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Sexual maturity, slaughter age, and sex on meat fatty acid composition of chickens raised in a free-range system

Joanna Oliveira Marçal [a], Giulia Piva Oliveira [a], Adriano Geraldo [b], Lidiany Mendonça Zacaroni Lima [a], Sara Wilhelmina Erasmus [c], Peter Bitencourt Faria [a,*]

[a] Federal University of Lavras, Trevo Rotatório Professor Edmir Sá Santos s/n, zip code 37203-202, Lavras, Minas Gerais, Brazil
[b] Federal Institute of Minas Gerais, Campus Bambuí, Fazenda Varginha, Rodovia Bambuí/Medeiros, Km 05 mailbox: 05, zip code: 38900-000, Bambuí, Minas Gerais, Brazil
[c] Food Quality and Design, Wageningen University and Research, P.O. Box 17, Wageningen, Gelderland 6700 AA, the Netherlands

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ABSTRACT

This study aimed to evaluate the effects of sexual maturity, slaughter age, and sex on the fatty acid composition of meat from free-range chickens. To measure the effect of sexual maturity and slaughter age, a total of 90 chickens from the Label Rouge lineage, 45 females and 45 males were selected and slaughtered for the study. Samples of breast and drumstick cuts were analyzed to determine the fatty acid profile. Chickens were slaughtered at 70, 90, 120, 150, and 180 days, and for sexual maturity, they were considered immature and mature according to sexual development. In a fatty acid profile in the breast, most effects found were through the influence of sexual maturity on sex, and for drumsticks, the effects of slaughter age on sex. Mature chickens showed an increase of polyunsaturated fatty acids (PUFA) in the breast and a reduction of monounsaturated fatty acids (MUFA) in drumstick. In both cuts, there was a decrease in saturated fatty acids (SFA) at 180 days, and an isolated effect of sex happened for drumsticks with a greater content of MUFA, C16:1, and C8:1n-9 C for females. Slaughter age and sexual maturity greatly influence fatty acid composition in free-range chickens, and meat from old birds tended to have the best nutritional characteristics in both cuts with decreasing atherogenicity and thrombogenicity indices.

1. Introduction

Modern consumers are increasingly looking for products considered 'natural' or 'organic' and considered healthier, especially regarding the content of fatty acids, which are considered beneficial to human health. Various metabolic processes in the body depend on the profile of fatty acids in the diet. A reduction or substitution of foods that are rich in saturated fatty acids (SFA) for others containing a higher percentage of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) can help to decrease the risk of coronary heart disease (Mozaffarian et al., 2010; Schwingshackl and Hoffmann, 2012; Chen and Liu, 2020).

Chicken meat could be included in a healthy diet since it contains more PUFA than meat from other species (Silva et al., 2017). Even so, the poultry industry has a growing desire for products from chickens raised using alternative production systems, such as outdoor or organic, as these are considered healthier. Previous studies have shown that chickens raised in a free-range system present meat with a better fatty acid profile than those raised under conventional conditions in an indoor system. Chicken meat from the free-range system contains higher amounts of PUFA, *n*-3, and *n*-6 fatty acids, a PUFA/SFA ratio, and a lower ratio of *n*-6/*n*-3 (Tougan et al., 2018; Giampietro-Ganeco et al., 2020).

In chicken production in a free-range system, many factors can affect the fat content of the meat, such as nutrition, genetics, sex, and age at slaughter (Cruz and Faria, 2019). The influence of these factors on enzyme activity involves the incorporation and synthesis of fatty acids in tissues, as well as Δ9-desaturases that act in the synthesis of MUFA and elongases that catalyze carbon extension of a fatty acid chain (Guillou et al., 2010; Ariel Igal and Sinner, 2021). Moreover, due to differences according to the proportion of muscle fiber type and their metabolism in the cuts, generally, there are differences in fatty acid composition between free range breast and leg (drumstick or thigh) (Tougan et al., 2018; Giampietro-Ganeco et al., 2020).

* Corresponding author.
E-mail address: peter@ufla.br (P.B. Faria).

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Recently, the Federal legislation in Brazil for chickens raised in a free-range system ("Caipira" or "Colonial" system) was amended. This updated legislation states that birds must be slaughtered between 70 and 120 days old (Brasil, 2020). Thus, birds must be slaughtered before reaching sexual maturity (Rose et al., 2016). In a previous study conducted by Li et al. (2021) on female chickens, a relationship was established between the age of the birds and their fatty acid composition. The findings indicated decreases in the levels of *n*-6 and *n*-3 fatty acids, total PUFAs, the *n*-6/*n*-3 ratios, and the ratio of PUFA/SFA for chickens slaughtered at 120 days or during the sexual maturity phase (Li et al., 2021).

With the aging process, fat accumulation in birds generally increases in different ways for male and female birds, and there are likely differences in fatty acid composition according to the level of sexual maturity or slaughter age of each sex. Male and female sex hormones can act on the activity of enzymes that synthesize lipid components, changing the fatty acid profile of the meat (Li et al., 2015; Zhang et al.,2017). These changes can affect the quality of food, considering the effects on health according to indices of atherogenicity and thrombogenicity based on their fatty acid profile (Chen and Liu, 2020; Carneiro et al., 2021).

Thus, to support the growth of the free-range chicken industry, it is imperative to explore the specific factors –within the free-range system– that impact the fatty acid composition of chicken meat and its nutritional benefits. A critical knowledge gap exists in understanding the nutritional advantages associated with such production methods. This study seeks to fill this gap by examining the fatty acid profile of free-range chicken meat, specifically investigating how variables like sexual maturity stage, age at slaughter, and sex impact the fatty acid composition of meat.

2. Materials and methods

2.1. Animals and diets

For the experiment, one-day-old Label Rouge – Pescoço Pelado chickens were acquired from a commercial hatchery (Globoaves®), and the chickens were kept in a masonry barn for 28 days and then housed in an area suitable for breeding free-range chickens, with separation of males and females and a maximum capacity of three-square meters per bird. The picket area was covered by Tifton grass (*Cynodon* spp.) and star grass (*Rynchospora* spp.) with a shelter lined with rice hulls. The chickens were fed *ad libitum* with a diet consisting of four formulations, according to the nutritional requirements of the Management Manual of Colonial Chickens (Globo Aves, 2015) at each growth stage (Table 1). According to the sanitary calendar, the chickens were vaccinated against Marek's disease, infectious bronchitis, Gumboro disease, Newcastle disease, and avian yaws. Deworming occurs at 49 and 120 days, and chickens were reared and slaughtered at different ages. For this study, 90 chickens were used, i.e., 45 males and 45 females of the Label Rouge (Naked Neck, *Pescoço Pelado*) lineage.

2.2. Experimental design

Aiming to evaluate the effect of age at slaughter was considered a completely randomized 5×2 factorial design, in which there were five slaughter ages (70, 90, 120, 150, and 180 days) and two sexes (Male and Female). For each slaughter age, nine females and nine males (totaling 18 birds) were slaughtered. The study was approved by the Animal Use Ethics Committee (AUEC) of the Federal Institute of Minas Gerais under permit number 04/2019.

In addition to the five slaughter ages, sexual maturity was also assessed. A completely randomized 2×2 factorial design, in which there were two sexual developments (immature or mature), and two sexes (Male and Female) was implemented with a variable number of replicates as it was dependent to the development of the gonads. The number of immature male chickens was 18, nine of them slaughtered at 70 days (n=9), and the remaining birds slaughtered at 90 days (n=9). In the case of mature males, a total of 27 birds were included, with slaughter taking place at 120 days (n=9), 150 days (n=9), and 180 days (n=9). Twenty-one were considered immature females, and they were slaughtered at 70 (n=9), 90 (n=9), and 120 days (n=3). Finally, 24 mature females were slaughtered at 120 (n=6), 150 (n=9), and 180 days (n=9).

The group of sexually immature animals was composed of chickens that did not show complete sexual development of the gonads. The degree of sexual maturity of males was determined according to Santos et al. (2012) through the evaluation of the testicles according to the diameter of the seminiferous tubules, and birds were considered mature if they presented a testicular diameter of 119.73–227.23 μm. In females, sexual maturity was defined visually, considering mature females to have macroscopically developed follicles. Thus, sexual maturity was defined as the age at which gonads were fully developed and capable of producing gametes.

Table 1
Components and composition of the initial, growth I, growth II, and final diets provided to the free-range chickens until slaughter at 70, 90, 120, 150, and 180 days.

Ingredients (kg)	Initial diet (1–28 days)	Growth I diet (29–49 days)	Growth II diet (50–70 days)	Final diet (71–180 days)
Corn	64.7	69.1	72.75	73.45
Soybean meal	31.7	27.7	23.85	22.8
Soybean oil	0	0	0.2	0.9
Kaolinite	0	0	0.2	0.2
Calcitic lime	0.1	0.2	0	0.15
Premix for free-range chickens[1]	3.5	3	3	2.5
Calculated values	**Initial diet (kg)**	**Growth I diet (kg)**	**Growth II diet (kg)**	**Final diet (kg)**
ME[2] (kcal/kg)	2949.2	2996.13	3047.97	3098.19
CP[3] (%)	20.07	18.5	17.01	16.5
Calcium (%)	1.05	0.9051	0.8663	0.7911
Phosphorus available (%)	0.4092	0.3609	0.3567	0.3102
Methionine + Cystine (%)	0.6911	0.637	0.643	0.5725
Lysine (%)	0.9609	0.8673	0.7789	0.751
Threonine (%)	0.6778	0.6273	0.5721	0.5522
Tryptophan (%)	0.2292	0.2081	0.1877	0.1816
Choline (mg/kg)	1153.96	1059.73	994.67	945.74
Sodium (mg/kg)	1848.39	1613.59	1611.59	1375.69
Chlorine (mg/kg)	3103.34	2744.98	2746.98	2381.02

[1]Guaranteed levels of the premix for free-range chicken: folic acid (min.) 23.33 mg/kg, pantothenic acid (min.) 333.33 mg/kg, Butylated hydroxytoluene (min.) 500 mg/kg, biotin (min.) 0.5 mg/kg, calcium (min.) 240 g/kg, calcium (max.) 270 g/kg, copper (min) 333 mg/kg, choline (min.) 6.000 mg/kg, iron (min.) 1.667 mg/kg, fluorine (max.) 497.8 mg/kg, phosphorus (min.) 51 g/kg, iodine (min.) 28.33 g/kg, lysine (min.) 10 g/kg, manganese (min.) 2.333 mg/kg, methionine (min.) 40 g/kg, niacin (min.) 1000 mg/kg, selenium (min.) 10 mg/kg, sodium (min.) 47.28 g/kg, vitamin A (min.) 159 IU/kg, vitamin B1 (min.) 33.33 mg/kg, vitamin B12 (min) 333.33 mcg/kg, vitamin B2 (min.) 133.33 mg/kg, vitamin B6 (min.) 66.67 mg/kg, vitamin D3 (min.) 50.000 IU/kg, vitamin E (min.) 266.667 IU/kg, vitamin K3 (min.) 53.33 mg/kg, zinc (min.) 2.000 mg/kg; [2]Metabolizable Energy; [3]Crude Protein.

2.3. Sample collection

Before slaughter, chickens were weighed and randomly selected according to the mean weight at each slaughter age. Chickens were fasted for 12 h, stunned electrically (110 V, 200 Hz) and bled manually, following the humanitarian method. The carcasses, after evisceration, were packaged, identified, and cooled in a cold chamber, where they remained for a period longer than three hours at a temperature of 5°C. After slaughter, the gonads (testicles and ovaries) from birds were

collected to carry out macroscopic and microscopic evaluations and determine the sexual maturity of each chicken.

After cooling the carcasses, cuts were made, and samples of the breast and drumstick were collected, followed by skinning and deboning procedures. The samples of these cuts were packaged, identified, and immediately frozen at −18 °C until analysis in the laboratory.

2.4. Fatty acid composition

Samples were taken from the breast (*pectoralis major*), and drumstick (*peroneus longus*) muscles (without skin) were used to determine the fatty acid profile of the meat. The samples were prepared according to the extraction methodology of Folch et al. (1957). The lipids were extracted from chicken meat samples using a chloroform-methanol mixture (2:1, v/v). Methyl esters were prepared according to the method of Hartman and Lago (1973), as modified by Maia and Rodrigues-Amaya (1993). The esterification of triglyceride acids was carried out using a series of steps. Initially, a lipid extract (approx. 50 mg of lipids) was concentrated in a threaded test tube by introducing 4.0 mL of a 0.5 N methanolic sodium hydroxide solution and subjected to a heating process at boiling temperature for 5 minutes, followed by cooling. The same procedure was repeated by adding 5.0 mL of an Esterification Solution containing methanol, ammonium chloride, and sulfuric acid to the test tube. Complete solubilization was achieved after the addition of 4.0 mL of saturated sodium chloride and 5.0 mL of n-hexane, with the material being agitated between each step. The test tube was allowed to rest for 15 minutes, and 3.0 mL of the supernatant was transferred to another test tube for vaporization of the liquid content using nitrogen gas. Finally, the residuals were resuspended with 1.0 mL of n-hexane and transferred to a 2.0 mL vial. The vials were sealed and stored at −18°C for subsequent chromatographic analysis.

The determination of the fatty acid profile was carried out using a gas chromatograph Shimadzu GC-2010 (Shimadzu Corporation, Kyoto, Japan) equipped with an automatic injector Shimadzu AOC-20i (Shimadzu Corporation, Kyoto, Japan), and the GC-FID flame ionization detector (Shimadzu Corporation, Kyoto, Japan) using a silica capillary column 100 mm long and 0.25 mm in diameter, with 0.2 μm thick Supelco film (SP-2560, Bellefonte, PA, U.S.) and helium as the carrier gas (2 mL/min). The operational parameters were as follows: the total run time was 60 minutes, with the initial column temperature of 140°C maintained for 5 minutes. Then, the temperature was increased by 4 °C/min to a temperature of 240°C, remaining constant for 30 minutes. The fatty acid profile was expressed in a chromatogram using GC Solution software and identified according to the Supelco 37® (Supelco 37 standard FAME Mix, Supelco Inc., USA) standard.

The quantification of fatty acids was performed using the internal standardization method, with methyl ester nonadecanoate (C19:0) as an internal standard (1 mg/mL) and correction factors such as the theoretical correction factor (TFC) and methyl ester to fatty acid conversion (FCEA), according to the methodology of Visentainer (2012). Estimates of the activity indices of the enzymes Δ9-desaturase^{C16}, Δ9-desaturase^{C18}, and elongase$^{C16\text{-}C18}$ were calculated according to the methodology of Metz et al. (2009), in which: activity index of Δ9-desaturase^{C16} = 100 [(C16:1) / (C16:1 + C16:0)]; activity index of Δ9-desaturase^{C18} = 100 [(C18:1ω9c) / (C18:1ω9c + C18:0)]; and activity index of elongase$^{C16\text{-}C18}$ = 100 [(C16:1 + C18:1ω9c) / (C16:0 + C16:1 + C18:0 + C18:1ω9c)]. The estimates of atherogenicity and thrombogenicity indices were calculated according to the methodology of Ulbricht and Southgate (1991), where: atherogenicity index = [(C12:0 + (4 x C14:0) + C16:0)] / (ΣMUFA + Σω3 + Σω6) and thrombogenicity index = (C14:0 + C16:0 + C18:0) / [(0.5 x MUFA) + (0.5 x Σω6) + (3 x Σω3) + (Σω3/Σω6)].

2.5. Statistical analysis

Three replicates were used (one replicate consisted of the mean of the parameters evaluated in three birds slaughtered at each age) to analyze the slaughter age effect. For the sexual maturity effect, the number of replicates was variable according to the level of gonadal development in birds. The data was analyzed using the SAS 9.4 software (SAS/STAT, SAS Institute Inc., Cary, NC, USA). The treatment and/or interaction variables with significant effects in the analysis of variance (F test) were submitted to the Tukey test with a significance level at 5% ($p<0.05$).

3. Results

The fatty acid composition of the broiler breast meat according to slaughter age, sexual maturity, sex, and interactions is shown in Table 2. The slaughter age influenced the fatty acid profile of the breast meat for C4:0, C10:0, C12:0, C14:0, C14:1, C15:0, C16:1, C17:1, C20:3*n*-6, C23:0 and C24:0 fatty acids (Table 2). These fatty acids had a lower mean value at 180 days ($p<0.05$). As shown in Table 2, the percentage of saturated fatty acids (SFA) in the breast meat was lower in the 180-day-old slaughtered chickens. There was a decrease in the thrombogenicity index at 180 days because of lower means of C12:0, C14:0, and C16:0 acids. The activity index of elongase$^{C16\text{-}C18}$ increased at 180 days, and lower values were found for birds slaughtered at 120 days.

The breast meat of chickens that had reached sexual maturity had the lowest average content of C4:0, C14:1, C16:1, C23:0, C20:3*n*-6 acids, and the highest content of C18:2*n*-6 C and C22:6*n*-3 acids (Table 2). The content of PUFA increased after birds reached sexual maturity, improving the PUFA/SFA ratio, which could be due to increases in the *n*-6 and *n*-3 fatty acid contents. This result could reduce the atherogenicity and thrombogenicity indices in breast meat from free-range chickens according to sexual maturity. In the breast meat, the estimate of Δ9-desaturase^{C16} and Δ9-desaturase^{C18} activities decreased when birds achieved sexual maturity.

Sex exerted an influence on *n*-3 content, the estimate of Δ9-desaturase^{C16}, and elongase$^{C16\text{-}C18}$ in the breast meat, with higher means observed in females compare to males. Conversely, female breast meat exhibited lower means of C20:4*n*-6 acids than males. For C10:0, C12:0, C14:0, C15:0, C17:1, C24:0, SFA, and SFA%, there were differences according to slaughter ages, while for C18:2*n*-6 C, C22:6*n*-3, PUFA content and percentage, *n*-6, PUFA/SFA ratio, Δ9-desaturase^{C18} and the atherogenicity index there was influence by sexual maturity. On the other hand, for C4:0, C14:1, C20:3*n*-6, C23:0, elongase$^{C16\text{-}C18}$, and the thrombogenicity index, there were main effects for both studied factors. Thus, these results showed that some fatty acids or parameters associated with breast meat fatty acid composition differed depending on the variable to be considered.

The fatty acid composition of the broiler drumstick meat according to slaughter age, sexual maturity, sex, and interactions is shown in Table 3. The fatty acids C10:0, C12:0, and C14:1 in the drumstick showed similar results according to slaughter age, with the greatest means at 150 days and the lowest means at 180 days. While for the fatty acids C17:0, C17:1, and C20:0, highest means were found at 120 and 150 days, respectively. Among the fatty acids in the drumstick, C16:1 and C20:3*n*-6 tended to decrease with increasing slaughter age, with the highest values found at 70 days and the lowest values found at 180 and 150 days, respectively. C18:0 showed high means at 120 days and low means at 90 days, and C24:0 was variable according to slaughter age and had the largest mean value at 150 days. The PUFA/SFA ratio increased while the activity index of Δ9-desaturase^{C16} decreased in the drumstick from chickens slaughtered from 70 to 180 days. The SFA content was influenced by slaughter age, with the largest mean values at 120 days and the lowest mean values at 180 days, while for the percentage of MUFA, high values were found at 90 days, and low values were found at 150 days. Thus, these results in the fatty acid profile were responsible for the low atherogenicity index found at 90 and 180 days in the drumstick (Table 3).

There were reductions in C16:1, C20:3*n*-6, C23:0 acids, the MUFA

Table 2
Fatty acid composition of breast meat from broilers of the Label Rouge lineage according to slaughter age (A), sexual maturity (M), sex (S), and their interactions.

Fatty acid profile	Slaughter age (A)					Sexual maturity (M)		Sex (S)		SEM	P value				
	70 d	90 d	120 d	150 d	180 d	Immature	Mature	Female (F)	Male (M)		A	M	S	A*S	M*S
	(n=18)	(n=18)	(n=18)	(n=18)	(n=18)	(n=18 M and 21 F)	(n=27 M and 24 F)	(n=45)	(n=45)						
Fatty acids (mg/g)															
C4:0	0.97[a]	0.53[a]	0.55[a]	0.65[a]	0.00[b]	0.76	0.40	0.56	0.55	0.08	**0.002**	**0.019**	0.936	0.754	0.511
C8:0	0.61	0.00	0.12	0.21	0.35	0.39	0.19	0.31	0.25	0.08	0.149	0.256	0.709	0.546	0.687
C10:0	2.08[ab]	2.19[ab]	3.82[a]	3.15[a]	0.14[b]	2.34	2.22	2.08	2.46	0.32	**0.001**	0.852	0.394	0.165	0.761
C12:0	5.77[a]	3.86[ab]	7.11[a]	5.40[a]	0.98[b]	5.29	4.23	4.44	4.94	0.59	**0.003**	0.404	0.571	0.066	0.787
C14:0	29.37[ab]	25.23[ab]	32.62[a]	25.96[ab]	11.66[b]	28.45	22.55	24.40	25.81	2.26	**0.023**	0.215	0.714	0.216	0.724
C14:1	3.11[a]	2.34[ab]	2.82[a]	2.48[ab]	0.70[b]	2.92	1.85	2.36	2.27	0.25	**0.014**	**0.035**	0.829	0.405	0.529
C15:0	3.34[a]	3.14[a]	3.72[a]	3.28[a]	0.89[b]	3.30	2.56	2.93	2.83	0.27	**0.001**	0.196	0.818	0.162	0.879
C16:0	249.86	259.05	252.25	248.48	195.98	255.56	229.54	237.69	243.94	7.33	0.051	0.085	0.647	0.598	0.431
C16:1	26.70[a]	25.02[a]	20.20[ab]	21.18[ab]	16.48[b]	25.95	18.93	23.58	20.36	1.14	**0.008**	**0.0004**	0.076	0.109	**0.041**
C17:0	2.89	2.93	3.35	3.23	2.11	2.94	2.87	2.79	3.01	0.16	0.114	0.842	0.452	0.384	0.439
C17:1	4.31[ab]	5.65[a]	5.42[a]	5.29[a]	2.29[b]	4.85	4.31	4.31	4.78	0.35	**0.007**	0.460	0.413	0.335	0.489
C18:0	101.22	88.78	103.55	106.25	87.01	96.37	98.53	94.36	101.20	2.88	0.089	0.670	0.193	0.227	0.178
C18:1*n*-9 T	2.31	2.38	2.01	1.88	1.94	2.32	1.93	1.93	2.27	0.14	0.734	0.165	0.240	0.483	0.607
C18:1*n*-9 C	269.50	273.05	248.28	279.68	262.23	272.39	261.87	274.18	258.68	6.93	0.661	0.431	0.270	0.239	**0.040**
C18:2*n*-6 C	120.18	94.58	122.00	132.47	153.09	109.91	137.10	129.56	121.08	6.48	0.120	**0.039**	0.507	0.741	0.596
C20:0	1.25	0.84	1.15	1.22	0.67	1.07	1.01	1.07	1.01	0.09	0.164	0.744	0.753	0.638	0.597
C18:3*n*-6	0.76	0.73	0.57	0.90	0.61	0.74	0.69	0.85	0.58	0.07	0.508	0.730	0.051	0.211	0.472
C20:1	1.88	1.38	1.23	2.04	5.26	1.67	2.91	1.82	2.93	0.64	0.272	0.346	0.380	0.447	0.409
C18:3*n*-3	4.50	3.80	5.39	5.30	5.58	4.28	5.44	5.43	4.44	0.32	0.376	0.057	0.118	0.258	0.114
C20:2	2.00	1.39	1.48	1.91	1.55	1.72	1.66	1.61	1.77	0.11	0.276	0.793	0.438	0.228	0.115
C22:0	0.72	1.13	1.26	0.83	0.80	0.94	0.93	1.10	0.77	0.11	0.421	0.982	0.132	0.462	0.782
C20:3*n*-6	5.44[a]	3.96[ab]	4.08[ab]	3.45[b]	2.86[b]	4.75	3.44	4.19	3.83	0.26	**0.006**	**0.012**	0.387	0.144	0.997
C20:4*n*-6	39.83	40.24	54.28	55.51	38.52	40.26	49.80	39.03	52.30	3.60	0.129	0.157	**0.021**	**0.007**	0.075
C23:0	0.32[a]	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.17	0.00	0.06	0.09	0.03	**0.001**	**0.009**	0.418	0.206	0.292
C24:0	0.04[b]	0.38[a]	0.17[ab]	0.32[a]	0.00[b]	0.19	0.15	0.21	0.12	0.04	**0.008**	0.670	0.102	0.360	0.553
C20:5*n*-3	1.59	0.44	0.27	0.42	1.33	1.05	0.70	1.08	0.61	0.21	0.145	0.412	0.251	0.813	0.997
C22:6*n*-3	5.13	4.85	6.03	7.74	6.20	4.63	7.04	6.52	5.47	0.46	0.348	**0.005**	0.271	0.719	0.223
Parameters															
SFA (mg/g)	398.45[a]	388.06[a]	409.69[a]	398.96[a]	300.59[b]	397.77	365.52	371.99	387.00	11.22	**0.006**	0.169	0.416	0.384	0.712
MUFA (mg/g)	307.82	309.82	279.94	312.54	288.89	310.11	291.80	308.18	291.29	7.71	0.589	0.219	0.281	0.270	**0.047**
PUFA (mg/g)	179.44	149.99	194.09	207.70	209.74	167.33	205.87	188.26	190.09	7.43	0.145	**0.009**	0.904	0.962	0.565
SFA%	44.87[ab]	45.73[a]	46.39[a]	43.41[ab]	37.63[b]	45.37	42.21	42.72	44.44	0.92	**0.014**	0.090	0.281	0.595	0.385
MUFA%	34.73	36.32	31.59	33.83	36.20	35.35	33.81	35.49	33.47	0.66	0.086	0.209	0.082	0.140	**0.036**
PUFA%	20.41	17.95	22.03	22.76	26.17	19.28	23.98	21.79	22.10	0.92	0.112	**0.010**	0.867	0.779	0.423
n-3 (mg/g)	11.21	9.09	11.68	13.46	13.10	9.95	13.18	13.03	10.52	0.63	0.228	**0.003**	**0.048**	0.734	**0.047**
n-6 (mg/g)	166.23	139.51	180.93	192.33	195.08	155.67	191.03	173.62	177.79	7.01	0.154	**0.011**	0.771	0.928	0.503
PUFA/SFA ratio	0.46	0.41	0.48	0.53	0.72	0.43	0.59	0.53	0.51	0.03	0.051	**0.024**	0.760	0.849	0.725
n-6/*n*-3 ratio	15.28	15.56	17.05	14.95	15.88	16.53	15.12	14.45	17.02	0.76	0.911	0.312	0.098	0.188	**0.018**
Δ9-dessaturase^{C16}	9.70[a]	8.81[ab]	7.41[b]	7.81[ab]	7.73[ab]	9.25	7.60	9.06	7.58	0.34	**0.029**	**0.005**	**0.005**	**0.014**	**0.039**
Δ9-dessaturase^{C18}	69.26	74.42	67.36	67.83	63.89	71.44	66.04	68.41	68.35	1.11	0.093	**0.014**	0.976	0.748	0.292
Elongase$^{C16\text{-}C18}$	46.07[ab]	45.63[ab]	42.89[b]	45.70[ab]	49.59[a]	45.82	46.10	47.32	44.63	0.69	**0.008**	0.779	**0.013**	**0.022**	**0.009**
ATHERO	0.80[a]	0.78[ab]	0.84[a]	0.69[ab]	0.50[b]	0.80	0.66	0.70	0.75	0.04	**0.032**	0.060	0.470	0.669	0.568
THROMBUS	1.42[ab]	1.51[a]	1.48[ab]	1.30[ab]	1.06[b]	1.46	1.26	1.29	1.41	0.05	**0.039**	**0.045**	0.199	0.840	0.370

Different lowercase letters in the same row per individual factor (Slaughter Age, Sexual Maturity, and Sex) indicate differences according to the Tukey test with $p<0.05$; Sexual maturity was determined by evaluation of gonad development; (SFA) Total saturated fatty acid content; (MUFA) Total monounsaturated fatty acid content; (PUFA) Total polyunsaturated fatty acid content; (%SFA) Percentage of saturated fatty acids; (%MUFA) Percentage of monounsaturated fatty acids; (%PUFA) Percentage of polyunsaturated fatty acids; (n-3) Total omega 3 fatty acid content; (n-6) Total omega 6 fatty acid content; (Δ9-desaturaseC16) Estimate of the activity index of Δ9-desaturaseC16; (Δ9-desaturaseC18) Estimate of the activity index of Δ9-desaturaseC18; (elongaseC16-C18) Estimate of the activity index of elongaseC16-C18; (ATHERO) Atherogenicity index; (THROMBUS) Thrombogenicity index; (SEM) Standard error of the mean.

Table 3
Fatty acid composition of drumstick meat from broilers of the Label Rouge lineage according to slaughter age (A), sexual maturity (M), sex (S), and their interactions.

Fatty acid profile	Slaughter age (A)					Sexual maturity (M)		Sex (S)		SEM*	P value				
	70 d (n=18)	90 d (n=18)	120 d (n=18)	150 d (n=18)	180 d (n=18)	Immature (n=18 M and 21 F)	Mature (n=27 M and 24 F)	Female (F) (n=45)	Male (M) (n=45)		A	M	S	A*S	M*S
Fatty acids (mg/g)															
C4:0	0.58	0.36	0.56	0.31	0.57	0.47	0.48	0.40	0.55	0.09	0.689	0.927	0.342	**0.044**	0.085
C8:0	0.69^{a}	0.04^{b}	0.32^{ab}	0.00^{b}	0.19^{b}	0.34	0.18	0.26	0.23	0.07	**0.0007**	0.270	0.753	**0.015**	0.475
C10:0	1.52^{bc}	1.89^{bc}	3.11^{ab}	3.83^{a}	0.00^{c}	1.68	2.37	1.67	2.47	0.32	**0.0001**	0.294	0.061	0.277	0.689
C12:0	4.71^{ab}	2.72^{bc}	5.98^{ab}	6.89^{a}	0.64^{c}	3.74	4.53	3.61	4.77	0.53	**0.0001**	0.482	0.118	0.723	0.997
C14:0	27.40^{a}	16.92^{ab}	30.78^{a}	32.36^{a}	8.68^{b}	22.26	23.97	20.97	25.49	2.32	**0.0010**	0.726	0.210	0.654	0.837
C14:1	2.87^{ab}	1.92^{bc}	2.83^{ab}	3.42^{a}	0.60^{b}	2.41	2.27	2.50	2.15	0.23	**0.0001**	0.779	0.247	0.384	0.691
C15:0	2.82	1.98	3.35	4.01	3.06	2.42	3.52	2.45	3.64	0.48	0.801	0.262	0.264	0.814	0.687
C16:0	224.59	210.75	238.78	239.47	193.23	220.81	221.79	224.85	217.87	6.04	0.060	0.937	0.522	0.262	0.210
C16:1	36.44^{a}	33.74^{a}	29.43^{ab}	24.65^{bc}	17.59^{c}	35.29	23.07	33.25	23.49	1.73	**<0.0001**	**<0.0001**	**<0.0001**	0.727	0.997
C17:0	2.45^{b}	2.12^{b}	3.93^{a}	3.75^{a}	2.84^{ab}	2.35	3.53	2.68	3.35	0.18	**0.0006**	**0.0003**	**0.015**	0.529	0.196
C17:1	1.57^{b}	2.19^{ab}	2.83^{a}	2.59^{a}	0.44^{c}	1.91	1.94	1.90	1.95	0.19	**<0.0001**	0.937	0.841	0.373	0.491
C18:0	94.66^{ab}	85.18^{b}	111.35^{a}	99.95^{ab}	92.57^{ab}	90.42	101.57	90.57	102.92	2.92	**0.019**	**0.036**	**0.012**	0.203	0.121
C18:1*n*-9 T	2.28	1.64	2.62	2.09	1.76	1.97	2.15	1.95	2.20	0.15	0.279	0.555	0.421	0.975	0.997
C18:1*n*-9 C	281.00	301.12	294.95	274.49	253.60	296.27	269.38	298.46	263.60	7.83	0.286	0.069	**0.024**	0.546	0.704
C18:2*n*-6 C	125.13	140.48	149.47	142.54	145.95	133.73	146.05	137.44	143.99	4.99	0.651	0.230	0.543	0.598	0.255
C20:0	0.94^{ab}	0.69^{ab}	1.07^{a}	1.13^{a}	0.50^{b}	0.82	0.89	0.91	0.82	0.07	**0.004**	0.623	0.367	0.172	0.567
C18:3*n*-6	1.10	1.20	0.76	0.78	1.06	1.12	0.87	1.10	0.85	0.08	0.207	0.136	0.086	0.067	0.729
C20:1	1.95	2.06	1.81	1.71	2.39	2.02	1.95	1.95	2.01	0.11	0.405	0.761	0.778	0.710	0.587
C18:3*n*-3	4.56	5.68	6.22	6.29	5.65	5.30	5.97	5.57	5.78	0.26	0.267	0.224	0.685	0.497	0.997
C20:2	1.72	1.54	1.45	1.48	1.36	1.59	1.45	1.30	1.72	0.10	0.687	0.431	**0.016**	**0.018**	**0.043**
C22:0	0.54	1.02	0.68	0.59	0.54	0.78	0.59	0.65	0.69	0.07	0.188	0.188	0.749	0.477	0.440
C20:3*n*-6	3.31^{a}	2.81^{ab}	2.78^{ab}	2.18^{b}	2.60^{ab}	3.01	2.52	2.71	2.76	0.11	**0.034**	**0.036**	0.829	0.483	0.684
C20:4*n*-6	28.09	29.93	34.70	28.28	37.39	28.46	34.14	25.50	37.85	2.78	0.260	0.259	**0.0008**	**<0.0001**	0.060
C23:0	0.46^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.21	0.00	0.05	0.13	0.04	**<0.0001**	**0.006**	0.084	**0.031**	0.131
C24:0	0.00^{b}	0.19^{b}	0.20^{b}	0.67^{a}	0.00^{b}	0.09	0.30	0.27	0.15	0.06	**<0.0001**	0.069	0.115	0.620	0.538
C20:5*n*-3	1.43^{a}	0.27^{b}	0.18^{b}	0.19^{b}	1.76^{a}	0.79	0.74	1.05	0.48	0.22	**0.0002**	0.913	**0.020**	**0.0001**	0.067
C22:6*n*-3	3.78	2.63	3.32	3.35	3.25	3.17	3.34	3.72	2.81	0.24	0.602	0.722	0.051	0.127	0.379
Parameters															
SFA (mg/g)	361.36^{abc}	323.88^{bc}	400.08^{a}	392.95^{ab}	302.81^{c}	346.40	363.72	349.34	363.09	9.74	**0.002**	0.401	0.382	0.844	0.855
MUFA (mg/g)	326.10	342.67	334.46	308.95	276.38	339.87	300.77	339.67	295.75	9.15	0.092	**0.019**	**0.010**	0.513	0.997
PUFA (mg/g)	169.12	184.54	198.87	185.08	198.99	177.16	195.08	178.40	196.24	6.37	0.4491	0.146	0.129	0.064	0.098
SFA%	42.19	38.26	42.96	44.51	38.91	40.21	42.25	40.24	42.49	0.89	0.113	0.259	0.186	0.444	0.325
MUFA%	37.97^{ab}	40.00^{a}	35.78^{ab}	34.83^{b}	35.50^{ab}	39.18	35.01	39.17	34.46	0.74	**0.028**	**0.0004**	**0.0002**	0.328	0.997
PUFA%	19.84^{b}	21.73^{ab}	21.27^{ab}	20.67^{ab}	25.60^{a}	20.61	22.74	20.58	23.06	0.75	**0.040**	0.145	**0.041**	**0.035**	0.268
n-3 (mg/g)	9.77	8.57	9.71	9.83	10.65	9.25	10.06	10.34	9.07	0.42	0.623	0.332	0.132	0.219	0.115
n-6 (mg/g)	157.63	174.42	187.70	173.78	186.98	166.32	183.53	166.75	185.45	6.22	0.408	0.146	0.096	**0.045**	0.074
PUFA/SFA ratio	0.48^{b}	0.57^{ab}	0.50^{ab}	0.48^{b}	0.67^{a}	0.52	0.56	0.52	0.56	0.03	**0.044**	0.483	0.293	0.067	0.163
n-6/*n*-3 ratio	17.12	20.38	19.37	17.91	21.63	18.58	19.82	16.77	21.79	1.32	0.721	0.614	**0.038**	**0.035**	**0.038**
Δ9-desaturase^{C16}	14.18^{a}	13.71^{ab}	11.22^{abc}	9.42^{bc}	8.31^{c}	13.85	9.47	13.01	9.72	0.67	**0.002**	**0.0001**	**0.002**	0.567	0.997
Δ9-desaturase^{C18}	69.28	68.04	66.62	66.75	63.49	68.87	65.28	68.60	65.07	0.85	0.215	**0.026**	**0.032**	0.408	0.997
Elongase$^{C16\text{-}C18}$	49.82	52.69	47.98	46.78	48.55	51.37	47.48	51.23	47.10	0.84	0.054	**0.004**	**0.002**	0.410	0.997
ATHERO	0.70^{ab}	0.55^{b}	0.70^{ab}	0.81^{a}	0.48^{b}	0.62	0.67	0.61	0.69	0.04	**0.009**	0.512	0.305	0.409	0.398
THROMBUS	1.29	1.12	1.33	1.43	1.12	1.20	1.30	1.19	1.32	0.05	0.186	0.340	0.181	0.503	0.580

Different lowercase letters in the same row per individual factor (Slaughter Age, Sexual Maturity, and Sex) indicate differences according to the Tukey test with $p<0.05$; Sexual maturity was determined by evaluation of gonad development; (SFA) Total saturated fatty acid content; (MUFA) Total monounsaturated fatty acid content; (PUFA) Total polyunsaturated fatty acid content; (%SFA) Percentage of saturated fatty acids; (%MUFA) Percentage of monounsaturated fatty acids; (%PUFA) Percentage of polyunsaturated fatty acids; (n-3) Total omega 3 fatty acid content; (n-6) Total omega 6 fatty acid content; (Δ9-desaturaseC16) Estimate of the activity index of Δ9-desaturaseC16; (Δ9-desaturaseC18) Estimate of the activity index of Δ9-desaturaseC18; (elongaseC16-C18) Estimate of the activity index of elongaseC16-C18; (ATHERO) Atherogenicity index; (THROMBUS) Thrombogenicity index; (SEM) Standard error of the mean.

content, the MUFA percentage, and the estimates of the activity index of Δ9-desaturase^{C16}, Δ9-desaturase^{C18} and elongase$^{C16\text{-}C18}$ in the drumstick meat of sexually mature chickens. The drumstick meat of mature birds exhibited the greatest means for the fatty acids C17:0 and C18:0.

There was an influence of sex on C16:1, C17:0, C18:0 C18:1*n*-9 C, C20:4*n*-6 acids, the content and percentage of MUFA, Δ9-desaturase^{C16}, Δ9-desaturase^{C18} and elongase$^{C16\text{-}C18}$ activity indexes independent of other variables. Females exhibited the highest means for C16:1, C18:1*n*-9 C, C20:5*n*-3 acids and the estimates of the activity index of elongase$^{C16\text{-}C18}$, while males exhibited the highest values for C17:0, C18:0, C20:2, and C20:4*n*-6 acids. There was an increase in C16:1 and C18:1*n*-9 C in meat from the drumsticks in females, which may have contributed to the difference between sexes found for estimates of the activity indexes of Δ9-desaturase^{C16}, Δ9-desaturase^{C18} and elongase$^{C16\text{-}C18}$ and the content and percentage of MUFA, with higher means in females as compared to males. However, males presented greater means than females for C17:0, C18:0, the percentage of PUFA, and the *n*-6/*n*-3 ratio in the meat from drumsticks.

The statistical analyses about the relation of age at slaughter or sexual maturity and sex showed interaction in the breast and drumstick for different parameters in the fatty acid composition (Tables 2 and 3). There was an interaction ($p<0.05$) between slaughter age and sex for C20:4*n*-6 and the estimates of the activity index of Δ9-desaturase^{C16} and elongase$^{C16\text{-}C18}$ in the breast meat. Moreover, there was an interaction of sexual maturity and sex for C16:1, C18:1*n*-9 C, MUFA content and percentage, *n*-3 content, the *n*-6/*n*-3 ratio, and the estimates of activity index of Δ9-desaturase^{C16} and elongase$^{C16\text{-}C18}$ (Table 2).

In the fatty acid composition of the drumstick meat, there was an interaction between age at slaughter and sex for C4:0, C8:0, C20:2, C20:4*n*-6, C23:0, C20:5*n*-3, PUFA%, *n*-6 content and the *n*-6/*n*-3 ratio (Table 3). In addition, for C20:2 and the *n*-6/n-3 ratio, there was an interaction between sexual maturity and sex. These results showed that increasing the age at slaughter or verification of the birds sexual maturity influence the parameters of fatty acid composition by modifying the fatty acid profile and other parameters that may be associated with it.

In breast meat, the interaction between slaughter age and sex for C20:4*n*-6 acid showed that in males, there were no differences according to slaughter ages, and higher means compared to females were found for birds slaughtered at 180 days. Females showed greatest means of this fatty acid at 120 and 150 days, decreasing levels at 180 days (Table 4). Slaughter age and sex affected estimates of Δ9-desaturase^{C16} activity index in the breast meat, with decreasing and lower means at 180 days for mature males, and there were no differences for females according to slaughter age. However, in the estimate of the elongase$^{C16\text{-}C18}$ activity index there were no significant differences for males according to slaughter age, while for females the largest means were observed at 180 days ($p<0.05$).

Sexual maturity and sex influenced the C16:1, C18:1*n*-9 C, *n*-3, MUFA content and percentage, and the estimates of the elongase$^{C16\text{-}C18}$

Table 4
Results for the interactions of slaughter age and sex, and sexual maturity and sex for the fatty acid composition of breast and drumstick muscles of Label Rouge broilers raised in a free-range system.

Muscle	Parameters	Sex	Slaughter age					Sexual maturity	
			70 d	90 d	120 d	150 d	180 d	Immature	Mature
Breast	C20:4*n*-6 (mg/g)	Female	40.60^{abA}	40.98^{abA}	54.48 ^{aA}	49.93 ^{aA}	9.26^{bB}	-	-
		Male	38.80 ^{aA}	39.75 ^{aA}	54.09 ^{aA}	61.09 ^{aA}	67.77 ^{aA}	-	-
	C16:1 (mg/g)	Female	-	-	-	-	-	25.08 ^{aA}	22.27 ^{aA}
		Male	-	-	-	-	-	26.96 ^{aA}	15.96^{bB}
	C18:1*n*-9 C (mg/g)	Female	-	-	-	-	-	264.18 ^{aA}	282.93 ^{aA}
		Male	-	-	-	-	-	281.97 ^{aA}	243.15^{bB}
	MUFA (mg/g)	Female	-	-	-	-	-	301.19 ^{aA}	314.29 ^{aA}
		Male	-	-	-	-	-	320.51 ^{aA}	271.82^{bB}
	MUFA%	Female	-	-	-	-	-	34.82 ^{aA}	36.08 ^{aA}
		Male	-	-	-	-	-	35.98 ^{aA}	31.79^{bB}
	n-3 (mg/g)	Female	-	-	-	-	-	10.28^{bA}	15.45 ^{aA}
		Male	-	-	-	-	-	9.56 ^{aA}	11.16^{aB}
	n-6/*n*-3 ratio	Female	-	-	-	-	-	17.05 ^{aA}	12.18^{bB}
		Male	-	-	-	-	-	15.93 ^{aA}	17.75 ^{aA}
	Δ9-desaturase^{C16}	Female	9.67 ^{aA}	8.72 ^{aA}	8.17 ^{aA}	8.11 ^{aA}	10.32 ^{aA}	9.21 ^{aA}	8.93 ^{aA}
		Male	9.73 ^{aA}	8.87 ^{aA}	6.64^{abA}	7.50^{abA}	5.13^{bB}	9.30 ^{aA}	6.42^{bB}
	Elongase$^{C16\text{-}C18}$	Female	46.45^{bA}	44.71^{bA}	44.69^{bA}	46.44^{bA}	54.29 ^{aA}	45.56^{bA}	48.86 ^{aA}
		Male	45.69 ^{aA}	46.55 ^{aA}	41.08 ^{aA}	44.95 ^{aA}	44.89^{aB}	46.12 ^{aA}	43.64^{aB}
Drumstick	C4:0 (mg/g)	Female	0.82 ^{aA}	0.34 ^{aA}	0.57 ^{aA}	0.28 ^{aA}	0.00^{aB}	-	-
		Male	0.35 ^{aA}	0.38 ^{aA}	0.55 ^{aA}	0.34 ^{aA}	1.14 ^{aA}	-	-
	C8:0 (mg/g)	Female	0.86 ^{aA}	0.07^{bA}	0.00^{bB}	0.00^{bA}	0.37^{abA}	-	-
		Male	0.52^{abA}	0.00^{bA}	0.63 ^{aA}	0.00^{bA}	0.00^{bA}	-	-
	C20:2 (mg/g)	Female	1.92 ^{aA}	1.40^{abA}	1.11^{abA}	1.43^{abA}	0.62^{bB}	1.58 ^{aA}	1.05^{bB}
		Male	1.51 ^{aA}	1.68 ^{aA}	1.78 ^{aA}	1.52 ^{aA}	2.09 ^{aA}	1.60 ^{aA}	1.80 ^{aA}
	C20:4*n*-6 (mg/g)	Female	32.93 ^{aA}	25.67^{abA}	30.25^{abA}	28.64^{abA}	10.02^{bB}	-	-
		Male	23.26^{bA}	34.19^{bA}	39.15^{bA}	27.92^{bA}	64.75 ^{aA}	-	-
	C23:0 (mg/g)	Female	0.27^{aB}	0.00^{bA}	0.00^{bA}	0.00^{bA}	0.00^{bA}	-	-
		Male	0.64 ^{aA}	0.00^{bA}	0.00^{bA}	0.00^{bA}	0.00^{bA}	-	-
	C20:5*n*-3 (mg/g)	Female	1.14^{bA}	0.30^{bA}	0.09^{bA}	0.20^{bA}	3.53 ^{aA}	-	-
		Male	1.72 ^{aA}	0.23^{abA}	0.26^{abA}	0.17^{bA}	0.00^{bB}	-	-
	PUFA%	Female	21.09 ^{aA}	20.52 ^{aA}	19.22 ^{aA}	21.06 ^{aA}	21.02^{aB}	-	-
		Male	18.60^{bA}	22.95^{abA}	23.31^{abA}	20.27^{bA}	30.17 ^{aA}	-	-
	n-6 (mg/g)	Female	168.70 ^{aA}	171.18 ^{aA}	166.69 ^{aA}	179.28 ^{aA}	147.92^{aB}	-	-
		Male	146.56^{bA}	177.65^{abA}	208.71^{abA}	168.28^{abA}	226.04 ^{aA}	-	-
	n-6/*n*-3 ratio	Female	19.26 ^{aA}	19.76 ^{aA}	17.69 ^{aA}	15.47 ^{aA}	11.69^{aB}	19.09 ^{aA}	14.75^{aB}
		Male	14.98^{bA}	21.01^{abA}	21.04^{abA}	20.36^{abA}	34.57 ^{aA}	17.99 ^{aA}	24.32 ^{aA}

Different lowercase letters in the same row indicate difference per slaughter age or sexual maturity, and different uppercase letters in the same column indicate differences per sex according to the Tukey test with p<0.05. (MUFA) Total monounsaturated fatty acid content; (MUFA%)- Percentage of monounsaturated fatty acids; (PUFA%) Percentage of polyunsaturated fatty acids; (n-3) Total omega 3 fatty acid content; (n-6) Total omega 6 fatty acid content; (Δ9-desaturaseC16) Estimate of the activity index of Δ9-desaturaseC16.

and Δ9-desaturase^{C16} activity indexes with the lowest means in the breast meat of mature male chickens (Table 4). While the *n*-6/*n*-3 ratio showed a decreasing in the breast meat of mature females.

For C4:0, C20:2, and C20:4*n*-6, acids in the drumstick cuts from female chickens decreased or were under the detection limit in samples of chickens slaughtered at 180 days (Table 4). The C23:0 acid content was found only in birds slaughtered at 70 days, and females showed the lowest means. There was a difference in C8:0 content according to sex for chickens slaughtered at 120 days, with higher means for males, while in females, the greatest means were found at 70 days. The female birds showed the highest C20:5*n*-3 content in the drumstick meat at 180 days, and in male birds, there was a reduction up to 150 days and below the detection limit at 180 days. In the drumstick meat of sexually mature females, a decrease in C20:2 was observed, and there was no difference between sexes for immature chickens. There were influences of slaughter age and sex on the percentage of PUFA, *n*-6, and the *n*-6/*n*-3 ratio of drumsticks, and males at 180 days showed greatest means. There were no differences in fatty acid composition for females according to slaughter age. For males, *n*-6 and the *n*-6/*n*-3 ratio showed higher means at 180 days and tended to increase with slaughter age. Thus, this effect could help explain the interaction between sexual maturity and sex for the *n*-6/*n*-3 ratio, which was higher for mature males than mature females.

4. Discussion

Sexual maturity, slaughter age, and sex influenced the profile of fatty acids in breast and drumstick cuts of chickens raised in the free-range system. Changes in C4:0, C10:0, C12:0, C14:0, C14:1, C16:1, C17:1, C18:1*n*-9 C, C20:3*n*-6, C23:0, C24:0, C20:4*n*-6, SFA content, MUFA content, MUFA percentage, PUFA percentage, PUFA/SFA ratio, *n*-6 content, *n*-6/*n*-3, Δ9-desaturase^{C16}, Δ9-desaturase^{C18}, elongase$^{C16\text{-}C18}$ and the atherogenicity index were observed in both muscle types.

The content of SFA decreased after birds reached sexual maturity, mainly in birds slaughtered at 180 days, which also showed decreases in the contents of C4:0, C10:0, C12:0, C14:0, C15:0, C20:0, and C24:0. Another saturated fatty acid, C23:0, had higher means at 70 days in both muscles. In the drumstick cuts, C18:0 increased with sexual maturity and was greater in males than females. The influence of bird aging was investigated by Li et al. (2021), who reported a lower SFA content in the breast of chickens slaughtered at 60, 150, and 180 days than in chickens slaughtered at 90 and 120 days of age and an increase in C14:0 up to 120 days and C17:0 up to 150 days. In the present study, the age at slaughter or sexual maturity showed similar influences on the fatty acid composition of some saturated fatty acids in the meat of free-range chickens.

In both cuts, there were reductions in the content and proportion of MUFA. On the other hand, a higher concentration of MUFA was reported by Li et al. (2021) for chickens slaughtered at 120, 150, and 180 days of age, which was attributed to a higher content of C18:1*n*-9 C in chickens slaughtered at these ages. However, in the present study, in both cuts, the reductions in the content and percentage of MUFA could be related to the influence of slaughter age, where there was a decrease in the means of C14:1, C16:1, C17:1, and C18:1*n*-9 C at 180 days.

About MUFAs, the content tended to decrease in drumsticks until 150 days, and for both muscles, there were low means for mature birds and high means for females. In breast meat from sexually mature males, low MUFA content and percentage were verified. Changes in the fatty acid percentage of breast and drumstick meat of chickens raised in a free-range system after sexual maturity may be due to alterations in the metabolism of these birds, causing changes in the amount and activity of enzymes that act in the biosynthesis of fatty acids, such as stearoyl-CoA desaturase, which participates in the production of MUFA through the introduction of double bonds in the C16:0 and C18:0 fatty acids (Nakamura and Nara, 2004). Furthermore, the hormones estradiol in females and testosterone in males affect the activity of these enzymes, activating and inhibiting them, respectively (Poureslami et al., 2010; Li et al., 2015; Zhang et al., 2017).

Such hormonal action may have caused the sexual effect on fatty acid percentages in both cuts, where females exhibited higher levels of C16:1 and C18:1*n*-9 C in both cuts, and it may have contributed to the increased estimated activity of Δ9-desaturase^{C16} and Δ9-desaturase^{C18}. Faria and Cruz (2019) reported a similar result in the drumstick cut of female chickens from different genotypes. These differences in the profile and biosynthesis of MUFAs according to the sex could be related to greater insulin sensitivity in adipose tissue in females, which influences the activity of Δ9-desaturase enzyme and increase the conversion of SFA into MUFA (Geer and Shen, 2009; Zhou et al., 2009).

Additionally, there was a reduction in the estimated activity of the enzymes Δ9-desaturase^{C16} and Δ9-desaturase^{C18} after the chickens had reached sexual maturity. The activity index estimates of these enzymes are directly associated with the fatty acid composition analysis results and help explain the change that occurs according to the variables studied. The Δ9-desaturase^{C16} enzyme is responsible for the conversion of C16:0 into C16:1, and the Δ9-desaturase^{C18} enzyme is responsible for the conversion of C18:0 into C18:1 through desaturation of the carbon chain. The elongase$^{C16\text{-}C18}$ enzyme, which is related to the process of elongating C16:0 and C16:1 to C18:0 and C18:1 fatty acid (Guillou et al., 2010; Cruz et al., 2017; Cruz and Faria, 2019), had similar results in the breast and drumstick, increasing in chickens slaughtered at 180 days and after sexual maturity. The present results are in accordance to those published by Popova et al. (2016), who reported an increase in the activity of elongase$^{C16\text{-}C18}$ with age in broilers.

The breast cuts from sexually mature chickens exhibited higher amounts and proportions of PUFA, while in drumstick cut, despite the lack of an influence of sexual maturity, a low percentage of PUFA was observed in females at 180 days. These increases in PUFA may be due to higher amounts of C22:6*n*-3, *n*-3, and *n*-6 content in the breast of chickens after sexual maturity. In both cuts, the PUFA/SFA ratio increased with the slaughter age or sexual maturity. This result could be related to a decrease in the SFA content and percentage according to the chickens' aging, which showed a reduction and lower means at 180 days. Li et al. (2021) reported reductions in PUFA, the PUFA/SFA ratio, the percentages of *n*-3 and *n*-6, and the *n*-6/*n*-3 ratio in the breast over 120 days in chickens raised in a free-range system and slaughtered at similar ages.

Most of the differences in fatty acid composition related to sexual maturity were decreases in contents according to an increasing slaughter age of the chickens, with a reduction mainly in the female contents of *n*-6, C20:2*n*-6, C20:4*n*-6, and *n*-6/*n*-3 ratio in both cuts and the percentage of PUFA in drumsticks at 180 days. Similar to the results found in the present study, Li et al. (2021) reported lower means for the total PUFA and *n*-6 contents in the breast meat of chickens slaughtered at 120, 150, and 180 days compared to 60 and 90 days of age. According to these authors, the total *n*-3 concentration and the proportions of C18:2*n*-6, C20:2, C20:3*n*-6, C20:4*n*-6, and C22:6*n*-3 acids decreased with increasing chickens' age. This effect would be associated with decreasing Δ6 desaturase activity with aging (Horrobin, 1981) that presents more affinity to bioconverted α-linolenic acid (C18 : 3*n*-3) into long-chain *n*-3 fatty acids.

On the other hand, in the present study, mature females showed high *n*-3 contents in breast meat despite no difference among slaughter ages. Changes in the fatty acid percentage of the breast and drumstick meat of chickens raised in an alternative system after sexual maturity may be due to alterations in the expression of fatty acid desaturases and elongases in the liver metabolism of these chickens with age (Jing et al., 2013). These cause changes in the amount and activity of enzymes that act in the biosynthesis of fatty acids, such as Δ6 desaturase, which has the power to catalyze stages of desaturation in the synthesis of polyunsaturated fatty acids (Nakamura and Nara, 2004; Van Dael, 2021).

In this study, female chickens had more *n*-3 than males, and mature females showed a higher *n*-3 content in the breast meat, while the drumstick meat of females slaughtered at 180 days showed lower

amounts of *n*-6. With the increase in *n*-3, mature female chickens exhibited a lower *n*-6/*n*-3 ratio in the breast and drumstick meat. Cruz et al. (2017) observed a similar result, reducing the *n*-6/*n*-3 proportion in female chickens regardless of the muscle (breast or drumstick). The differences in the present study according to slaughter age and sexual maturity may suggest that the activity of the Δ6 desaturase enzyme was different in each sex. In females, its activity increases mainly after sexual maturity and at slaughter age (180 days), as the Δ6 desaturase enzyme has a higher affinity for forming *n*-3 fatty acids (Palmquist, 2009). This hypothesis could be verified because there was no influence of the studied factors on the contents of C18:2*n*-6 (linoleic) and C18:3*n*-3 (linolenic), and they are precursors for the synthesis of other fatty acids, such as C20:4*n*-6 and C20:5*n*-3, through Δ6 desaturase enzyme activity. There was a difference between sexes, with females having a lower C20:4*n*-6 content in both muscles, a decrease at 180 days, and a higher mean C20:5*n*-3 content in the drumstick, while for male chickens, there was an increase in the C20:4*n*-6 content in both cuts and the *n*-6 content in the drumstick at 180 days.

With sexual maturation, fatty acid synthesis is increased in the female liver through the activation of enzymes that participate in fatty acid biosynthesis (Li et al., 2015; Zhang et al., 2017; Vignale et al., 2018; Zheng et al., 2018; Shi et al., 2020). However, in females, these fatty acids are synthesized in the liver, secreted into the bloodstream, and directed to egg yolk formation, mainly altering the content of PUFA and *n*-6 fatty acids (Lešić et al., 2017; Zheng et al., 2018; Cui et al., 2020). Thus, the reduction in the *n*-6 content in mature female meat may be due to their targeting of egg yolk formation at 180 days when laying has begun.

Furthermore, the increase, mainly in the amount of *n*-3 fatty acids, is of great importance, given that this compound has an antithrombotic and antiatheromatous role, helping to reduce cardiovascular diseases in addition to regulating leukotrienes, prostaglandins, and thromboxane synthesis through activation of cytosolic phospholipase A_2 ($cPLA_2$), cyclooxygenase 2, and the production of PGE_2, as a $cPLA_2$ inhibitor, to modify the metabolic pathway of arachidonic acid (Nakamura and Nara, 2004; Oppedisano et al., 2020; Van Dael, 2021).

In the present study, the PUFA/SFA ratio was 0.59 for mature chickens and 0.43 for immature chickens in the breast, while in drumsticks, it increased with slaughter age, with higher means at 180 days (0.67) and lower means at 70 and 150 days (0.48). These values are lower than those reported by Li et al. (2021), who found a mean of 0.70 for females slaughtered at 120, 150, and 180 days of age and a mean of 0.92 for those slaughtered at 60 and 90 days. According to the World Health Organization - WHO. 2003, the PUFA/SFA ratio must be more than 0.4; in this study, the ratio increased in birds with slaughter age.

The *n*-6/*n*-3 ratio detected in the chicken breast muscle ranged from lower means for mature females (12.18) to higher for mature males (17.75). These results were similar to the means found by Li et al. (2021), who reported breast values for females from 15.64 to 18.83, with a lower mean for birds slaughtered after 120 days. In the drumstick, lower means were found for mature females (14.75) and chickens slaughtered at 180 days (11.69), and higher means for mature males (24.32) and chickens slaughtered at 180 days (34.57). Similarly, in meat from different breeds, Cruz and Faria (2019) reported a lower *n*-6/*n*-3 ratio in female drumstick cuts. The results in the present study for chickens raised in the free-range system are still higher than the 4/1 ratio recommended by Simopoulos (2002). Cruz and Faria (2019) reported similar values for drumsticks, who presented results between 20.97 and 35.10 for chickens slaughtered at 105 days, while in the breast, there was variation between 16.27 and 53.59 and no difference between sexes.

The atherogenicity and thrombogenicity indexes decreased in both cuts after birds reached sexual maturity, mainly in chickens slaughtered at 180 days. These indexes are related to the fatty acid composition that influenced this index, mainly because of the reduction in C10:0, C12:0, C14:0, and SFA content at this slaughter age, in contrast to the increase in other fatty acids, such as C20:5*n*-3, C22:6*n*-3, and content and percentage of PUFA according to sexual maturity.

5. Conclusion

The fatty acid composition of meat from chickens raised in a free-range system was altered by sexual maturity and slaughter age. There was an increase in polyunsaturated fatty acids in the breast muscles and monounsaturated fatty acids in the drumstick cut after the birds reached sexual maturity. The findings of this study suggest that free-range chickens slaughtered after reaching sexual maturity or at higher ages will have a modified fatty acid composition in both muscles, and females will have meat with better nutritional characteristics than males. Female birds had higher *n*-3 contents and a lower *n*-6/*n*-3 ratio than males. Therefore, slaughtering chickens after sexual maturity or at older ages could be a strategy to produce free-range chicken meat with less risk of promoting cardiovascular disease, and with better value for their products.

CRediT authorship contribution statement

Joanna Oliveira Marçal: Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization. **Giulia Piva Oliveira:** Methodology, Formal analysis. **Adriano Geraldo:** Supervision, Project administration. **Lidiany Mendonça Zacaroni Lima:** Methodology, Formal analysis. **Peter Bitencourt Faria:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation. **Sara W. Erasmus:** Writing – review & editing, Visualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Peter Bitencourt Faria reports financial support was provided by Fundação de Amparo à Pesquisa do Estado de Minas Gerais.

Data availability

Data will be made available on request.

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