Determinant production factors to the in vitro organic matter digestibility and protein oxidation of poultry by-product meal

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ABSTRACT The quality of poultry by-product meal (**PBM**) is not standardized in the industry. Several factors are detrimental to PBM and compromise its nutritional value and shelf life. Therefore, this study was conducted to determine the main PBM production factors that directly affect its in vitro organic matter digestibility (**IVDOM**) and protein oxidation (**POX**). Data on the processing of PBM samples (n = 100) were recorded in a rendering plant. Two types of PBM were used: 1) Low ash (LA, n = 66) with mineral matter (**MM**) content of 11% and 2) High ash (HA, n = 34) with MM above 11%. Processing traits and chemical composition of PBM were considered independent variables. The IVDOM and POX were determined in each sample and considered dependent variables. Data on independent variables were submitted to factorial and principal components (**PC**) analyses. In vitro organic matter digestibility data were clustered ($P = 0.001$) in low (778.92 g/kg), average (822.85 g/kg), and high (890.06 g/kg). The best arrangement was composed of six independent variables distributed in two PC, which explained 82.10% of the total variation. The ash concentration, oil to raw material ratio, collagen, and crude protein comprised PC1 with greater relevance and explained 58.46% of the total variance. The PC2 was composed of the processing time and temperature and explained 23.64% of the total variance. Protein oxidation data were clustered ($P < 0.001$) in low (265.19 nmol/mg CP), average (393.07 nmol/mg CP), and high (524.40 nmol/mg CP). Based on our results, the composition of the raw material from the slaughterhouse holds most of the information on PBM composition and digestibility. Developing improvements in the slaughtering or in the screening of the raw material that will be used by the rendering process is important to obtain a more nutritionally standardized ingredient.

Key words: animal by-products meal, in vitro digestibility, processing traits, protein carbonyl, poultry product

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INTRODUCTION

A balanced diet should be composed of ingredients or raw materials that favor high digestibility, to meet the nutritional requirements of animals. That nutrient composition and nutritional value of feedstuff are important factors is well established in the literature. However, information on digestibility is usually limited (Biagi et al., 2016). In this sense, physical-chemical composition analyses of ingredients and apparent digestibility coefficient calculations are techniques capable of predicting feedstuff nutritional value and quality. Thus, an adequate feed ingredient should provide greater nutrient content and high digestibility for optimal assimilation of nutrients (Berchielli et al., 2005).

Therefore, in vivo and in vitro digestibility analyses are important criteria for evaluating the availability of animal- or plant-derived feedstuffs individually or in diets (Malafaia et al., 2002). Besides helping to cope with the lack of resources to perform in vivo studies, the in vitro technique has contributed to the determination of digestibility with relevant acceptance by the scientific community (Volpato et al., 2022). Even though it is less reliable than in vivo, the in vitro technique reproduces the ability of digestive enzymes to hydrolyze proteins

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(Hervera et al., 2009). Estimates of protein digestibility among in vitro techniques were highly correlated to the pepsin enzyme determined via the in vivo technique in fish (Lewis et al., 2019)

Variations in the quality of ingredients, as well as in feed processing, have beneficial and detrimental effects on nutrient availability (Zentek et al., 2004) due to lipid or protein oxidation. In fact, lipid oxidation has been known over the years to reduce diet quality due to undesirable rancid odors and flavors developed during storage. Besides serious health-related problems, the causes and effects of protein oxidation have been extensively studied because they are not fully understood yet (Laudadio and Tufarelli, 2011; Ahmed et al., 2016).

Therefore, this study was conducted to determine the main poultry by-product meal production factors that directly affect its in vitro digestibility and protein oxidation.

MATERIAL AND METHODS

Two types of PBM were used: 1) Low ash (LA, n = 66, <11% MM) PBM produced from the viscera and intestines of poultry and 2) High ash (HA, n = 34, >11% MM) PBM produced from mechanically separated meat residues (**MSMR**). This classification was based on Abinpet guidelines (2019). The identification of the factors affecting PBM quality, from the raw material to the final product, was performed by following up manufacturing process in a rendering plant inspected by the Brazilian Federal Inspection Service located in Rolândia, PR, Brazil.

Processing Steps

The rendering plant produced its own raw material obtained from poultry slaughtering. The ingredients were previously washed using a rotational sieve (2.5 cm diameter) and then kept in a hopper until the processing.

During processing, the raw material was transported to a cooker (Julian D 500, Jaú, SP, Brazil) via an endless screw device. Quantities were based on the company's formula. Subsequently, a load of oil from the storage tank was added to the cooker to reach its maximum capacity (5,000 L). Then, the raw material was cooked at 110°C. Variations followed in the production flow and pressure.

Temperature and pressure data were recorded every 15 min. Thereafter, the material was released into the percolator and oil flowed by gravity. The still-hot material (70°C−90°C) was transported to the press and the excessive oil was removed. The material was then cooled, ground, and stored in silos.

Sampling and Analytical Procedures

A total of 100 PBM samples were collected as cakes without antioxidants. Samples were ground through a knife mill (Modelo R-TE-650/1, Piracicaba, SP, Brasil) equipped with a 1-mm at the Animal Nutrition Laboratory of the State University of Maringá and stored at −20°C for further analyses. Each PBM sample was obtained from one single batch (5,000 kg). Whenever two batches were used, the production of cookers 1 and 2 were released to the percolator at the same time to comprise one single sample.

Information related to the ingredients was recorded before thermal processing (raw material), during processing (cooking in cookers), and for the PBM product. An infrared digital thermometer (ST-700 Infrared digital thermometer, Incoterm, Porto Alegre, RS, Brazil) was used to determine the temperature of raw material, water, and visceral material when transferred to the cooker.

The processing time was considered as the interval between the beginning of thermal processing and the output from the cooker to the percolator. The processing temperature and pressure in kilogram-force (kgf) were recorded at every 15-min by sensors installed in the cookers.

Poultry by-product meal samples (n = 100) were analyzed according to methods described by the Association of the Official Analytical Chemists (AOAC, 2005) as follows: moisture (method 930.15), dry matter (**DM**), MM (method 942.05) organic matter, crude protein (**CP**, method 954.01), ethereal extract (**EE**) via acid hydrolysis (method 954.02), and in vitro digestibility. Residual concentration of synthetic antioxidants (BHA, BHT, and etoxiquin) that were added to the ingredients was determined as previously described by Yang et al. (2002) using a gas chromatograph equipped with a flame ionization detector (**GC-FID**). Collagen analysis was performed as previously reported by Ramos and Gomide (2017). Water activity (**AW**) was determined using specific equipment (Aqualab Pawkit - Decagon, Washington), with precision of ±0.02 and resolution capacity of ±0.01.

The in vitro digestibility coefficient of organic matter (**IVDOM**) was determined according to the method of Hervera et al. (2007) with a modification. Briefly, 0.50 g PBM was used instead of 0.75 g due to the amount of protein substrate to be digested. Protein oxidation was determined as described by Reznick and Packer (1994) and Özer and Seçen (2018) with some modifications. Briefly, 1 g PBM was weighed and placed in a 15 mL Falcon-type tube containing 9 mL sodium phosphate buffer solution (Synth, pH 6.5). The mixture was vortex-mixed and centrifuged (Excelsa, Baby II 206 R, Brazil) at 4,500 *g* for 1 min.

Approximately 2 mL supernatant were sampled and a 200 μL subsample were placed into a microtube containing 400 μL 2,4-dinitrophenylhydrazine (DNPH, Sigma-Aldrich) freshly prepared as described by Reznick and Packer (1994). A blank was prepared for each sample. Samples were kept in the dark for 1 h and, in the meantime, they were shaken every 15 min, for 10 s. Thereafter, 1 mL 20% trichloroacetic acid (**TCA**, Dynamic brand) was added to stop the reaction, and microcubes were then kept on ice for 60 min.

Subsequently, samples were centrifuged at 9,000 *g* for 10 min (MPW, 351R, Poland). Washing was performed

three times with ethanol:ethyl acetate solution (1:1) followed by centrifugation at 9,000 *g* for 4 min. Then, the supernatant was carefully discarded within a 10 min interval.

After the last washing, 900 μL 6M guanidine solution (Sigma-Aldrich) were added to the samples and microtubes were incubated in a 37°C water bath for 5 min. Thereafter, microtubes were vortexed for 10 s to have the pellet suspended and then centrifuged at 9,000 *g* for 10 min. Finally, absorbance was measured using an ultraviolet−visible spectrophotometer (Bioplus, Bio 2000, Brazil). Carbonyl content was expressed as nmol/mg protein with a 22,000 M^{-1} cm^{-1} extinction coefficient.

Statistical Procedures

Data were submitted to multivariate analysis to establish the relationship between independent variables and dependent variables. Data were analyzed using common factorial analysis, principal component (**PC**) analysis, Kaiser-Meyer-Olkin test, and Bartlett sphericity test (Lebart, 2000). Components with factor loading lower than |0.60| were discarded based on 60% minimum cumulative variance criteria (Favero et al., 2009; Hair et al., 2009). The number of components retained in the analysis was defined based on the minimum cumulative variance (60%) and eigenvalues >1.0 (Favero et al., 2009; Hair et al., 2009). A factorial indicator was generated for each PC.

Dependent variables were submitted to hierarchical clustering analysis using the Statistical Package for Social Science. Hierarchical clusters were submitted to variance analysis and Duncan's test. Significant differences were set at $P \leq 0.05$. Factorial indicators obtained from PC were used at first. Thereafter, components were unfolded, and values of independent variables were used.

Results related to processing variation, average, and types of PBM were analyzed via descriptive statistics.

RESULTS

Poultry by-product meal$_{LA}$ and PBM$_{HA}$ showed average values of 942.20 and 926.20 g DM/kg, respectively (Table 1).

Table 2. Principal component (PC) and percentage of variance explained by the components (% PC variation) of the process variables.

Component[†]	Eigenvalues	% of variance	% PC variation (Cumulative)
1	4.51	58.46	58.46
2	1.42	23.64	82.10
3	0.49	8.10	90.19
4	0.38	6.28	96.47
5	0.17	2.82	99.29
6	0.04	0.71	100.00

[†]Extraction method: principal component analysis.

Table 3. Eigenvectors associated with factor analysis of raw material characteristics, process parameters and quality of poultry by-product meal.

	Principal component (PC)[†]	
Variables	PC1 Composition	PC2 Processing
Ash	**0.96**	0.07
Oil to raw material ratio	**0.94**	0.16
Crude protein	**−0.92**	−0.08
Collagen	**0.82**	0.05
Time to processing	−0.01	**−0.88**
Average processing temperature	0.16	**0.85**

[†]Principal components forming the common factor analysis. The specific variables used in each PC are in bold in the same column.

Principal component results in multivariate analysis showed that the best arrangement was with 6 independent variables, distributed in 2 PC (Table 2).

The first PC was named composition and was comprised of ash, oil to raw material ratio, CP, and collagen. The second PC was named processing and was comprised of processing time and average processing temperature (Table 3).

The dependent variables, IVDOM and POX, were ordered in hierarchical clusters. In vitro digestibility coefficient of organic matter was clustered based on digestibility coefficients as follows: 1) low (778.92 g/kg), 2) average (822.85 g/kg), and 3) high (890.07 g/kg). After clustering, averages among the variables of each component within clusters were compared to identify the factors that most affected the IVDOM (Table 4).

The principal component composition influenced ($P < 0.001$) the dependent variable IVDOM (Table 4).

Table 1. Descriptive statistics of the chemical composition of poultry by-product meal.

	Maximum [g/kg]		Minimum [g/kg]		Average [g/kg]		CV [%][§]	
Variables	LA[†]	HA[*]	LA	HA	LA	HA	LA	HA
Dry matter	964.40	944.00	920.00	907.30	942.20	926.20	1.17	1.11
Ash	109.30	299.00	57.10	160.30	82.90	227.20	11.45	15.82
Crude protein	815.10	736.60	675.10	549.80	751.10	639.70	3.74	6.41
Ethereal extract	176.80	142.40	75.80	97.40	119.60	117.40	15.94	8.61
Water activity	0.53	0.62	0.26	0.28	0.39	0.45	17.94	16.57

[†]LA: low ash, n = 66.
[*]HA: high ash, n = 34.
[§]CV: coefficient of variation.

Table 4. In vitro digestibility values of organic matter (IVDOM) after grouping for the factorial indicators of the principal components and independent variables.

	Groups[†]					
Parameter	Low (n = 10)	Moderate (n = 54)	High (n = 36)	Total (n = 100)	SEM[*]	*P*-value
IVDOM [g/kg]	778.92[C]	822.85[B]	890.07[A]	842.66	4.171	<0.001
Components[§]	Groups[†]				SEM[*]	*P*-value
	Low (n = 10)	Moderate (n = 54)	High (n = 36)	Total (n = 100)		
Factor loading of composition on digestibility	-0.76[B]	-0.45[B]	0.88[A]	<0.001	0.100	0.001
Ash [g/kg]	81.28[B]	99.53[B]	197.58[A]	133.00	7.184	0.001
Oil to raw material ratio	0.14[B]	0.15[B]	0.24[A]	0.18	0.006	0.001
Crude protein [g/kg]	760.80[A]	734.32[A]	665.59[B]	712.22	6.258	0.001
Collagen [g/kg]	180.85[B]	200.43[B]	293.52[A]	231.99	9.083	0.001
Factor loading of processing on digestibility	0.01	0.07	-0.12	<0.001	0.100	0.650
Time [min]	85.10	84.13	82.31	83.57	1.996	0.887
Temperature [°C]	97.84	98.07	98.92	98.35	0.263	0.260

[†]Classification of groups according to in vitro digestibility of organic matter.
[*]Standard error of the mean.
[§]Principal components.
[A–C]Averages followed by different capital letters in the line, differ from each other by Duncan's test ($P < 0.05$).

Moreover, although all variables of this PC influenced ($P < 0.001$) the IVDOM, the PBM with the high ash and collagen concentrations showed the highest IVDOM.

The variables processing time ($P = 0.887$) and processing temperature ($P = 0.260$) of PC processing did not influence digestibility among the clusters. The average processing temperature was 99.1°C with a CV below 2% (Figure 1). The processing temperature averaged 98.4°C (ranging from 92.4°C and 104.4°C) for PBM_{LA}.

The IVDOM in PBM_{LA} was also not affected and averaged 821.50 g/kg (the lowest value was 745.50 g/kg and highest of 898.00 g/kg). In vitro digestibility coefficient of organic matter in PBM_{HA} was higher and average values were similar to the highest IVDOM (880.20 g/kg) observed in PBM_{LA} (Figure 2).

The processing time averaged 94 min (ranging from 61 and 125 min) and 79 min (ranging from 9 to 149 min) for PBM_{LA} and PBM_{HA}, respectively (Figure 3). This variable also did not affect nutritional quality of PBM.

Like IVDOM, protein oxidation was hierarchically clustered, on a DM basis, as follows: 1) low (265.19 nmol/g CP), 2) average (393.07 nmol/g of CP), and high (524.40 nmol/g of CP) (Table 5). After clustering, averages were compared among variables of each component within clusters in order to identify the most influential factors on POX.

Our results suggest that the processing did not affect ($P = 0.231$) POX. The processing time was correlated (r = 0.196, $P = 0.050$) with POX, according to a simple Pearson correlation. The results indicated that residual antioxidant from the raw material was negatively associated (r = −0.249, $P = 0.013$) with POX formation.

DISCUSSION

Poultry by-product $meal_{HA}$ showed lower CP content than PBM_{LA} due to the lower initial number of bones (Johnson and Pearson, 1997) that makes CP inversely

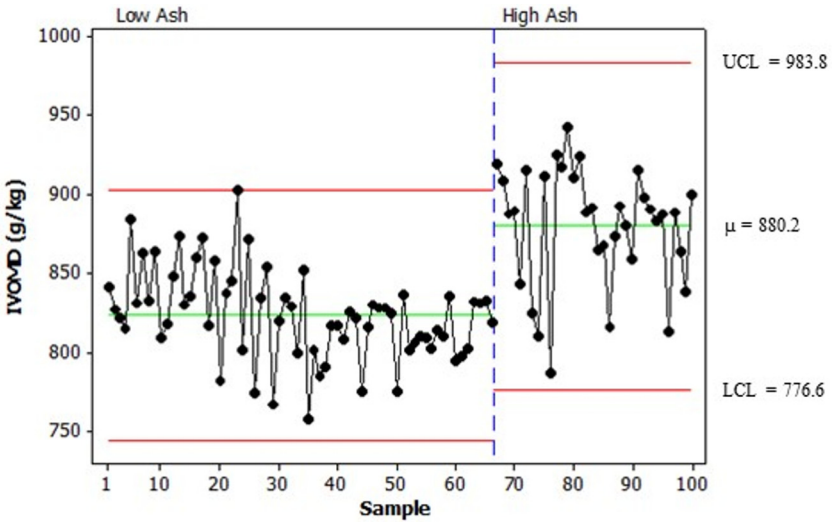

Figure 1. Processing temperature (°C) of obtaining the poultry by-product meal from the cookers.

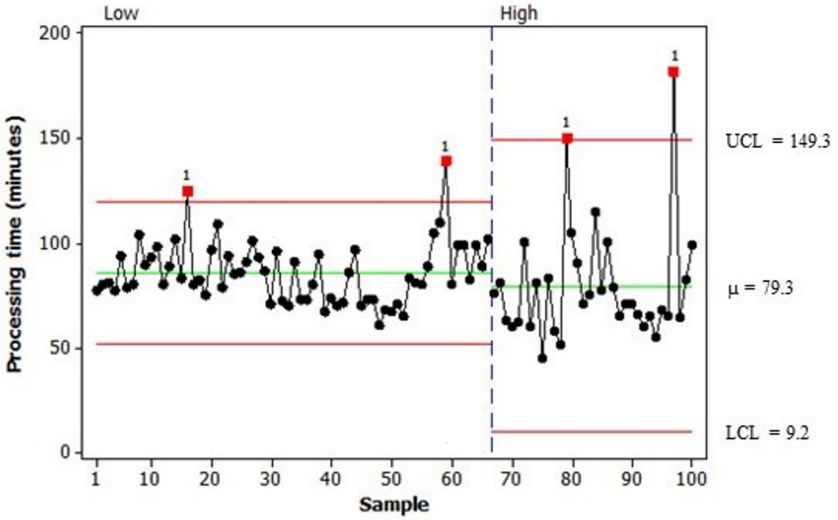

Figure 2. Values of in vitro digestibility of organic matter (g/kg) of the two types of poultry by-product meal.

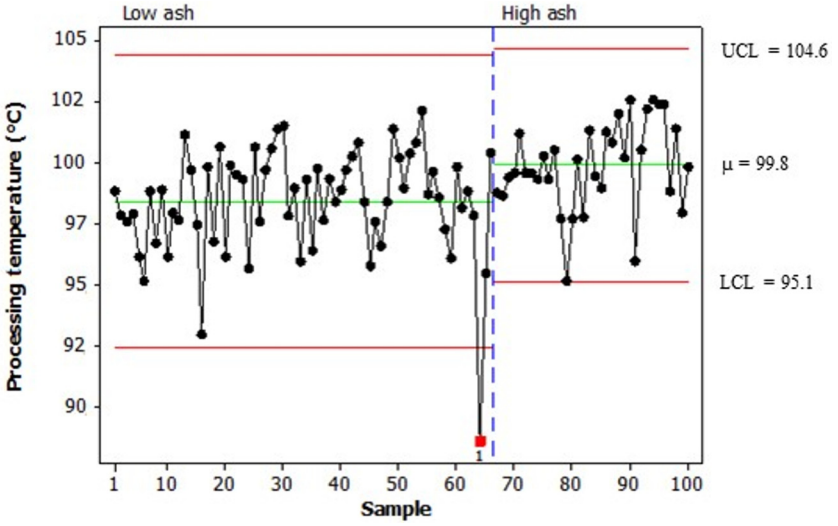

Figure 3. Relationship between processing time (min) and crude protein, mineral matter and in vitro digestibility values (g/kg) of poultry by-product meal.

Table 5. Carbonylated protein values after grouping for the factorial indicators of the main components and independent variables.

	Groups[†]					
Parameter	Low (n = 35)	Moderate (n = 51)	High (n = 14)	Total (n = 100)	SEM[*]	*P*-value
PCO [nmol/g CP DM][§]	265.19[C]	393.07[B]	524.40[A]	366.70	9.460	0.0001
Components[Δ]	Groups[†]				SEM[*]	*P*-value
	Low (n = 35)	Moderate (n = 51)	High (n = 14)	Total (n = 100)		
Factorial load of the composition	0.03	−0.08	0.10	<0.001	0.100	0.787
Ash [g/kg]	134.19	129.86	136.47	133.00	7.184	0.947
Oil to raw material ratio	0.18	0.18	0.18	0.18	0.006	0.926
Crude protein [g/kg]	709.15	722.98	696.51	712.22	6.258	0.361
Collagen [g/kg]	228.64	236.18	233.69	231.99	9.083	0.929
Factorial load of processing	−0.13	0.23	-0.10	<0.001	0.100	0.231
Time to processing [min]	85.93	79.50	85.17	83.57	1.996	0.319
Average process temperature [°C]	98.06	98.85	98.16	98.35	0.263	0.364

[†]Classification of groups according to protein oxidation in dry matter.
[*]Standard error of the mean.
[§]Protein carbonyl.
[Δ]Principal components.
[A–C]Averages followed by different capital letters in the line, differ from each other by Duncan's test ($P < 0.05$).

proportional to the content of ash (Nunes et al., 2005; Zarei et al., 2014). Poultry by-product meal$_{LA}$ and PBM$_{HA}$ showed 82.90 and 227.20 g ash/kg, respectively. Eyng et al. (2011) reported CP to ash ratio of 683 to 137 g/kg for fish meal and 561 to 239 g/kg for meat and bone meal. Similarly, Najafabadi et al. (2007) reported that PBM from three industries had a maximum of 634 g CP/kg with 79 g ash/kg and a minimum of 565 g CP/kg and 117 g ash/kg.

Ethereal extract average value was 110 g/kg. However, CV was 15.94% for PBM$_{LA}$ and 8.61% for PBM$_{HA}$. Nascimento et al. (2002) reported EE values ranging from 101.40 to 142.00 g/kg in PBM. The high variation in PBM$_{LA}$ could be explained by the type of raw material, the product formula, and even the press maintenance status. Indeed, Silva et al. (2010) reported that such variation may be related to the type of raw material when MSMR residues or only visceral material are used in the formula due to the content of water and bones. The type and conditions of the processing could also affect the chemical composition because may there be variations in pressure, temperature, and oil amount (Zhang et al., 2021). Thus, the chemical composition can be impaired by the processing (Abraha et al., 2018) and by the press capacity (kgf).

The average values of AW were 0.39 in PBM$_{LA}$ and 0.45 in PBM$_{HA}$. These values are within the reference range to prevent microbial growth. Water activity values up to 0.65 ensure safe feed storage. In fact, microbe growth is unlikely to occur when AW values are below 0.65 since the ingredient does not have enough water (Thomas et al., 1998; Hemmingsen et al., 2008). However, in a few PBM$_{HA}$ samples, average values of 0.62 AW, which is close to the reference value, were observed.

The percentages of explained variance and the extraction of PC corroborates those reported by Ribeiro et al. (2019), who also studied oxidative stability and nutritional values of PBM. The two PC explained 82.10% of the cumulative variance. Composition of the material that constitutes PC1 explained 58.46% of total variance. The variable of larger correlation showed that this characteristic affects PBM quality because they are totally different raw materials. In another study, Johnson et al. (1998) used processing temperature and ash concentration as two main factors that directly affect the quality of an ingredient. In this study, the composition of the ingredients was the factor that most affected the quality of PBM showing high eigenvalue (4.51).

The processing temperature, included in the second PC, was a contributing factor to the explanation of total variance. However, a low eigenvalue (1.42) was observed for it. This suggests processing temperature was well controlled within this industry whose this data was colleted and had low effect on PBM quality. This result differs from those found by Ribeiro et al. (2019) who conducted a similar study in 4 different industries. These authors reported that high temperature and processing time can be detrimental to IVDOM. Despite these differences, data from the present study showed that in an industry, where the processing conditions are more controlled, variations in the composition of the raw material are the main contributor to variation of the ingredient digestibility. This is corroborated by the low eigenvalue we observed for processing variables.

However, IVDOM was highly influenced by the chemical composition of the raw material. High ash and collagen concentration and high oil to viscera ratio had a positive association with IVDOM. This was expected since the amount of collagen is related to the inclusion of bone residues in the raw material entering the cooker (Macelline et al., 2020). Furthermore, despite having a low biological value (Bryan and Classen, 2020), collagen is a highly digestible protein component (Reutersward et al., 1985). A high oil to viscera ratio is also related to an increased IVDOM. This is due to the high-fat digestibility in non-ruminants, usually greater than 90% (Carciofi et al., 2009; Maria et al., 2017).

Unlike collagen and supplemental fat, the increase in CP reduced IVDOM. The fibrous protein concentration in PBM is important to be highlighted. Collagen is found in the skin and visceral sheaths and its digestibility is reduced when overheated during processing (Johnson and Pearson, 1997; Johnson et al., 1998; Hicks and Verbeek, 2016; Park et al., 2020). This could explain the effect on CP digestibility we observed in the present study. Thus, the raw material quality and the processing conditions must be closely controlled to reduce variations in the quality of the final ingredient. The variation in PBM composition depends on the traits and quality of raw materials (Ribeiro et al., 2019). The results suggest that the PBM samples classified as high digestibility are PBM$_{HA}$.

In the present study, we observed PBM from low and average IVDOM groups were classified as PBM$_{LA}$. The PBM$_{HA}$ were all ranked in the group of high IVDOM. Due to this, we cannot state that this classification was only due to higher level of ash in PBM$_{HA}$ but also related to the quality of raw tissues. It is possible that greater digestibility of PBM$_{HA}$ is affected by the type of raw material used for its production which is rich in collagen that comes from the bones, and muscle tissues that come from the deboning processing. Both are high digestible tissues, and probably presented a high contribution to include the PBM$_{HA}$ samples in the high IVDOM group.

Chang et al. (2011) observed greater solubility of collagen when heated to 90°C. This could be associated with the conversion of collagen into gelatin, which occurs in this temperature range. However, the digestibility and bioavailability of collagen from bone are still unknown and its structural components, such as secondary and tertiary structures, are especially susceptible to processing factors (i.e., pressure and temperature). Indeed, heating collagen over 100°C promotes complete unfolding of collagen matrix that along with grinding methods should be considered (Nawaz et al., 2020).

The lack of standardization of ingredients leads to different processing loads and conditions for each batch. This significantly affects the quality and digestibility of

PBM (Cramer et al., 2007; Kawauchi et al., 2014; Zarei et al., 2014; Ribeiro et al., 2019). Thus, in the present study, the oil to raw material ratio, as well ash and collagen concentration, correlated positively with IVDOM, where CP content presented a negative impact on IVDOM. Ribeiro et al. (2019) reported chicken oil ranging from 131.3 ± 80.6 to 161.4 ± 99.3 g/kg during the cooking process improved the IVDOM. This is corroborated by Ferroli et al. (2001) who reported that overheating and immediate frying of ingredients can occur when oil is not used or when a low quantity of oil is used.

In the rendering plant where the present study was performed, the classification relative to the type of PBM is based on the raw material having MSMR for PBM_{HA} and viscera (intestine and organs) for PBM_{LA}. Therefore, studies focused exclusively on factors that directly affect the quality of raw materials and process control procedures are required and should be performed. Bhaskar et al. (2014) analyzed the chemical composition of visceral material (60.67% CP, 8.93% ash, and 12.05% EE) and intestine (53.77% CP, 6.25% ash, and 10.41% EE) of poultry used as raw material for PBM production and obtained consistent PBM nutritional composition.

In the present study, the results indicated that the temperature was well controlled and did not affect the processing quality. However, Ribeiro et al. (2019) observed a direct influence on rendering time. They reported that increasing rendering was detrimental for the IVDOM in PBM. The greater initial temperature variation could be attributed to the final temperature of the previous batch. As this is a continuous process, the interval between unloading and new load in the cooker may be not enough to cool the cooker down.

In addition, nutritional composition was not impaired by processing temperature. Thus, the temperature was not a variable of interference to explain the total variation of the final product quality data. This variation was random according to changes in type and quality of ingredients. Similar studies were developed by Wang and Parson (1998) who also did not observe the effect of processing temperature on nutrient composition. Thus, similarly to the present study, the processing temperature did not explain lower digestibility of meals. These authors reported that the processing time was longer among meals, that is, the variable time was partially responsible for digestibility.

Awonorin et al. (1995) found that the temperature ranging from 130°C to 150°C and processing time from 90 to 120 min were ideal for production of a >56% CP PBM with reduced amino acid loss (<50%). Ribeiro et al. (2019) observed greater IVDOM in PBM when the average and maximum processing temperatures were coupled with longer processing times, and that the processing temperature should range from 100.6 ± 2.05°C and 106.0 ± 1.02°C.

Predicting what affected processing time is difficult since the processing, type of material, amount of steam, or even the operator of equipment may have contributed to this variation. This variable showed that quality and type of ingredients used are of great influence on the final product traits (Nascimento et al., 2002; Nunes et al., 2005; Carciofi, 2008). Ferroli et al. (2001) reported a processing time (140 min) about twofold longer than the one we observed for PBM_{HA} (79 min) when cookers with the same capacity (5,000 L). Thus, we cannot say there is an ideal processing time for PBM production. However, conditions of each equipment and cooker loading rate are to be considered.

Johnson et al. (1998) studied PBM_{LA} as a protein source in dog food and reported lower digestibility and availability of amino acids with greater processing time making PBM standardizing difficult. Therefore, manufacturers face a hard time controlling the variation in ingredients used for PBM production and also setting a method to choose an ingredient since this variability impairs digestibility and amino acid profile (Murray et al., 1998; Yamka et al., 2003).

The variations of protein carbonyl were not explained by the two PC because they did not present a direct effect on this variable. It was hypothesized that processing-related factors, especially temperature and time, would be correlated with POX formation. However, our results suggest that the processing did not affect POX. The quantitative differences we observed for oxidative-sensitive proteins in PBM were possibly due to the variation of the raw material. However, the amino acid content and their oxidized products (not analyzed in the present study) could help explain the POX formation.

The processing time was correlated with POX, according to a simple Pearson correlation. Cooking stimulates the formation of reactive oxygen species which can increase POX formation (Traore et al., 2012). The effects of temperature coupled with heating time may be related to antioxidant defense mechanism as protein denaturation increases. This provides higher iron releasing and free radical production. As a result, greater POX concentration at higher cooking temperatures is observed (Soladoye et al., 2015). In the present study, we observed that residual antioxidant from the raw material was negatively associated with POX formation, that is, the greater the residual antioxidant concentration in PBM the lower POX concentration. This highlights the importance of using antioxidants during processing as a protection against oxidative damage.

In recent years, the DNPH method has been applied to feed analysis even with a few restrictions. Carbonyl compounds are not only formed during protein oxidation, but also during lipid peroxidation, and Maillard reaction, and hence, protein oxidation could be overestimated. Besides, proteins must be soluble for spectrophotometric assays (Soglia et al., 2016; Hellwig, 2019).

The standardization complexity of raw material for PBM production is well established in the literature. Large carcass fractions, feet, and heads from poultry are added to PBM because rendering plants usually do not have a proper destination for them. Therefore, mixing it with visceral material is the fasted disposal option. In addition, the meal producing process is slow compared to abattoirs supplying capacity contributing to such

mixture or longer storage of ingredients. Altogether these may impair the quality of the final product.

Based on the criteria assessed in this study, the processing condition was less relevant for the IVDOM as suggested by PC2 (processing time and temperature) which accounted only for 23.64% of the PBM digestibility. On the other hand, PC composition was the most influential and showed that the composition and characteristic of raw material are the main contributors to nutritional composition and digestibility of the final product. Since all the samples were collected from the same rendering plant, it is important to consider that, regardless of the percentage contribution of the composition and processing PCs obtained in this study, both are important and, possibly, if the study were conducted in more than one rendering plant, the greater variations in processing between them could have a greater influence on the quality of the finished product. The assessment of protein oxidation by the 2,4-dinitrophenylhydrazine method was not able to show the effect of processing on carbonyls formation.

DISCLOSURES

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. All authors declare that they have no competing interests. All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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