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ANIMAL PRODUCTION

Gender, lineage, and age on muscle morphometry traits and meat tenderness of free-range chickens

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ABSTRACT: *This study assessed whether the meat tenderness of broilers raised in a free-range system varies by sex, lineage, and age at the time of slaughter. Physicochemical parameters were measured to adequately assess breast and thigh muscle morphometry, including shear force (SF), muscle fibre diameter (MD), histological connective tissue (HCT) content, and total and soluble collagen contents (TCC and SCC, respectively). The experiment was conducted with a completely randomized design using two lineages (Pescoço Pelado and Paraíso Pedrês), with specimens of both sexes raised in a free-range system. Randomly selected broilers were slaughtered at 65, 75, 85, and 95 days old. A total of 192 birds were analysed. SF values correlated positively with age. The highest SF values were measured in thighs of the Pescoço Pelado lineage. Age also showed a positive correlation with MD and HCT content of the breast regardless of lineage. Gender did not have any significant correlation with physical parameters; although, higher values were measured in thighs of the Paraíso Pedrês than in those of the Pescoço Pelado lineage. There were no significant differences among treatments regarding the SCC and TCC of thighs and breasts. Principal component analysis (PCA) revealed relationships between SF and the rest of the measured attributes. PCA showed that age was positively correlated with the SF, MD, and HCT content of thighs and breasts. In contrast, SCC and TCC had negative correlations. The greatest breast and thigh MD values were associated with the least tender meat. Thus, age at the time of slaughter proved to be the most significant parameter impacting the physical characteristics of muscle morphometry and meat tenderness of breasts and thighs of broilers raised in a free-range system.*

Key words: *birds, collagen, connective tissue, lineages, muscle fibre.*

Associação de fatores de produção e de morfometria muscular sobre a maciez da carne de frangos caipiras

RESUMO: *Objetivou-se com este estudo avaliar os fatores relacionados a maciez da carne de frangos criados em sistema alternativo. Para o experimento foi avaliado a força de cisalhamento, diâmetro das fibras musculares do peito e da coxa, conteúdo de tecido conjuntivo através de histologia e teor de colágeno solúvel e total. O experimento foi executado em delineamento inteiramente casualizado (DIC) com uso de duas linhagens (Pescoço Pelado e Paraíso Pedrês) de ambos os sexos (macho e fêmea) e abatidos em quatro idades (65, 75, 85 e 95 dias), criados em sistema alternativo, sendo utilizado um total de 192 aves. Para força de cisalhamento (FC), ocorreu aumento nos valores em ambos os cortes em função da idade de abate e houve interação entre linhagem e sexo, com as maiores médias para os machos da linhagem Pescoço Pelado e diferença entre sexo para Paraíso Pedrês. A idade de abate influenciou o diâmetro das fibras musculares e o conteúdo de tecido conjuntivo do peito e da coxa, havendo aumento linear destes parâmetros em função da idade ao abate. O sexo não afetou o diâmetro das fibras musculares da coxa, enquanto na linhagem Paraíso Pedrês foram verificadas maiores médias em relação à Pescoço Pelado. A análise de componentes principais demonstrou a relação entre a força de cisalhamento e as demais variáveis analisadas. Assim, o aumento do diâmetro das fibras musculares do peito e da coxa também está relacionado à redução da maciez da carne de frangos caipiras, conjuntamente com o aumento da quantidade de tecido conjuntivo, teor de colágeno total e redução de sua solubilidade.*

Palavras-chave: *aves, colágeno, tecido conjuntivo, linhagens, fibra muscular.*

INTRODUCTION

The alternative production of broiler chickens is characterized by lower stocking rates and older age at slaughter, as well as lineages with a lesser degrees of genetic improvement; this type of production system associated with improved animal welfare, meat sensory quality, and consumer health (MADEIRA et al., 2010; STADIG et al., 2016). The rearing system, along with muscle type, location, age, sex, genetics, and environment, are factors that affect the composition of muscle tissue in terms of the type

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of fibre, type and amount of collagen, lipid content, enzyme, and tenderness (YOUNG & BRAGGINS, 1993; PICARD et al., 2002; SILVA et al., 2017; IKUSIKA et al., 2020).

Genetic selection for growth alters the quantitative and qualitative characteristics of muscle fibres, which are higher in number and have a higher rate of hypertrophy in fast-growing lineages than slow-growing lineages (DRANSFIELD & SOSNICKI, 1999; SARTORI et al., 2003; MADEIRA et al., 2006). The number of muscle fibres after birth does not change, and muscle growth occurs due to hypertrophy of muscle cells, especially glycolytic fibres, which predominate in the muscle tissue of birds (ONO et al., 1993; SARTORI et al., 2003; NAKAMURA et al., 2004). However, the different types of fibres in birds undergo changes after birth (SMITH & FLETCHER, 1988; SARTORI et al., 2001) depending on the conditions in which the animals live, and these changes may affect the sensory characteristics of the meat (SARTORI et al., 2003).

Free-range chicken meat tends to be less tender than conventional poultry (CRABONE et al., 2005); the main factors associated with reduced tenderness are associated with the older age at slaughter and the consequent decrease in collagen solubility (GOMIDE et al., 2013, SILVA et al., 2017). However, in the literature, factors related to the production system have had variable associations with the parameters considered in the evaluation of muscle morphometry of broilers raised in alternative systems, and there is no consensus regarding their relationship with tenderness. Thus, the objective of this study was to assess whether the meat tenderness of broilers raised in a free-range system varies by gender, lineage, and age at the time of slaughter.

MATERIALS AND METHODS

The experiment included 192 chickens and was conducted over 95 days at the Federal Institute of Mato Grosso (IFMT), São Vicente Campus. The experiment was organized with a completely randomized 2 × 2 × 4 factorial design with two lineages (Pescoço Pelado, slow growth; and Paraíso Pedrês, fast growth), two sexes (male and female), and different ages at slaughter (65, 75, 85, and 95 days), with three replicates per treatment (each experimental unit was composed of four birds). The linear model includes mean values (μ), residual error (e_{ijkl}), the effects of lineage (L_i = 1, 2), age (A_j = 1, 2, 3 and 4), sex (S_k = 1, 2), lineage and age interaction (LA_{ij}), lineage and sex interaction (LS_{ik}), age and sex interaction (AS_{jk}), lineage, age (A) and sex j and sex k interactions (LAS_{ijk}):

$$Y_{ijkl} = \mu + L_i + I_j + S_k + LA_{ij} + LS_{ik} + AS_{jk} + LAS_{ijk} + e_{ijkl}$$

The birds were subjected to two diets: an initial diet (day 1-28) and a final diet (day 29 until slaughter). Diet feed was produced in the Animal Feed Plant of the Animal Science sector of the IFMT São Vicente Campus, and the compositions of the diets are shown in table 1.Chickens were slaughtered by mechanical stunning (*i.e.,* concussive impact in the head) on the premises of the IFMT São Vicente Campus, followed by cutting of the blood vessels near the occipital and atlas bones to allow complete bleeding of the birds. The samples for morphometric analysis were removed immediately after slaughter, and samples were collected from the *pectoralis major* muscles of the breast and *fibularis longus* muscle of the thigh, from which 1 × 2 × 1 cm pieces were taken and fixed in 10% formaldehyde solution. This procedure was performed on all samples at different ages at slaughter, and these samples were taken from the middle portions of the cuts. After the fixation step, the muscle samples were processed and stained. The morphological structure of the muscle fibre was evaluated by haematoxylin–eosin (HE) staining according to LILLIE (1954). The slides for the identification of connective tissue were prepared with picrosirius staining according to JUNQUEIRA et al. (1979).

Morphometric measurements of muscle fibre diameter (MD, μm) and connective tissue quantification were performed by digital image analysis. The captured images were transferred to a computer and analysed with ImageJ® software. The degree of muscle cell development (hypertrophy) was measured using the cross-section of the muscle fibre internal diameter (μm) and a 10× magnification objective, and the images of the muscle fibres of each sample were analysed. A total of 100 fibres inside the bundles of each sample were analysed, and the area of each fibre was measured in μm². Then, each fibre diameter (μm) was calculated using the equation $D^2 = (A \times 4)/\pi$, where D = fibre diameter; A = fibre area, and π = 3.14.

The determination of collagen was performed by cross-sectioning the muscles, and quantification was performed under a 10× magnification objective. The number of fields for the determination was set by calculating the cumulative mean according to the method recommended by REIS et al. (2001).

After evisceration, the carcasses were packaged, separated into groups of four, identified, and cooled to 0 °C for 24 hours; then, the breast and thigh muscles were collected to determine

Table 1 - Compositions and values of the initial and final feeds calculated for broilers raised in an alternative system.

Ingredients	Initial feed (%)	Final feed (%)
Mashed corn	61.0	67.5
Soybean meal (46%)	34.0	27.5
Vitamins and minerals	5.0[1]	5.0[2]
---Values calculated---		
ME, (kcal/kg)	2.860	2.934
CP (%)	20.71	18.31
Lysine (%)	1.10	0.93
Methionine + cystine (%)	0.66	0.60
Calcium (%)	1.19	1.17
Available phosphorus (%)	0.49	0.48

[1]Levels of guarantee per kg of product: calcium: 212 g; phosphorus: 74.7 g; sodium 40 g; selenium: 6.8 mg; nicotinic acid: 866 mg; copper: 3125 mg; calcium pantothenate: 237.5 mg; biotin: 4 mg; manganese: 1906 mg; DL-methionine (minimum): 42.5 g; iodine: 32.5 mg; coccidiostat: 2750 mg; antioxidants: 100 mg; choline: 15 g; growth promoter: 1250 mg; Vit. A: 175,000 IU; Vit. B1: 44.5 mg; Vit. B2: 240 mg; Vit. B6: 86.6 mg; Vit. B12: 250 mcg; Vit. D3: 7500 IU; Vit. E: 625 mg; Vit. K: 24.5 mg; zinc: 2281 mg; fluoride (max): 747 mg.
[2]Levels of guarantee per kg of product: calcium: 212 g; phosphorus: 65 g; sodium 40 g; selenium: 6.8 mg; nicotinic acid: 866 mg; copper: 3125 mg; calcium pantothenate: 237.5 mg; biotin: 4 mg; manganese: 1906 mg; DL-methionine (minimum): 33.7 g; iodine: 32.5 mg; coccidiostat: 2750 mg; antioxidants: 100 mg; choline: 15 g; growth promoter: 1250 mg; Vit. A: 175,000 IU; Vit. B1: 44.5 mg; Vit. B2: 240 mg; Vit. B6: 86.6 mg; Vit. B12: 250 mcg; Vit. D3: 7500 IU; Vit. E: 625 mg; Vit. K: 24.5 mg; zinc: 2281 mg; fluoride (max): 650 mg.

shear force (SF) and total and soluble collagen content. The texture was determined using a TA-XT2 texturometer® (Texture Technologies Corp./ Stable Micro Systems) according to the method of FRONING & UIJTTENBOOGARTE (1988), and the results are expressed in kgf. Collagen and its fractions were quantified by measuring the amino acid hydroxyproline and collagen solubility described by RAMOS & GOMIDE (2017).

The study data were analysed with the support of the SISVAR® program. The variables with significant effect responses in the analysis of variance for the factors of lineage, gender, and age at slaughter and any interaction between these variables were subjected to the Scott–Knott means test or regression ($\alpha = 0.05$). To test for the existence of a binary correlation between the variables in the thigh and breast cuts, the Pearson or Spearman binary correlation test was performed; the latter was performed when the distribution of the variables was determined to be non-normal by the Kolmogorov–Smirnov test.

To test the multivariate relationship between the same variables subjected to binary correction, multivariate principal component analysis (PCA) was performed on the correlation matrix with factor extraction by the principal component method followed by Varimax rotation. The Kaiser–Meyer–Olkin test, Bartlett's sphericity, gross commonality, and percentage of variance retained in the axes (components) were used, which yielded eigenvalues equal to or greater than 1; this was in consonance with the scree plot to measure the quality of the correlations between the variables and the model fit. All analyses were performed with SPSS 20.0 software.

RESULTS AND DISCUSSION

The analysis of the SF of breast meat revealed no effect of lineage or sex but showed a significant effect of age. There was no significant interaction between these variables for breasts and thighs of the studied birds. For thigh samples, there were significant effects of lineage and age at slaughter (Table 2). There was no difference in the tenderness of the breast meat between the lineages studied. CASTELLINI et al. (2002b), compared fast- and slow-growing lineages and reported no difference between Ross, Kabir, and Robusta maculata lineages reared in an organic system. However, differences in breast tenderness among different free-range lineages including Paraíso Pedrês, Super Pesadão, and Cobb (SOUZA et al., 2011); Redbro cou nu (Label Rouge); Redbro plume (Pesadão), and Gris Barre plume (Carijó) (SOUZA et al., 2012); Índio Gigante; New Hampshire and Gigante Negra de Jersey (CRUZ et al., 2018) have been reported in the literature.

Table 2 - Mean values related to the muscle tissue of free-range broilers as a function of lineage (L), sex (S), and age at slaughter (A).

Traits		-----SF (kgf)-----		-----MD (um)-----		-----HCT (%)----		-----SCC (%)----		-----TCC (%)---	
		Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigl
Lineages (L)	Pelado[3]	2.83	2.41[a]	40.61	33.21[b]	1.10	1.09	0.22	0.61	0.42	1.89
	Pedres[4]	3.12	2.21[b]	41.02	36.67[a]	1.03	1.09	0.18	0.60	0.46	1.51
Sex (S)	Male	3.07	2.28	41.17	35.03	1.14	1.21[a]	0.23	0.57	0.48	1.63
	Female	2.88	2.34	40.45	34.85	0.99	0.98[b]	0.16	0.65	0.40	1.77
Age at Slaugter[1] (A)	65	2.36	1.87	36.58	32.94	0.88	0.80	0.19	0.61	0.49	1.87
	75	2.73	2.31	38.35	31.33	0.96	1.04	0.19	0.70	0.38	1.84
	85	3.67	2.56	42.64	36.84	1.11	1.23	0.23	0.50	0.49	1.59
	95	3.14	2.50	45.68	38.64	1.30	1.32	0.19	0.64	0.42	1.50
Significance[2]	L	n.s.	*	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	S	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.
	A	**a	***b	**c	**d	*e	***f	n.s.	n.s.	n.s.	n.s.
	LxS	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	LxA	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.
	SXA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	LxSxA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Parameters spans all columns after Traits.

[1]In days; [2]Scott–Knott test; [3]Pescoço Pelado; [4]Paraíso Pedrês; [a]SF_{breast}= 0.0327x + 0.3541 (P=0.001, R^2= 0.57); [b]SF_{thigh} = 0.021x + 0.598 (P > 0.0001, R^2= 0.77); [c]MD_{breast} = 0.3161x + 15.526 (P > 0.0001, R^2=0.97); [d]MD_{thigh}=0.226x + 16.846 (P > 0.0001, R^2=0.57); [e]HCT_{breast}= 0.0141x – 0.0655 (P > 0.0001, R^2= 0.97); [f]HCT_{thigh} = 0.3778x+0.0089 (P=0.008, R^2 = 0.87). SF – shear force, MD – muscle fibre diameter, HCT - histological connective tissue; SCC - soluble collagen content by chemical analysis; TCC - total collagen content by chemical analysis. $^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$.

The mean SF values of the thigh was different between the lineages, and the Pescoço Pelado lineage had the highest mean (Table 2). Similarly, SOUZA et al. (2012) reported a higher SF in Pescoço Pelado birds than in Pesadão and Carijó birds, and CRUZ et al. (2018) found differences between different lineages and their crosses.

In the literature, similar to the finding in this study, no effect of gender and tenderness of chicken breast meat has been reported (LONERGAN et al., 2003; SANTOS et al., 2005; FARIA et al., 2012). Conversely, FANATICO et al. (2005), CHEN et al. (2006) and CRUZ et al. (2018) reported higher SF values in the meat of male animals, and there was an indication of lower meat tenderness in comparison to female broilers.

The SF of both cuts increased linearly with age at slaughter (Table 2). SOUZA et al. (2012) also reported an effect of age at slaughter on the SF values of the breast meat of free-range chickens, reporting lower values at 70 and 85 days than at 110 days. CASTELLINI et al. (2002a) also reported increased meat hardness, with increased SF values, for chickens raised in an organic system from 56 to 81 days. A similar trend was observed for thighs, similar to the result of SOUZA et al. (2011), for which meat hardness was a function of age at slaughter. TOURAILLE et al. (1981b), by using a panel of judges to measure the effect of age on the sensory characteristics of free-range chicken meat, reported variations in texture and indicated a reduction in tenderness from the 8th to the 14th week, accompanied by a reduction in breast collagen content. In another study, TOURAILLE et al. (1981a) reported an effect of increasing age on tenderness reduction from the 9^{th} to the 16^{th} week, as reported by tasters and supported by an increase in the amount of collagen measured. In contrast, NAKAMURA et al. (2004) reported few differences in collagen contents (mg/g) in breast and thigh muscles of broilers ranging from 02 to 14 weeks old. Variations in collagen content in the muscles were associated with the presence of pyridinoline, a crosslinking amino acid of collagen fibres.

The finding of lower meat tenderness in the thighs of free-range broilers in the present study may be related to the increase in the amount of collagen and the reduction in the solubility of this protein as the age at slaughter increased (TOURAILLE et al., 1981a;b; ZANUSSO & DIONELLO, 2003; GOMIDE et al., 2013).

Breast and thigh MDs (μm) were not significantly affected by sex, but an effect of age at slaughter was observed, which showed a linear increase in the regression analysis (Table 2). Increasing fibre diameter with increased age was also observed by ONO et al. (1993) in different types of muscle fibres of the *iliotibialis lateralis* and *femorotibialis medius* muscles and by NAKAMURA et al. (2004) in the *iliotibialis lateralis* muscle.

The finding regarding the pectoralis major muscle (breast) is in agreement with results reported by ONO et al. (1993), who reported a progressive increase in the MD in birds from 1 to 35 weeks of age. DRANSFIELD & SOSNICKI (1999) in slow- and fast-growing broilers at 0, 11 and 55 weeks of age; and NAKAMURA et al. (2004) in broilers at 1, 2, 5, 11, and 14 weeks of age. Although, the total number of muscle fibres in this species remains unchanged after birth, the increase in muscle mass is due to the increase in fibre size by the hypertrophy process through the incorporation of satellite cells. They are the source of new nuclei that are incorporated into muscle fibres (ONO et al. 1993; DRANSFIELD & SOSNICKI, 1999; REHFELDT et al., 2000).

There was no difference between the broiler lineages in breast MD (Table 2). These results are in discordance with those of DRANSFIELD & SOSNICKI (1999), who observed larger MDs in the *pectoralis major* muscles in fast-growing broilers than in slow-growing broilers. SARTORI et al. (2003) reported that fibre size correlated with glycolytic metabolism in birds with fast growth rates; *viz.*, fast-growing birds are more inclined to develop muscle mass with glycolytic fibres –hypertrophy of fast-oxidative-glycolytic type fibres (intermediate), or fast-glycolytic type fibres (white)– than slow-growing broilers.

No significant differences were found in the breast or thigh MD as a function of sex (Table 2). SMITH & FLETCHER (1988) and MADEIRA et al. (2006) observed similar results for the respective cuts.

There was a significant difference in the thigh MD between the lineages, with a mean of 33.21 μm in the Pescoço Pelado lineage and 36.67 μm in the Paraíso Pedrês lineage (Table 2). The results in the literature indicated larger MDs in lineages with greater growth potential, an effect that is mainly observed for glycolytic fibres (MADEIRA et al., 2006).

In the thigh, analysis of variance revealed that the variables sex and age at slaughter had significant effects on the histological percentage of histological connective tissue (HCT), and there was an interaction between the factors of lineage and age at slaughter (Table 2). The HCT content of the thigh positively correlated with age (Figure 1). Nevertheless, the Pescoço Pelado lineage had a stronger correlation than the Paraíso Pedrês lineage at 65 to 95 days old. This may help explain the differences among lineages regarding thigh meat tenderness.

The histological evaluation of the connective tissue of the pectoralis major muscle of the breast revealed a linear increase with increasing age at slaughter, whereas no significant differences were observed for sex or lineage (Table 2). NAKAMURA et al. (2004), evaluated the architecture of the pectoralis major muscle and reported higher perimysium thickness values from the 11th to 14th weeks. According to PURSLOW (2005), this, along with an increase in the MD due to the hypertrophic process, occurs during animal growth.

Determination of the collagen content by the quantification of hydroxyproline and soluble fraction: Total collagen content (TCC) and soluble collagen content (SCC) in the breast and thigh meat were not significantly different between the bird lineages, sexes, or ages at slaughter (Table 2).

TOURAILLE et al. (1981a; 1981b) reported an increase in the percentage of connective tissue of meat from free-range chickens, accompanied by a reduction in tenderness with increasing age at slaughter. Thus, for the thigh cut, the differences in tenderness between the lineages could be related to the amount of collagen as determined by the histological technique because most of the connective tissue evaluated in the present study was composed of the perimysium structure, which represents more than 90% of intramuscular collagen tissue (McCORNICK, 1994). According to LEPETIT (2007), the increase in the amount of collagen and perimysium thickness may be related to a higher SF and tensile strength of chicken meat, which would explain the finding observed in the present study.

The effect of sex on the percentage of HCT in the thigh was verified (Table 2), and the male animals showed higher values. According to SOUZA et al. (2011), this is related to higher growth rates in males than in females.

We observed a moderate correlation of SF with the age at the time of slaughter ($P = 0.006$; $r = 0.389$) and a weak correlation with MD ($P = 0.039$; $r = 0.299$) in the thighs. Moderate correlations were reported for MD and SCC ($P = 0.032$; $r = -0.309$), TCC ($P = 0.033$; $r = -0.308$), and age at the time of

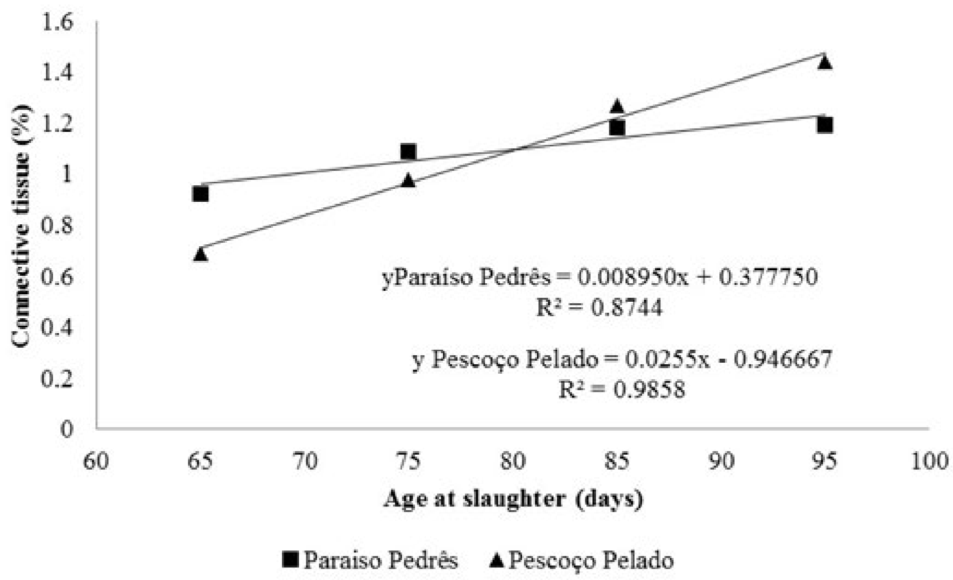


Figure 1 - Percentage of histological connective tissue (HCT) (%) in the *fibularis longus* muscle of the free-range chicken thighs as a function of age at slaughter (P = 0.0095).

slaughter (P = 0.001; r = 0.473). SCC in thighs was highly significantly correlated and TCC in thighs was moderately correlated with age at the time of slaughter (P = 0.001; r = 0.652; P = 0.027; r = -0.318, respectively). Regarding breast meat, weak correlations were reported for age with SF (P = 0.044; r = 0.292), SCC with SF (P = 0.045; r = 0.290), and SCC with MD (P = 0.04; r = 0.404), whereas strong correlations were observed for age with MD (P = 0.001; r = 0.616) and medium correlation to the SCC (P = 0.001; r = 0.490).

Although, no effect of the variables on the TCC or SCC chemically determined through the quantification of hydroxyproline was observed in the present study, the correlation analysis showed relationships between these variables and tenderness, which confirmed the results of LIU et al. (1996), who reported a strong correlation between the texture, TCC, and perimysium thickness of chicken meat.

Table 3 shows the factor weights of each variable in each component retained in the thigh and breast cuts, as determined by PCA. For thighs, two components were retained, accounting for 60.8% of the variability in the model. For breasts, three components accounted for 73% of the variability in the model. The number of components retained for each cut (*i.e.,* breast or thigh) followed the criterion of component selection when eigenvalues were greater than 1 (Figure 2). Initially, high values of commonality were observed for all variables, demonstrating that the three retained components were appropriate to describe the correlational structure between the variables.

Specifically, for thighs, it seemed that the first component imposed a high factor weight on the age of the birds, MD, and connective tissue. That is, the MD and the connective tissue content evaluated by histology tended to increase with increasing age. When analysing the second component, SCC tended to decrease as TCC and SF increased in the Paraíso Pedrês and Pescoço Pelado lineages in both sexes.

Comparison of the connective tissue and SF data revealed that an increase in the amount of connective tissue was accompanied by a reduction in the tenderness value of each cut. Thus; although, the results showed an increase in the connective tissue content in the cuts, the reduction in tenderness was related to the reduction in collagen solubility. This occurs because several factors are associated with the process of meat hardening, which is a function of collagen content, the presence of crosslinked muscle fibres, and MD (GOMIDE et al., 2013). The progressive increase in the percentage of total

Table 3 - Factor values and the percentage of variance explained for each item in the three retained components after performing the multivariate principal component analysis.

Cut	Item	Factors[1]			Commonality
		1	2	3	
Thigh[2]	Age at slaughter	**0.878**	0.061	-	0.774
	Shear force	**0.515**	**0.524**	-	0.540
	Diameter	**0.734**	-0.093	-	0.547
	Connective tissue	**0.715**	-0.127	-	0.528
	Soluble collagen by chemical analysis	-0.001	**-0.862**	-	0.742
	Total collagen by chemical analysis	-0.399	**0.597**	-	0.516
	Variance explained (%)	37.5	23.3	-	-
Breast[3]	Age at slaughter	**0.874**	-0.036	0.148	0.787
	Shear force	0.110	0.019	**0.892**	0.808
	Diameter	**0.809**	-0.035	-0.340	0.772
	Connective tissue	**0.743**	0.010	0.213	0.597
	Soluble collagen by chemical analysis	0.113	**-0.706**	-0.365	0.644
	Total collagen by chemical analysis	0.055	**0.853**	-0.197	0.770
	Variance explained (%)	33.5	21.8	17.6	-

[1]in bold: the highest factor weights for each component; [2] two factors retained; [3]three factors retained.

collagen with increasing age at slaughter and the reduced solubility of this protein were also reported by TOURAILLE et al. (1981a) and ZANUSSO & DIONELLO (2003) and in poultry meat.

Specifically, for thighs, the PCA showed that the SF also had a high factor weight for factor 1, which reflects the fact that the hardness of the meat depends on the age of the bird and the meat MD (Figure 3).

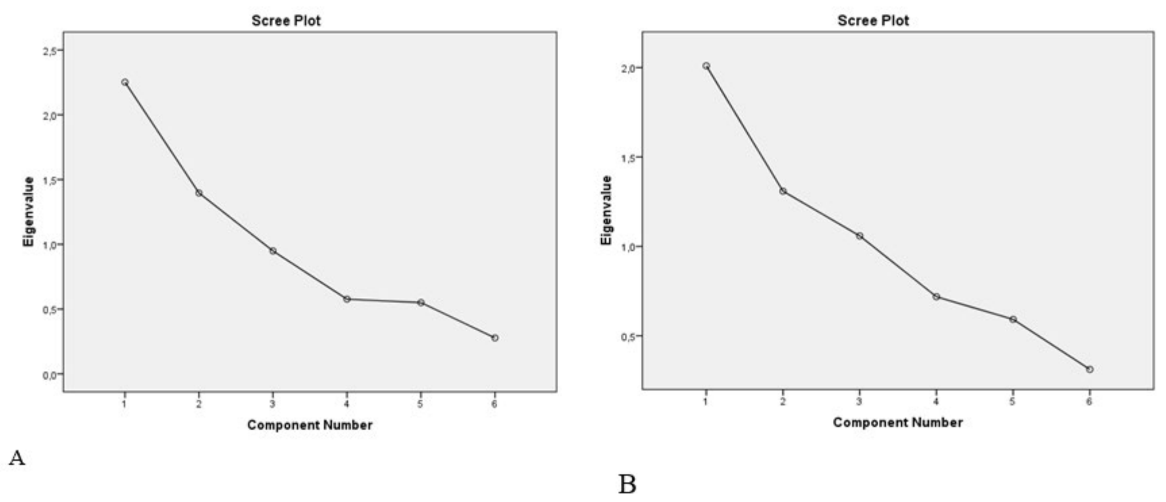
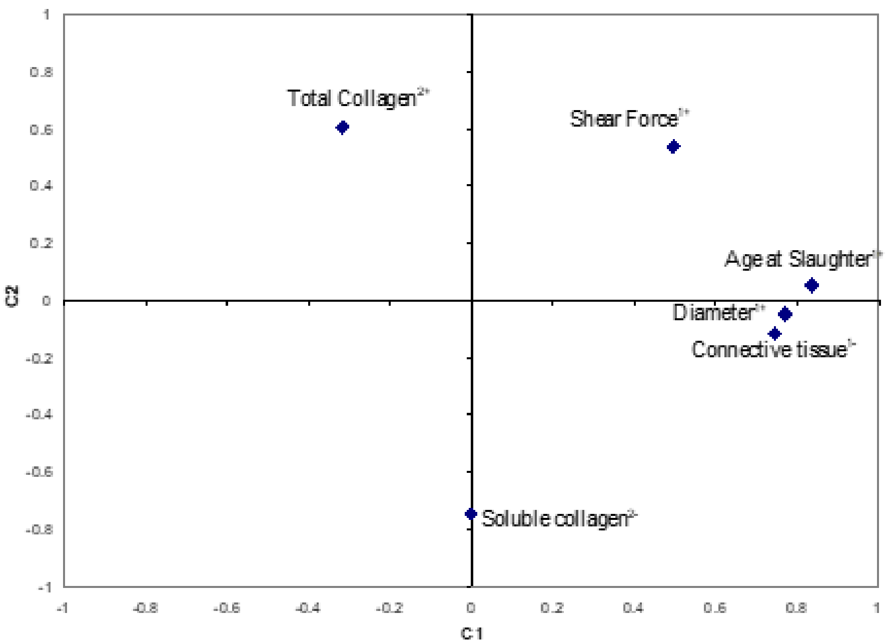


Figure 2 - Scree plot of the principal components of the factors related to the thigh (A) and breast (B) cuts of the Paraíso Pedrês and Pescoço Pelado lineages.



Cumulative inertia up to the 2nd component: 60.8%; Kaiser–Meyer–Olkin: 0.62; Bartlett <0.01. [1]variable mainly related to the 1st component; [2]variable mainly related to the 2nd component; [+]variable with positive sign in the component; [-]variable with negative sign in the component TOTAL COLLAGEN = determination of total collagen content as a function of the hydroxyproline level; SOLUBLE COLLAGEN = determination of the soluble collagen content as a function of the hydroxyproline level; CONJUNCTIVE TISSUE = determination of collagen content through histological analysis of the tissue by the picrosirius technique; DIAMETER = mean muscle fibre diameter; AGE AT SLAUGHTER = age of the birds of the different lineages at slaughter; SHEAR FORCE = determination of meat tenderness as a function of maximum cutting force.

Figure 3 - Main components of the factors related to the thigh cuts of birds of the Paraíso Pedrês and Pescoço Pelado lineages.

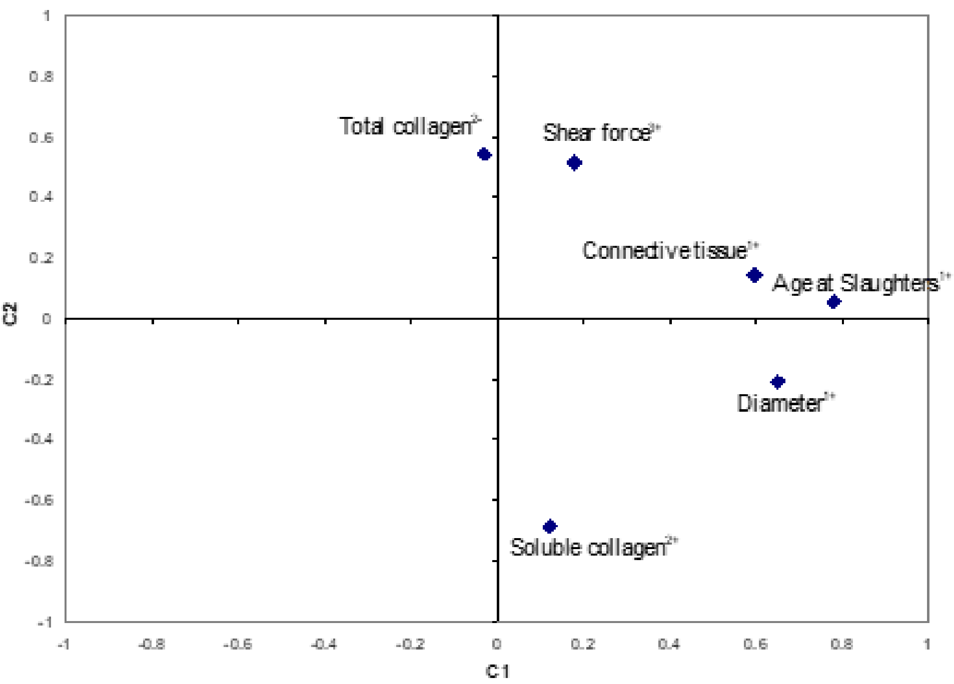
LAWRIE & LEDWARD (2006) stated that the amount of connective tissue in other species (cattle, pigs, and sheep) does not increase with age; although, an increase in meat hardness is evident and is related to reduced collagen solubility. YOUNG & BRAGGINS (1993) evaluated the relationship between tenderness and collagen amount and solubility and reported that collagen concentration was the main factor affecting the sensory outcome, while collagen solubility was best observed by objective analysis.

For breasts, the results were similar to those for thighs, except for the relationship between SF and the other variables, which were less pronounced in the breast than in the thigh since it was the only variable that still had a high factor weight for the third component (Figure 4).

As the MD increased, more connective tissue, which had a more fibrous appearance, was present. This effect is related to animal growth, where the muscle fibres increase in size due to hypertrophy. The endomysium and perimysium must be remodelled to accompany this development so that they do not limit the growth or the diameter increase, contributing to the formation of non-reducible bonds and an increase the tensile strength of the meat (LAWRIE & LEDWARD, 2006; HADLICH et al., 2006).

CONCLUSION

Age at the time of slaughter proved to be the most significant parameter affecting the physical



Cumulative inertia up to the 3rd component: 73.0%; Kaiser–Meyer–Olkin: 0.56; Bartlett <0.01. [1]variable mainly related to the 1st component; [2]variable mainly related to the 2nd component; [3]variable mainly related to the 3rd component; [+]variable with positive sign in the component; [−]variable with negative sign in the component

TOTAL COLLAGEN = determination of total collagen content as a function of the hydroxyproline level; SOLUBLE COLLAGEN = determination of the soluble collagen content as a function of the hydroxyproline level; CONJUNCTIVE TISSUE = determination of collagen content through histological analysis of the tissue by the picrosirius technique; DIAMETER = mean muscle fibre diameter; AGE AT SLAUGHTER = age of the birds of the different lineages at slaughter; SHEAR FORCE = determination of meat tenderness as a function of maximum cutting force.

Figure 4 - Principal components of the factors related to the breast cuts of the Paraíso Pedrês and Pescoço Pelado lineages.

characteristics of muscle morphometry and meat tenderness of breasts and thighs of broilers raised in a free-range system. Age was positively correlated with SF, muscle growth development, and connective tissue content in both thigh and breast cuts. Male specimens had a higher connective tissue content in the thigh than female specimens. Lineage differences in the tenderness of thigh meat were observed due to differences in the connective tissue characteristics between each lineage. Overall, low meat tenderness in free-range chickens is associated with larger MD, high connective tissue content, high TCC, and low SCC.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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