Chemical, Physical and Oxidative Characteristics of Broilers Meat Supplemented with Passion Fruit Seed Oil

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Summary

Residues that could be discarded and transformed in high biological value protein is a measure of environmental preservation combined with the sustainability of animal production. This study evaluated the effects of the addition of passion fruit seed oil (PFSO) on broiler diet under the resistance of skin, physical-chemical characteristic, fatty acid profile and lipid oxidation (under storage) of breast meat obtained from these animals. There was improvement in the condition of skin rupture and meat tenderness; apart from that, oxidative reactions decreased, as PFSO was added to the diet (P<0.05). On the other hand, no effect on colour, pH, water retention capacity and weight loss on cooking (P>0.05) was observed. There was a linear effect increasing only for the margaric (C17:0) and cis10-Heptadecanoic (C17:1; P<0,05) fatty acids. However, due to the low amount of these fatty acids in PFSO, the found content was still low in all evaluated treatments. However, the evaluated levels did not contribute to the increase of the polyunsaturated fatty acid profile (P>0.05). Thus, it can be concluded that PFSO improves the resistance of broiler skin, which becomes softer and less susceptible to oxidative effects due to the oil's antioxidant action, suggesting that it may be an ingredient that brings substantial benefits in the poultry industry.

Keys words: by-products, poultry, PUFA, TBARS, tenderness

1. INTRODUCTION

Amongst wastes and residual materials derived from agro-industrial processing, citrus peels are obtained in large amounts from fruit juice producers in many countries [1, 2]. This waste represents a relevant management cost for juice companies and, in some cases, an environmental problem due to illegal disposal [2]. Otherwise, its appreciation could represent an opportunity for environmental protection linked to sustainability, through which the transformation of residues into proteins of high biological value for the consumer occurs [3].

The passion fruit (*Passiflora edulis*) wastes due to their properties, may act as a phytogenic additive, which are products composed of essential oils and / or plant extracts used in animal feed to improve animal performance and meat characteristics without use. of medications [4]. With Brazil's current export position as a world leader and driven by the demands of external demand for antibiotic use, the use of these plant additives may be a step towards the modernization of the poultry sector, with potential for supply environmentally friendly products produced within a sustainable poultry industry [5].

Passion fruit peel and seed residues evaluated in the diet of broilers and laying hens have already demonstrated benefits in meat quality. In contrast, they were detrimental to intestinal quality because of the high level of fiber, especially pectin [6, 7, 8].

Thus, a new proposal to continue benefiting from the properties of passion fruit residue, without harming the intestinal quality of animals, would be the extraction of oil from its seed, in order to use this byproduct separately from the fibers that make up the seed.

This oil, extracted from fruit seeds, has a light texture, high content of linoleic acid (55 to 66%), oleic acid (18 to 20%) and palmitic acid (10 to 14%), as well as a large amount of carotenoids, 63 mg β-carotene per 100 g of oil), vitamin C, vitamin A, vitamin E and various B vitamins, as well as minerals such as phosphorus, zinc and iron [9, 10]. However, there are few studies with PFSO in animal feed, which leaves some doubts as to the potential of its use in the final product.

Thus, the objective of this work was to evaluate the effects of the addition of PFSO oil on broilers on poultry skin resistance and the physical and chemical composition, fatty acid profile and lipid oxidation (during storage) of meat chest of these animals.

2. MATERIALS AND METHODS

The experiment was carried out in accordance with ethical principles for animal tests (Protocol No. 03/2016), determined by the Ethics Committee on Animal Use (ECAU) Faculty of Veterinary Medicine and Animal Science – UNESP, Botucatu, Brazil.

Experimental procedure and samples

A total of 1440 male Cobb chicks housed in an air-conditioned shed, distributed in 56 boxes of 2.0 m², with 30 birds in each box and with each box equipped with nipple drinkers, tubular feeder. Passion Fruit Seed Oil (PFSO) used in the experimental rations was purchased from a commercial company with posterior analysis of the product's characteristics (Table 1).

The design was completely randomized, with six treatments: control treatment (0.0%), five levels of PFSO inclusion (0.1; 0, 2.0, 0.3, 0.4 and 0.5%) and eight replicates per treatment. The experimental rations were deprived of antibiotics as performance improver.

Table 1. Characterization, determination of antioxidant potential and fatty acid profile of passion fruit seed oil and soybean oil based on the literature.

Parameter	Quantity
Saponification index, mg KOH/g	194.33
Iodine index, g I_2/100g	236.77
Total carotenoids, mg of β-carotene/100g of oil [10]	75.63
Total phenolic compounds, g GAE/100g of oil [10]	1.47
[1]EC_{50}, mg/ml [35]	7.20
[2]ORAC, mM equivalent of TE/100g of oil [36]	416

Passion fruit seed oil**			**Soybean oil [37, 38, 39]**		
Formula	Name	Quantity (%)			
C14:0	Myristic	0.09±0.01	-	-	-
C16:0	Palmitic	10.99±0.03	10.70	10.40	11.00
C16:1	Palmitoleico	0.17±0.01	-	-	-
C17:0	Margárico	0.07±0.00	-	-	-
C18:0	Stearic	2.89±0.02	1.80	3.70	4.00
C18:1n9	Oleic	14.89±0.06	22.30	21.60	23.40
C18:1n7	Vaccinium	0.96±0.04	-	-	-
C18:2n6	Linoleic	69.14±0.01	51.90	50.60	53.20
C18:3n3	Linolenic	0.39±0.01	7.00	11.80	7.80
C20:0	Eicosanoic	0.12±0.02	-	-	-
C20:1	Eicosenoic	0.11±0.04	-	-	-
C22 :0	Docosanoic	0.06±0.01	-	-	-
C22:1	Erucic	0.05±0.01	-	-	-
C24:0	Lignoceric	0.06±0.00	-	-	-
	Saturated	14.28±0.01	12.60	14.50	15.20
	Monounsaturated	16.19±0.02	22.30	21.60	23.40
	Polyunsaturated	69.53±0.02	58.90	62.40	61.00

Analyzes carried out at the Faculty of Pharmaceutical Sciences of the Department of Food and Experimental Nutrition of the University of São Paulo with a sample of the oil used in this experiment.

[1]$EC_{50:}$ Efficient Concentration, concentration of the sample necessary to sequester 50% of DPPH

[2]ORAC: "Oxygen Radical Absorbance Capacity"

The diets were formulated on the basis of maize and soybean bran for medium-performing male broilers1[11]. The feeding program was divided into four phases: pre-initial (1 to 7 days), initial (8 to 21 days) (Table 2), growth phase (22 to 35 days) and final phase (36 to 42 days) (Table 3).

Table 2. Nutrition composition calculated of experimental diets (Pre-initial and Initial).

Ingredients	Pre-initial						Initial					
	0.0	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Maize	55.57	55.58	55.59	55.61	55.62	55.63	59.66	59.67	59.68	59.70	59.71	59.7
SM 45%	38.1	38.1	38.1	38.1	38.1	38.1	34.6	34.6	34.6	34.6	34.6	34.6
Soybean oil	2.02	1.91	1.80	1.69	1.58	1.47	2.00	1.89	1.78	1.67	1.56	1.45
PFSO	-	0.10	0.20	0.30	0.40	0.50	-	0.10	0.20	0.30	0.40	0.50
DP	1.90	1.90	1.90	1.90	1.90	1.908	1.49	1.49	1.49	1.49	1.49	1.49
Limestone	0.80	0.80	0.80	0.80	0.80	0.80	0.82	0.82	0.82	0.82	0.82	0.82
DL-Methionine	0.35	0.35	0.35	0.35	0.35	0.35	0.28	0.28	0.28	0.28	0.28	0.28
L-Lysine HCl	0.28	0.28	0.28	0.28	0.28	0.28	0.21	0.21	0.21	0.21	0.21	0.21
L-Threonine	0.11	0.11	0.11	0.11	0.11	0.11	0.06	0.06	0.06	0.06	0.06	0.06
Antibiotic[1]	-	-	-	-	-	-	-	-	-	-	-	-
Anticoccidian[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.06	0.06
supplements[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Mineral[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100
Nutrition composition calculated												
ME (kcal kg^{-1})	2.95	2.95	2.95	2.95	2.95	2.95	3.00	3.00	3.00	3.00	3.00	3.00
Crude protein (%)	22.2	22.2	22.2	22.2	22.2	22.2	20.8	20.8	20.8	20.8	20.8	20.8
Calcium (%)	0.92	0.92	0.92	0.92	0.92	0.92	0.82	0.82	0.82	0.82	0.82	0.82
Phosphorus (%)	0.47	0.47	0.47	0.47	0.47	0.47	0.39	0.39	0.39	0.39	0.39	0.39
Potassium (%)	0.86	0.86	0.86	0.86	0.86	0.86	0.81	0.81	0.81	0.81	0.81	0.81
Sodium (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Chlorine (%)	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Lysine (%)	1.31	1.31	1.31	1.31	1.31	1.31	1.17	1.17	1.17	1.17	1.17	1.17
Methionine (%)	0.64	0.64	0.64	0.64	0.64	0.64	0.56	0.56	0.56	0.56	0.56	0.56
Met + Cys (%)	0.94	0.94	0.94	0.94	0.94	0.94	0.85	0.85	0.85	0.85	0.85	0.85
Threonine (%)	0.85	0.85	0.85	0.85	0.85	0.85	0.76	0.76	0.76	0.76	0.76	0.76

SM 45% = Soybean meal 45%; PFSO = Passion fruit seed oil; ME = Metabolizable energy; DP = Dicalcium phosphate; DL-Methionine = DL-Methionine 99%; L-Lysine HCl = L-Lysine HCl 78.4%; Lysine = Digestible lysine (%); Methionine = Digestible methionine (%); Met + Cys = Digestible Met + Cys (%); Threonine = Digestible threonine (%). [1]Enramycin (10 ppm); [2]Salinomycin (66 ppm); [3]Vitamin supplement (guarantee levels/kg ration): A=13,500 U.I.; D3=3,750 U.I.; E=30 U.I.; K3=3.75 mg; B1=3 mg; B2=9 mg; Pantothenic acid=18 mg; B6=4.5 mg; B12=22.5 µg; Nicotinic Acid=52.5 mg; Folic acid=2.25 mg; Biotin=0.15 mg; Selenium=0.375 mg. [4]Mineral supplements (guarantee levels/kg ration): Fe=50 mg; Cu=10 mg; Mn=65 mg; Co=1 mg; Zn=65 mg; I=1 mg.

Calculated based on Brazilian tables for poultry and swine.[11]

Nutrition composition calculated of experimental diets (Growth and final).

Ingredients	Growth						Final					
	0.0	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**
Maize	62.27	62.29	62.30	62.31	62.33	62.34	67.08	67.09	67.11	67.12	67.13	67.15
Soybean meal 45%	31.46	31.46	31.45	31.45	31.45	31.45	27.23	27.22	27.22	27.22	27.22	27.21
Soybean oil	2.99	2.88	2.77	2.66	2.55	2.44	2.79	2.67	2.56	2.45	2.34	2.23
PFS	-	0.10	0.20	0.30	0.40	0.50	-	0.10	0.20	0.30	0.40	0.50
DP	1.25	1.25	1.25	1.25	1.25	1.25	1.04	1.04	1.04	1.04	1.04	1.04
Limestone	0.78	0.78	0.78	0.78	0.78	0.78	0.71	0.71	0.71	0.71	0.71	0.71
DL-Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.23	0.23	0.23	0.23	0.23	0.23
L-Lysine HCl	0.19	0.19	0.19	0.19	0.19	0.19	0.23	0.23	0.23	0.23	0.23	0.23
L-Threonine 98.5%	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.05
Antibiotic[1]	-	-	-	-	-	-	-	-	-	-	-	-
Anticoccidian[2]	0.05	0.05	0.05	0.05	0.05	0.05	-	-	-	-	-	-
Choline	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04
Supplements[3]	0.12	0.12	0.12	0.12	0.12	0.12	0.08	0.08	0.08	0.08	0.08	0.08
Mineral[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100
	Nutrition composition calculated											
ME (kcal kg^{-1})	3.10	3.10	3.10	3.10	3.10	3.10	3.15	3.15	3.15	3.15	3.150	3.150
Crude protein (%)	19.5	19.5	19.5	19.5	19.5	19.5	18.0	18.0	18.0	18.0	18.0	18.0
Calcium (%)	0.73	0.73	0.73	0.73	0.73	0.73	0.64	0.64	0.64	0.64	0.64	0.64
Phosphorus (%)	0.34	0.34	0.34	0.34	0.34	0.34	0.30	0.30	0.30	0.30	0.30	0.30
Potassium (%)	0.76	0.76	0.76	0.76	0.76	0.76	0.69	0.69	0.69	0.69	0.69	0.69
Sodium (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Chlorine (%)	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Lysine (%)	1.08	1.08	1.08	1.08	1.08	1.08	1.01	1.01	1.01	1.01	1.01	1.01
Methionine (%)	0.52	0.52	0.52	0.52	0.52	0.52	0.49	0.49	0.49	0.49	0.49	0.49
Met + Cys (%)	0.79	0.79	0.79	0.79	0.79	0.79	0.74	0.74	0.74	0.74	0.74	0.74
Threonine (%)	0.70	0.70	0.70	0.70	0.70	0.70	0.66	0.66	0.66	0.66	0.66	0.66

PFSO = Passion fruit seed oil; DP = Dicalcium phosphate; ME = Metabolizable energy; DL-Methionine = DL-Methionine 99%; L-Lysine HCl = L-Lysine HCl 78.4%; Lysine = Digestible lysine (%); Methionine = Digestible methionine (%); Met + Cys = Digestible Met + Cys (%); Threonine = Digestible threonine (%). [1]Enramycin (10 ppm); [2]Salinomycin (66 ppm);

[3]Vitamin supplement (guarantee levels/kg ration): A=13,500 U.I.; D3=3,750 U.I.; E=30 U.I.; K3=3.75 mg; B1=3 mg; B2=9 mg; Pantothenic acid=18 mg; B6=4.5 mg; B12=22.5 µg; Nicotinic Acid=52.5 mg; Folic acid=2.25 mg; Biotin=0.15 mg; Selenium=0.375 mg.

[4]Mineral supplements (guarantee levels/kg ration): Fe=50 mg; Cu=10 mg; Mn=65 mg; Co=1 mg; Zn=65 mg; I=1 mg.

Calculated based on Brazilian tables for poultry and swine.[11]

At 42 days of age, two birds per experimental unit (n=96) were chosen randomly, subjected to an eight-hour fasting period, weighed individually and later desensitized by electronarcosis and slaughtered at the experimental slaughterhouse of the Faculty of Veterinary Medicine and Zootechnics. After boning and removal of the skin from the thigh, the whole breast fillets were individually packed in plastic bags (18μ).

Physical analyzes were performed 24 hours after slaughter. For the chemical analyzes, the samples remained frozen at -20ºC for 30 days. Lipid oxidation was performed at 30, 60, 90 and 120 days after slaughter, and the samples from the same bird were subdivided into four parts, which also remained frozen at -20ºC until taken out for analysis.

Skin resistance

Skin resistance (value of the breaking force required to break the skin) was measured using the upper thigh skin samples of 16 birds per treatment, each sample measuring 4cm × 4cm (width and length). The samples were submitted to the flexural test at constant strain rate for viscoelastic material using a fixture for drilling test adapted to the texturometer (CT3, Brookfield, Middleboro, MA, USA) [12].

Colour and pH

The colour (*L**, *a** and *b**) was measured in three different locations in the inner part of the *Pectoralis major* muscle immediately after deboning using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan) [13]. The pH of samples was measured using a digital pH meter HI 99163 (Hanna Instruments, Woonsocket, RI, USA) with a penetration electrode.

Water holding capacity (WHC), Cooking weight loss (CWL), Shear force (SF)

The WHC was determined using 2 g of samples, which were placed between two filter papers and acrylic plates and subjected to a pressure of 10 kg for 5 min. Subsequently, samples were reweighed to determine WHC expressed as a percentage: (final weight × 100)/initial weight [14].

The CWL was determined in samples with similar size and weight were weighed, packaged, and cooked in a water bath (85ºC) for 30 min. After cooling at room temperature, the samples were reweighed to determine the CWL, which is expressed as a percentage: (initial weight – final weight) × 100/initial weight [15].

Three subsamples with a cross-sectional area equal to 1 cm^2, and a length equal to 3 cm, were obtained from each cooked sample, placed with the fibers oriented perpendicularly to a Warner–Bratzler shear device coupled to a texture analyzer (CT3, Brookfield®, Middleboro, MA, USA), to determine SF [16]. The force required for shearing the samples was expressed in Newton (N).

Fatty acid profile and lipid oxidation

The fatty acids were isolated (extracts the lipid phase from the sample) and esterification using a Shimadzu 14B gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a flame ionization detector and a fused silica capillary column (Omegawax 250), with H_2 used as the carrier gas. The identification of peaks was made by comparison with standard retention times of known compositions [17].

Lipid oxidation was determined by a thiobarbituric acid reactive substance (TBARS) [18]. At each storage stage, 10 g of meat samples were homogenized for 2 minutes using an Ultra-Turrax mixer and added to 50 ml of 7.5% trichloroacetic acid solution. This mixture was then filtered and a 5.0 ml aliquot was mixed with 5.0 ml of TBA solution (0.020 mol/L), then placed in a water bath (100°C) for 30 minutes. The absorbance of the samples was measured twice at 532 nm in a spectrophotometer (FEMTO 600, São Paulo, SP, Brazil) and expressed in milligrams of malonaldehyde (MDA) per kilogram of meat using a standard curve made with tetraethoxypropane (TEP) as a base.

Chemical composition

The chemical composition was evaluated by determining protein, fat, moisture and ash contents according to the methods 977.14, 991.36, 950.46 and 920.153, respectively [19].

Statistical analyses

The data obtained in the experiment were tested for normality of distribution and were subsequently submitted to regression analysis between the different inclusion levels of PFSO (0.0, 0.1, 0.2, 0.3; 0.4 and 0.5%). In order to compare mean values of the proposed treatments with those of the diet containing no PFSO (0.0%), a variance analysis was performed and, when a significant value was obtained, the Dunnett test was performed at a 5% probability. The lipid oxidation was analyzed in a 4 × 6 factorial scheme (four storage periods and six PFSO inclusion levels), with 10 replications each, and the interactions were analyzed using the Generalized Linear Model (GLM) of the Minitab® statistical software (Minitab Version 18, Minitab Inc., State College, PA).

3. RESULTS AND DISCUSSION

There was improvement in the skin rupture condition, with greater strength needed to break the skin as the PFSO was included in the diet (Table 4).

Table 4. Mean values of **s**kin resistance, lightness (*L**), red intensity (*a**) and yellow intensity (*b**), pH, water-holding capacity (WHC), cooking weight loss (CWL), shear force (SF) of broilers meat supplemented with passion fruit seed oil.

Items	Level (%)						*P*-value	SE
	0.0	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**		
Skin resistance (kg)	4.70	5.10	5.28	5.31*	5.42*	5.80*	0.010[1]	0.294
L*	52.23	50.90	51.17	52.15	51.44	52.10	0.705	0.612
a*	4.47	5.46	4.89	4.65	5.60	4.89	0.443	0.349
b*	5.34	6.24	5.70	5.62	5.16	5.67	0.547	0.329
pH	6.05	6.07	6.02	5.71	6.09	6.05	0.821	0.132
WHC (%)	89.63	89.10	87.85	87.84	92.58	90.41	0.187	1.220
CWL (%)	21.97	22.27	23.72	23.55	24.51	23.27	0.123	1.005
SF (N)	21.83	19.76*	19.52*	19.39*	19.19*	18.82*	0.027[2]	0.943

SE=Standard error. * Differs from 0.0% by the Dunnett test ($P<0.05$). [1]Y= 1.839x + 4.813; R^2=0.913; [2]Y= - 4.8203x + 20.961; R^2=0.817

The greater resistance of the skin can be explained by the large amount of oleic fatty acid (C18:1) present in the oil (Table 1), which has as its main function antioxidant action that reduces lipid peroxidation. The higher lipid peroxidation causes irreversible loss of the fluidity and elasticity of the cellular membranes and, in extreme cases, may even lead to rupture of the cell. This damage is caused by an increase of free radicals that accompany the process of lipid peroxidation [20].

In addition, these oxidations involving the formation of free radicals also accelerate the aging phenomenon by causing damage to the DNA and by acting on protein glycation, which involves the loss of the biological functions of proteins, such as collagen, that result in structural alterations of the membranes and increased skin flaccidity [21]. Given the antioxidant action of PFSO, these oxidative reactions were avoided as more oil was added to the feed.

Regarding meat quality, no effect was observed on colour, pH, WHC and CWL. According to the literature sources, the pH of poultry meat after slaughter is ~7.2 and stabilizes between 5.7 and 5.9 after rigor mortis [22]. Thus, the pH of the meat of broilers fed diets containing different levels of PFSO was slightly higher than the one suggested in the literature. Although it is expected that the pH of the meat would be within the suggested range due to the calming properties of passion fruit, no changes in meat were observed regarding this variable. It is worth mentioning that the soothing effect of passion fruit is concentrated mostly in the leaves, where a greater quantity of indole alkaloids can be found, which have value in medicine as tranquillizers [23], and not in the seed that was the byproduct used for the extraction of the oil.

However, the slightly high pH did not influence the colour of the meat, which can be seen as falling squarely within the normal colour pattern, since PSE meat, commonly found in poultry, presents low WHC, high CWL and L *> 53 [24]. Thus, absence of effect on WHC and CWL also explain the proper coloring of the meat.

On the other hand, there was a difference in shear force (Table 4), with a linear reduction in the force needed to break the muscle fibers as PFSO was included in the diet.

As in the skin, the antioxidant action capable of preserving polyunsaturated fatty acids (PUFAs) is related also to meat tenderness, with these compounds seemingly involved indirectly in the synthesis of collagen, reducing the cross-links of the same and thus influencing the texture of the meat [25]. In general, the amount of crosslinking is more directly linked to the age of the animals, with older animals having greater crosslinks between the collagen fibers than the younger ones, making the latter more tender [26].

However, studies have shown that there is also an influence of fatty acids on the tissues of broiler chickens, with a lower level of collagen cross-linking in tissues of birds fed with soybean oil rich in PUFA n-6 [25]. This fact may explain the increase in chicken meat tenderness as the level of PUFA-rich PFSO increased (Table 1) in the diets.

Although there was no increase in the levels of PUFA in broiler meat in the different treatments evaluated (P>0.05, Table 5), the amount of these fatty acids was significantly higher compared to red meat [27]. Furthermore, if we consider the shelf-life of the product, evaluated at 60, 90 and 120 days, respectively, as a function of PFSO inclusion in the diet (Table 6), we will note that there was greater protection against lipid oxidation of the meat, indicated by the decrease of malonaldehyde contents as the

level of PFSO inclusion in the diet increased (P<0.05; Figure 1). This protection mainly refers to PUFAs that are more sensitive to oxidation in periods of storage compared to saturated fatty acids (SFAs) [28].

Within the food-producing industries, oxidation is an undesirable process, since lipid decomposition and production of volatile compounds cause sensory changes, especially in taste, thus reducing the nutritional value of foods [29]. This means that the use of PFSO in the feeding of broilers attenuates the oxidative effects of meat stored for long periods due to the antioxidant action of this product.

There was an increasing linear effect only for the margaric (C17:0) and cis10-Heptadecanoic (C17:1) fatty acids. However, due to the low amount of these fatty acids in PFSO, the content found was still low in all evaluated treatments (Table 5).

The main saturated fatty acid found in chicken breast meat was palmitic (C16:0) fatty acid. Among other abundant monounsaturated fatty acids were oleic acid (C18:1), followed by palmitolic (C16:1) acid. In the available sources, the same profile was observed in chicken meat fed exclusively with soybean oil [30]. However, when considering the effects of the addition of PFSO in the diet in the present study, where this oil substituted proportional levels of soybean oil, it is important to note that the amount of these fatty acids in both oils is similar (Table 1), thus not altering their profile in the meat.

Table 5. Fatty acid composition (% of total fatty acids) of broilers meat supplemented with passion fruit seed oil.

Fatty acid	Level (%)						*P*-value	SE
	0.0	0.1	0.2	0.3	0.4	0.5		
C4:0	1.075	0.058	0.230	0.186	0.329	0.458	0.642	0.437
C6:0	0.116	0.053	0.078	0.156	0.128	0.076	0.244	0.032
C8:0	0.015	0.010	0.010	0.022	0.005	0.029	0.572	0.009
C10:0	0.011	0.010	0.013	0.023	0.006	0.007	0.330	0.005
C11:0	0.014	0.008	0.014	0.008	0.011	0.003	0.407	0.004
C12:0	0.015	0.015	0.012	0.017	0.016	0.012	0.339	0.002
C13:0	0.006	0.006	0.012	0.012	0.004	0.007	0.388	0.003
C14:0	0.306	0.309	0.308	0.370	0.298	0.289	0.606	0.034
C14:1	0.073	0.090	0.102	0.133	0.069	0.065	0.500	0.026
C15:0	0.041	0.041	0.044	0.047	0.040	0.041	0.414	0.004
C15:1	0.037	0.039	0.032	0.052	0.049	0.043	0.961	0.015
C16:0	22.324	21.796	22.219	21.427	22.293	22.155	0.740	0.477
C16:1	3.399	4.009	3.743	4.034	3.628	3.651	0.952	0.524
C17:0	0.087	0.068	0.164	0.381	0.492	0.334	0.003[1]	0.099
C17:1	0.028	0.020	0.153	0.304	0.332	0.229	0.004[2]	0.078
C18:0	5.698	5.325	5.283	5.206	5.586	6.036	0.829	0.483
C18:1n9c	29.130	30.129	29.549	27.117	30.995	29.127	0.979	0.712
C18:1n9t	3.507	3.630	3.127	2.936	3.032	3.269	0.329	0.246
C18:2n6c	26.611	26.789	26.431	26.623	26.361	25.640	0.902	0.731
C18:3n6	0.226	0.257	0.281	0.171	0.198	0.165	0.212	0.037
C18:3n3	1.247	1.260	1.301	1.221	1.294	1.180	0.759	0.062
C20:0	0.056	0.060	0.054	0.040	0.082	0.053	0.093	0.009
C20:1n9	0.172	0.174	0.177	0.171	0.177	0.180	0.769	0.006

C20:2	0.267	0.281	0.237	0.250	0.219	0.286	0.898	0.046
C20:3n6	0.564	0.655	0.535	0.545	0.481	0.488	0.833	0.097
C21:0	0.008	0.008	0.010	0.011	0.010	0.010	0.424	0.003
C20:4n6	2.930	2.966	3.086	3.160	3.150	3.806	0.139	0.391
C20:3n3	0.019	0.023	0.037	0.012	0.007	0.017	0.319	0.009
C20:5n3 (EPA)	0.088	0.097	0.110	0.142	0.102	0.129	0.551	0.023
C22:0	0.010	0.007	0.018	0.012	0.010	0.010	0.699	0.004
C22:1	0.008	0.014	0.024	0.009	0.010	0.010	0.149	0.006
C22:2	0.259	0.340	0.505	0.300	0.321	0.387	0.891	0.149
C23:0	0.669	0.676	0.664	0.695	0.582	0.721	0.977	0.121
C24:0	0.325	0.331	0.319	0.470	0.313	0.478	0.650	0.096
C22:6n3 (DHA)	0.230	0.180	0.172	0.171	0.116	0.310	0.170	0.049
C24:1	0.025	0.012	0.016	0.025	0.022	0.024	0.934	0.012
SFA	30.778	28.783	29.452	29.084	30.207	30.710	0.599	0.986
MUFA	36.380	38.118	36.923	34.782	38.314	36.591	0.190	1.011
PUFA	31.928	32.245	31.967	32.061	31.713	31.736	0.700	0.754
n-6	30.344	30.686	30.347	30.514	30.194	30.100	0.676	0.716
n-3	1.496	1.463	1.510	1.405	1.417	1.507	0.736	0.065
MUFA/SFA	1.182	1.324	1.254	1.196	1.268	1.192	0.662	0.068
PUFA/SFA	1.037	1.120	1.085	1.102	1.050	1.033	0.536	0.043
n-6/n-3	20.279	20.975	20.091	21.722	21.304	19.970	0.783	0.769

SE=Standard error. DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids. [1]$Y = 0.05971429 + 0.77714286x$ ($R^2 = 0.7092$); [2]$Y = 0.02933333 + 0.59200000x$ ($R^2 = 0.6889$).

Table 6. Mean values of lipid oxidation (TBARS) of broilers meat supplemented with passion fruit seed oil in different periods of storage.

Period (days)	Level (%)	TBARS (mg MDA/kg)
30		0.253
60		0.234
90		0.277
120		0.290
	0.0	0.292
	0.1	0.269
	0.2	0.263
	0.3	0.247
	0.4	0.245
	0.5	0.239
	P-value	
Period		0.015
Level		0.023
Period*Level		0.001
EP		0.537

TBARS=Substances reactive to thiobarbituric acid; SE=Standard error.

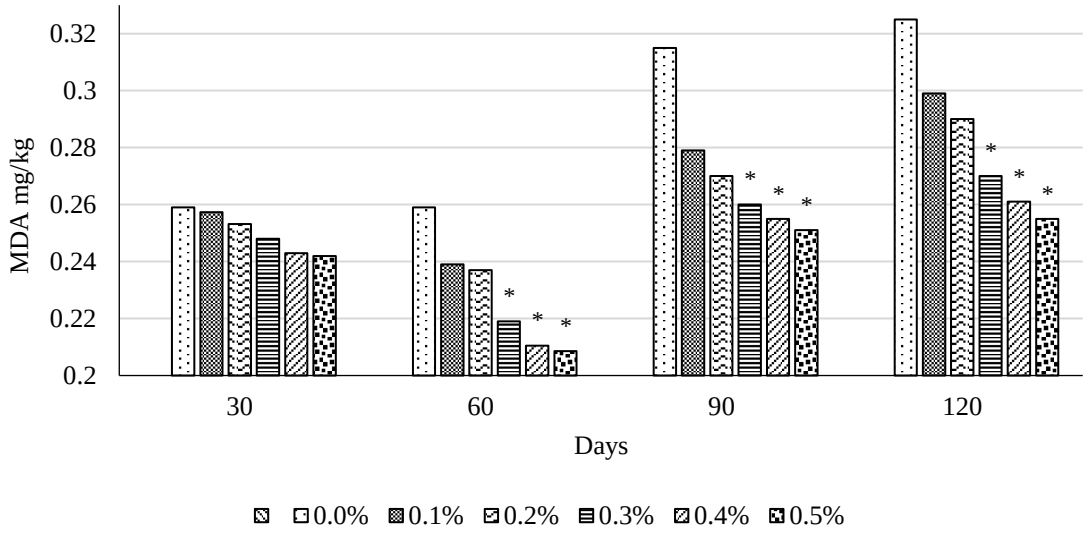

Figure 1. Unfolding lipid oxidation (TBARS) of broilers meat supplemented with passion fruit seed oil in different periods of storage.

30 days: *P*=0.132. SE=0.212

60 days: *P*=0.043. SE=0.301; Y=-0.0893x + 0.2473. R^2= 0.82

90 days: *P*=0.031. SE=0.223; Y=-0.112x + 0.2997. R^2= 0.80

120 days: *P*=0.027. SE=0.356; Y=-0.1234x + 0.3159. R^2 = 0.88

SE=Standard error. * Differs from 0.0% by the Dunnett test (*P*<0.05).

On the other hand, a greater quantity of linoleic fatty acid was expected in the breast meat of the chickens, due to its large quantity in the PFSO. Although the levels were relatively high in the meat, there was no effect with the evaluated inclusion levels. In addition, it was also expected that the content of arachidonic acid in broiler meat (C20:4n6) would increase, since the latter is synthesized from linoleic acid [31]. However, only numerical increase was observed with the increase of the PFSO in the diet, presenting no statistical effect. Possibly, the absence of these results is due to the evaluated levels, which presented an important contribution in the lipid oxidation, but did not alter the fatty acid profile.

It is important to note also that the proportion of polyunsaturated n-6 to n-3 was ~20: 1 in broiler chicken breast in all evaluated treatments. The recommended ratio of n-6 to n-3 is 6:1, which helps to prevent cardiovascular disease [32]. However, the observed proportion is common in chicken meat, also reported by literature [30]. On the other hand, the PUFA/SFA ratio in all treatment was above the value recommended for human diet (0.45), capable of preventing cardiovascular problems [33].

In addition, although there was no effect on the centesimal composition of broiler meat in relation to PFSO inclusion in the diet (Table 7).

Table 7. Mean values of moisture, ash, protein and fat content of broilers meat supplemented with passion fruit seed oil.

Items	Level (%)						*P*-value	SE
	0.0	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**		
Moisture (%)	73.58	74.26	74.40	73.86	73.85	74.46	0.095	0.249
Ashes (%)	1.24	1.20	1.16	1.21	1.55	1.25	0.624	0.050
Protein (%)	23.43	24.16	23.83	24.10	24.12	23.44	0.197	0.264
Lipid (%)	0.84	0.85	0.74	0.82	0.71	0.86	0.820	0.092

SE=Standard error.

Low fat and high protein content could be observed in the meat, which is considered as lean and good quality, since the fats are mainly mono- and polyunsaturated fats, while at the same time the meat was rich in indispensable amino acids with high biological value that can be recommended for consumption by all age groups [34].

4. CONCLUSION

Passion fruit seed oil can be a beneficial ingredient for the poultry industry because it improves resistance of poultry skin. In addition, broilers fed with 0.5% of passion fruit seed oil present softer meat, which is less susceptible to oxidative effects due to the antioxidant action of the used product. However, the evaluated levels did not contribute to an increase in the polyunsaturated fatty acids content.

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