (El-Bahr et al. 2020). This may indicate that when AC combines with linseed oil, lysine may be guided to other metabolic activities, in addition to its key function in muscle building. In particular, it is well known that lysine is one of the limiting amino acids for broiler growth and it is required to maintain many metabolic processes, such as carnitine synthesis (Vaz and Wanders 2002) and obligatory oxidation (Ball et al. 2007). The reverse trend observed in the current study between measured L-carnitine and lysine content of different treatments, particularly those augmented by AC, confirms this interpretation.

### ***Oxidative stress biomarkers and antioxidants in liver and muscle tissues***

Present endogenous enzymatic antioxidant and oxidative stress markers including SOD, GSH, GSSG, 8-OHdG, and MDA levels in the liver and muscle support the potential antioxidant activity of AC observed earlier when complemented to broiler diet (El-Bahr et al. 2020). Negative oxidative indices of breast muscle produced from broilers complemented with LSO have also been noted due to high PUFA content (Kalakuntla et al. 2017). Furthermore, levels of MDA and OHdG were found to be higher in the muscle of broilers fed LSO-diet than SFO-diet, confirming prior reports that n-3 PUFA is more susceptible to oxidation than n-6 PUFA (Cortinas et al. 2005; Li et al. 2013), though the reasons for this remain unknown. High levels of carotenoids and phenolics, assessed in a previous study on the same AC microalgae (El-Sayed et al. 2018), seems to have engaged in a variety of biological activities, especially potent antioxidant activity against free radicals and peroxidation reactions (El-Sayed et al. 2018) in the broiler, that perhaps contributed also to the promotion of antioxidant activity in the current study.

The addition of AC to broiler diets, irrespective of the oil source, was associated with a slight increase in hepatic non-enzymatic antioxidants CoQ10 and vitamin E contents. In addition, linseed oil-containing diets had similarly incorporated higher levels of these antioxidants (vitamin E) than sunflower oil diets. The high level of coenzyme Q10 addressed in the current study may be correlated with its primary role as effective lipid antioxidants (Geng et al. 2004) or its involvement in the synthesis of adenosine triphosphate ATP (mitochondrial oxidative phosphorylation) (Saini 2011). Both attributions may be enhanced by AC supplementation. The correlation between high vitamin E muscle content and AC supply can be due to its ability to boost dietary vitamins and mineral absorption. Furthermore, AC may be a source of these bioactive compounds, including  $\alpha$ -tocopherol, vitamin E, and vitamin C (El-Sayed et al. 2018; Pestana et al. 2020). Current results are consistent with previous work (Long et al. 2018), reported that dietary microalgae increased the non-enzymatic antioxidant potential of chicken meat and were attributed to vitamin A (2.04%) and vitamin E (0.07%) of these microalgae, which of course play an important role in protecting endogenous lipids from peroxidation and oxidation. Earlier studies on flaxseeds for estimation of potential antioxidants showed high levels of total tocopherols, total phenolic content, and total flavonoids in addition to essential PUFA oils, which may explain the reason

for the improvement of non-enzymatic antioxidants in the liver from broilers that received linseed containing diets (Goyal et al. 2014). Current findings, therefore, indicated that high levels of non-enzymatic antioxidants, CoQ10 and vitamin E, may have played a role in lowering concentrations of MDA and 8-OHdG and shared enzymatic antioxidants in promoting oxidative stability of broiler liver and meat even in the presence of high n-3 PUFA. So far it has undoubtedly led to the improved growth of broiler chickens, LSAC comes from its ability to neutralise oxidative damage and mitigate lipid peroxidation in both the plasma and skeletal muscles (Gao et al. 2010), and it will, of course, enhance the preservation of meat (Goñi et al. 2007).

Carnitine is primarily involved in the transport of long-chain fatty acids to mitochondria to initiate the citric acid cycle for  $\beta$ -oxidation and subsequent energy generation (Vaz and Wanders 2002), where coenzyme A exchange for carnitine with mitochondrial carnitine palmitoyltransferase I (CPT I) to promote the transfer of acyl groups to mitochondria for  $\beta$ -oxidation (Power and Newsholme 1997). Thus, the level of hepatic carnitine could indirectly predict the level of integrated PUFA and related metabolic activities from different rations of the current study. High DHA or PUFA content of broiler muscle diets supplied with AC and/or linseed (SFAC, LSO, LSAC) are shown to indicate high levels of hepatic carnitine. Whereas low lysine content in the muscles of the same broiler groups may also be due mainly to their increased metabolic role in endogenous carnitine biosynthesis, including protein-bound lysine methylation (Vaz and Wanders 2002).

### ***Meat quality parameters***

The currently estimated meat quality traits of different broiler groups were not influenced by dietary oil sources but were clearly influenced by AC fortification. The insignificant changes in breast meat pH reported in the current study were logically reflected as insignificant changes in technical and tenderness characteristics, including drip loss, thawing loss, cooking loss, WBSF, and colour, as all of them are related and affected by pH change (El Rammouz et al. 2004). Exceptionally, breast meat yellowness ( $b^*$ ) and Chroma ( $c^*$ ) decreased in birds fed a diet containing AC, SFAC, and LSAC. According to these criteria, meat from AC-fed broiler groups, SFAC and LSAC, would be less yellow than meat from AC-free diets, SFO and LSO. However, broilers fed AC-containing diets, particularly SFAC, had low redness and chroma values as

well as slightly higher hue meat values ( $P > .05$ ), indicating mildly less red meat. In contrary, high levels of dietary incorporation of microalgae such as *Spirulina platensis* (2.5 – 15%) were always associated with yellow lower meat, which was attributed to its high carotenoid content (Raach-Moujahed et al. 2011; Pestana et al. 2020). However, the levels of AC used in the current study (0.1%; 1 g/kg) or that of *Spirulina* (0.25, 0.5, 0.75, and 1%) used in other studies (Park et al. 2018) may provide low carotenoid levels that interpret the observed less yellow or even unchanged colour of breast muscle under investigation of both studies. Even though the current study's thawing loss, WBSF, and cooking loss values were statistically insignificant, similar to our previous research (El-Bahr et al. 2020), it was observed that some values of these technological indices were found to be more favourable and were directly linked to broilers fed AC supplemented diets (SFAC and LSAC) and diet-containing linseed oil. In comparison to previous experiments that tested sunflower oil replacement with linseed oil (Kalakuntla et al. 2017) and assessed the incorporation of linseed oil into broiler chicken diet at different levels of 7%, 4%, 3% and 2% (López-Ferrer et al. 2001; Zelenka et al. 2008; Panda et al. 2015; El-Bahr et al. 2021), the present investigation reached the same conclusion that the oil source had little effect on instrumental meat quality parameters.

## Conclusion

Dietary supplementation of *Amphora coffeaeformis* (AC) at 1 g/kg with either SFO or LSO diets improved broiler chickens' performance, fatty, and amino acid profiles, oxidative stability, and meat quality. The contents of SFAs declined whilst values of MUFA remained unchanged or slightly increased by the dietary application of AC. Remarkably, the levels of muscular lysine were inversely linked to the hepatic ATP and l-carnitine concentration of each broiler group, especially those of AC and/or linseed diets.

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## Ethical approval

Institutional Animal Care and Use Committee Research Ethics of Benha University, Faculty of Veterinary Medicine,

Egypt reviewed and approved all experimental protocols (BUFVTM, 07032020) actually conducted in this study.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## References

- Abd-Elrazek AM, Ahmed-Farid OAH. 2018. Protective effect of L-carnitine and L-arginine against busulfan-induced oligospermia in adult rat. *Andrologia*. 50(1): 1–8.
- Abudabos AM, Okab AB, Aljumaah RS, Samara EM, Abdoun KA, Al-Haidary AA. 2013. Nutritional value of green seaweed (*Ulva lactuca*) for broiler chickens. *Ital J Anim Sci*. 12(2):e28.
- Ahmed-Farid OAH, Nasr M, Ahmed RF, Bakeer RM. 2017. Beneficial effects of curcumin nano-emulsion on spermatogenesis and reproductive performance in male rats under protein deficient diet model: enhancement of sperm motility, conservancy of testicular tissue integrity, cell energy and seminal plasma amino acids content. *J Biomed Sci*. 24(1):66–66.
- Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, Khafaga AF, Taha AE, Tiwari R, Yatoo MI, Bhatt P, Khurana SK, et al. 2019. Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. *Animals*. 9(8):573.
- Ali EH, Elgoly AH. 2013. Combined prenatal and postnatal butyl paraben exposure produces autism-like symptoms in offspring: comparison with valproic acid autistic model. *Pharmacol Biochem Behav*. 111:102–110.
- Alwaleed EA, El-Sheekh M, Abdel-Daim MM, Saber H. 2020. Effects of *spirulina platensis* and *Amphora coffeaeformis* as dietary supplements on blood biochemical parameters, intestinal microbial population, and productive performance in broiler chickens. *Environ Sci Pollut Res*. 28: 1–11.
- AMSA. 2012. Meat color measurement guidelines. Champaign (Illinois): American Meat Science Association; p. 1–135.
- AMSA. 2015. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat. Second Edition (version 1.0) ed. Champaign (IL): American Meat Science Association.
- Ball RO, Urschel KL, Pencharz PB. 2007. Nutritional consequences of interspecies differences in arginine and lysine metabolism. *J Nutr*. 137(6 Suppl 2):1626S–1641S.
- Bhosle NB, Evans L, Edyvean R. 1993. Carbohydrate production by *amphora coffeaeformis*, a marine fouling diatom. *Biofouling*. 7(1):81–91.
- Bou R, Guardiola F, Barroeta AC, Codony R. 2005. Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat. *Poult Sci*. 84(7):1129–1140.
- Calder PC. 2010. Omega-3 fatty acids and inflammatory processes. *Nutrients*. 2(3):355–374.
- Calder PC. 2012. Mechanisms of action of (n-3) fatty acids. *J Nutr*. 142(3):592S–599S.
- Cortinas L, Barroeta A, Villaverde C, Galobart J, Guardiola F, Baucells MD. 2005. Influence of the dietary

- polyunsaturation level on chicken meat quality: lipid oxidation. *Poult Sci.* 84(1):48–55.
- Crespo N, Esteve-Garcia E. 2001. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. *Poult Sci.* 80(1):71–78.
- El Rammouz R, Babilé R, Fernandez X. 2004. Effect of ultimate pH on the physicochemical and biochemical characteristics of turkey breast muscle showing normal rate of postmortem pH fall. *Poult Sci.* 83(10):1750–1757.
- El-Bahr SM, Shousha S, Alfattah MA, Al-Sultan S, Khattab W, Sabeq II, Ahmed-Farid O, El-Garhy O, Albusadah KA, Alhojaily S. 2021. Enrichment of broiler chickens' meat with dietary linseed oil and lysine mixtures: influence on nutritional value, carcass characteristics and oxidative stress biomarkers. *Foods.* 10(3):618.
- El-Bahr S, Shousha S, Shehab A, Khattab W, Ahmed-Farid O, Sabike I, El-Garhy O, Albokhadaim I, Albosadah K. 2020. Effect of dietary microalgae on growth performance, profiles of amino and fatty acids, antioxidant status, and meat quality of broiler chickens. *Animals.* 10(5):761.
- El-Beltagi H, Salama Z, El-Hariri D. 2007. Evaluation of fatty acids profile and the content of some secondary metabolites in seeds of different flax cultivars. (*Linum Usitatissimum L.*). *General Applied Plant Physiol.* 33(3–4): 187–202.
- El-Katcha MI, El-Kholy ME, Soltan MA, EL-Gayar AH. 2014. Effect of dietary omega-3 to omega-6 ratio on growth performance, immune response, carcass traits and meat fatty acids profile of broiler chickens. *Poultry Sci J.* 2(2): 71–94.
- Elokil AA, Imbabi TA, Mohamed HI, Abouelezz KFM, Ahmed-Farid O, Shishay G, Sabike II, Liu H. 2019. Zinc and copper with new triazine hydrazone ligand: two novel organic complexes enhanced expression of peptide growth factors and cytokine genes in weaned V-line rabbit. *Animals.* 9(12):1134.
- El-Sayed AE-KB, Aboulthana WM, El-Feky AM, Ibrahim NE, Seif MM. 2018. Bio and phyto-chemical effect of *Amphora coffeaeformis* extract against hepatic injury induced by paracetamol in rats. *Mol Biol Rep.* 45(6):2007–2023.
- Enwereuzoh U, Onyeagoro G. 2014. A novel aeration method for the Preparation of algae (*Dunaliella Salina*) biomass for biofuel production. *Am J Eng Res.* 3(9):209–214.
- Gao J, Lin H, Wang XJ, Song ZG, Jiao HC. 2010. Vitamin E supplementation alleviates the oxidative stress induced by dexamethasone treatment and improves meat quality in broiler chickens. *Poult Sci.* 89(2):318–327.
- Geng AL, Guo YM, Yang Y. 2004. Reduction of ascites mortality in broilers by coenzyme Q10. *Poult Sci.* 83(9): 1587–1593.
- Goñi I, Brenes A, Centeno C, Viveros A, Saura-Calixto F, Rebolé A, Arija I, Estevez R. 2007. Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. *Poult Sci.* 86(3):508–516.
- Gonzalez L, Moreno T, Bispo E, Dugan ME, Franco D. 2014. Effect of supplementing different oils: linseed, sunflower and soybean, on animal performance, carcass characteristics, meat quality and fatty acid profile of veal from "Rubia Gallega" calves. *Meat Sci.* 96(2):829–836.
- Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. 2014. Flax and flaxseed oil: an ancient medicine & modern functional food. *J Food Sci Technol.* 51(9):1633–1653.
- Honikel KO, Hamm R. 1994. Measurement of water-holding capacity and juiciness. In: Pearson AM, Dutson TR, editors. *Quality attributes and their measurement in meat, poultry and fish products.* Boston (MA): Springer US; p. 125–161.
- Honikel KO. 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Sci.* 49(4):447–457.
- Horwitz W, AOAC. 2000. *Official methods of analysis of AOAC International.* 17th ed. Gaithersburg (MD): AOAC International.
- Hughes MC, Kerry JP, Arendt EK, Kenneally PM, McSweeney PL, O'Neill EE. 2002. Characterization of proteolysis during the ripening of semi-dry fermented sausages. *Meat Sci.* 62(2):205–216.
- Ibrahim D, El-Sayed R, Khater SI, Said EN, El-Mandrawy SAM. 2018. Changing dietary n-6:n-3 ratio using different oil sources affects performance, behavior, cytokines mRNA expression and meat fatty acid profile of broiler chickens. *Anim Nutr.* 4(1):44–51.
- Jameel YJ, Sahib AM. 2014. Study of some blood parameters of broilers fed on ration containing fish oil. *J Biol Agric Healthc.* 4(7): 67–71
- Kalakuntla S, Nagireddy NK, Panda AK, Jatoth N, Thirunahari R, Vangoor RR. 2017. Effect of dietary incorporation of n-3 polyunsaturated fatty acids rich oil sources on fatty acid profile, keeping quality and sensory attributes of broiler chicken meat. *Animal Nutrition.* 3(4):386–391.
- Karpińska J, Mikołuc B, Motkowski R, Piotrowska-Jastrzebska J. 2006. HPLC method for simultaneous determination of retinol, alpha-tocopherol and coenzyme Q10 in human plasma. *J Pharm Biomed Anal.* 42(2):232–236.
- Kishawy ATY, Amer SA, Abd El-Hack ME, Saadeldin IM, Swelum AA. 2019. The impact of dietary linseed oil and pomegranate peel extract on broiler growth, carcass traits, serum lipid profile, and meat fatty acid, phenol, and flavonoid contents. *Asian-Australas J Anim Sci.* 32(8): 1161–1171.
- Kralik G, Kralik Z, Grčević M, Hanžek D. 2018. Quality of chicken meat. In: Yuçel B, editor. *Animal husbandry and nutrition.* Intechopen, Turkey; p. 63.
- Lee SA, Whenham N, Bedford MR. 2019. Review on docosa-hexaenoic acid in poultry and swine nutrition: consequence of enriched animal products on performance and health characteristics. *Anim Nutr.* 5(1):11–21.
- Lee S-H, Karawita R, Affan A, Lee J-B, Lee K-W, Lee B-J, Kim D-W, Jeon Y-J. 2009. Potential of benthic diatoms *Achnanthes longipes*, *Amphora coffeaeformis* and *Navicula sp.*(Bacillariophyceae) as antioxidant sources. *Algae.* 24(1):47–55.
- Li H, Fan Y-w, Li J, Tang L, Hu J-n, Deng Z-y. 2013. Evaluating and predicting the oxidative stability of vegetable oils with different fatty acid compositions. *J Food Sci.* 78(4):H633–H641.
- Li K, Sun Q. 2010. Simultaneous determination of free and total carnitine in human serum by HPLC with UV detection. *J Chromatogr Sci.* 48(5):371–374.
- Long SF, Kang S, Wang QQ, Xu YT, Pan L, Hu JX, Li M, Piao XS. 2018. Dietary supplementation with DHA-rich microalgae improves performance, serum composition, carcass

- trait, antioxidant status, and fatty acid profile of broilers. *Poult Sci.* 97(6):1881–1890.
- Long S, Liu S, Wu D, Mahfuz S, Piao X. 2020. Effects of dietary fatty acids from different sources on growth performance, meat quality, muscle fatty acid deposition, and antioxidant capacity in broilers. *Animals.* 10(3):508.
- López-Ferrer S, Baucells MD, Barroeta AC, Galobart J, Grashorn MA. 2001. n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: linseed oil. *Poult Sci.* 80(6):753–761.
- Marklund S, Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem.* 47(3):469–474.
- Meydani SN, Lichtenstein AH, White PJ, Goodnight SH, Elson CE, Woods M, Gorbach SL, Schaefer EJ. 1991. Food use and health effects of soybean and sunflower oils. *J Am Coll Nutr.* 10(5):406–428.
- Mirshekar R, Boldaji F, Dastar B, Yamchi A, Pashaei S. 2015. Longer consumption of flaxseed oil enhances n-3 fatty acid content of chicken meat and expression of FADS2 gene. *Eur J Lipid Sci Technol.* 117(6):810–819.
- Panda AK, Sridhar K, Lavanya G, Prakash B, Rama Rao SV, Raju MVLN. 2015. Growth performance, carcass characteristics, fatty acid composition and sensory attributes of meat of broiler chickens fed diet incorporated with linseed oil [Article. *Indian J Anim Sci.* 85(12):1354–1357.].
- Park JH, Lee SI, Kim IH. 2018. Effect of dietary *Spirulina* (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. *Poult Sci.* 97(7):2451–2459.
- Pestana JM, Puerta B, Santos H, Madeira MS, Alfaia CM, Lopes PA, Pinto RMA, Lemos JPC, Fontes CMGA, Lordelo MM, et al. 2020. Impact of dietary incorporation of *Spirulina* (*Arthrospira platensis*) and exogenous enzymes on broiler performance, carcass traits, and meat quality. *Poult Sci.* 99(8):4100.
- Power GW, Newsholme EA. 1997. Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. *J Nutr.* 127(11):2142–2150.
- Raach-Moujahed A, Hassani S, Zairi S, Bouallegue M, Darej C, Haddad B, Damergi C. 2011. Effect of dehydrated *Spirulina platensis* on performance and meat quality of broilers. *Res Opin Anim Vet Sci.* 1(8):505–509.
- Radwan OK, Ahmed RF. 2016. Amendment effect of resveratrol on diclofenac idiosyncratic toxicity: augmentation of the anti-inflammatory effect by assessment of Arachidonic acid and IL-1 $\beta$  levels. *J Appl Pharm Sci.* 6(12):170–177.
- Ribeiro T, Lordelo MM, Costa P, Alves SP, Benevides WS, Bessa RJB, Lemos JPC, Pinto RMA, Ferreira LMA, Fontes CMGA, et al. 2014. Effect of reduced dietary protein and supplementation with a docosahexaenoic acid product on broiler performance and meat quality. *Br Poult Sci.* 55(6):752–765.
- Rodriguez-Leyva D, Dupasquier CMC, McCullough R, Pierce GN. 2010. The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid. *Can J Cardiol.* 26(9):489–496.
- Saini R. 2011. Coenzyme Q10: the essential nutrient. *J Pharm Bioallied Sci.* 3(3):466–467.
- Salah AS, Ahmed-Farid OA, El-Tarabany MS. 2019. Carcass yields, muscle amino acid and fatty acid profiles, and antioxidant indices of broilers supplemented with synbiotic and/or organic acids. *J Anim Physiol Anim Nutr.* 103(1):41–52.
- Sokoła-Wysoczańska E, Wysoczański T, Wagner J, Czyż K, Bodkowski R, Lochyński S, Patkowska-Sokoła B. 2018. Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders—a review. *Nutrients.* 10(10):1561.
- Tang PH, Miles MV, DeGrauw A, Hershey A, Pesce A. 2001. HPLC analysis of reduced and oxidized coenzyme Q10 in human plasma. *Clin Chem.* 47(2):256–265.
- Vaz FM, Wanders RJ. 2002. Carnitine biosynthesis in mammals. *Biochem J.* 361(Pt 3):417–429.
- Vossen E, Raes K, Van Mullem D, De Smet S. 2017. Production of docosahexaenoic acid (DHA) enriched loin and dry cured ham from pigs fed algae: Nutritional and sensory quality. *Eur J Lipid Sci Technol.* 119(5):1600144.
- Wiley J. 2007. *The meat buyer's guide.* North American Meat Processors Association. Dietary fatty acids and human health. *Annales de zootechnie; 2000: EDP Sciences, John Wiley and Sons, NJ, USA.*
- Wood JD, Richardson RI, Nute GR, Fisher AV, Campo MM, Kasapidou E, Sheard PR, Enser M. 2004. Effects of fatty acids on meat quality: a review. *Meat Sci.* 66(1):21–32.
- Yalçın H, Ünal MK. 2010. The enrichment of hen eggs with omega-3 fatty acids. *J Med Food.* 13(3):610–614.
- Yan L, Kim IH. 2013. Effects of dietary  $\omega$ -3 fatty acid-enriched microalgae supplementation on growth performance, blood profiles, meat quality, and fatty acid composition of meat in broilers. *J Appl Anim Res.* 41(4):392–397.
- Zelenka J, Schneiderova D, Mrkvicova E, Dolezal P. 2008. The effect of dietary linseed oils with different fatty acid pattern on the content of fatty acids in chicken meat. *Veterinari Medicina.* 53(No. 2):77–85.