

Effect of Different Sources of Dietary Starch on Meat Quality, Oxidative Status and Glycogen and Lactate Kinetic in Chicken *pectoralis* Muscle

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Abstract We studied the effects of different dietary starch sources fed to poultry on the quality attributes and oxidative damage in fresh and aged chicken *pectoralis* muscle. In a corn-soya diet, 300 g.kg⁻¹ of the starch from ground corn was replaced by starch from broken corn, ground sorghum or pure starch, and fed for 11 days prior to slaughter to male broilers. In *pectoralis* muscle, pH rate, colour, drip loss, glycogen and lactate were measured at 10, 45, 90 minutes and 24 hours *postmortem*. Protein, lipid oxidation and haem iron were measured in fresh and aged meat. Sorghum starch caused a lower initial pH, while broken corn and pure starch gave higher pH in the *pectoralis* muscle. Ground corn and pure starch sources showed the lowest pH (45 min *postmortem*) indicating faster decline curves. Ground sorghum produced a lower level of residual glycogen in muscle and a lower rate of protein oxidation while the highest glycogen and a higher protein oxidation rate was observed with pure starch. Type of starch sources in diet received prior to slaughter affect the quality parameters in poultry meat. Particularly, ground sorghum improved meat quality whereas pure starch provoked a higher protein oxidation.

Keywords: *pectoralis*, poultry, starch sources, meat quality, lipid and protein oxidation

Cite This Article: Marta del Puerto, Alejandra Terevinto, Ali Saadoun, Roberto Olivero, and M. Cristina Cabrera, "Effect of Different Sources of Dietary Starch on Meat Quality, Oxidative Status and Glycogen and Lactate Kinetic in Chicken *pectoralis* Muscle." *Journal of Food and Nutrition Research*, vol. 4, no. 3 (2016): 185-194. doi: 10.12691/jfnr-4-3-9.

1. Introduction

Poultry meat is a high biological value protein food with a relatively low cost, which has led to increased consumption worldwide. Thus, poultry meat is a valuable alternative to red meat, especially in regions where under-nutrition or low-income conditions are increasing [1]. This demand has led to an increase in poultry production and the development of new "ready to eat" products with implications for meat quality as an important economic factor in the industry [2]. Quality attributes affect marketing, conservation and technological aptitude, while nutritional quality impacts the consumer [3,4,5]. The rate of pH decline during the *post mortem* period and its final value (ultimate pH) influence meat attributes (water holding capacity, colour) directly and the technological aptitude for processing indirectly. These attributes are defined in a very short period of time after slaughter and depend on the type of muscle, its metabolism and fibre composition, genetic factors, *peri-mortem* factors, pre-slaughter handling and nutritional status, with dietary glycogen as one of the main factors [2,6,7,8,9,10]. In avian pectoral muscle a fast pH drop is associated with slaughter conditions due to the glycolytic metabolism and

the type of fibres (Type IIB), which causes protein denaturation in the early post-mortem stages, with the result of poor water retention and less texture [2]. Also, in pigs, a high glycogen content at the moment of slaughter may lead to a rapid decrease in pH *post mortem* and a low pH_u, which thus reduces meat quality. However, a low muscle glycogen content *pre mortem* may result in a limited decrease in pH, a high pH_u and also DFD (Dark, firm and dry) meat. Thus, the optimal muscle glycogen content is somewhere between these two extremes [11,12].

The nutritional practices, specifically those related to the glycogen content prior to slaughter could be a short-term strategic approach to improve the nutritional quality of poultry meat. It was shown that cereals, the main starch source for birds, affect pH and colour of meat probably through the glycogen metabolism. In spite of the fact that Garcia *et al*, [13] show that the replacement of 500 g.kg⁻¹ of maize by sorghum lowered the pH of poultry meat and that Bee *et al*, [14] obtained a dark colour of meat of pigs with a diet low in digestible carbohydrates, no studies have been carried up with different sources of starch in diet at the time of slaughter and the impact on the parameters of quality and oxidative stability of poultry meat.

The objective of our research was evaluate the effects of the different available starch source in the diet (ground

and broken corn, ground sorghum and pure starch) fed prior to slaughter on the glycogen and lactate content, quality attributes, oxidative damage and nutritional compounds in fresh and aged chicken *pectoralis* muscle.

2. Material and Methods

2.1. Animals and Management

The animal care and handling were approved by the Honorary Committee on Experimental Animals of the Universidad de la República, Montevideo, Uruguay (CHEA) before the experiments started. The trial was conducted at the Agronomy Faculty de la Universidad de la República (UDELAR, Montevideo, Uruguay), following the human animal care and handling procedures, according to the accepted protocol. One-day-old male birds (Ross) obtained from a commercial hatchery were reared until forty-five days of age on a litter floor in a climate-controlled room with a photoperiod of 23 hours of light. They were fed *ad libitum* with a commercial corn-soya diet (212 g.kg⁻¹ CP; 13.35 MJ.kg⁻¹ of ME). Fresh water was provided *ad libitum*. At forty-five days, twenty-six birds were selected by weight and health appearance

and assigned randomly into four groups of six or seven birds each individually located in pens with floor litter (one bird/pen). Each group was fed with one of the experimental diets until sacrifice. All birds received water and food *ad libitum* throughout the whole period. At fifty-six days old, all the birds were sacrificed in an experimental abattoir. Pre-harvest handling and transportation (transportation time was 3 minutes) were in accordance with good animal welfare practices. Slaughtering procedures followed the CHEA accepted protocols.

2.2. Experimental Diets

A corn-soya based diet was formulated to meet the nutrient requirements for finishing male broilers following INRA, [15] and Larbier & Leclercq, [16] using ground corn as a starch source and was considered as one test diet (n=6). The other three diets were formulated by replacing 300 g.kg⁻¹ of the starch from the ground corn with starch from broken corn (n=7), ground sorghum (n=7) or pure starch (n=6) (Table 1). All experimental diets were iso-nitrogenous and iso-caloric. Dry matter, CP, crude fat, and crude fibre analyses of feed were carried out according to AOAC methods [17].

Table 1. Ingredient composition (g.kg⁻¹ as-fed basis) and chemical composition of experimental diets

Ingredient	Experimental diets			
	Ground sorghum	Broken corn	Ground corn	Pure starch
Ground corn	380.7	392.7	590.7	350.7
Broken corn	0.0	198.0	0.0	0.0
Ground sorghum	210.0	0.0	0.0	0.0
Pure starch	0.0	0.0	0.0	139.0
Wheat bran	0.0	0.0	0.0	95.0
Soybean meal	280.0	280.0	280.0	273.0
Meat bone meal	56.0	56.0	56.0	54.0
Blood meal	15.0	15.0	15.0	24.0
Vegetal fat	40.0	40.0	40.0	46.0
Calcium carbonate	4.0	4.0	4.0	4.0
Dicalcium phosphate	5.0	5.0	5.0	5.0
NaCl	3.0	3.0	3.0	3.0
DL-Methionine	1.8	1.8	1.8	1.8
Anticoccideal	1.5	1.5	1.5	1.5
VM premix ^(*)	3.0	3.0	3.0	3.0
Chemical composition				
Moisture	879.2	877.6	877.6	884.0
Crude protein	199.1	201.2	201.2	197.9
ME (MJ.kg ⁻¹) ⁽¹⁾	13.1	13.3	13.3	13.1
Crude fiber	23.0	22.5	22.5	26.4
Starch ⁽¹⁾	358.8	366.6	366.6	356.0
Sugar ⁽¹⁾	3.6	4.0	4.0	4.6
Crude fat	77.6	78.2	78.2	79.4
Calcium ⁽¹⁾	10.8	10.8	10.8	10.5
Available Phosphorus ⁽¹⁾	4.0	4.0	4.0	4.0

^(*)VM premix, Vitamin Mineral premix: Contained the following vitamins per 1.5 kg: 12,000,000 U.I. of vitamin A ; 2,000,000 U.I. of vitamin D3; 25,000 U.I. of vitamin E; 7.6 g of vitamin K; 5 g of vitamin B2; 10 g of Ca-D-pantothenate; 30 g of niacin; 0.5 g of folic acid; 13 mg of vitamin B12; 500 g of choline chloride; 0.5 g of vitamin B1 and 1 g of vitamin B6. Contained the following minerals per 1.5 kg: 90 g of manganese; 35 g of zinc, 25 g of iron; 2 g of copper; 2 g of iodine; 0.1g of cobalt and 0.1g of selenium. ⁽¹⁾ Calculated nutritional value (INRA, 1989).

2.3. Animal performance Parameters

Individual body weight at 45 and at 56 days of age was determined, and weight gain for the experimental period was estimated as the difference between the two measures. Individual daily food consumption and conversion efficiency (kg feed / kg gain) were also calculated. At 56

days, all the birds were slaughtered after fasting for 4 hours according to standards established by CHEA, making the sacrifice by cutting the jugular vein until total bleeding (3 min) to cause the least possible stress to the animal. Immediately after exsanguination, the liver was extracted (3 min) and the gallbladder was removed. The total fresh weight of each organ was measured followed by immediate storage at -80 °C until analysis.

2.4. Meat quality Parameters

The pH was determined at 10, 45, 90 minutes and 24 hours *post mortem* (maintained at 4°C) in the *Pectoralis* muscle from both sides, using a penetration pHmeter LT Lutron pH-201. Meat colour was determined (after 30 min bloom time) using the CIELAB method L*, a* b* at 10, 45 minutes and 24 hours *post mortem* (at 4°C) in the *Pectoralis* muscle from both sides (two readings on each) using a Minolta Lab CR-10 colourimeter. Measurement conditions were with illuminant D₆₅, an observation angle of 10° and an aperture size of 0.31 inches. The chroma ($C^* = \sqrt{a^{*2} + b^{*2}}$) and hue ($H^\circ = \arctan b^*/a^*$) were also calculated [18]. Water drip loss was determined by the weight difference in 2.5 g of *Pectoralis* muscle samples at 15, 45, 90 minutes and 24 hours *post mortem* from both sides [19]. Glycogen and lactate content were measured in *Pectoralis* muscles from both sides at 10, 45, 90 minutes and 24 hours *post mortem* (maintained in freezer at -80°C until analysis) and at 3 min *post mortem* for the liver. For both determinations, an 8 g meat sample was homogenized and extracted with 8 ml of HCl acid (4 N) for 2 hours at 100°C, filtered and neutralized by adding NaOH (4 N) until pH reached 6.5 – 7 [20]. Glycogen was determined as glucose total equivalents following Bergmeyer *et al.*, [21] using colourimetric diagnostic kits (1001201, Spinreact, Spain) and expressed as mg glucose/kg fresh meat. Lactate was assayed in the same hydrolysed slurry using commercially available enzymatic colourimetric diagnostic kits (LO-POD; 1001330; Spinreact, Spain) and expressed as mg lactate/kg fresh meat.

For the oxidation of lipids and proteins and Fe haem determination, samples of *Pectoralis* muscle (both sides at 24 hours *post mortem*) were stored in polyethylene bags at -30°C until analysis (fresh meat) or were vacuum packed, kept at 4°C for 5 days and then frozen at -30°C until determination (aged). Lipid oxidation was determined by the TBARS method with some modifications [22,23]. A 1.5 g sample of *Pectoralis* muscle was homogenized (1 minute, 8000 g) with 30 ml KCl (Mallinckrodt 6858), 750 µl EDTA and 300 µl BHT. An aliquot of 4 ml was taken and frozen at -20°C to determine protein oxidation. The rest was centrifuged at 2000 g, 10 min, 4°C. One millilitre of the supernatant was taken, added to 1 ml TBA-TCA and boiled for 30 min. It was then placed on ice for 5 min to stop the reaction and left at room temperature for 45 min. Then, 2 ml N-butanol ppa was added, vortexed and centrifuged at 3000 g for 3 min. The supernatant was measured colourimetrically at 535 nm. The results were given as mg of MDA/kg of fresh meat. Protein oxidation was estimated by the reactions between carbonyls and DNPH (2,4-dinitrophenylhydrazine) with the resulting formation of a Schiff base, which produces the corresponding hydrazone, quantified spectrophotometrically at 360 to 385 nm [23]. The determination was performed using the method of Mercier *et al.*, [24] with some modifications. From the homogenized mixture, 2 ml was taken and centrifuged at 2000 g for 10 min at 4 °C. Then, 2 ml of 20 mM DNPH in 2 M HCl was added, and the mixture was incubated for 1 hour at room temperature, vortexed every 10 min. Next, 2 ml of 200 g.l⁻¹ TCA was added and the mixture allowed to stand 15 min, vortexed every 5 minutes, then centrifuged at 2000 g for 10 minutes

and the supernatant removed. The pellet was washed 3 times with 4 ml ethanol-ethyl acetate (1:1) and centrifuged after each washing. After the third wash, the pellet was dissolved in 6 ml of guanidine 6 M HCl with 0.02 M KH₂PO₄ and incubated at room temperature for 15 min with regular stirring. The resulting mixture was centrifuged at 2400 g for 10 min, and the absorbance of the supernatant was measured at 370 nm. The concentration of DNPH was calculated using its molar extinction coefficient of 22.000 M⁻¹ cm⁻¹, and the results were expressed as nmol DNPH/mg protein. Protein content was determined at 280 nm in the homogenized mixture using bovine serum albumin (BSA) from Sigma Chemical Co. (St. Louis, USA) as a protein standard, as described by Stoscheck, [25]. For the Fe haem determination, the Hornsey, [26] method was followed with some modifications [5,27]. Samples of 1 g *Pectoralis* muscle were macerated in a mixture of acidified acetone in a glass tube, capped to prevent evaporation, and vortexed for 1 minute. After an hour of incubation at room temperature in darkness, the mixture was filtered through an ashless paper and the absorbance measured at 640 nm against acidified acetone. The Fe haem concentration was calculated from the content of haematin with a factor of 0.082.

2.5. Statistical Analysis

Data were analysed as a complete randomized design using the General Linear Model (GLM) procedure of NCSS (NCSS software release 2006, 329 North 1000 East, Kaysville, UT 84037) with the individual bird as the experimental unit. The model used for the ANOVA of oxidation measures and haem iron included diet, process (fresh or aged) and diet x process interactions as fixed effects. The data on pH, drip loss, glycogen and lactate in muscle at different times *post mortem* were analysed by repeated measures ANOVA with diet as a between-animal factor and time *post mortem* as a within-animal factor. Interactions are not shown if p>0.05. To determine differences for each parameter due to the diet at each time *post mortem*, we used one-way ANOVA. To analyse the performance parameters, we used one-way ANOVA. When appropriate, the Tukey-Kramer *post hoc* multiple comparison test (p<0,05) was used.

3. Results and Discussion

3.1. Animal Performance Parameters

To evaluate the effect on the animal response of different starch sources in diet prior to slaughter for a period between 45-56 days of age, 300 g.kg⁻¹ of the ground corn in a standard corn-soya diet was replaced with starch from broken corn, ground sorghum or pure corn starch, and the growth parameters were measured. The results are shown in Table 2. No significant effects were observed due to the source of starch in the parameters considered (p>0.05). Fernández *et al.*, [28], García *et al.*, [13] and Nyannor *et al.*, [29] reported no changes in weight gain, feed conversion and mortality in birds that received diets with replacement of corn by sorghum in proportions from 0 to 100 %, likely due to the similarity in nutritional value, crude protein and

metabolisable energy of the two cereals, which assisted in formulating rations with similar nutritional bases. A possible explanation for both the previous and current

results is that a lesser sensitivity to tannins is found in the later periods of the life production of the bird.

Table 2. Effect of dietary starch source on animal performance parameters.

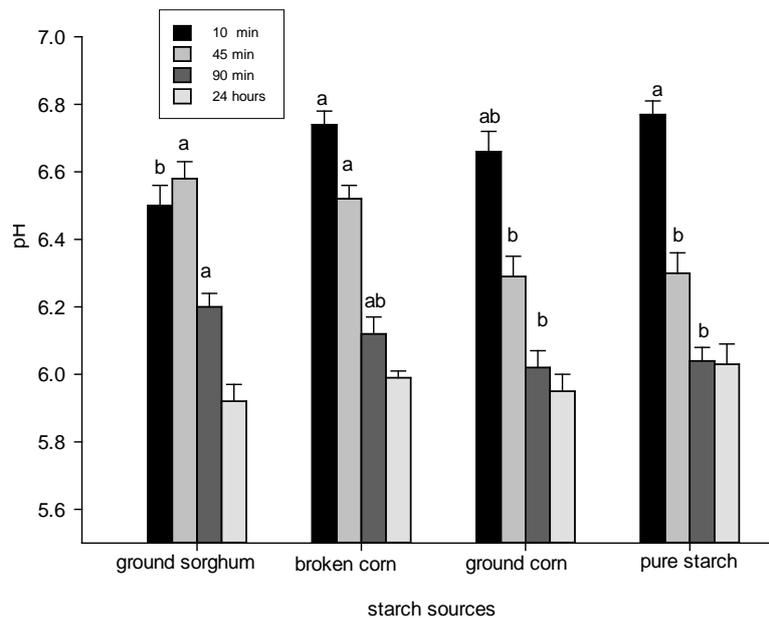
Source	BW G	FI g/d	WG g/d	FC	LW g	LW %
GS	2962±183	201.5±9.6	96.9±14.5	2.07±0.51	43.6±3.9	1.46±0.05
BC	2783±145	193.1±12.1	90.6±19.8	2.13±0.51	38.7±2.7	1.38±0.05
GC	3403±90	192.4±9.5	137.7±13.7	1.39±0.25	43.0±3.1	1.30±0.09
PS	3016±206	216.3±11.2	98±27	2.20±0.80	42.1±1.8	1.41±0.06
P	ns	ns	ns	ns	ns	ns

Data represent mean ± SEM of n = 6 (ground corn, GC; pure starch, PS) or 7 birds (ground sorghum, GS; broken corn, BC). ns = p>0.05. BW, body weight at 56 days old; FI, feed intake and WG, weight gain and FC, feed conversion, kg food/kg gain, at 45-56 days; LW, weight of liver in g and as a percentage of body weight, at 56 days old.

3.2. Meat Quality Parameters

3.2.1. pH. The pH was measured at 10, 45, 90 minutes and 24 hours *post mortem* in the *Pectoralis* muscle, in response to the starch sources received during the last 11 days before slaughter, and the data are shown in Figure 1. According to the results obtained and considering all *post mortem* times, the birds receiving broken corn exhibited a significantly higher average pH (6.34, p<0.05) than the birds receiving ground corn (6.23, p<0.05). Ground sorghum and pure starch showed a similar intermediate average pH (6.28 and 6.30, respectively). We can also observe a clear effect of time on the pH drop, as the values reached were all significantly different at 10, 45, 90

minutes and 24 hours (6.67, 6.42, 6.10 and 5.97, respectively). At 10 minutes *post mortem*, the muscles of the birds receiving broken corn and pure starch have the highest pH (6.74 and 6.77, p<0.05), whereas sorghum sources show the lowest (6.5, p<0.05). At 45 minutes, the source of sorghum and broken corn resulted in significantly higher pH in the muscles (6.58, 6.52, respectively; p <0.05) than the other two sources and, as the same results were found at 90 min *post mortem*, these sources could produce a slower pH descent curve. In birds, the decline in pH for the *pectoralis* muscle, rich in white fibres, can continue until 6-10 hours *post mortem*, and the pH_u rises to 5.4-5.6 [30].



Main effects:

Starch source (S) p<0,028

6,30 ± 0,03ab 6,34 ± 0,03a 6,23 ± 0,03b 6,28 ± 0,03ab

Time (T) p<0,001

10:6,67 ± 0,02a 45: 6,42 ± 0,02b 90:6,1 ± 0,02c 24:5,97 ± 0,02d

S x T p<0,0001.

Figure 1. Effect of dietary starch source on pH evolution at 10, 45, 90 min and 24 hours *post mortem* in the *Pectoralis* muscle. Data are the mean ± SEM for n=6 birds (ground corn, pure starch) or 7 birds (ground sorghum, broken corn). a,b,c,d, indicate significant differences by Tukey-Kramer test (p<0,05). Bars within a time group with a different letter above them differ significantly by one-way ANOVA and Tukey-Kramer test (p>0.05)

In our work, pure starch, which is highly digestible, induced a more rapid fall in pH, whereas starch from sorghum induced a slow fall. Garcia *et al*, [13] also observed lower initial pH values in the *pectoralis* muscle when birds were fed with diets containing more than 75 %

sorghum. No differences were found at 24 hours *post mortem* time between the sources. At this time, the individual values of the final pH ranged between 5.6 and 6.0, which was consistent with the values reported by Duclos *et al*, [7], Jones & Grey, [31] and Sams & Mills,

[32]. These values could indicate normal glycolysis [33]. The pH value at 45 minutes would be related to other quality parameters such as colour and water retention [33]. As the pH at 10 and at 45 min would be key to the relationship with water retention and colour, we expected that the two sources that led to slower pH fall also improved water retention and reduced colour loss. The rapid decline in pH in the *pectoralis* muscle from groups fed with pure starch or ground corn (at 10 and 45 minutes *post mortem*) was similar to the decline found in birds supplemented with glucose, which could indicate a greater amount of liver glycogen due to feeding with more rapidly degradable starch sources [34].

However, Bee *et al.*, [14] found no difference in the decline of pH when comparing diets with high and low carbohydrate digestibility. Animals fed with ground sorghum or broken corn presented a pH decrease curve similar to the one found in animals not supplemented with glucose, which leads us to think that both cereals behave as slower availability starch sources, as observed through *in vitro* digestibility tests (starch availability of 85.30 % for pure starch and 34.16 % for ground sorghum) [34,35]. It would be expected that, if the pH decrease curves are sufficiently fast, water retention would be adversely affected as the pH falls below 6 in meat that is still warm, which causes protein deterioration. The early pH drop may be due to a higher rate of degradation of the glycogen deposited in the animals fed diets of ground corn or pure starch or to an alteration in muscle buffer capacity. Henckel *et al.*, [36] proposed that significant effects on the

meat quality in pigs due to glycogen concentrations at slaughter could be expected when the level is below a critical threshold of 53 $\mu\text{mol.g}^{-1}$ of wet tissue.

The ability of sorghum to modulate the pH drop could be due to the action of tannins slowing down the starch digestion in broilers or to the protein in sorghum grain, whose amino acid cysteine in β - and γ -kafirins form disulphide bridges in the mature grain, reducing the digestibility of protein [37,29,38,39]. The effect on pH of broken corn could be due to the lesser digestibility of coarse particles of corn [40].

3.2.2. Glycogen and lactate kinetic. Glycogen and lactate produced in the *pectoralis* muscle were measured at 10, 45, 90 min and 24 hours *post mortem*, and the data are shown in Table 3. There is a clear effect of the sources on glycogen content, where the pure starch sources show a higher amount of glycogen (3.52 g glucose.kg⁻¹ of meat), whereas the ground sorghum gives the lowest value (2.09 g glucose.kg⁻¹ meat). There is also a time effect, showing a permanent decrease in glucose content from 10 minutes to 24 hours *post mortem*. No interaction effect was found between sources and time for these parameters. For each time, differences due to starch sources were found at 24 hours *post mortem*, resulting in higher glycogen with a pure starch source and lower glycogen with ground sorghum (1.29 and 0.67 g glucose.kg⁻¹ meat, respectively, $p < 0.05$). This higher amount of glycogen in the muscles of birds fed with a highly digestible source could be the cause of a lower pH at 45 min *post mortem*.

Table 3. Effect of dietary starch source on glycogen (g glucose.kg⁻¹ fresh meat) and lactate (g lactate.kg⁻¹ fresh meat) content in *Pectoralis* muscle at 10, 45 and 90 min and 24 hours *post mortem*

	Time p.m.	STARCH SOURCES			
		Ground sorghum	Broken corn	Ground corn	Pure starch
Glycogen (g.kg ⁻¹ meat)	10 min	2.99 ± 0.29	4.37 ± 1.02	3.54 ± 0.80	4.92 ± 0.32
	45 min	2.45 ± 0.71	3.76 ± 0.28	3.14 ± 0.43	4.31 ± 0.45
	90 min	2.24 ± 0.32	2.77 ± 0.59	2.90 ± 0.18	3.56 ± 0.50
	24 h	0.67 ± 0.10b	1.04 ± 0.13ab	0.92 ± 0.17ab	1.29 ± 0.20a
	S	2.09 ± 0.24b	2.98 ± 0.37ab	2.63 ± 0.28ab	3.52 ± 0.33a
Main effects	T	10 min	45 min	90 min	24 h
		3.95 ± 0.33ab	3.42 ± 0.27b	2.87 ± 0.22bc	0.98 ± 0.08c
Lactate (g.kg ⁻¹ meat)	10 min	0.62 ± 0.06	0.68 ± 0.08	0.71 ± 0.05	0.84 ± 0.06
	45 min	0.70 ± 0.08	0.81 ± 0.12	0.67 ± 0.09	0.75 ± 0.08
	90 min	0.83 ± 0.07	0.83 ± 0.06	0.85 ± 0.06	0.79 ± 0.12
	24 h	0.93 ± 0.07	0.83 ± 0.04	0.91 ± 0.06	0.81 ± 0.05
	S	0.77 ± 0.03	0.79 ± 0.03	0.78 ± 0.04	0.80 ± 0.04
Main effects	T	10 min	45 min	90 min	24 h
		0.71 ± 0.01b	0.73 ± 0.04b	0.82 ± 0.04ab	0.87 ± 0.02a

Data are the mean ± SEM of n=6 birds (ground corn, pure starch) or 7 birds (broken corn, ground sorghum). S=source; T=time. a, b, c, indicate significant differences among starch sources or each time by Tukey-Kramer test ($p < 0.05$). At each time, different lowercase indicate significant differences between sources by one-way ANOVA and Tukey-Kramer test ($p < 0.05$).

The data obtained for lactate (Table 3) show no differences among the sources and no interaction effects, but there was a clear effect of time on the lactate content in the *pectoralis* muscle. In liver (data not shown), the glycogen content at 3 min post exsanguination was higher in the ground corn diet (7.66 g.kg⁻¹ meat) and lower in the ground sorghum diet (5.85 g.kg⁻¹ meat; $p < 0.05$), while the lactate content did not differ between diets ($p > 0.05$). The *pectoralis* muscle has a high buffer capacity, demonstrated

by the fact that the same quantity of lactate is accumulated in muscle with different pH_u values [41].

However, in this work, pH_u did not differ between diets. Rosenvold *et al.*, [11] reported that a small decrease in muscle glycogen stores induced by a diet rich in low digestible carbohydrates resulted in a significant increase in pH_i and concluded that the diet, rather than the glycogen store in the muscle, is the main factor in the glycogen metabolism and/or composition of the muscle. In pigs, it was reported that a diet poor in available starch

could decrease the protein content and increase the lipid content in muscle [42].

3.2.3. Drip loss. Table 4 shows the effect of including different sources of starch in the diet of chickens on the water loss parameter in the *pectoralis* muscle, in samples obtained at 10, 45, 90 minutes and 24 hours after slaughter.

Table 4. Effect of dietary starch sources on drip loss (%) at 10, 45, 90 min and 24 hours post mortem in the Pectoralis muscle

Source	Time post mortem			
	10 min	45 min	90 min	24 hours
GS	5.48±0.77	3.91±0.97	2.56±0.35	4.41±0.47
BC	5.63±0.64	3.23±0.31	3.17±0.27	3.94±0.34
GC	4.98±0.64	2.80±0.68	3.67±0.72	4.32±0.45
PS	5.42±1.23	3.6±0.37	3.45±0.40	3.58±0.26
Main effects				
	GS	BC	GC	PS
S	4.09 ± 0.5	4.00 ± 0.36	3.94±0.48	4.03 ± 0.53
T	10 min	45 min	90 min	24hours
	5.38 ± 0.40a	3.40 ± 0.37b	3.21±0.25b	4.06±0.20b

Data are mean ± SEM of n = 6 birds (ground corn, GC; pure starch, PS) or 7 birds (ground sorghum, GS; broken corn, BC). S=source; T=time. a, b, indicate significant differences among each time by Tukey-Kramer test (p<0.05).

There was no source effect on the drip loss (p>0.05). There was a time effect (p=0.001), as water loss is clearly higher in the first 10 minutes post slaughter (5.38 %) versus 45 to 90 minutes and 24 hours (3.40, 3.21 and 4.06 %, respectively). The water holding capacity and the

aptitude for transformation are largely influenced by the final pH and its decay rate after the slaughter, as the decline of pH to its lowest value (isoelectric point of myofibrillar proteins; 5.4-5.6) is accompanied by a decrease in the net charge of proteins, causing a loosening of the myofibrillar protein network related to the decrease in electrostatic forces of repulsion between the protein filaments [43,44]. This development results in a transfer of water from the intracellular space to the extracellular space [45].

Consequently, a detrimental effect is noted on the taste of fresh or processed products in terms of juiciness and tenderness due to the loss of soluble proteins and haem groups [43,46,47]. The expected impact of the different sources of starch on the water loss as a consequence of the effect on the pH was not observed in this work. Although the pH decrease was moderated by the replacement of corn with ground sorghum or broken corn, the effect was not sufficient to reduce the water drip loss (up to 24 hours post slaughter). Garcia *et al.*, [13] reported an effect of sorghum on the drip loss when introduced at levels of 500g.kg⁻¹ and higher. A possible explanation of the lack of effect found in our work could be that the substitution was only of 300g.kg⁻¹.

3.2.4. Colour. The variation of colour in the *pectoralis* muscle of animals that received different sources of starch was measured immediately after slaughter and at 10, 45 minutes and 24 hours post mortem (Table 5).

Table 5. Effect of starch source included in diet on the colour, measured as L*, a*, b* in muscle Pectoralis at three post mortem times (10, 45 min and 24 hours)

Starch source	Time post mortem											
	10 min			45 min			24 hours					
	L	a*	b*	L	a*	b*	L	a*	b*			
Ground sorghum	43.8±0.4	0.78±0.55	22.2±0.2b	43.0±0.3	0.78±0.17	22.5±0.2	44.4±0.54	2.44±0.2	23.7±0.4b			
Broken corn	44.0±0.5	1.21±0.23	23.1±0.2a	43.5±0.5	1.32±0.36	23.7±0.3	45.8±0.54	2.78±0.2	25.1±0.4a			
Ground corn	43.5±0.2	0.80±0.14	22.7±0.2ab	43.2±0.2	0.93±0.19	22.8±0.2	45.1±0.31	2.27±0.2	24.0±0.3b			
Pure starch	43.80±3	0.77±0.17	22.1±0.3b	43.24±0.3	0.93±0.1	22.3±0.4	44.2±0.54	2.44±0.2	23.7±0.4b			
Main effects												
Source	Ground sorghum			Broken corn			Ground corn			Pure starch		
L	43.8 ± 0.3			44.5± 0.3			44.0 ± 0.2			43.8 ± 0.2		
a*	1.31 ± 0.2			1.76 ± 0.2			1.34 ± 0.2			1.55 ± 0.2		
b*	22.8 ± 0.2 b			23.8 ± 0.2 a			23.2 ± 0.2 ab			22.7 ± 0.2b		
Time	10 min			45 min			24 hours					
L	43.8 ± 0.2 b			43.3 ± 0.2 b			44.9 ± 0.3 a					
a*	0.90 ± 0.1 b			0.99 ± 0.1 b			2.60 ± 0.1a					
b*	22.5 ± 0.1 b			22.7 ± 0.1b			24.2 ± 0.2 a					

Data are mean ± SEM of n = 6 birds (ground corn, pure starch) or 7 birds (ground sorghum, broken corn). a, b, indicate significant differences among sources or each time by Tukey-Kramer test (p<0.05). At each time, different lowercase indicate significant differences between sources by one-way ANOVA and Tukey-Kramer test (p<0.05).

In poultry as well as in other species, colour is the single most important sensory attribute that affects consumer purchasing decisions. The colour of fresh or cooked meat is a very important factor in the buying decision made by the consumer, and this colour is often seen by consumers as an indicator of the freshness and overall quality of the meat [48,49]. The colour of meat is generally characterized by its chromaticity (pigment haem) and its surface brightness. Chromaticity depends on the physico-chemical state of the pigment, and the pigment concentration is dependent on biological factors, while the brightness depends on extrinsic factors (pre-slaughter conditions and manipulations after slaughter) [50,51]. Colour is usually expressed by three components: brightness, L*; redness, a*; and yellowness, b*. According to the data

obtained in our work for the pectoral muscle, the different starch sources in finishing diets for broilers did not change the brightness or redness of chicken meat at any of the times considered (p>0.05). The values found for redness, a*, increase with time (p<0.05), which could be explained by the chemical changes undergone by the tissues to establish rigor mortis. The a* values for the *pectoralis* muscle for all dietary starch sources evaluated correspond to the values reported by Fletcher *et al.*,⁴⁸, a* = 1.7-2.2 at 24 hours post slaughter.

As for the yellowish-green colour, determined by the variable b*, we observed that broken corn contributes favourably to the emergence of more yellow at 10 minutes and 24 hours post mortem (p> 0.05), perhaps because the larger particle size remains longer in the animal's digestive

tract and promotes the action of enzymes to improve absorption of the corn pigments. In the study of the proportion of red and yellow through the Hue parameter (Table 6), we note that in the *pectoralis* muscle, there was no effect of the source at any of the times considered. It is

noteworthy that the values found are in the same order (76.45 ± 1.63 to 76.03 ± 2.74 , in chickens fed diets containing $680\text{g}\cdot\text{kg}^{-1}$ corn and $244\text{g}\cdot\text{kg}^{-1}$ sorghum) as the results found by Ponsano *et al.*, [52].

Table 6. Effect of dietary starch source (ground sorghum, broken corn, ground corn, pure starch) on colour saturation measured as chroma (C^*) and color tone measured as angle hue (H°) in *Pectoralis* muscle at three times *post mortem* (10 min, 45 min and 24 hours)

Starch source	Time <i>post mortem</i>					
	10 min		45 min		24 hours	
	C^*	H°	C^*	H°	C^*	H°
Ground sorghum	22.2 b ±0.2	88.0±0.4	22.5±0.2	87.7±0.4	23.8 b ±0.4	83.9±0.4
Broken corn	23.1 a ±0.2	86.9±0.6	23.2±0.3	86.7±0.6	25.3 a ±0.4	83.8±0.5
Ground corn	22.7 ab ±0.2	87.9±0.4	22.8±0.2	87.7±0.5	24.2 ab ±0.33	84.6±0.5
Pure starch	22.2 b ±0.3	87.9±0.3	22.3±0.4	87.4±0.3	23.9 b ±0.3	82.9±0.7

Main effects				
Source	Ground sorghum	Broken corn	Ground corn	Pure starch
C^*	22.9 ± 0.2 b	23.9 ± 0.2 a	23.2 ± 0.2 b	22.8 ± 0.2 b
H°	86.6 ± 0.4	85.8 ± 0.5	86.8 ± 0.4	86.1 ± 0.5
Time	10 min	45 min	24 hours	
C^*	22.6 ± 0.1 b	22.7 ± 0.1 b	24.3 ± 0.2 a	
H°	87.7 ± 0.2 a	87.4 ± 0.3 a	83.8 ± 0.3 b	

Data are mean ± SEM of n = 6 birds (ground corn, pure starch) or 7 birds (ground sorghum, broken corn). a, b, indicate significant differences among sources or each time by Tukey-Kramer test ($p < 0.05$). At each time, different lowercase letters indicate significant differences between sources by one-way ANOVA and Tukey-Kramer test ($p < 0.05$).

With respect to saturation or purity of colour, the coordinate Chroma, both at 10 min and 24 hours *post mortem*, broken corn induced significantly higher values (23.13 and 25.29, respectively) than the other sources ($p < 0.05$) in the *pectoralis* muscle. One possible explanation is that the carotenoid pigments in broken corn would be more available in the digestive tract, resulting in a more vivid colour in the meat [52]. Ground sorghum and pure starch produced the lowest colour saturation in the *pectoralis* muscle. Garcia *et al.*, [13] studied the inclusion of sorghum replacing corn in broiler feeds and found a significant negative correlation between meat pH decrease and corn replacement. Sorghum inclusion also affected the meat colour, promoting paler meat.

According to Bressan & Beraquet, [53] meat colour is the factor most affected when replacing corn with sorghum. Garcia *et al.*, [13] reported no differences in the brightness parameter (L^*), but found decreases in redness (a^*) and yellowness (b^*) with increasing replacement of corn by sorghum. Although the colour in carcasses of chickens fed with sorghum could be affected by the lack of pigments, recent studies have shown that the tannins in the diet may slow the deterioration of colour in lamb under prolonged storage, although in this case the reason would be a

retarding effect on the appearance of methaemoglobin [54]. However, the study of Bee *et al.*, [14] showed that using diets with low-digestibility carbohydrates produced darker meat and less yellow colouration in pigs subjected to either transport or increased stress before slaughter but differentially affected different muscles according to their respective glycolytic potential.

3.2.5. Lipid and protein oxidation. Another aspect of our study was the oxidation of lipids and proteins in fresh and aged meat with respect to the source of starch in diet. There was no effect of starch source on the lipid oxidation (Table 7) regardless of the process of preserving poultry meat, expressed as TBARS ($\text{mg MDA}\cdot\text{kg}^{-1}$ meat). These results differ from the findings of Rajasekar *et al.*, [55] in rats fed diets with high added fructose, as that study recorded changes in lipid metabolism (significant increase in cholesterol, triglycerides and free fatty acids, lower level of phospholipids) and oxidative stress in their tissues (higher content of TBARS, lipid hydroperoxide and conjugated diene). To test this hypothesis, a profile fatty acids study could be desirable. In cereals, it is likely that a slower release starch allows assimilation and utilization, preventing the accumulation of glucose in plasma and thus reversing the previous effect.

Table 7. Effect of dietary starch source and process (fresh and aged) on the lipid oxidation, measured as TBARS ($\text{mgMDA}\cdot\text{kg}^{-1}$ meat) on the *Pectoralis* muscle

Process	Starch sources			
	Ground sorghum	Broken corn	Ground corn	Pure starch
Fresh	0.37±0.02	0.34±0.01	0.45±0.09	0.38±0.01
Aged	0.35±0.02	0.37±0.02	0.45±0.09	0.44±0.04

Main effects				
Sources	Ground sorghum	Broken corn	Ground corn	Pure starch
	0.36 ± 0.01	0.35 ± 0.01	0.39 ± 0.06	0.41 ± 0.05
Process	Fresh		Aged	
	0.38 ± 0.02		0.37 ± 0.02	

Data are mean ± SEM of n = 6 birds (ground corn, pure starch) or 7 birds (ground sorghum, broken corn). No significant effects due to sources or process were observed ($p < 0.05$). No significant differences between sources within each process were observed ($p > 0.05$).

Tannins in diets for rats reduced lipid oxidation almost as effectively as vitamin E supplements [56,57]. Du *et al.*,

[58] found that birds fed high-tannin sorghum (Sorghum Red Valpo) had lower TBARS values, so that the presence

of sorghum improved the oxidative stability of raw meat, probably due to the polyphenols that act as antioxidants. However, in our work, all of the treatments had the same effect on lipid oxidation. In relation to the conservation process, lipid oxidation is not affected by the conservation process regardless of the dietary source ($p > 0.05$). Poultry meat in cold storage (4°C) or in vacuum packaging has been reported to develop increased TBARS values even within a few hours post slaughter [59,60,61].

However, in our work, the vacuum produced a protective effect on lipid oxidation, showing equal TBARS values to fresh meat. In the study of the effect of starch sources on the oxidative deterioration of proteins (Table 8), it was observed that the diets with pure starch resulted in greater protein oxidation ($1.06 \text{ nmol DNPH}\cdot\text{mg}^{-1} \text{ protein}$), while broken corn and ground sorghum yielded significantly lower values (0.83 and $0.75 \text{ nmol DNPH}\cdot\text{mg}^{-1} \text{ protein}$) regardless of the conservation process. There was no effect of ageing on muscle protein degradation ($p > 0.05$). In fresh meat, pure starch sources showed higher values of protein oxidation, while broken corn had the lowest value (1.08 and 0.60 , $p < 0.008$). It could be concluded that the diets containing more available starch promote oxidative damage to the proteins in muscle. The processes of protein oxidation are linked to pro-oxidant factors similar to the ones observed for the oxidation of lipids, resulting in the loss of muscle function associated with a higher water loss and less stable gels [62,63]. As these oxidation processes are affected by the same factors as lipid oxidation, it is expected that the

increased availability of starch in the diet favours increased glucose in blood plasma and therefore increased protein oxidative damage [64]. In rat skeletal muscle with a low state of glycogen, Philp *et al.*, [65] found an increased lipid utilization and the transcription of genes regulating mitochondrial β -oxidation. This could be save the muscle protein oxidation as an adaptation mechanism to do face to stress at the time of slaughter. Then, we can hypothesize that a diet high in carbohydrates and a high level of glycogen in muscle immediately post slaughter lead to increased muscle protein oxidation. These findings are original results that show an effect of ground sorghum and broken corn incorporated in pre-slaughter diets on meat quality, perhaps related to the reduced availability of starch and not only the presence of tannins in sorghum.

3.2.6. Haem iron. Haem iron is an indicator of nutritional content, as it is the most bioavailable form of iron in meat and is also an indicator of loss of pigment during poultry meat processing [66]. There was no effect of diet on the haem iron content found in the *pectoralis* muscle (Table 9) ($p > 0.05$). We had expected a similar effect of the starch source on haem iron to the case of proteins, i.e., a lower presence of haem iron in meat chickens fed with pure starch, as in meat haem is mainly found in iron-dependent proteins. However, the alteration in muscle protein is not reflected in the haem iron, probably because the deterioration was not sufficient to alter these values. Luciano *et al.*, [54] found clear effects of dietary tannins on the conservation of haem iron, but the same result was not found in this work.

Table 8. Effect of dietary starch source and process (fresh and aged) on the protein oxidation ($\text{nmol DNPH}\cdot\text{mg}^{-1} \text{ protein}$) of the *Pectoralis* muscle.

Process	Starch source			
	Ground sorghum	Broken corn	Ground corn	Pure starch
Fresh	$0.81 \pm 0.03\text{ab}$	$0.60 \pm 0.12\text{b}$	$0.89 \pm 0.05\text{ab}$	$1.08 \pm 0.03\text{a}$
Aged	0.86 ± 0.07	0.90 ± 0.03	0.82 ± 0.04	1.04 ± 0.04
Main effects				
Source (S)	$0.83 \pm 0.05\text{b}$	$0.75 \pm 0.08\text{b}$	$0.85 \pm 0.04\text{ab}$	$1.06 \pm 0.03\text{a}$
Process (P)	Fresh 0.84 ± 0.06		Aged 0.91 ± 0.03	
SxP $p < 0.05$				

Data are mean \pm SEM of $n = 6$ birds (ground corn, pure starch) or 7 birds (ground sorghum, broken corn). a,b, indicate significant differences between sources, by a Tukey-Kramer test ($p < 0.05$). No significant differences between processes were observed ($p > 0.05$). Within each process, different lowercase letters indicate significant differences between sources, by one-way ANOVA and Tukey-Kramer test ($p < 0.05$).

Table 9. Effect of dietary starch source on Fe haem content ($\text{mg}\cdot\text{kg}^{-1}$ fresh meat) in the fresh and aged *Pectoralis* muscle

Process	Starch source			
	Ground sorghum	Broken corn	Ground corn	Pure starch
Fresh	3.99 ± 0.41	4.53 ± 0.36	3.66 ± 0.49	3.26 ± 0.56
Aged	5.08 ± 0.43	3.56 ± 0.33	3.85 ± 0.29	4.51 ± 0.71
Source	4.53 ± 0.32	4.04 ± 0.27	3.76 ± 0.29	3.82 ± 0.47
Process	Fresh 3.86 ± 0.23		Aged 4.25 ± 0.24	

Data are mean \pm SEM of $n = 6$ birds (ground corn, pure starch) or 7 birds (ground sorghum, broken corn). No significant differences due to source or process were observed ($p > 0.05$). Within each process, no significant differences between sources were observed by one-way ANOVA and Tukey-Kramer test ($p > 0.05$).

4. Conclusions

The effects of different starch sources in a pre-slaughter poultry diet (ground and broken corn, ground sorghum and pure starch) on quality attributes and oxidative damage in fresh and aged poultry *pectoralis* muscle were

studied. The starch source did not affect growth or feed consumption ($p > 0.05$), but several impacts on meat were observed. The replacement of $300\text{g}\cdot\text{kg}^{-1}$ corn starch with sorghum starch caused a lower initial pH (6.5 , $p < 0.05$), while broken corn and starch gave higher pH (6.74 and 6.77 , $p < 0.05$). After $45 \text{ min post mortem}$, ground corn and pure starch sources showed the lowest pH (6.33 and

6.29, $p < 0.05$), indicating faster decline curves. Colour was not altered by the starch source in terms of the L^* or a^* parameters, but broken corn gave higher b^* values at 10 min and 24 h post mortem. There was no effect on drip loss at 24 h. The pure starch source replacing ground corn was more deleterious in meat, and an increase in protein oxidation was observed in meat at 24 hours *post mortem*, which could be related to a rapid decrease in pH in the early stages *post mortem*, most likely due to a higher glycogen reserve in the liver at the pre-slaughter stage. Interestingly, ground sorghum and broken corn produced a slow fall in pH in the early stages *post mortem*, lower glycogen and lower protein oxidation. This effect of different sources of starch in the diet could be associated with the digestible carbohydrates for the chicken and could thus serve as a tool to modulate the post-slaughter responses of technological and oxidative parameters that affect the qualitative attributes of chicken meat.

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