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Quality of Chicken Feather Processed in Different Conditions

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Abstract. The objective of this research was to evaluate the hydrolyzed chicken feather based on pepsin digestibility and nutrient content, after physico-chemical and biological process. It was carried out by experimental methods at feed and nutrition laboratory. The treatments were hydrolyzed feather meals immersed in 0.5% NaOH and Na_2S solution for 0, 2, 4, 6 and 8 hours, each treatment was repeated three times. The results showed that chemical treatment (NaOH-Na_2S) in various time of incubation at 60^oC followed by fermentation using *Bacillus* sp. MTS at 37^oC for four days decreased the protein of hydrolyzed feather (78.88 to 73.06%), but increased the keratin fiber (1.9 to 3.26%). Pepsin digestibility informed that the increasing incubation time from 0, 2, 4, 6 to 8 hours resulted in higher solubility than that of control (30.2% at 8 hours vs 15.4% at 0 hours). Processing chicken feather by 0.5% NaOH and Na_2S solution at 60^oC for 6 hours followed by fermentation increased the value of pepsin digestibility.

Key words: hydrolyzed, *Bacillus* sp. MTS, feather, solubility

Abstrak. Penelitian ini bertujuan mengevaluasi kualitas nutrien tepung bulu ayam hasil proses hidrolisis secara fisiko-kimia dan biologis menggunakan *Bacillus* sp. MTS. Metode eksperimental digunakan dalam penelitian yang menggunakan dua tahap proses hidrolisis yaitu tahap 1: setelah perebusan bulu dalam larutan NaOH maka bulu direndam dalam larutan 0.5% NaOH dan Na_2S pada 60^0C dan tahap 2: fermentasi bulu selama empat hari pada suhu 37^0C. Perlakuan berupa waktu inkubasi yaitu 0, 2, 4, 6 dan 8 jam diterapkan pada tahap kedua dengan ulangan sebanyak tiga kali. Perlakuan fisiko-kimia yang dilanjutkan fermentasi menggunakan bakteri spesifik penghasil enzim-enzim pendegradasi keratin bulu menurunkan kadar protein tepung bulu (78,88% menjadi 73,06%) dan meningkatkan kadar serat tepung bulu (1,9 menjadi 3,26%). Uji kelarutan protein tepung bulu dalam pepsin menginfromasikan bahwa proses tahap 1 menghasilkan nilai kelarutan protein tepung bulu yang meningkat dua kali dibanding kontrol (30,2% pada 8 jam vs 15,4% pada 0 jam inkubasi) atau enam kali dibanding tepung bulu tanpa hidrolisis (5%). Pengolahan bulu ayam menggunakan cara pemanasan, perendaman dalam larutan NaOH dan Na_2S selama 6 jam pada 60^0C serta fermentasi menghasilkan tepung bulu dengan daya larut dalam pepsin lebih baik dibanding tanpa pengolahan.

Kata kunci: hidrolisis, tepung-bulu, *Bacillus* sp. MTS, kelarutan

Introduction

Insoluble and hard-to-degrade animal proteins are ubiquitously present throughout animal bodies e.g. nails, horns, hair, wool and feather. Feather wastes are generated in large quantities as a byproduct of commercial poultry processing. Feathers represent 5-7% of the total weight mature chickens and made up primarily of keratin (Rahayu et al., 2010). Statistics Indonesia (BPS) reported there were 1.076 million broilers in 2008. Provided that a 1.5 kg broiler produces roughly six percent feather, there were 96.830 ton feather waste at the same year. Feather contains about 80% protein made up of keratin and its resistant to common proteolytic enzymes and poorly digested by most organism. Feather meal contains nutrients essential for animal namely protein, fat, energy and minerals (Ca, P, K, and Na), however feather meal utilization as animal feed was yet optimal due to its low

solubility and digestibility. Although feather contains high protein, it is also comprised of hard polypeptide difficult to digest (Kim and Patterson 2000, Zerdani et al. 2004). While amino acids of feather meal is similar to those of fish meal (Sarmwatanakul and Bamrongtum, 2000; Arunlertaree and Moolthongnoi, 2008).

Optimizing digestibility of feather meal is therefore needed for maximum feed production. Keratin has unique structure unlike others which are solely formed by peptide bonds. Disulfide bonds are mostly found in keratin because it contains sulfur amino acids or cysteine. Accordingly, degrading keratin requires protease (keratinase) and disulfide reductase or reducing agents. Physical-chemical technique will loosen polypeptide bonds and disulfide bonds and dissolve wax coat on the feather to make it crumby and easy to grind.

Coward-Kelly et al. (2006) reported that 80% of feather keratin will dissolve after 25 minute heating at 150^{o} C or 300 minute heating at 100^{o}C. A number of microorganisms have been reported could degrade different sources of keratin, mainly bacteria, actinomycetes, saprophytic and dermatologist fungi have been reported to exhibit keratinolytic properties. Keratinases from many bacteria have been isolated and characterized. Some researchers informed that fermenting feather waste with specific keratinase-producing microbe can improve broiler performance (Odetallah et al.. 2003), essential amino acids, and protein (Williams et al., 1991; Bertch and Coello, 2005), lower keratin fiber fractions (Belewu et al.. 2008), and substitute fish meal (Arunlertaree and Moolthongnoi, 2008). Supplementation of feather meal with PWD-1 keratinase can alter keratin structure to increase digestibility and chicken's growth (Odetallah et al., 2003). We had screened and isolated a feather degrading bacteria from Tangkuban Perahu crater West Java Indonesia and based on its morphology and biochemical reactions, the isolate was grouped as a Bacillus species and tentatively refered to as *Bacillus* sp. MTS. The aerobic mesophillic bacteria was very effective in degradation of whole chicken feather and this appeared to be related to activity of the six extracellular keratinases and three disulfide reductases enzymes (Rahayu et al., 2010). The purified enzymes of *Bacillus* sp. MTS worked optimally at alkaline pHs, for keratinase at pH 8–12, and for disulfide reductase at pH 8–10. Optimum temperature for the extracellular keratinase was within 40–70^{0}C, while that for disulfide reductase was 35^{0}C (Rahayu et al., 2012).

In this research we hydrolyzed chicken feather using NaOH and reducing agent (Na_2S) followed by *Bacillus* sp. MTS fermentation and evaluated the quality of proceesed feather meal.

Materials and method

This research was comprised of two stages; the first is physico-chemical hydrolysis of chicken feathers using NaOH and Na_2S. The first stage applied experimental method with three repetitions and the observed variables were protein content, fiber and protein solubility in pepsin.

Physico-chemical hydrolysis. Feather waste collected from various slaughterhouse. After being washed and cleaned, the feathers were then boiled in NaOH solution for 45 minutes. In the next stage, the feathers were drenched in 0.5% NaOH and Na2S solution and incubated at 60% for 0, 2, 4, 6 and 8 hours, resulting in five types of feathers coding K0, K2, K4, K6 and K8 each was repeated three times. Afterwards, the feathers underwent neutralization by washing under flowing water and then oven drying to make up 60% water content.

Biological hydrolysis. At this stage, keratin meals coded K0, K2, K4, K6 and K8 were fermented with *BacillusSp.* MTS at 37^{o} C for four days using 16-20 hour inoculum with 10^{6} cell/ml density. Inoculum was made by growing *Bacillussp*. MTS in media containing various minerals and 1% feather meal substrate (modification Macedo et al.. 2005). Nutrient composition of hydrolyzed feathers was analyzed using AOAC procedure (1994) and protein solubility test in pepsin (Kim and Patterson, 2000)

Results and Discussion

Without processing, keratin proteins in feather is protein depleted since keratin is difficult to hydrolize by protease in digestive tracts. Keratin's resistance against proteolisis was because its structure was tightly enclosed in peptide bonds, polypeptide hydrogen bonds, hydrophobic interaction and stabilized by supercoiled polypeptid chains (Onifade et al., 1998).

Heating and pressure followed by soaking in NaOH and reducing agent significantly affected (P>0.05) the protein content of hydrolyzed feather. In this research, two stages of hydrolysis generally decreased nutrient content of dry matter, protein, fat and ash but increased the fiber of hydrolyzed feather (Table 1). The total decrease of protein and fat was 10% and 46%, respectively, and fiber increased by 41%. Fiber in Table 1 is the residual keratin fraction unhydrolyzed by physical, chemical and biological treatments.

After hydrolysis, a part of nutrient component in feather became soluble. Heating and pressure (autoclave) in NaOH solution for 45 minutes (first hydrolysis) dissolved the wax and opened the keratin structure of the feather. The first hydrolysis resulted in a more tender and easier to cut feathers. This stage however lowered protein content in feather (unhydrolyzed feathers) from 81 to 78.88% (0 hour of incubation).

In the second hydrolysis, feathers were macerated in solution containing NaOH and reducing agent of Na2S at 60% for 0, 2, 4, 6 and 8 hours. Observation result informed that longer maceration period caused protein to decrease (Figure 1) but hydrolyzed fiber to increase (Figure 2).

Heating and pressure continued by drenching in solution of NaOH and reducing agent significantly affected (P>0.05) the fiber content of feathers. Hydrolysis observation indicated that physical and chemical hydrolysis has opened the structure of keratin and dissolve some keratin. NaOH is known as chemical to open natural material structure like rice hay and chicken feather. Sodium sulfide (Na_2S) is reducing agent to open disulfide bonds in keratin structure. The second hydrolysis produced tender hydrolyzed feathers and the feather's protein content decreased during neutralization under flowing water. According to Coward-Kelly et al. (2006), chemical and heating treatments with high

Table 1. Nutrient content of hydrolyzed feather meal

Incubation time (hours)	Dry Matter (%)	Crude Protein (%)	Crude Fiber (%)	Fat (%)	Ash (%)
0	93.79±2.03	78.88±1.55	1.90±0.93	2.13±0.27	0.95±0.15
2	90.04±1.56	75.50±1.82	2.41±0.87	2.05±0.32	0.81±0.30
4	89.14±1.04	76.19±0.56	2.62±0.16	1.88±0.10	0.78±0.27
6	87.52±2.27	76.71±1.68	3.26±0.79	1.25±0.22	0.68±0.33
8	84.09±0.52	73.06±5.67	3.26±1.22	1.14±0.43	0.65±0.21

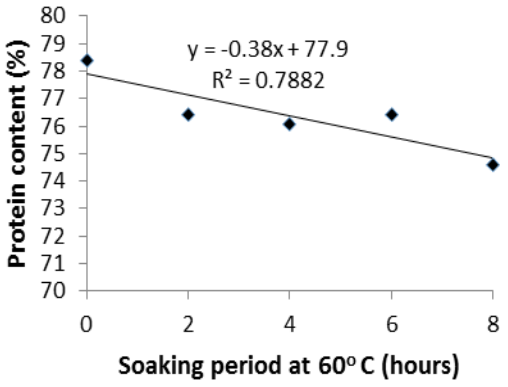

Figure 1. Protein content of hydrolyzed feathers

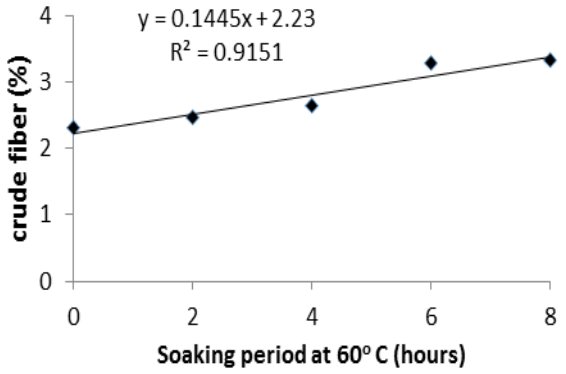

Figure 2. Fiber content of hydrolyzed feathers

protein content would result in small peptides and free amino acids. Hydrolyzing keratin into soluble protein at 50°C was estimated as much as 2-6%. Accordingly, the neutralization was assumed to cause small peptides dissolved and carried by the water. In this research heating-chemical and biological hydrolysis caused decrease in crude protein as much as 5.8%, namely from 78.88% (0 hour incubation) to 73.06% (8 hour incubation). Bertsch and Coello (2005) reported similar tendency in which protein decreased in feather meal fermented by *K. rosea* compared to the non-fermented feather meal (71.4% vs 88.2%). In this research, protein decrease due to soluble protein loss caused the increasing fiber of hydrolyzed feather.

Solubility test of hydrolyzed feather informed that physical-chemical and biological hydrolysis could increase solubility two fold (Figure 3). The effect was highly significant (P>0.01) and gave quadratic response although on the other hand induced protein decrease. Steiner et al. (1983) reported that various levels of NaOH or H3PO4 used in processing feather can increase keratin solubility in pepsin in vitro. While Papadopoulos (1989) stated that feather processing using 0.2-0.6% NaOH and maxatase indicated that both treatments could break disulfide bonds, increase solubility and open feather structure to increase the affinity between pepsin and keratin.

Incubating feather in NaOH-Na_2S at 60°C for eight hours followed by biological hydrolysis resulted in the highest keratin solubility in pepsin namely 30.2%. While the lowest, 15.4%, was found in feather solely hydrolyzed with NaOH-autoclave (0 hour incubation). Observation result was not different from that of Bertsch and Coello (2005) reporting that keratin solubility in pepsin fermented with *K. rosea* was significantly higher than that of non-fermented feather mill. Tiwary and Gupta (2012) stated that in-vitro digestibility of feather meal was found to be digestible by pepsin and trypsin by

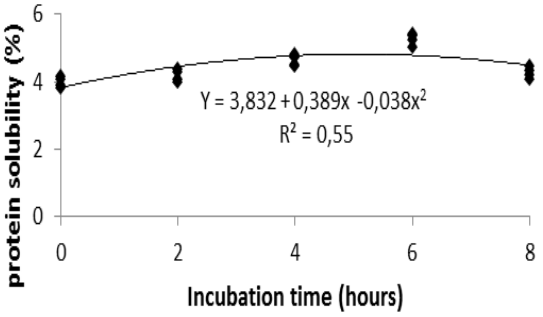

Figure 3. Solubility of feather's keratin protein in pepsin

releasing 670 mg protein/g feather meal after 18h of enzyme digestion from *Bacillus licheniformis* ER-15. Kim and Patterson (2000) informed that keratin solubility in pepsin was affected by heating-pressure (autoclave) process, in that keratin solubility of feather processed with enzyme and NaOH without autoclave was 33.25% and 17.64%, while non-processed feather was 5.98%. Feather that underwent autoclave before processing with enzyme and NaOH produced similar pepsin solubility.

Conclusions

1. Chemical hydrolysis ((NaOH-Na_2S) of feather followed by *Bacillus sp.* MTS fermentation resulted in decreasing protein and crude fiber of hydrolyzed feather
2. Chemical hydrolysis (NaOH-Na_2S) of feather for six hour followed by *Bacillus sp.* MTS fermentation was the best process to increase keratin solubility

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