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# Research Article
# Effects of Autoclaving, Addition of Sodium Hydroxide and Their Combination on Protein Content and *in vitro* Digestibility of Chicken Feathers

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## Abstract

**Objective:** The aim of this study was to determine the effects of NaOH addition, autoclaving and their combination on the protein and pepsin digestibility of chicken feathers. **Methodology:** The first experiment consisted of the following treatments: (1) The 2 h treatment with 1.0 M of NaOH, (2) The 12 h treatment with 1.0 M of NaOH, (3) The 24 h treatment with 1.0 M of NaOH and (4) Control, consisting of raw feathers incubated with only 100 mL of distilled water for 24 h at 37°C. The second experiment consisted of the following treatments: (1) Raw chicken feathers autoclaved at $2.5*10^5$ Pa, at a temperature of 121°C for 30 min and (2) Raw chicken feathers soaked in 0.5% NaOH solution for 24 h, followed by autoclaving at $2.5*10^5$ Pa and 121°C for 30 min. **Results:** The prolonged treatment (24 and 12 h) with NaOH improved feather solubility but resulted in lower protein retention, whereas the addition of NaOH followed by autoclaving resulted in higher protein content and increased *in vitro* pepsin digestibility. **Conclusion:** The addition of NaOH followed by autoclaving is recommended as a treatment for processing of chicken feathers.

Key words: Chicken feather, sodium hydroxide, autoclave, pepsin digestibility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Feathers are a waste product in poultry processing plants but have become of interest in nutritional studies because of their high protein content. The volume of this poultry industry waste represents a serious rendering or disposal challenge. Discarding this material is becoming more difficult due to restrictive laws that have been enacted to eliminate the current practices of landfill dumping and burning[1]. Instead, feathers are being degraded to produce feather meal that is used as animal feed, organic fertilizers and feed supplements because it contains more than 90% protein and is rich in hydrophobic amino acids and important amino acids such as cysteine, arginine and threonine[2,3]. Due to the complex structure of keratin proteins that contain cystine disulfide bonds[4,5], feathers must be treated to permit digestion by animals because in their natural state, feathers have no nutritive value. Keratin is a fibrous protein consisting of typical long-chain peptides, is insoluble in water and is difficult to digest[6].

At present, despite the wide utilization of feather meal in feeds for different animal production industries, it has not been used in fish feeds. This could be mainly attributed to the lack of information on nutritional data and inadequate economic methods of handling, storage and conversion into acceptable feed ingredients. However, encouraging results in several countries under laboratory and practical conditions have shown that it is possible to replace fishmeal in aquatic animal diets with different types of feather and poultry by-product meals[1,7].

Rendered animal proteins are a potential source not only of digestible protein but also of essential amino acids, vitamins and minerals[8,9]. Hydrolysed feather meal (HFM) is a by-product of the poultry industry. An increase in HFM incorporation in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) diets has been described in North America[10]. HFM is an economical protein source with relatively high digestible protein content for fish[11]. Studies found that replacing fish meal with limited amounts of feather meal in practical trout or salmon diets did not negatively impact fish growth and feed utilization[12], while specific types of fish, such as Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio* L.), can thrive on diets with up to 50% replacement of fishmeal (FM) by HFM[13,1]. Mendoza *et al.*[1] indicated that white shrimp can be fed with a practical diet containing 20% of enzymatically treated feathers coextruded with soy-bean meal (EHF-SBM) at a 2:1 ratio without impairing growth or food conversion. The use of 20% EHF-SBM (2:1) allowed the fish meal portion to be reduced by nearly 55%. A 6 week feeding trial using feeds formulated with 20% poultry by-product meal (PBM), no fishmeal or 3% of blood meal (BLM), anchovy fishmeal (AFM), fish hydrolysate (FHD), squid liver meal (SLM), or krill meal (KRL) showed that the attractiveness of the feeds was improved by including SLM and KRL. However, palatability and growth were improved only with KRL[14].

The most popular method of HFM production is through a hydrothermal process where feathers are cooked at high pressure up to $7.2*10^5$ Pa[16] and high temperature up to 400°C[16]. However, in feather meal, hydrothermal treatment results in destruction of essential amino acids such as methionine, lysine, tyrosine and tryptophan, as well as relatively poor digestibility and low nutritional value[4, 17]. Because of this problem, this study was conducted to determine the effects of sodium hydroxide (NaOH) addition, autoclaving and the combination of these two treatments on the protein content and pepsin digestibility of chicken feathers.

## MATERIALS AND METHODS

**Feather treatments:** Raw chicken feathers were collected from local farms after the chickens were slaughtered at 6 weeks of age. Then, a mixture of all parts of fluff, down, wing tail and feathers were washed in tap water and placed in a circulating air-drying oven at 60°C for 24 h. Following the method of Kim *et al.*[18], four feather treatments were employed: (1) NaOH for 2 h, (2) NaOH for 12 h, (3) NaOH for 24 h and (4) control. All treatments were replicated three times. Each replicate contained 4.0 g of whole feathers. The control was raw feathers incubated with only 100 mL of distilled water for 24 h at 37°C. For the 2, 12 and 24 h NaOH treatments, feathers were incubated with 50 mL of 1.0 Mol reagent-grade NaOH for 2, 12 and 24 h at 37°C, respectively. Feathers were incubated in an end-over-end type agitator that held twelve 224 mL brown screw-cap bottles. After all treatments were incubated, digested feathers were filtered through Whatman No. 4 filter paper to separate solubilized and unsolubilized fractions.

Another treatment for raw feathers was by autoclaving after addition of 0.5% reagent-grade NaOH, following the procedure of Wiradimadja *et al.*[19]. There were two NaOH feather treatments: (1) Raw feathers autoclaved at 2.5*10^5 Pa for 30 min at 121°C and (2) NaOH pre-treatment plus autoclaving at $2.5*10^5$ Pa for 30 min at 121°C. All treatments had three replicates. For the NaOH treatment, feathers were soaked in 0.5% reagent-grade NaOH for 24 h at room temperature. After soaking, feathers were autoclaved for 30 min at 121°C. For the control, raw feathers were washed in tap water and then autoclaved for 30 min at 121°C.

**Analytical procedures:** The proximate composition of the raw feathers and treated feathers was determined using standard methods[20]. The crude protein content was determined by using the Kjeldahl method (Kjeltec Analyzer Unit 2300, Sweden), which includes three steps: Digestion, distillation and titration. Total lipid extraction was determined by using Soxhlet Ether Extraction. Dry matter was determined using a freeze-drier (VIRTIS GENESIS SQ 12) for five days. The ash content was determined by burning 1 g of samples in clean, weighed silica crucibles overnight in a muffle furnace (GALLEN KAMP, size 2, UK) at 600°C.

***In vitro* digestibility:** The *in vitro* digestibility of the ingredients in each treatment was evaluated using single-enzyme assay with pepsin-digestible nitrogen (N) using the procedure of the AOAC[20]. This was done using 0.2% pepsin (activity 1:10,000) in 0.075 N of HCl. In this assay, 0.5 g samples were incubated in 150 mL of pepsin solution at 45°C for 16 h. The end-over-end type agitator used for this determination held twelve 224 mL brown screw-cap bottles and the temperature was maintained in a circulating air-drying oven.

**Statistical analysis:** All analyses were performed in duplicate and they were presented as the mean values±S.D. The mean values were analyzed by one-way ANOVA at $p<0.05$ to detect significant differences among groups. The results of raw and treated feathers with NaOH were evaluated using Student's t-test ($p<0.05$) to determine the significance of differences between the mean values obtained from the proximate analysis. After ANOVA, significant differences among means were determined by Tukey's multiple range test.

## RESULTS

**Protein content and pepsin digestibility:** The protein contents and pepsin digestibility of the chicken feathers treated with NaOH are given in Table 1. Incubation with 1.0 M of reagent-grade NaOH for 2, 12 and 24 h solubilized most of the feathers. This treatment improved solubility and indicated that prolonged incubation with 1.0 Mol of NaOH increased the solubility of feathers.

The pepsin digestibility of solubilized feathers in the 2 h treatment had the highest digestibility compared to other treatments. However, its protein content was lower than the control and raw feathers. The raw feathers showed the highest protein content (87.47%) followed by the insolubilized control feathers with a value of 86.05% protein, but they are indigestible. The 12 and 24 h treatments showed the lowest protein contents (4.90 and 5.78%, respectively) and the lowest pepsin digestibility percentages (11.25 and 15.53%, respectively). There were significant differences ($p<0.05$) in protein content in the original sample and the pepsin digestibility of the chicken feathers treated with NaOH among all experimental groups.

**Proximate composition of raw and treated feathers by autoclaving:** The proximate composition of raw and treated feathers by autoclaving with NaOH addition are shown in Table 2. The crude protein of treated feathers was higher (98%) than in the raw feathers (87%), while the crude lipid was higher in raw feathers. Treated feathers had higher ash and fibre content (4.95 and 1.06%, respectively) than the raw feathers. There were significant differences ($p<0.05$) in the proximate composition of raw and treated feathers among the experimental groups.

The protein contents and pepsin digestibility of the chicken feathers treated by autoclaving only and NaOH addition followed by autoclaving are given in Table 3. The use of autoclaved treatment in feathers showed very high protein content and pepsin digestibility compared with the raw feathers with values of 98.96 and 97.99% protein for autoclaved and autoclaved plus NaOH, respectively. However, feathers that were soaked in 0.5% reagent-grade NaOH for 24 h at room temperature followed by autoclaving for 30 min at 121°C were more digestible than feathers autoclaved only.

Table 1: Protein content and pepsin digestibility of chicken feathers treated with NaOH. Values are means and standard deviations of three replicates. Means in a column with different superscripts are significantly different ($p<0.05$)

| Sample names | Protein content (%) | Pepsin digestibility (%) |
|---|---|---|
| Raw feather | $87.47\pm0.01^{a}$ | $14.54\pm0.02^{d}$ |
| Control | $86.05\pm0.05^{b}$ | $19.56\pm0.10^{b}$ |
| 2 h treatment | $10.61\pm0.43^{c}$ | $78.47\pm0.01^{a}$ |
| 12 h treatment | $4.90\pm0.75^{e}$ | $11.25\pm0.01^{e}$ |
| 24 h treatment | $5.78\pm0.13^{d}$ | $15.53\pm0.01^{c}$ |

Table 2: Proximate composition of raw and treated feathers by autoclaving with NaOH addition. Values are means and standard deviation of three replicates. Means in a column with different superscripts are significantly different ($p<0.05$)

| Sample names | Crude protein (%) | Crude lipid (%) | Ash (%) | Fibre (%) | Dry matter (%) |
|---|---|---|---|---|---|
| Raw feathers | $87.47\pm0.01^{b}$ | $1.90\pm0.01^{a}$ | $1.50\pm0.10^{b}$ | $0.42\pm0.10^{b}$ | $93.31\pm0.05^{a}$ |
| Treated feathers (autoclaved+NaOH) | $97.99\pm0.02^{a}$ | $1.01\pm0.07^{b}$ | $4.95\pm0.04^{a}$ | $1.06\pm0.53^{a}$ | $84.01\pm0.07^{b}$ |

Table 3: Protein contents and pepsin digestibility of the chicken feathers treated with autoclaving and NaOH

| Sample names | Protein content (%) | Pepsin digestibility (%) |
|---|---|---|
| Raw feather | 87.47±0.01 | 14.54±0.02 |
| Autoclave only 30 min | 98.96±0.28 | 39.23±0.02[b] |
| Autoclave+NaOH-30 min | 97.99±0.02 | 58.01±0.07[a] |

## DISCUSSION

This experiment demonstrated that NaOH treatment and autoclave treatment after NaOH pre-treatment of feathers improved nitrogen (N) solubility, pepsin digestibility and *in vitro* amino acid digestibility. NaOH treatments improved the solubility of feathers and dissolved all the feathers over time. Other researchers had also indicated that NaOH is an efficient agent for feather hydrolysis[18]. Both treatments (NaOH and autoclaving) improved the *in vitro* digestibility of raw feathers.

Proteins in chicken feathers can be physically and covalently bound. Covalently bound proteins can be degraded by chemical treatment, i.e., dissolution in strong alkaline solutions or with biological treatment[21]. Low protein content with addition of NaOH resulting from hydrolysis (Table 1) was reported by Wiradimadja *et al.*[19]. Hydrolysis by strong bases causes depolymerization due to excessive cuts in the molecular structure of proteins, vitamins and minerals. In this study, 1 Mol of NaOH (40 g $L^{-1}$) was used for chemical hydrolysis of feathers, following the procedure of Kim *et al.*[18]. However, a strong base such as NaOH should not be over-used for the hydrolysis process. Another concern with NaOH treatment for feather hydrolysis is that the reagent can degrade the protein quality of the end product meal, although it does improve the digestibility of feathers[18]. Papadopoulos *et al.*[22] and Papadopoulos[23] reported that prolonged processing time and high concentrations of NaOH reduced amino acid digestibility of feather meal. These studies also found that prolonged processing times and high concentrations of NaOH affected amino acid concentrations. Moreover, Yang and Reddy[24] found that feathers displayed gradual degradation and consistent release of nitrogen as ammonia up to 10 days following treatment with alkaline potassium persulfate solution. Pepsin digestibility decreased at 12 and 24 h of incubation with NaOH (Table 1) because the protein content was low in the solution analyzed for pepsin digestibility.

In raw feathers autoclaved after the addition of 0.5% reagent-grade NaOH, the crude protein, ash and fibre were higher than in raw feathers but displayed reduced lipid content. This implies that autoclaving of chicken feathers may have altered the protein structure in such a way that NaOH digestion was hindered[25]. Findings of current study agree with Kim *et al.*[18] who indicated that NaOH with autoclaving significantly diluted the crude protein and crude lipid extract. Sodium levels of the autoclaved NaOH-treated feathers in the present study were 2.9 g (wet weight) of 0.5% NaOH stock solution, which was higher than in raw feathers. Higher sodium content in NaOH-treated feathers has been reported in other studies. Papadopoulos[25] indicated that the higher ash concentration in feather meal treated with NaOH was a result of sodium retained in the samples. Shafer and Carey[26] preserved fully feathered broiler carcasses using a 1:1 ratio (wt/wt) of carcasses and 2 M of NaOH. They also indicated that the end product meal had a high level of sodium (Na) (12.30%). Therefore, the Na level should be considered carefully when NaOH-treated feathers are used as a feed ingredient.

As shown in Table 3, the chemical compositions of crude protein in feathers treated with, or without, NaOH were affected by autoclaving and pepsin digestibility was significantly enhanced. Before autoclaving, the crude protein and pepsin digestibility of raw feathers were 87.47 and 14.54%, respectively. After autoclaving, both feather products, with and without NaOH treatment, had higher crude protein content and digestibility. Other studies[23,27,28] also indicated that NaOH treatment significantly improved pepsin digestibility of feather meal following autoclaving. Steiner *et al.*[28] evaluated the effect of various concentrations of NaOH and phosphoric acid ($H_3PO_4$) on feather digestibility. Pepsin digestibility of feather meal increased as chemical contact time increased from 0-16 h and concentrations of NaOH or $H_3PO_4$ increased from 0-9.0%. Improved pepsin digestibility of feather meal with increased incubation time and NaOH concentrations was also observed by Papadopoulos[23]. That study indicated that prolonged processing time at higher NaOH concentrations increased pepsin digestibility and resulted in a significant reduction in crude protein. The present study showed that there was no reduction in the crude protein level after autoclaving with NaOH treatment. In this study, pepsin digestibility of feather meal increased from 39.23-58.01% with NaOH addition and autoclaving.

In this study, all treatments were autoclaved at $2.5*10^5$ Pa and 121°C for 30 min. These processing conditions were appropriate for feathers pre-treated with or without NaOH. The conditions (pressure, temperature and time) might be reduced for enzyme- or NaOH-pretreated feathers[29]. Other researchers[27,28] have indicated that enzymes or sodium hydroxide pre-treatment could reduce processing time, temperature, or pressure. Munch and Stein[30] reported that

the addition of acid or alkaline could facilitate a decrease in pressure or processing time required to reach a given level of pepsin digestibility of hair. Woodgate[31] indicated that the addition of enzyme allowed the processing temperature to be reduced from 105-50°C in the first phase and from 155-125°C in the second phase, with better results. The pepsin digestibility of Woodgate's control treatment was 70.7% when feathers were cooked at 105°C for 20 min in the first phase and at 155°C for 20 min in the second phase. The pepsin digestibility results of enzyme-treated feathers were 74.3% when cooked at 50°C for 30 min and 125°C for 20 min in the second phase.

## CONCLUSIONS

Prolonged treatment (24 and 12 h) with NaOH improved feather solubility but resulted in lower protein retention, whereas NaOH addition followed by autoclaving resulted in higher protein and pepsin digestibility. Therefore, NaOH addition followed by autoclaving is recommended in chicken feather treatment in poultry processing factories.

## SIGNIFICANT STATEMENT

This study discovers the possible effect of NaOH addition, autoclaving and their combination on the protein and amino acid content and pepsin digestibility of chicken feathers. This study will help the researcher to treat the chicken feathers on an optimum treatment with highest protein and digestibility that many researchers were not able to explore. Thus, a new theory on these treatments combination and possibly other combinations, may be arrived at.

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