Hydrolysis of chicken feathers for their use as a protein additive in cattle feed

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ABSTRACT

Objective: To evaluate diverse protein hydrolysis methods of chicken feathers meal on ruminal digestibility for its possible use in the elaboration of protein additives for animal consumption.

Design/Methodology/Approach: Four protein hydrolysis methods of chicken feathers meal were evaluated: thermal hydrolysis (TH), chemical hydrolysis (CH), acid-enzymatic hydrolysis (AH) and allkaline-enzymatic hydrolysis (BH). These methods were compared to chicken feathers meal without any treatment as a control into a completely randomized design.

Results: All hydrolysis methods reduced the protein content in feathers meal when compared to control ($p<0.05$). Crude protein contents were 97.3, 70, 74.1, 75.5 and 87.7 for Control, TH, CH, AH and BH, respectively. However, the highest value in digestibility was observed in CH ($p<0.05$); whereas the other methods showed digestibility values lower to 20% ($p<0.05$).

Implications: Given results show that CH provide higher contents of soluble and digestible protein, as well as higher hydrolysis.

Conclusion: These results demonstrate that chicken feathers meal hydrolyzed by chemical methods shows optimal conditions which makes it suitable for elaboration of protein additives to animal consumption.

Keywords: Keratin, ruminal fermentation, Drought, Chicken feathers meal, Protein additive.

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INTRODUCTION

The impacts of climate change are reflected in the agricultural sector in various ways (Kogan, Guo, & Yang, 2019). Prolonged drought periods, coupled with excessive overgrazing, have deteriorated pastures, leading to a reduction in their availability and nutritional value (Allen *et al.*, 2018). In Mexico, 36 million heads of cattle are produced under different systems. However, the droughts experienced in 2023 are atypical and have caused damages not seen in decades (SMN, 2023). Thus, the low nutritional quality of the pastures has forced producers to supplement the diet with protein and energy additives

to increase daily weight gains (Itavo *et al.*, 2008; Carvalho *et al.*, 2009). As a result, the demand for protein and energy additives has increased, offering products with a wide range of costs and utilizing a variety of ingredients for their formulation.

On the other hand, the poultry industry in Mexico has experienced significant growth in recent years, reaching a production of 4.6 million tons of poultry meat in 2020 (SIAP, 2022). Consequently, the waste or by-products of the industry have also increased. Such is the case with chicken feathers, which have a production of 192,000 tons per year (Florida-Rofner, 2019; UNA, 2021); these can represent up to 5.2% of the total weight of chicken meat. The main nutrient in feathers is protein, as they contain up to 83% on a dry matter basis; keratin constitutes between 85% and 90% of the total protein found in feathers (Alzamora *et al.*, 2018). However, despite feathers being a potential protein source, their high keratin content limits their use (Parzanese, 2018). Keratin is a fibrous protein that is insoluble and indigestible for monogastric animals, including humans. Additionally, it cannot be broken down by common proteolytic enzymes such as trypsin, pepsin, and papain (Adetunji *et al.*, 2012). However, it can be hydrolyzed by various methods, which may facilitate its utilization in the agricultural industry, particularly in ruminants (Morris *et al.*, 2020). Hydrolysis involves the breaking of the peptide bonds in the protein, leading to the generation of new proteins with lower molecular weight (Sánchez-Villafuerte, 2018). In this sense, hydrolyzed keratin can be used as a source of protein. Based on the above, the objective of this study was to evaluate various methods of hydrolyzing chicken feathers and their potential use as a protein feed for cattle.

MATERIALS AND METHODS

Study Site

The study was conducted in the laboratory of the Faculty of Veterinary Medicine and Zootechnics (FMVZ) at the Juárez University of the State of Durango (N 23° 57' 21.535", W 104° 34' 24.419") and in the Graduate Unit for Research and Technological Development at the Technological Institute of Durango, in Durango, Mexico (N 24° 03' 60.756", W 104° 64' 87.926").

Obtaining and Treatment of Feathers

Chicken feathers were collected from a poultry farm located in the municipality of Durango, Durango. After being washed with running water and commercial detergent, they were dried in a convection oven (FE-294A, Felisa, Mexico) at 55 °C for 48 hours. Subsequently, they were ground using a Willey Mill 4 (Thomas Scientific, USA) and sieved with a 2 mm mesh to obtain feather meal, which was used in the different hydrolysis methods. Additionally, untreated ground feather meal was used as a control.

Thermal Hydrolysis

To carry out thermal hydrolysis (TH), the feather meal was subjected to a sterilization process for 5 hours at 115 °C and 1 atm of pressure. Subsequently, the meal was dried in a convection oven (FE-294A, Felisa, Mexico) for 48 hours at 55 °C for further analysis (Papadopoulos, 1985).

Chemical Hydrolysis

In the chemical hydrolysis (CH) process, 150 g of feather meal were immersed in 600 ml of 1 M sodium hydroxide (NaOH) for 36 hours at 25 °C. Subsequently, the pH was neutralized with a 1 M acetic acid solution (Bauza *et al.*, 2009). The meal was then dried in a convection oven (FE-294A, Felisa, Mexico) for 48 hours at 55 °C for further analysis.

Enzymatic Hydrolysis

Commercial proteolytic enzymes donated by a local company (ENZIQUIM, Mexico) were used. According to the technique described by Viloria *et al.* (2019), the chicken feather meal was immersed in a 0.1 N NaOH solution (pH=8) for alkaline enzymatic hydrolysis (BH) and in a 20% v/v H_2SO_4 solution (pH=3) for acid enzymatic hydrolysis (AH), respectively. For BH, 380 μL of alkaline enzyme (Protease HA 2x, ENZIQUIM, Mexico) was added for every 10 g of feather meal, while for AH, 380 mg of acid enzyme (Acid Protease 25,000, ENZIQUIM, Mexico) was added for every 10 g of feather meal. Both hydrolysis processes were incubated with agitation (200 rpm) at a controlled temperature of 55 °C for 4 hours; the pH was adjusted every 30 minutes during each process. Once the incubation and agitation were completed, the meals were filtered and dried at 55 °C for 48 hours for further analysis.

Protein Determination

The products obtained from the different hydrolysis processes were subjected to crude protein analysis (AOAC, 2001). Additionally, the feather meal was also analyzed for organic protein using the Bradford method, with bovine serum albumin (BSA) used for the calibration curve (Bradford, 1976).

All hydrolyzed meals were also analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) in a Mini-PROTEAN® Tetra cell electrophoresis chamber. The applied potential difference for the electrophoretic run was 25 volts, and the time employed was 2.5 hours. Protein staining was performed using Coomassie Brilliant Blue R-250, and destaining was conducted with acetic acid and methanol diluted in distilled water (Hernández *et al.*, 2012).

***In Vitro* Digestibility**

To evaluate the *in vitro* dry matter digestibility (IVDMD), a Daisy incubator (Ankom Technology, USA) was used. Samples were placed in F57 nylon bags (ANKOM, USA) submerged in a mixture of ruminal fluid and buffer solutions at a 1:2 ratio for 48 hours. The ruminal fluid was donated by two Creole cattle with ruminal fistulas, fed a maintenance diet consisting of alfalfa and concentrate (50:50). The analyses were conducted in triplicate and followed the methodology proposed by the manufacturer (Ankom Technology, USA).

Statistical Analysis

The obtained data were analyzed using a completely randomized design. The Shapiro-Wilk test was used to check for normality, and means were compared using Tukey's test ($p<0.05$).

RESULTS AND DISCUSSION

According to Table 1, the different hydrolysis methods decreased crude protein content compared to feathers with no hydrolysis treatment (Control) ($p<0.05$). The BH treatment recorded the highest protein content, with a value of 87.7%. Conversely, the treatment that lost the most nitrogen and protein was TH ($p<0.05$). These changes can be attributed to the formation of ammonia during the hydrolysis process, which is released as gas (Duong *et al.*, 2019). Consequently, TH shows nitrogen losses close to 30% ($p<0.05$).

In contrast, the other hydrolysis processes (CH, AH, and BH) recorded a lower loss of nitrogen in the form of ammonia, which suggests a greater degree of hydrolysis to lower molecular weight proteins. This is consistent with the findings of Beaubier *et al.* (2019), who published the characteristics and nitrogen losses in the form of ammonia during the hydrolysis of chicken feathers. In addition, the enzymatic hydrolysates exhibited very low keratin degradation. This may be due to a low degree of specificity of the proteolytic enzymes used; the enzymes employed are exo and endopeptidases, but they are not keratinases, which significantly reduces their effectiveness in this type of assay (Guo *et al.*, 2016).

On the other hand, the Bradford technique is a sensitive method that involves the interaction of Coomassie Brilliant Blue G-250 dye with a protein rather than with nitrogen as an element (Kielkopf, Bauer, & Urbatsch, 2020). Therefore, the organic protein values obtained in this study were higher in CH and BH, with 17.3% and 16.6% organic protein, respectively ($p<0.05$); whereas the control treatment recorded 5.3% organic protein ($p<0.05$). Organic protein values indicate a higher content of highly degradable protein in the rumen, as well as a high solubility of the protein. In this regard, Valencia-Andrade (2018) obtained approximately 14% organic protein in feathers using specific keratinase enzymes. This suggests that, although the enzymes used in this study were not specific keratinases, the degree of hydrolysis is similar to that of other assays.

On the other hand, the degree of hydrolysis affects the ruminal digestibility of dry matter ($p<0.05$). The increase in dry matter digestibility observed can be attributed to an increase in keratin hydrolysis in the chemical treatment; conversely, the lower digestibility observed in enzymatic hydrolysis may be caused by the concentration of keratin in

Table 1. Protein and total nitrogen contents in hydrolyzed chicken feather meals by different methods.

Treatments	Crude Protein (%, DM)	Organic Protein (%, DM)	Total Nitrogen (%, DM)	IVDMD (%)
Control	97.3 ± 0.81^{a}	5.3 ± 0.04^{c}	15.5 ± 0.06^{a}	11.6 ± 0.01^{d}
TH	70.0 ± 1.30^{d}	2.3 ± 0.01^{d}	11.0 ± 0.02^{d}	19.9 ± 0.44^{b}
CH	74.1 ± 0.84^{cd}	17.3 ± 0.19^{a}	11.8 ± 0.13^{c}	86.8 ± 0.22^{a}
AH	75.5 ± 1.96^{c}	1.7 ± 0.04^{d}	11.8 ± 0.09^{c}	10.4 ± 0.07^{d}
BH	87.7 ± 0.91^{b}	16.6 ± 0.12^{a}	14.0 ± 0.14^{b}	16.2 ± 0.41^{c}
SEDM	1.05	0.11	0.08	0.28

^{abcd} Different letters in the same column indicate significant differences ($p<0.05$); DM: Dry Matter; IVDMD: *In vitro* Dry Matter Digestibility; TH: Thermal Hydrolysis; CH: Chemical Hydrolysis; AH: Acid Enzymatic Hydrolysis; BH: Alkaline Enzymatic Hydrolysis; SEDM: Standard error of the difference among means.

the sample (Sypka, Jodłowska, & Białkowska, 2021). Additionally, it is known that the microorganisms contained in the rumen are not capable of degrading keratin. Thus, since keratin is the most abundant component in chicken feathers (approximately 70% DM), its hydrolysis involves the breaking of disulfide bonds contained in keratin and the release of soluble proteins and lower molecular weight proteins, as well as the reduction of amino acid chains capable of being degraded in the rumen (Machuca-Loja *et al.*, 2016). For the above reasons, the chemical hydrolysis (CH) was the treatment that showed the greatest keratin degradation compared to the other treatments ($p<0.05$). However, the other hydrolysis treatments also improved the percentage of digestibility.

Figure 1 shows the results of the SDS-PAGE analysis of the hydrolysates. As can be seen, in lanes 2 and 3 of Figure 1a, a faint band at 75 kDa is observed, which represents the keratin content in the untreated feathers (control); it is worth mentioning that keratin has an approximate molecular weight of 70 kDa (Sypka, Jodłowska, & Białkowska, 2021). In lanes 6 and 7, the degradation bands obtained in the chemical hydrolysis (CH) are shown. In these bands, a greater pattern of degradation or a sweep of proteins with lower molecular weight can be observed. The staining in these lanes indicates a higher

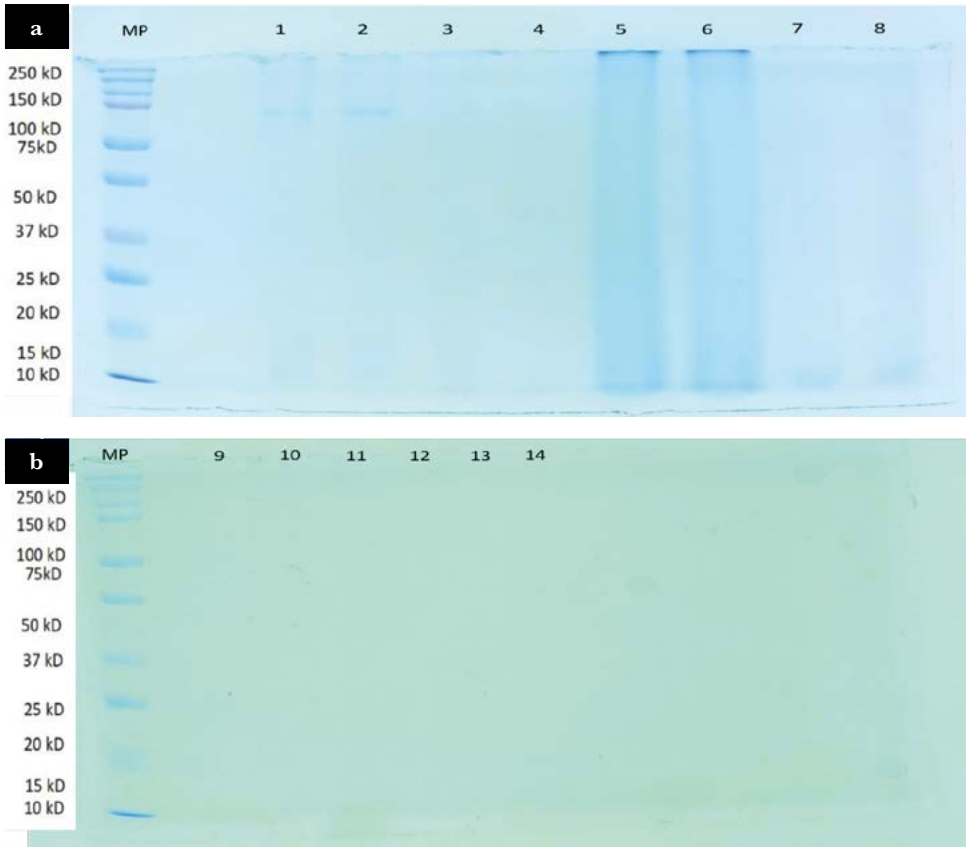

Figure 1. SDS-PAGE of the different hydrolyzed feather treatments. 1a: Lane MP: Standard protein marker; Lane 1and 2: whole feathers; Lane 3 and 4: thermal hydrolysates; Lane 5 and 6: chemical hydrolysates; Lane 7 and 8: alkaline enzymatic hydrolysates. 1b: Lane MP: Standard protein marker; Lane 9 and 10: acidic enzymatic hydrolysates; Lane 11 and 12: liquid alkaline enzymatic hydrolysates; Lane 13 and 14: liquid acidic enzymatic hydrolysates.

concentration of peptides, resulting from a greater degree of hydrolysis. Electrophoresis is a technique that allows for an accurate description of proteins. In this regard, Beaubier *et al.* (2019) stated that an important parameter of protein hydrolysis is the molecular weight distribution of the peptides in the hydrolysate, as revealed in SDS-PAGE gels. However, it is worth noting that the sweeps presented in the migration lanes of SDS-PAGE correspond to soluble protein. That is, even if some lanes do not show degradation or coloration as an effect of hydrolysis, it does not imply the absence of protein, as demonstrated by the presence of total nitrogen and crude protein.

The use of these technologies for obtaining protein from keratin represents a sustainable and viable alternative in bovine feeding. Thus, hydrolyzed chicken feather meals could be incorporated into the diets of confined or even pasture-based cattle as feed supplements that provide the necessary amounts of protein for the proper productive and reproductive development of cattle. In this way, the benefits for producers can be reflected in increased weight gain and reduced costs in protein supplementation. However, it is essential to conduct a feasibility economic analysis to substantiate this.

CONCLUSIONS

The chemical hydrolysis process (CH) proved to be more effective in keratin hydrolysis, showing a clear degradation or degradation pattern; in addition, it maintained the highest amount of total nitrogen or crude protein. Furthermore, CH exhibited a digestibility of over 80%, indicating that it is the technique that provides the highest content of soluble and digestible protein for use in the production of additives for ruminant feed.

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