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Synthesis of Feather Concentrate from Broiler Feather Waste using Different Chemical Hydrolysis Process and Effect on Its Properties

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Abstract: Feather waste, a resultant of livestock industry, has annually increased, but its existence has not been optimally utilized. Production of feather concentrate (Fc) is regarded a beneficial application to minimze the waste. The objective of the study was to evaluate the use of NaOH and HCl as a hydrolyzing agent in Fc preparation. The results showed that microstructural changes occurred in filament molecules in keratin protein as exhibited in T_0 and T_1 treatments. Keratin molecules underwent denaturation and degradation, resulting in molecular changes of their structure. After hydrolysis reaction, *in-vitro* protein digestibility was increased and the highest digestibility value was achieved at T_1 treatment (21.76%). The treatments showed no significant effects on Fc yield compared to the control, with exception of T_3 treatment. Yield could indicate the preparation efficiency, in which the value seemed to decrease a result of denaturation. The relative protein content was not different from the control (T_0) especially on the T_1 and T_2 treatments showed no significant effects on relative protein content compared to control T_0. The highest pH of product (9.76) was attributed to T_1 treatment using NaOH, while the lowest pH was found at HCl. Different types of hydrolysis process showed significant effects ($p<0.05$) on *in-vitro* digestibility of protein, yield and protein content. Application of NaOH (T_1) is the best treatment compared to T_2, T_3 and T_0.

Keywords: NaOH, HCl, Hydrolysis, Concentrate, Feather, Broiler

Introduction

The production of waste generated from livestock industry has annually increased significantly. The environmentally friendly waste disposal process is a problem in today's modern world. This is due to the increasingly difficult waste dumps. Economic and environmental pressures have increased interest in the use of renewable and sustainable feed stocks, in addition to reduce dependence on non-renewable petroleum resources. Such condition encouraged the industry to find a better way to overcome the waste. The use and development of by-products of livestock have been largely carried out by researchers lately. By-product utilization, in addition to feed, has also been developed as an environmentally friendly packaging material (Tesfaye *et al*., 2017; Said *et al*., 2016; 2011). The poultry industry produces about 6 million tons per year of feathers as a by-product. The feather was mostly composed by keratin protein, accounting for 80-90% (Mazotto *et al*., 2017). By the live weight of broiler chickens produced about 37% are not consumed directly by humans (Meeker and Hamilton, 2006). In 2010, approximately 25 billion kg of broiler and turkey meat is produced by the US (USDA, 2010).

A total of 44% of the weight of non-fat waste generated by the US sewage treatment industry comes from poultry. Of this amount, more than one-third is a feather waste. In 2008, the processing industry produced 604 million kg of feather meal. A total of >90% is used domestically (Swisher, 2009). The feather meal was used as organic fertilizer (Hadas and Kautsky, 1994),

biodiesel feedstock (Kondamudi *et al*., 2009) and as an additional feed for poultry (Elboushy *et al*., 1990), pig (Van-Heugten and Van-Kempen, 2002), ruminants (FAOUN, 2011) and fish (Arunlertaree and Moolthongnoi, 2008; Jamil *et al*., 2007).

One of the many livestock wastes produced by the poultry slaughtering industry is feather waste (Darah *et al*., 2013). Feathers are regarded as waste disposal and even though the amount is small. These wastes are often processed into valuable products such as feed and fertilizer (Veerabadran *et al*., 2012; Stingone and Wing, 2011). Uncontrolled waste disposal contributes to environmental damage and disease transmission (Tronina and Bube, 2008) and also potentially as a source of harmful arsenic toxins if not managed properly (Nachman *et al*., 2012). The poultry waste management system through the combustion process can have an impact on the environment (Nachman *et al*., 2005; 2008).

Feather waste needs to reduce using some beneficial applications such as feed ingredient. Protein source from chicken feather waste has remained a great challenge mainly related to its low digestibility. This characteristic is associated with presence of disulfide bonding components (S-S) in the keratin structure that compose the feather (Pruekvimolphan and Grummer, 2011). Keratin proteins are the main types of protein guided in feather wastes (Riffel and Brandelli, 2006). The utilization of feather wastes as animal feed especially ruminants has now become a consideration (Hasni *et al*., 2014).

Feed digestibility remarkably affected carrying capacity of feed for livestock. In form of feather concentrate (Fc), the feather digestibility could be improved. The use of acid and base compounds is widely applied in hydrolysis process of feather to produce Fc. Therefore, researches pertaining their effectiveness need to be carried out.

The results before of the study show that Fc can be an alternative protein source in feed for both ruminant and non-ruminants (Scholljegerdes *et al*., 2005). This study aimed to evaluate the microstructural changes, *in-vitro* protein digestibility (Iv-PD), yield and protein content of Fc produced using chemical process method (NaOH and HCl).

Materials and Methods

Research Materials

Broiler feather waste (BFW) was obtained from poultry slaughterhouse at Daya Village, Makassar, South Sulawesi. Other materials included distilled water, NaOH 1 M (10 and 20%, w/v), HCl 1M (10 and 20%, v/v) of HCl 1M. Supporting research equipment such as the Scanning Electron Microscope (SEM) (*Tescan Vega 3SB*), oven (*Memmert*) and grinder (Kirin).

Feather Hydrolysis

Broiler feather waste (50 g) was washed using running water and dried using oven for 15 h at 60°C. The feather was then hydrolized using different hydrolyzing agents, i.e., HCl 1M (10 and 20%, v/v) and NaOH 1M (10 and 20%, w/v) for 4 h at room temperature. Hydrolized feather was washed with running water and dried using oven at 60°C for 24 h. The dried sample was then milled for further analysis.

Parameters observed in this study included (1) micrustructure, (2) *in-vitro* protein digestibility (Iv-PD), (3) yield, (4) protein content and (5) pH value. The research was conducted experimentally based on completely random design (CRD) pattern undirectional for 4 treatments (T_0 = Control/without hydrolysis, T_1 = 20% (w/v) NaOH 1M, T_2 = 20% (v/v) HCl 1M, T_3 = 10% (w/v) NaOH 1M + 10% (v/v) HCl 1M) and 5 repetitions.

Data Analysis

The data obtained were evaluated using analysis of variance (ANOVA) in SPSS Version 15.0 statistical program. Significant difference between means was compared using Duncan's Multiple Range Test (DMRT) at 5% level (Steel and Torrie, 1991). Meanwhile, microstructure data were descriptively evaluated:

- *Microstructure analysis*. Microstructure analysis of feather waste analyzed by Scanning Electron Microscope (SEM) (*Tescan Vega 3SB*)
- *In-vitro protein digestibility (%) (In-VtPD)* (AOAC International, 1997); (Swaisgood and Catignani, 1991). In-VtPD can predict the digestibility of protein or by product accurately by minimizing costs. This method was imitated the digestive function of livestock (Moyano *et al*., 2015). In this study, Iv-PD of Fc was determined using pepsin method. Sample (1 g) was placed in mortar, added with pepsin acid solution (25 ml) and incubated for 72 h at 50°C in shaking incubator. After incubation, sample was filtered using crucible no 2, dried overnight at 103°C and heated at 520°C for 3 h. In-VtPD value was calculated by following formula: In-VtPD = 100% - % DII, where DII (%) = B-C/A x 100%, DII = dry ingested ingredients; A = sample weight, B = crucible weight after dried and C = initial crucible weight
- *Yield (%)*. Yield was determined using previous method of (Giménez *et al*., 2005) with the following formula: Yield = A/B x 100%, where, A = weight of Fc (g) ; B = weight of BFW (g)
- *Proximate analysis (%)*. The protein content (%) was determined by proximate analysis method (AOAC International, 1997)
- *pH value*. The pH value was determined using pH meter (AOAC International, 1997). Sample (0.5 g) was dissolved in aquadest. The cathode end of the pH meter was dyed into the Fc solution and then the result was determined

Results and Discussion

Microstructure of Broiler Feather Waste

The appearance of microstructural differences of the broiler feather waste before and after the chemical hydrolysis process was presented in Fig. 1.

Figure 1 shows the comparison of protein molecule bond structure in broiler feather waste before and after chemical hydrolysis process. The results showed that during the process of hydrolysis, protein was denaturated, yielding remarkable structural changes. Denaturation is the process by which proteins or nucleic acids lose their quaternary structures, tertiary structures and secondary structures. The denaturation process can occur due to exposiure to the physical treatments (pressure, radiation and heat) and chemical treatments (strong, basic acids, concentrated inorganic salts, organic solvents e.g alcohol or chloroform). Denaturation of proteins is also a consequence of cell death. The denatured protein may exhibit a variety of characteristics, ranging from conformational changes and loss of solubility to aggregation due to the role of hydrophobic groups (Samson *et al*., 2012; 2016). The appearance of pores in the keratin structure can be due to the dual diffusion process between keratin filaments (Ma *et al*., 2016).

In-vitro Protein Digestibility (Iv-PD)

Comparison of *in-vitro* protein digestibility (Iv-PD) of Fc in different chemical hydrolysis processes was presented in Fig. 2.

Statistical analysis revealed that different hydrolysis process applied showed significant effect (p<0.01) on Iv-PD. In this case, T_1 treatment (20% w/v NaOH 1M) significantly improved the digestibility of Fc (21.76%± 0.79^{d}) compared with T_0 (10.40%±1.05^{a}), T_2 (13.65%±0.30^{b}) and T_3 (15.39%±0.45^{c}) (Sukma, 2017). Hydrolysis using NaOH can improve the digestibility of chicken feather meal compared to the control. Hydrolyzing agent NaOH could promote degradation of bonds on the feather component (Kim and Patterson, 2000). Said *et al*. (2017) found that protein content of Fc was comparable with the protein concentrate of the skin of the Bali cattle. The Fc contains a number of ccysteine amino acids which is the most dominant composition in the structure (Klemesrud *et al*., 2000). The use of Fc as a feed on pigs can be considered as much as 8% (Van-Heughten and Van-Kempen, 2002) with a protein content of 63.46% (Keegan *et al*., 2004).

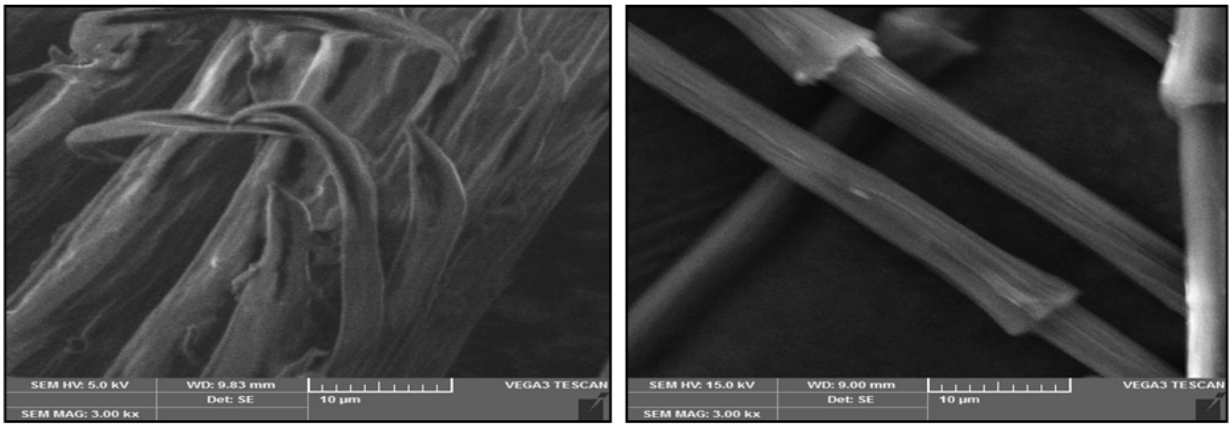
Yield

The yield was closely related to the efficiency of production process and provided a great effect on mass scale production. Giménez *et al*. (2005), that the yield is the number of products that produced a number of raw materials. Comparison of Fc yield produced by different chemical hydrolysis processes was presented in Fig. 3.

Statistical analysis exhibited that different hydrolysis processes showed significant effects (p<0.05) on the yield. The results showed that no difference was observed among T_0, T_1 and T_2 treatments. Hydrolysis using a single hydrolyzing agent (NaOH or HCl) did not affect the yield of Fc products. Additionally, T_0 and T_3 indicated a difference related to the value of yield (Sukma, 2017). This result is acceptable because T_3 treatment involves two types of hydrolyzing agent, leading to enhancement of the yield. Kołodziejska *et al*. (2007) found that the resulting yield was dependent on the process used. A larger yield indicates that the production process becomes more efficient.

Protein Content

Protein content of feed ingredient fundamentally affected the feed quality, since it was essential for increasing livestock productivity. Protein content of Fc produced from different hydrolysis processes was presented in Fig. 4.



(A) (B)

Fig. 1: Microstructural changes of broiler feather waste observed under SEM; A = Without hydrolysis (Magnification 3000×) (T_0); B = after hydrolysis (T_1) (Magnification 3000×)

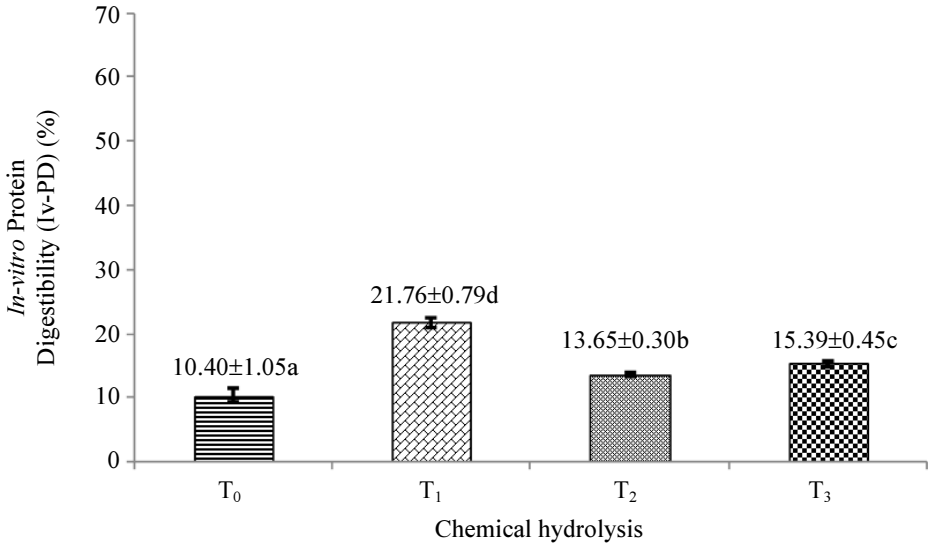


Fig. 2: Comparison of *in-vitro* protein digestibility (Iv-PD) (%) of Fc in different chemical hydrolysis process. [ab,c,d] Different superscripts following means showed significant differences (p<0.05)

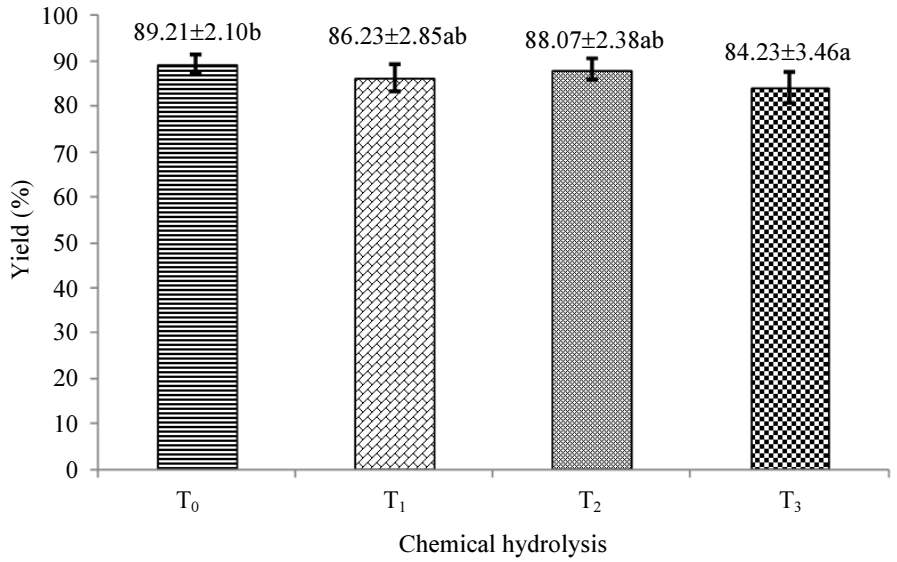


Fig. 3: Comparison of yield (%) of Fc in different chemical hydrolysis processes. [a,b,c,d] Different superscripts following means showed significant differences (p<0.05)

Statistical analysis showed that the use of different hydrolyzing agents significantly affected (p<0.05) protein content of Fc, ranging from 83.69-92.24% (Sukma, 2017). We also found that T_0, T_1 and T_2 treatments showed no different effects on the protein content. However, T_3 treatment showed the significant difference in protein levels compared to control. The combination of hydrolyzing agent significantly decreased protein levels, which might be associated with raised level of protein denaturation. Combination of acid (HCl) and base (NaOH) compounds allows the continued denaturation process to the protein component of Fc, thus decreasing protein content. The denaturation process can occur at temperature of >60°C. The denaturation process is a function of water content and temperature (Atuonwu *et al*., 2017) and enables to alter its biological activity. Fc produced by hydrolysis reaction has better performance in term of nutritional aspect when compared with similar feed that does not contain chicken feathers as additional ingredient.

pH Value

The pH value of each chemical hydrolysis process is completely different as shown in Fig. 5.

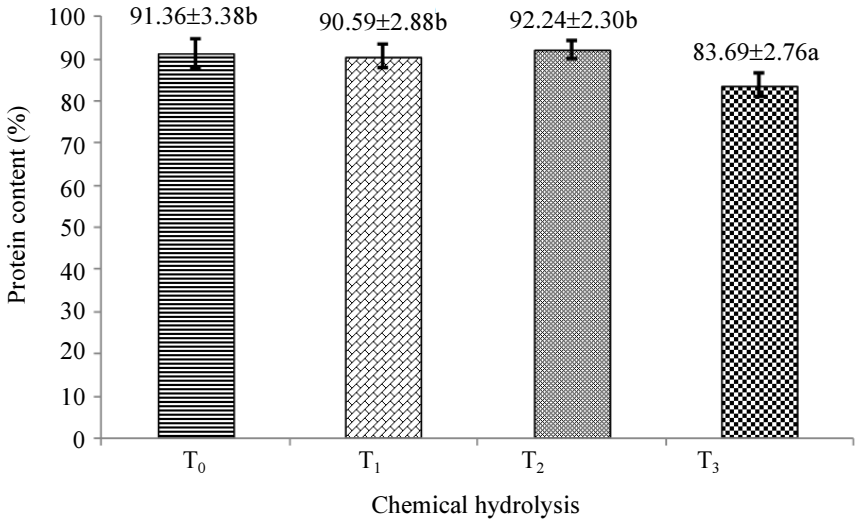


Fig. 4: Comparison of protein content (%) of Fc in different chemical hydrolysis processes: [a,b] Different superscripts following means showed significant differences (p<0.05)

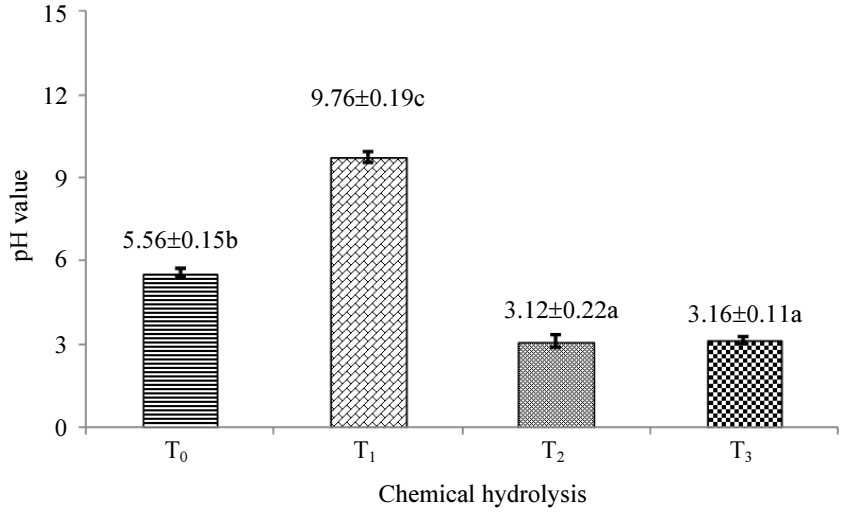


Fig. 5: Comparison of pH value of Fc in different chemical hydrolysis process: Note: [a,b,c] different superscripts showed significant differences (p<0.05)

Figure 5 showed that differences in chemical hydrolysis process affected pH value of Fc. Treatment T_1 showed the highest pH value compared to T_0, T_2 and T_3. This is because T_1 treatment uses alkaline NaOH (pH>7). The NaOH is a chemical compound having alkaline properties resulting in a higher pH. Feather waste can be hydrolyzed at pH 5.5-7.0 (Pedersen *et al*., 2012).

Conclusion

In the feather microstructure, changes occur in keratin protein filament molecules. The application of hydrolysis process enhances the *in-vitro* protein digestibility (Iv-PD) significantly. Combination of hydrolyzing agents (T_3 treatment) decreased yield significantly compared to the control. An application of 10% (w/v) NaOH 1M (T_1 treatment) as a hydrolysis agent resulted in the best characteristic of Fc in comparison with T_2, T_3 and T_0 treatments.

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Author Contribution

Muhammad Irfan Said: Participating in all experiments, coordinated the data-analysis and contributed to the writing of the manuscript.

Effendi Abustam: Designing the experiments and assisting data analysis.

Wempie Pakiding: Writing the manuscript and analyzing the data.

Muhammad Zain Mide: Participating in all experiments, collecting and analyzing data.

Midiawati Sukma: Collecting data and providing assistance in the writing of the manuscript.

Ethics

This article is original and has not been published or presented elsewhere. All the authors have approved the manuscript and agree with submission to this journal. There is no conflict interest to be declared.

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