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Solubility of Dry Matter and Protein of Hydrolyzed Feather Meal Fermented by *Lactobacillus Casei* Shiota

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Abstract: This study aims to determine the solubility of dry matter and protein of hydrolyzed feather meal (HFM) fermented by *Lactobacillus casei* Shiota on different levels. Most of the keratin-degrading bacteria are Bacillus sp, but publications on the potential of Lactobacillus as a producer of keratinase enzymes are still very limited. Materials used in the study were a feather, coconut meal, Yakult as *L. casei* Shiota resource, instruments of fermentation, instruments of analysis of the dry matter, and protein solubility. The experiment was arranged in a completely randomized design with 5 treatments and 4 replicated. The treatments were P0 = Feather fermented with 0% *L. casei* Shiota, P1 = Feather fermented with 5% *L. casei* Shiota, P2 = Feather fermented with 10% *L. casei* Shiota, P3 = Feather fermented with 15% *L. casei* Shiota, P4 = Feather fermented with 20% *L. casei* Shiota. The data were analyzed by variance analysis and, if progression was present, by Duncan's multiple range test. The study results showed that hydrolyzed feather meal fermented by *L. casei* Shiota on different levels significantly affected ($P < 0,05$) to decreased biomass, pH, dry matter, and protein solubility, but not significantly ($P > 0,05$) to characteristic and bulk density of HFM. This study concludes that hydrolyzed feather meal fermented by *L. casei* Shiota up to level 20% could not increase the solubility of dry matter and protein.

Keywords: dry matter solubility, protein, hydrolyzed feather meal, *L. casei* Shiota.

由干酪乳杆菌城田发酵的水解羽毛粉的干物质和蛋白质的溶解度

摘要：本研究旨在测定由干酪乳杆菌 城田 发酵的水解羽毛粉不同水平的干物质和蛋白质的溶解度。大多数角蛋白降解细菌是芽孢杆菌，但关于乳杆菌作为角蛋白酶生产者的潜力的出版物仍然非常有限。研究中使用的材料是羽毛、椰子粉、作为干酪乳杆菌城田 资源的养乐多、发酵仪器、干物质分析仪器和蛋白质溶解度。试验采用完全随机设计，5个处理，4个重复。处理为磷0 = 用 0%干酪乳杆菌城田 发酵的羽毛，磷1 = 用 5%干酪乳杆菌城田 发酵的羽毛，磷2 = 用 10%干酪乳杆菌城田 发酵的羽毛，磷3 = 用 15%干酪乳杆菌城田 发酵的羽毛，磷4 = 用 20%干酪乳杆菌城田 发酵的羽毛。数据通过方差分析进行分析，如果存在进展，则通过邓肯多系列检验进行分析。研究结果表明，干酪乳杆菌城田 不同水平发酵的水解羽毛粉对生物量、酸碱度、干物质和蛋白质溶解度的降低有显著影响（磷 <0.05 ），但对生物量、pH 值、干物质和蛋白质溶解度的降低没有显著影响（磷 >0.05 ）。水解羽毛粉的特性和堆积密度。该研究得出的结论是，由干酪乳杆菌发酵至 20% 的水解羽毛粉不能增加干物质和蛋白质的溶解度。

关键词：干物质溶解度，蛋白质，水解羽毛粉，干酪乳杆菌城田。

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1. Introduction

Chicken feathers are one of the types of chicken slaughtering waste, especially broiler chickens, which can be used as a source of protein feed. Based on data from BPS – Indonesia [1], broiler chicken production in 2018 reached 3,409,558.00 tons and increased to 3,495,090.5 tons in 2019. It is assumed that the weight of chicken feathers produced is 6% of the live weight, then the production of feathers in Indonesia in 2019 reached 236,705.4 tons. However, the chicken feather waste is currently not optimally used and significantly pollutes the environment because the decomposition process is quite long. Based on these conditions, it is necessary to manage and handle waste which is also a government policy in preserving environmental functions, as stated in the Constitution of the Republic of Indonesia Number 23 of 1997 concerning Environmental Management. In waste management, it is necessary to apply environmentally sound technology to overcome environmental problems by preserving environmental functions. Environment preservation needs zero waste, reducing or minimizing environmental pollution by utilizing waste chicken feathers. It can be used as an alternative animal feed [2] because of its high protein content of 80-90% of dry matter, exceeding the crude protein content of soybean meal (42.5%) and fish meal (66.5 %) [3].

The problem is that the protein contained in chicken feathers is a type of keratin that is difficult to digest by poultry. Keratin is a fibrous protein that is rich in sulfur amino acids. However, ultimate analyses resulted in carbon (64.47%), nitrogen (10.41%), oxygen (22.34%), and sulfur (2.64%) [4], so that chicken feather protein is difficult to dissolve in water, insoluble by digestive enzymes, and resistant to physical and chemical treatment.

For chicken feathers to be used as a source of quality protein, one alternative that can be done is by fermentation using bacteria that produce the enzyme keratinase. These keratinolytic enzymes are secreted by different types of microorganisms found in soil, water, and various keratin-rich sources; several types of bacteria identified that produce keratinase are derived from bacteria such as *Bacillus licheniformis*, *B. subtilis*, and *Stenotrophomonas maltophilia* [5].

Currently, publications on the potential of *Lactobacillus* as a producer of keratinase enzymes are still very limited. One type of *Lactobacillus* bacteria that have not been identified as having keratinolytic potential is *L. casei* Shirota. *L. casei* Shirota is a type of *Lactobacillus* probiotic that can maintain the health of the digestive tract of living things. This *Lactobacillus* is found in one of the probiotic drinks, namely Yakult, with a 6.5×10^9 CFU/ml concentration. Besides acting as a probiotic, *L. casei* Shirota also can break down protein and carbohydrates in food.

Based on the explanation above, a study was conducted to determine the potential of *L. casei* Shirota

bacteria in hydrolyzing the keratin contained in chicken feathers. This study aimed to examine the effect of the level of use of *L. casei* Shirota in fermenting chicken feathers on the solubility of dry matter and crude protein HFM produced.

2. Research Methods

2.1. Research Material

The materials used in this study included: chicken feathers, coconut cake, *L. casei* Shirota bacteria, water, 1 kg plastic bag, label paper, rubber bands, and a set of materials for solubility analysis of DM and CP. The tools used in this study include digital scales, insulation, buckets, pressure cookers, stoves, gas cylinders, 5 cc syringes, 50 cc syringes, fish grinding machines, digital pH meters, and a set of tools for solubility analysis of DM and CP.

2.2. Processing Stage

Fermentation of chicken feathers in this study followed the method carried out by Hendalia et al. [6]. Chicken feathers are obtained from chicken slaughterhouses in traditional markets. Chicken feathers are cleaned of other parts other than feathers, washed and then drained using a washing machine, steamed with a pressure cooker for 1 hour, and ground using a fish grinder machine. The ground chicken feathers were weighed as much as 450 grams (90%) and added 50 grams of steamed coconut cake (10%), then stirred until homogeneous. Furthermore, the chicken feather mixture was added with *L. casei* Shirota at different levels, namely 0%, 5%, 10%, 15%, and 20%. After adding *L. casei* Shirota, all the ingredients were stirred until homogeneous, then put into a 1 kg plastic bag, compacted, and fermented anaerobically for 7 days.

2.3. Testing Stage

After 7 days of fermentation, the feathers were weighed, and the percentage of biomass shrinkage was calculated; pH, availability, and organoleptic tests were measured by scores. After that, the HFM was dried in an oven at 60°C, the dry matter solubility was measured [7] and crude protein solubility [8].

2.4. Research Design

The study used a completely randomized design with five treatments and four replications, where:

P0 = 90% chicken feather + 10% soybean meal + 0% *L. casei* Shirota

P1 = 90% chicken feather + 10% soybean meal + 5% *L. casei* Shirota

P2 = 90% chicken feather + 10% soybean meal + 10% *L. casei* Shirota

P3 = 90% chicken feather + 10% soybean meal + 15% *L. casei* Shirota.

P4 = 90% chicken feather + 10% soybean meal + 20% *L. casei* Shirota + 15% *L. casei* Shirota.

The variables observed in the treatment were biomass shrinkage, pH, organoleptic, absorption, dry matter solubility, and crude protein of HFM produced.

2.5. Data Analysis

Table 1 Average biomass shrinkage, acidity, and HFM absorption for each treatment

Variable	Treatments				
	P0	P1	P2	P3	P4
Biomass Shrinkage (%)	0.6093 ± 0.1656 ^a	0.3395 ± 0.1871 ^b	0.3687 ± 0.1507 ^b	0.3552 ± 0.0901 ^b	0.254 ± 0.0972 ^b
Acidity (pH)	6.4850 ± 0.2144 ^a	5.0275 ± 0.2923 ^b	4.0500 ± 0.4614 ^c	3.8750 ± 0.2748 ^c	3.8025 ± 0.1056 ^c
Bulk Density (Kg/M ³)	0.4587 ± 0.0103	0.4615 ± 0.0157	0.4498 ± 0.089	0.4493 ± 0.009	0.4430 ± 0.0113
Solubility of DM in water(%)	18.8507 ± 3.2916 ^a	14.7557 ± 0.3108 ^b	15.0135 ± 0.2025 ^b	16.6447 ± 0.0836 ^{ab}	17.1695 ± 0.0274 ^{ab}
Solubility of DM in McDougall (%)	31.5336 ± 0.2898 ^a	21.3392 ± 0.8033 ^b	19.5546 ± 1.7431 ^{bc}	22.1946 ± 3.4803 ^b	18.1670 ± 1.2027 ^c
Solubility of CP (%)	2.2230 ± 0.0096 ^a	2.1316 ± 0.0080 ^b	1.6456 ± 0.0039 ^c	1.2332 ± 0.0012 ^d	0.9372 ± 0.0004 ^e

Notes: Different superscripts in the same row indicate a significant difference in each treatment.

3.1. Biomass Shrinkage

Biomass shrinkage is the weight lost when the material is fermented so that the final weight after fermentation will be reduced. Depreciation of the biomass of a fermented feed ingredient occurs due to the biochemical activity that occurs in the breakdown of carbohydrates and proteins. The analysis of variance showed that the treatment had a significant effect ($P < 0.05$) on biomass shrinkage, which is presented in Fig. 1.

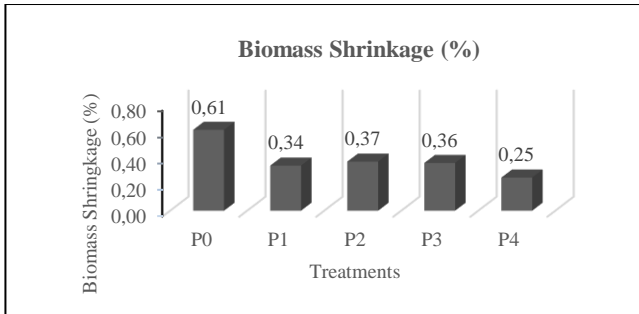


Fig. 1 Average biomass shrinkage

The use level of *L. casei* Shirota in chicken feather fermentation resulted in relatively the same biomass shrinkage but significantly different from the biomass shrinkage of HFM, which fermented without *L. casei* Shirota. The biomass shrinkage in each treatment was thought to be due to the decomposition of carbohydrates and proteins from added coconut meals.

3.2. Degree of Acidity (pH)

The degree of acidity is the acid-base level of a solution measured on a scale of 0-14. The degree of acidity is used to express the level of acidity or alkalinity possessed by a solution. Based on the results of the analysis of variance, it was seen that the treatment had a very significant effect ($P < 0.01$) on the degree of acidity (Fig. 2). The above means that the higher the level of *L. casei* Shirota added, the more acidic the HFM produced. *Lactobacillus* spp is lactic acid bacteria that produce lactic acid, showing

The data obtained were analyzed of variance; if there was a significant effect, it was continued with the Duncan's Multiple Range Test.

3. Results and Discussion

The average biomass shrinkage, acidity, and HFM absorption resulting from each treatment can be seen in Table 1.

interesting fermentation capacities [9]. The acid produced will reduce the pH value of the growth environment and cause a sour taste. A similar opinion *lactic acid* converts environmental conditions to acid (pH around 3.5). This low environmental pH does not match the optimum pH for keratinase activity. The keratinolytic enzymes show that most proteases work optimum reaction conditions at neutral to high alkaline pH from 7.0 to 12.5 [5]. With the low pH, the hydrolysis process of chicken feathers keratin in this study could not run well.

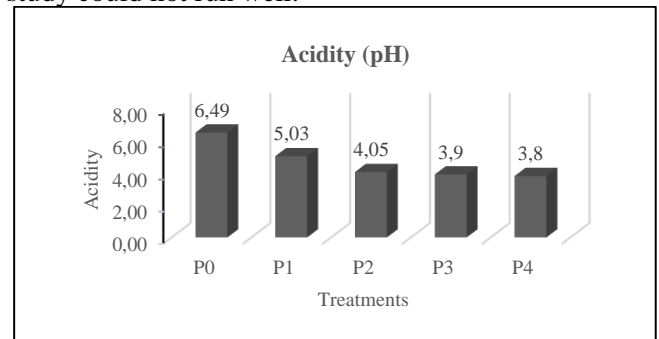


Fig. 2 Average acidity of HFM

3.3. Bulk Density

Bulk density is a general property of fibrous feed. HFM that has high quality is not bulky or has a high density [10]. The results of observations on the bulk density of HFM for each treatment can be seen in Fig. 3 below.

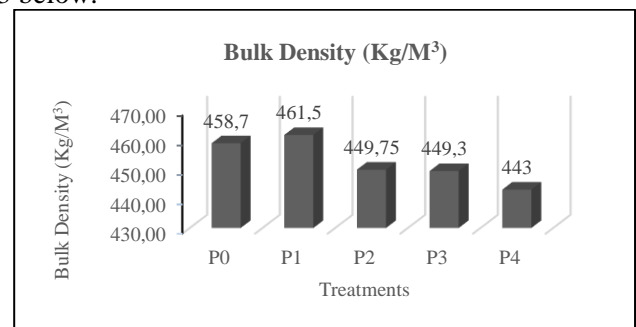


Fig. 3 The average value of bulk density

Based on variance analysis, it was found that the treatment had no significant effect ($P < 0.05$) on the HFM density value. In Table 1, it can be seen that the use of *L. casei* Shirota did not cause a change in the HFM density value, presumably because *L. casei* Shirota cannot hydrolyze the keratin in chicken feathers. Hence, the density values in each treatment are relatively the same. The value obtained in this study is lower than the optimal HFM density value reported by Moritz and Latsaw [10], which is 483 kg/m^3 .

3.4. Organoleptic Test

The organoleptic test showed the physical quality of the HFM produced. The results of observations on the organoleptic HFM can be seen in Table 2.

Table 2 Average results of physical observations of HFM

Physical Observation	Treatments					
	P0	P1	P2	P3	P4	
Color	3.00	2.80	2	2.98	3.13	2.98
Smell	3.25	3.25	3	3.28	3.30	3.38
Shape	3.60	3.30	3	3.18	3.58	3.40

Notes: Color (1. Gray white, 2. Yellowish white 3. Light brown, 4. Dark brown). Smell (1. Odorless, 2. Characteristic of feathers, 3. Fermented, 4. Ammonia). Shape (1. Very pliable, 2. Clay, 3. Slightly brittle, 4. Brittle).

The analysis of variance showed that the color, smell, and shape of HFM after fermentation in each treatment were relatively the same ($P > 0.05$). Before fermentation, HFM was gray-white, but after fermentation, treatment P0 was light brown, P1, P2, and P4 were yellowish-white. In contrast, treatment P3 was a dark brown caused by the browning reaction that occurs during the fermentation process. Some parameters of organoleptic properties of food quality, such as color and protein functionality, are affected by Maillard reactions. Unique aroma profiles are developed dependent on temperature–time profiles used during food processing [11].

HFM aroma had a pungent smell of fermentation in all treatments, indicating that the added inoculum produced the smell of butyrate fermentation. Increased NH_3 levels cause the pungent aroma of fermentation during fermentation. Process metabolism of a variety of compounds in all body tissues occurs ammonia production. Ammonia is produced by the metabolism of amino acids and other compounds which contain nitrogen [12]. The hydrolysis process affects increasing levels of NH_3 (ammonia) [13].

The shape of HFM in the P0 and P3 treatments was more brittle than P1, P2, and P4 ($P > 0.05$). The slightly brittle shape is thought to be caused by microbes' reshuffling of food substances during the fermentation process. The activity of enzymes that break the bonds in proteins, lipids, and amylase causes the breakdown of these components making the shape smooth and crumbly [14].

3.5. Solubility of Dry Matter and Crude Protein

The dry matter solubility of feed ingredients shows the number of feed ingredients that can be degraded in the digestive tract. The above means that the higher the solubility of the feed ingredients, the higher the digestibility of the feed ingredients. The following is a picture of the average solubility of DM presented in Fig. 4.

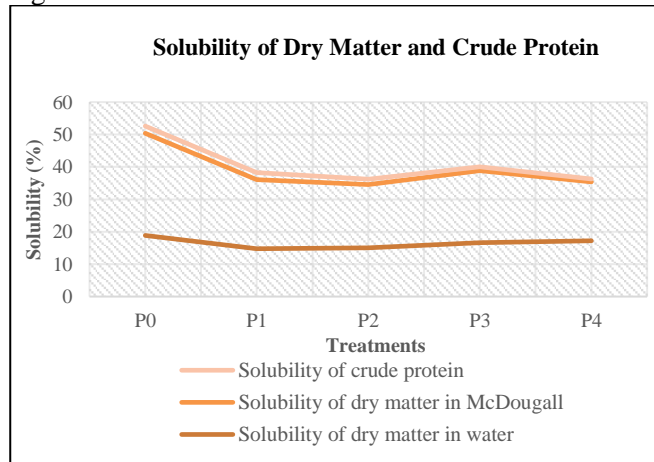


Fig. 4 Solubility of dry matter and crude protein of HFM

The analysis of variance showed that the treatment had a very significant effect ($P < 0.01$) on the dry matter solubility of HFM. The solubility of DM of HFM in McDougall was highest in treatment P0 and lowest in treatment P4. The solubility of DM in McDougall decreased with the addition of the level of *L. casei* Shirota. It increased again at the addition to 15% level. The above proves that the resulting HFM can still dissolve in water even though the value obtained is smaller than the solubility of DM in McDougall. The solubility percentage of DM in each treatment showed a higher solubility value of DM compared to the solubility of commercial DM, which was 11.6% [7].

The solubility of HFM protein in this study was smaller than that of chicken feather protein without treatment, namely 5.98% [15] that may be due to the heat treatment at the time of steaming the feathers. The solubility of keratin protein ranges from 2 to 6% at 50°C [16]. Chicken feathers were ground at a size of 1 mesh and stored at 4°C to obtain a protein solubility of 8.05%. Cold storage treatment only can increase the solubility of chicken feather protein below 30% [17]. The low DM solubility value of commercial HFM treated using high temperature is thought to be due to much protein being denatured, crystallizing so that the solubility decreases [13]. Broiler chickens grown on feed containing chicken feather hydrolysate with lactobacillus fermentation have a higher protein digestibility than bacillus fermentation [6].

4. Conclusion

This study examined the solubility of dry matter and crude protein hydrolyzed of chicken feathers fermented by *L. casei* Shirota on different levels. This study also examined the organoleptic, pH, biomass shrinkage, and

bulk density of HFM. So far, the use of *L. casei* Shirota is a protein-breaking bacteria, and its benefits have not been reported in breaking down keratin composed of cysteine and sulfur. Publications on the potential of Lactobacillus as a producer of keratinase enzymes are still very limited. Based on the study results, it can be concluded that chicken feathers fermented with *L. casei* Shirota to a level of 20% cannot increase the solubility of dry matter and crude protein HFM produced. Publications about the potential of Lactobacillus as a producer of keratinase enzymes are still very limited. Based on the study results, it can be concluded that fermented chicken feathers with *L. casei* Shirota at a level of 20% could not increase the solubility of dry matter and crude protein of HFM produced. However, in measuring the dry matter solubility of HFM fermented with *L. casei* Shirota, there is a tendency to increase dry matter digestibility along with the increase in the level of addition of *L. casei* Shirota, so further studies on the optimal level of addition are needed.

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