R. Bras. Zootec., 53:e20220108, 2024
https://doi.org/10.37496/rbz5320220108

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Blood addition and processing conditions: Improving protein content and digestibility value of hydrolyzed feather meal

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Received: August 23, 2022
Accepted: October 18, 2023

How to cite: Sinhorini, M. R.; Balbinot-Alfaro, E.; Aguiar, W.; Ferreira, E. S.; Sbardelotto, P. R. R. and Alfaro, A. T. 2024. Blood addition and processing conditions: Improving protein content and digestibility value of hydrolyzed feather meal. Revista Brasileira de Zootecnia 53:e20220108.
https://doi.org/10.37496/rbz5320220108

Editor:
Mateus Pies Gionbelli

ABSTRACT - The study aimed to optimize the processing conditions for hydrolyzed feather meal (HFM) with the addition of coagulated blood. Factorial design and response surface methodology were used to optimize processing conditions in function of protein content (%) and digestibility value (%) of HFM. Higher protein contents were obtained when the upper levels of the variables pressure (kgf.cm^{-2}), hydrolysis time (min), and blood addition (%) were used in the process. Longer hydrolysis time and a higher percentage of blood result in higher digestibility values. Mathematics models can be used to estimate the protein content and digestibility value of HFM with coagulated blood. Hydrolyzed feather meal with higher protein content and digestibility value can be produced by adding coagulated blood and appropriate processing parameters.

Keywords: byproducts, coagulated blood, HFM, process optimization

1. Introduction

Brazilian chicken meat production in 2021 was 13.7 million tons (IBGE, 2022). Currently, Brazil is the world's second-largest producer of chicken meat and the largest exporter (ABPA, 2022).

The steady increase in the production volume and chicken slaughter raises questions about the appropriate destination of slaughterhouse waste (Tesfaye et al., 2017; Bezus et al., 2021). According to the Brazilian Animal Recycling Association, about 28% of the live weight of slaughtered birds is destined for recycling (ABRA, 2019). In this context, economic activities focused on the processing of animal byproducts are highlighted (Prajapati et al., 2021). In addition to representing an excellent business opportunity, the activity allows slaughterhouses to add value to the production chain and comply with environmental laws and policies (Sharma et al., 2018).

Hydrolyzed feather meal (HFM) is among the main proteinaceous materials obtained from poultry processing byproducts (Jayathilakan et al., 2012). Feather meal is a global commodity used to feed terrestrial and aquatic animals (Love et al., 2012) and to produce *Bacillus* spp.-fermented mixtures (Hong and Wu, 2021). However, the main limitation of its use as an ingredient in animal feed is the wide composition variation, protein quality, and aminoacids (Brumano et al., 2006). According to

Pfeuti et al. (2019), several studies report that the composition and digestibility of HFM are highly variable, making precise feed formulation difficult.

The protein content and digestibility value (DV) of HFM are the main attributes used to determine its commercial value and use as an ingredient in the feed formulation. The protein content of the feed influences productivity and profitability, which are essential in the supply of proteins and aminoacids in adequate quantity and quality for the animals (Rombola et al., 2008). Similarly, in the formulation of feeds, the percentage of protein digestibility is very important, as greater digestibility leads to better nutritional results, as well as a decrease in the loss of nutrients by excretion.

According to Cancherini et al. (2005), the aminoacid profile of a feed can be optimized when byproducts of animal origin are used. These byproducts have an aminoacid pattern similar to the animal's needs. Coagulated blood, an abundant byproduct in slaughterhouses, is a widely used alternative for this purpose.

It is known that the protein content and digestibility of HFM aminoacids are strongly influenced by process variables such as temperature, cooking time, and drying of the material (Sinhorini et al., 2021). The quality of HFM also depends on the proportion of raw materials, as it significantly affects its final composition (Latshaw, 1990). Therefore, determining the optimal processing parameters, the adequate percentage of blood added, as well as studying the combination of these variables in the process is vital to produce better quality HFM.

The objective of the present study was to optimize the processing conditions of HFM with the addition of coagulated blood, aiming at obtaining higher protein contents and DV.

2. Material and Methods

The experiment was conducted in Enéas Marques, PR, Brazil (25°56'45.1" S, 53°10'15.4" W). The HFM was produced with chicken and turkey feathers. Coagulated blood of poultries from slaughterhouses was used. All reagents used were of analytical grade (P.A.).

2.1. Characterization of coagulated blood

The protein content and the DV of coagulated blood were determined according to the Brazilian Compendium of Animal Nutrition (Sindirações, 2013).

2.2. Processing of HFM

The chicken feathers were processed in a digester (Prestatti, model DDP 13000) at pressures of 2.0, 2.25, and 2.5 $kgf.cm^{-2}$ and hydrolysis times of 20, 30, and 40 min. After each process, the material was subjected to a pre-drying step in the digester for 20 min. Subsequently, the material was unloaded in a percolator and conducted in a helical thread to the primary rotary dryer (Prestatti, model SPF 3000). Then, it went to the secondary rotary dryer (Prestatti, model SRP 5000). In the last step of the process, the material was milled in a hammer mill (Prestatti, capacity 4000 kg/h).

2.3. Dosages of coagulated blood

After loading the feathers, the measurement of coagulated blood was carried out in the digester. The added blood concentrations were 10, 15, and 20%. The proportion of feathers and coagulated blood used were: 5.400 kg of feathers for 540 kg of coagulated blood (10%), 5.100 kg of feathers for 765 kg of coagulated blood (15%), and 4.800 kg of feathers for 960 kg of coagulated blood (20%).

2.4. Hydrolyzed feather meal sampling

After packaging in 1500-kg capacity bags, the sampling of the HFM with the addition of blood was carried out. With a hand probe, 50 g of flour from 10 different points were removed. The quartering

method was used to reduce the sample of 500 g. The result of the sampling process provided an analytical sample of 75 g.

2.5. Protein content of HFM

The protein content of the feather meal was determined by the Kjeldahl method, recommended by the Brazilian Compendium of Animal Nutrition (Sindirações, 2013).

2.6. Digestibility value

The DV was performed following the guidelines of the Brazilian Compendium of Animal Nutrition (Sindirações, 2013). About 1 g of HFM sample was used to determine the DV. The sample and 75 mL of pepsin solution (0.02%) were added to an Erlenmeyer flask. The material was homogenized and incubated for 16 h at 45 °C under stirring. Subsequently, it was centrifuged for 10 min and filtrated. A 15-mL aliquot of the filtered material was transferred to a Kjeldahl macro tube. Then, the protein content was determined according to the Kjeldahl method. The percentage of crude protein in the supernatant (Equation 1) and the DV (Equation 2) were calculated.

$$CPS\ (\%) = \frac{[(V_1 \times CF_1 \times M_1) - (V_2 \times CF_2 \times M_2)] \times 6.25 \times 14 \times 100}{W} \quad (1)$$

in which CPS = % crude protein in the supernatant of the sample; V_1 = volume of 0.2M NaOH used in titration, in mL; CF_1 = 0.2M NaOH correction factor; V_2 = volume of 0.1M H2SO4 used in titration, in mL; CF_2 = 0.1M H2SO4 correction factor; M_1 = molarity of NaOH solution; M_2 = molarity of H2SO4 solution; 6.25 = conversion factor of N in protein; 14 = molar mass of N; W = sample weight in aliquot, in mg (1.00 g/75 mL × 15 mL).

Digestibility in pepsin:

$$\text{Digestibility in pepsin} = \frac{CPS \times 100}{CP} \quad (2)$$

in which CP = % crude protein of the sample.

2.7. Experimental design and statistical analyses

The experimental design and RSM techniques were used to verify the relationships between process variables and experimental responses. The variables, as well as their respective levels, were defined based on preliminary tests (not shown).

An experimental design 2^3 with three replicates of the central point (Barros Neto et al., 2007) was applied, a total of 11 trials. The independent variables were pressure ($kgf.cm^{-2}$), hydrolysis time (min), and added blood (%). The dependent variables (responses) were protein content (%) and DV (%) (Table 1).

Table 1 - Real values of the independent variables and their coded levels applied in the processing of the hydrolyzed feather meal with the addition of coagulated blood

Variable/level	−1	0	+1
Pressure ($kgf.cm^{-2}$) (X_1)	2.0	2.25	2.5
Hydrolysis time (min) (X_2)	20	30	40
Blood (%) (X_3)	10	15	20

The experimental data were analyzed with the Statistica 8.0 program for Windows (Statsoft™, Inc., Tulsa, USA). The statistical significance (P<0.05) of the 2nd order model was evaluated by the analysis of variance. The response surface plots were generated from the nonlinear model (Equation 3), represented as a function of two independent variables, while the other independent variable was kept at the central point.

$$Y = \beta_0 + \sum_{i=1}^{n} bixi + \sum_{i=1}^{n} biixi^2 + \sum_{i \neq 1}^{n} bijxixj + \varepsilon \quad (3)$$

in which Y is the dependent variable (protein content or DV), β_0 is the model coefficient, bi is the linear coefficient, bii is the quadratic coefficient, bij is the interaction coefficient, xi and xj are the independent variables in coded levels, n is the number of independent variables, and ε is the model error.

The deviations (Equation 4) and relative deviations (Equation 5) between the experimental values and those predicted by the models for the optimum conditions of each variable response were calculated:

$$\text{Deviations} = Y - \hat{Y} \quad (4)$$

$$\text{Relative deviations} = \left(\frac{Y - \hat{Y}}{Y}\right) \times 100 \quad (5)$$

in which Y = experimental response and $\hat{Y}$ = model predicted response.

3. Results

According to analytical tests, coagulated blood had a protein content of 89.50±0.01% and a protein DV of 92.34±0.09%. The protein content and DV of the HFM were evaluated according to factorial design (Table 2).

Three independent variables showed significant influence (P<0.05) on the protein content (Figure 1A). The DV of HFM was significantly influenced (P<0.05) by hydrolysis time and blood percentage and the quadratic terms of the pressure (Figure 1B).

The pressure variable (X_1), hydrolysis time (X_2), and percentage of added blood (X_3) significantly influenced the protein content. While the DV of HFM was significantly influenced by variables hydrolysis time, percentage of blood, and the quadratic term of pressure (Table 3).

Table 2 - Factorial planning matrix with results obtained for the protein content and digestibility value of the hydrolyzed feather meal with the addition of coagulated blood

Essay	Pressure (kgf.cm^{-2})	Hydrolysis time (min)	Blood (%)	Hydrolyzed feather meal[1]	
				PC	DV
1	2.0 (−1)	20 (−1)	10 (−1)	78.82	39.58
2	2.5 (+1)	20 (−1)	10 (−1)	79.2	40.12
3	2.0 (−1)	40 (+1)	10 (−1)	81.5	45.1
4	2.5 (+1)	40 (+1)	10 (−1)	83.2	45.39
5	2.0 (−1)	20 (−1)	20 (+1)	79.98	41.28
6	2.5 (+1)	20 (−1)	20 (+1)	80.74	41.73
7	2.0 (−1)	40 (+1)	20 (+1)	83.5	47.23
8	2.5 (+1)	40 (+1)	20 (+1)	84.56	47.96
9	2.25 (0)	30 (0)	15 (0)	80.45	41.55
10	2.25 (0)	30 (0)	15 (0)	80.92	41.68
11	2.25 (0)	30 (0)	15 (0)	81.35	42.6

[1] All results are presented in % and represent the average values for three determinations.

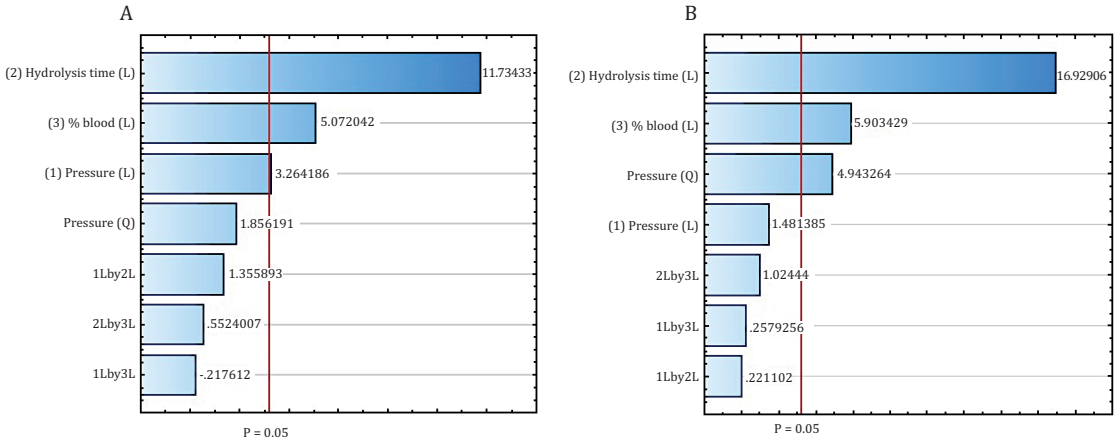


Figure 1 - Pareto chart of the effects of pressure (kgf.cm^{-2}), hydrolysis time (min), and percentage of blood (%) on the (A) protein content (%) and (B) digestibility value (%) of hydrolyzed feather meal.

Table 3 - Estimated effects on the protein content and digestibility value of hydrolyzed feather meal (HFM) with the addition of coagulated blood

Factor	Protein content of HFM	Digestibility value of HFM
X_1	0.97500*	0.50250
X_1X_1	1.06167	3.21083*
X_2	3.50500*	5.74250*
X_2X_2	-	-
X_3	1.51500*	2.00250*
X_3X_3	-	-
X_1X_2	0.40500	0.00750
X_1X_3	−0.06500	0.08750
X_2X_3	0.16500	0.34750

X_1 - pressure (kgf.cm^{-2}); X_2 - hydrolysis time (min); X_3 - % blood.
* Significant (P<0.05).
% explained variation for protein content of HFM; R^2 = 98.3.
% explained variation for digestibility value of HFM; R^2 = 99.1.

The analysis of variance (ANOVA) was performed for the experimental tests of protein content and DV. The F-test ensured the validity of the models to predict the protein content and DV of HFM (Table 4).

Table 4 - Analysis of variance (ANOVA) of the experimental design for the protein content and digestibility value of hydrolyzed feather meal

	Variation source	Sum of squares	Degrees of freedom	Mean squares	F-value calculated
Protein content	Regression	31.06	3	10.35	
	Residues	1.54	7	0.22	47.04
	Total	32.60	10		
Digestibility value	Regression	79.59	3	26.53	
	Residues	1.45	7	0.20	132.65
	Total	81.04	10		

*$F_{3;7;0.05}$ = 4.34.

The model (Equation 6) was generated in coded form, representing the protein content (PC) of HFM as a function of significant variables (P<0.05).

$$PC = 69.37523 + 1.95000\ (X_1) + 0.17525\ (X_2) + 0.15150\ (X_3) \quad (6)$$

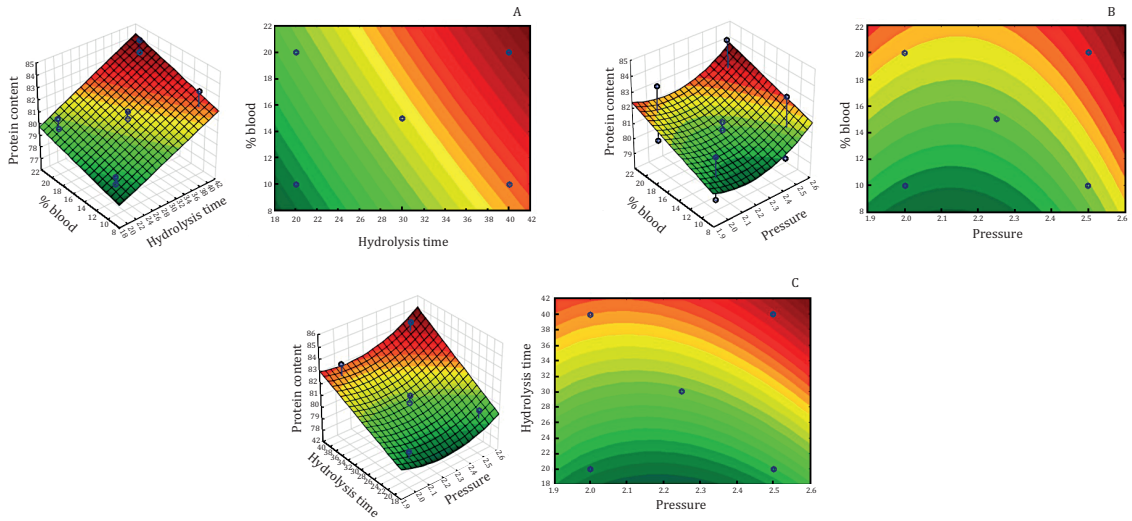
The increase in hydrolysis time and pressure, as well as the addition of more amount of blood, contributed to the rise in the protein content of HFM (Figure 2).

The model (Equation 7) for the DV generated as a function of the significant variables (P<0.05) is:

$$DV = 30.24324 + 0.28713\ (X_2) + 0.20025\ (X_3) \quad (7)$$

The increase in the hydrolysis time and the addition of higher percentages of blood promoted an increase in the DV. Lower DV of the HFM were observed at intermediate pressure values (Figure 3).

The predicted result in the optimal conditions (essay 8) was determined to assess the predictive ability of the model. The values predicted were similar to the experimental values, demonstrating that the models can be used to estimate the protein content and DV of HFM when obtained under these conditions (Table 5).

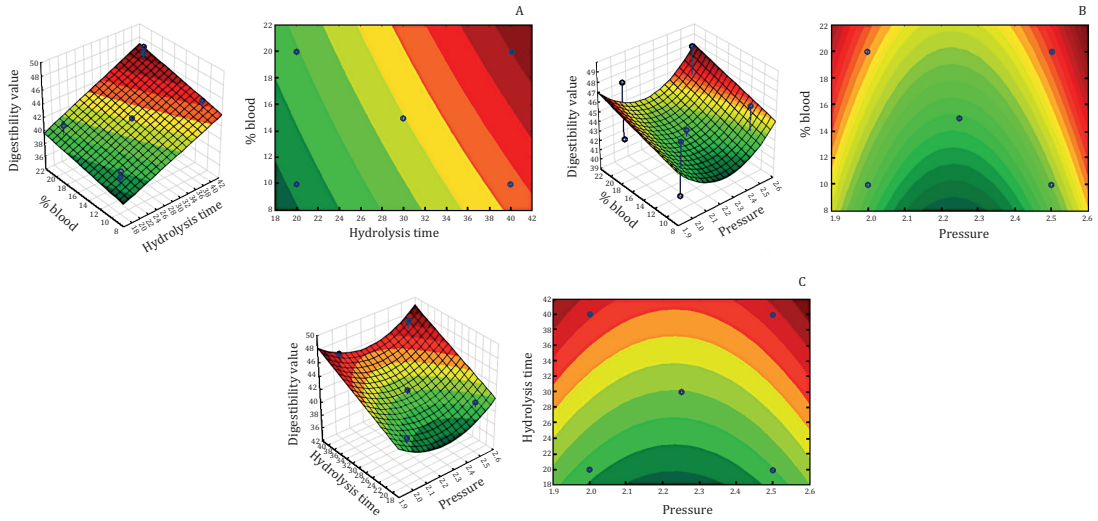


HFM - hydrolyzed feather meal.

Figure 2 - Response surface and contour diagram for (A) hydrolysis time (min) and percentage of added blood (%) (pressure fixed at 2.25 kgf.cm^{-2}) as a function of the protein content of the HFM; (B) pressure (kgf.cm^{-2}) and percentage of added blood (%) (hydrolysis time set at 30 min) as a function of the protein content of HFM; (C) hydrolysis time (min.) and pressure (kgf.cm^{-2}) (% blood set at 15%) as a function of the protein content of HFM.

Table 5 - Experimental results, predicted by the models, deviations, and relative deviations under optimal process conditions for protein content and digestibility value of hydrolyzed feather meal

Essay 8	Experimental results (%)	Predicted by the models (%)	Deviation	Relative deviation (%)
Protein content	84.56	84.29	0.27	0.31
Digestibility value	47.96	45.73	2.23	4.64



HFM - hydrolyzed feather meal.

Figure 3 - Response surface and contour diagram for (A) hydrolysis time (min) and percentage of added blood (%) (pressure set at 2.25 kgf.cm^{-2}) as a function of the HFM digestibility value; (B) percentage of added blood (%) and pressure (kgf.cm^{-2}) (hydrolysis time set at 30 min) as a function of the HFM digestibility value; (C) hydrolysis time (min) and pressure (kgf.cm^{-2}) (% blood set at 15%) as a function of the HFM digestibility value.

4. Discussion

The high protein content gives coagulated blood desirable characteristics for its use as a protein source in the production of HFM. However, the percentage of added blood must be controlled. According to the Brazilian Compendium of Animal Nutrition (Sindirações, 2013), the addition of blood is allowed provided its inclusion does not significantly alter the average composition of the finished product, as this inclusion would characterize another class the feather meal and blood.

The highest protein content (84.56%) and DV (47.96%) for HFM were obtained in essay 8, using the upper levels for pressure, hydrolysis time, and percentage of blood (Table 2). Within this context, it is observed that essay 1, which used the lower variable levels, presented the lowest protein content (78.82%) and DV (39.58). The effects of the variables and interactions on the protein content of HFM are observed in the Pareto graph (Figure 1A). The linear terms of the three independent variables showed significant influence ($P<0.05$), while the quadratic term of the pressure variable and the interactions of the variables did not significantly influence the protein content ($P<0.05$). The quadratic terms of hydrolysis time and percentage of blood showed redundant effects and were not estimated.

Similar to the results verified for the protein content, the highest DV (47.96%) for the HFM were obtained when upper levels of the variables pressure, hydrolysis time, and percentage of blood were used in the process (Table 2). The linear terms of the variables hydrolysis time and blood percentage and the quadratic terms of the variable pressure showed significant influence ($P<0.05$) on the DV of HFM (Figure 1B). The quadratic terms of the variables hydrolysis time and percentage of blood showed redundant effects and were not estimated.

The protein content of HFM was also significantly ($P<0.001330$) influenced by the variable hydrolysis time (X_2) (Table 3). The increase in hydrolysis time resulted in an average increase of 3.50% in the protein content of HFM (Figure 2A). Feathers are composed of approximately 1% fat, 9% water, and 90% structural proteins, mainly keratin (Sinhorini et al., 2021). According to Prajapati et al. (2021), keratin is a fibrous protein that is insoluble in water and plays a basically structural role. The mechanical

stability and resistance of feathers result from the packaging of protein chains in the forms of α and β keratin (Sharma et al., 2018), in addition to the formation of disulfide bonds between cysteine residues of adjacent polypeptide chains, thus increasing the resistance of the fibers (Said et al., 2018). The longer time possibly allowed complete hydrolysis of the feathers, resulting in higher protein content. According to Ghosh et al. (2016), insufficient times can lead to incomplete hydrolysis of the feathers, resulting in HFM with lower protein content.

The percentage of added blood (X_3) significantly influenced ($P<0.014800$) the protein content of HFM (Table 3). The addition of higher blood percentages increased the protein content of HFM by an average of 1.51% (Figure 2B). It is known that blood plasma is a good source of proteins of high biological value (Pereira et al., 2012), and therefore their addition not only increases the protein content, but also improves the protein quality of the HFM.

The pressure variable (X_1) used in processing had a significant influence ($P<0.046983$) on the protein content of HFM. The increase in pressure generated an increase, on average, of 0.97% in the protein content of HFM (Figure 2C). The increase in pressure possibly led to a more pronounced hydrolysis of the rigid fraction of the feathers, making it possible to break the bonds and, consequently, obtain a higher protein content. According to Pérez-Calvo et al. (2010), the protein content variability in feather meal is a result of differences in processing conditions, including the pressure used.

Hydrolysis time had a significant influence ($P<0.000449$) on the DV (Table 3). The increase in hydrolysis time contributed to the gradual increase in the DV of HFM, resulting in an average increase of 5.74% (Figure 3A). According to Said et al. (2018), protein digestibility is affected by the hydrolysis parameters used in the processing of feather meal.

The percentage of blood also had a significant influence ($P<0.009706$) on the DV (Table 3). The addition of higher blood percentages led to an average increase of 2% in the DV of HFM (Figure 3B). The high DV of coagulated blood (92.34%) may have contributed to the higher DV of the HFM that contained larger amounts of blood.

The DV of HFM was significantly influenced ($P<0.015881$) by the pressure variable (quadratic term). With the gradual increase in pressure, a decrease in the DV is observed, which at higher pressures increases again, reaching the highest values (Figures 3B and 3C). Thus, it appears that only the quadratic pressure term has a significant influence on the DV of HFM. Higher working pressures lead to an average increase of 3.21% in the DV (Table 3). Overall, it is noted that regardless of the applied pressure range, the DV tends to be higher with increasing blood percentage and hydrolysis time (Figures 3B and 3C).

In the analysis of variance (ANOVA) for the protein content of HFM (Table 4), the F-test ensured the validity of the model, since the calculated F presented a value 10 times higher than the listed value, for a 95% confidence interval. Therefore, it can be stated that the model is significant and predictive. Evaluating the statistical analysis results for the protein content variable, it can be seen that the linear terms of the variables pressure, hydrolysis time, and blood percentage were significant. The other variables and the interaction effects were not significant at a 95% confidence level. The model (Equation 6) was generated without the statistically non-significant parameters, which had an explained variation percentage (R2) of 95.2%.

In the analysis of variance (ANOVA) for the experimental design tests, with the DV of the HFM as the dependent variable (Table 4), the F-test ensured the validity of the model, since the calculated F was considerably higher than the tabulated F, for a 95% confidence interval. The model (Equation 7) for the DV was generated without the statistically non-significant parameters, presenting a percentage of explained variation (R2) of 92%.

The mathematical model presented a value of 84.29% for the protein content of the HFM, with a deviation of 0.27 from the experimental value (84.56%) (Table 5). The relative deviation between the experimental and predicted values for the protein content of the HFM was 0.31%. The protein content predicted by the model was very similar to the value obtained experimentally, thus demonstrating that under these conditions, the model can predict the protein content of the HFM.

For the DV, the value predicted by the model was 45.73%, with a deviation of 2.23 from the experimental value (47.96%). The relative deviation of 4.64% is acceptable for a mathematical model prediction. Therefore, we can infer that the model can be used to estimate the DV of HFM when obtained under these conditions.

5. Conclusions

The variables pressure, hydrolysis time, and percentage of blood have a significant influence on the protein content, while the hydrolysis time and percentage of blood have a significant influence on the value of digestibility of hydrolyzed feather meal. The values predicted by the models are similar to the experimental values, indicating that the models can be used to estimate the protein content and digestibility value of hydrolyzed feather meal. The addition of coagulated blood and suitable processing parameters increase the protein content and the digestibility value of hydrolyzed feather meal.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Sinhorini, M. R.; Aguiar, W. and Alfaro, A. T. **Investigation:** Sinhorini, M. R.; Balbinot-Alfaro, E.; Aguiar, W.; Ferreira, E. S.; Sbardelotto, P. R. R. and Alfaro, A. T. **Methodology:** Sinhorini, M. R. **Project administration:** Alfaro, A. T. **Resources:** Sinhorini, M. R. **Supervision:** Balbinot-Alfaro, E. and Alfaro, A. T. **Validation:** Sinhorini, M. R.; Ferreira, E. S. and Sbardelotto, P. R. R. **Writing – original draft:** Sinhorini, M. R.; Balbinot-Alfaro, E.; Aguiar, W.; Ferreira, E. S.; Sbardelotto, P. R. R. and Alfaro, A. T. **Writing – review & editing:** Sinhorini, M. R. and Alfaro, A. T.

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