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The effects of chicken feather silage substitution for fish meal in the diet on growth of saline tilapia fingerlings (*Oreochromis niloticus*)

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Abstract. The objectives of this research were to study the effects of silage substitution of chicken feathers for fish meal in the diet on growth of saline tilapia fingerlings (Oreochromis niloticus). The fish sample used in the study was saline tilapia with the weight average of 3.21±0.04 g/fish. Completely Random Design was employed in the study with 5 (five) treatments and 3 repetitions. The treatments of the study were by replacing fish meal with the silage of chicken feathers. Those treatments were in various doses, namely; A treatment (0% of chicken feather silage), B treatment (12,5% of chicken feather silage), C treatment (25% of chicken feather silage), D treatment (37,5% of chicken feather silage), and E treatment (50 % of chicken feather silage). Parameters observed were Specific Growth Rate (SGR), Diet Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Efficiency of Diet Utilization (FEU), Protein Digestibility (ADCp), Survival Rate (SR) of Saline Tilapia Fingerlings and water quality. The results of the study show that the effects of the substation of silage chicken feathers for fish meal were highly significant (P<0,01) on SGR, FCR, PER, EFU, ADC_P; however, it was insignificant on survival rate of saline tilapia fingerlings. The results also show that B treatment (12,5% of chicken feather silage) was the best dose to increase growth of saline tilapia fingerlings. During study, the water quality was in viable condition.

1. Introduction

Saline Tilapia (*Oreochromis niloticus*) has several advantages, such as resistance to high salinity, high adaptability to the environment, fast growth, and carnivore. Saline tilapia industry faces several problems, such as high price of the diet; it is due to imported raw material of the diet. An excess demand for diet as main source of protein in the long run forced farmers to find an alternative of protein source [1].

Chicken feather is one of protein sources to substitute fish meal as an ingredient diet. Chicken feather is a waste of chicken industry. It is abundant and keeps growing as chicken industry growing. Chicken feather has high content of protein (80-90%) that is higher than soy meal (42.5%) and fish meal (66.5%) [2]. Although chicken feather has high protein, the protein is difficult to digest due to keratin type protein [3]. Keratin is hardened epidermal fiber such as nail, hair, and feather containing sestein and cysteine [4]. Moreover, keratin is made of 14% disulphide bond; therefore, it becomes stabile, rigid, and also difficult to digest by proteolithic enzymes such as tripcyne, pepsin, and papain in the intestine [5]. Ruminansia animal is only able to digest protein at the keratin type around 5.8% [6]. Low digestibility of protein in the chicken feather is one of constraints as protein source of fish

diet. To solve the problem, chicken feather was first made as flour and then fermented. The fermentation used microorganism, one of them was *Bacillus subtilis* [7]. *B. subtillis* can decompose keratin in the chicken feather because *B. subtillis* can produce keratonic enzyme. The fermentation using B. Subtillis was able to increase digestibility and improve the protein. The fermentation was to grow micro-organism which can produce enzyme. In turn, the enzyme was to change the substance that was difficult to digest into the form easy to digest; therefore, it improved diet quality and flavor [7]. Keratinase has good ability to transform keratin chemical cell membranes, unbind hydrogen and disulphide [2][8]. With chemical treatment, chicken feather can be a source of amino acids containing sulphur [7]. Chicken feather has a rich of essential amino acids, such as Sistine, threonine, and arginine and also contains protein that is difficult to digest (75-87%) [2].

Studies on substitution of fish meal with silage of chicken feather were done in several species, such as on the studies of *Onchorhynchus tshawytscha* [9], *Oncorhynchus mykiss* [10], Hybrid Clarias Catfish [11], *Cromileptes altivelis* [12] *Clarias gariepinus* [13], and *Oreochromis niloticus* [8][14][15]. However, there was a lack of studies of saline Tilapia on the fish meal substitution with chicken that in the diet; therefore, it needs study the effects of silage substitution of chicken feathers for fish meal in the diet on protein digestibility, efficiency of diet utilization, and growth of saline tilapia fingerlings (*Oreochromis niloticus*)

2. Research Methods

The study was conducted at the Center for Brackish Water Aquaculture in Jepara, Central Java, Indonesia from January until April 2018. The study used saline tilapia fingerlings (*O. niloticus*). The average weight of the fingerlings was between 3.17 and 3.25 gram per fingerling. The samples were obtained from Center for Brackish Water Aquaculture in Jepara, Central Java, Indonesia. The samples were selected based on size, weight, body normality, and physical health [16].

Diet used in the study was in the form of granule. It contained 30% protein (ios-protein) and 2.47 kcal energy per gram diet (iso-energy). The treatment in the study was substituted fish meal with silage of chicken feather. The treatments consisted of various doses of substitution, those were treatment A (0% of chicken feather silage), treatment B (12.5% of chicken feather silage), treatment C (25% of chicken feather silage), treatment D (37.5% of chicken feather silage), and treatment E (50% of chicken feather silage).

In the study, silage of chicken feather and fish meal were main sources for animal protein, while soy meal was as a source of plant based protein. Carbohydrate source came from corn meal, rice bran, and wheat flour. Fat was obtained from fish oil and corn oil. Minerals and vitamin mix were as sources for mineral and vitamin. CMC was used as binder. Cr2O3 was an indicator of digestibility 0,5% [17]. The composition and type of diet were analyzed by proximate analysis.

Process of diet production was initiated by fermenting chicken feather. Before fermentation, chicken feather was sterilized at 121 °C, 1 kg/cm2 for 15 minutes. The objective of the fermentation was to get rid of bad micro-organism or to sterilize contaminant produced by other organisms. First, 2 gram flour of chicken feather was mixed with *B. subtilis* liquid as much as 5, 10, and 15 mL into Erlenmeyer at pH 8.5 and incubated at 55°C for 72 hours. The results were called chicken feather hydrolisate [18].

The ingredients of the diet were shown in the Table 1. To produce diet was by mixing the ingredients, starting with least amount ingredient needed to the most amounts. After mixing all the ingredients, the diet was dried by putting it into the oven. After the diet dried, it was produced in the granule form. The results of proximate analysis can be seen in the following table.

Table 1. Diet Composition

| Diet Ingredient (g/100g diet) | Composition (%) | | | | | |
|-------------------------------|-----------------|-------|-------|-------|-------|--|
| | A | В | C | D | E | |
| Fish meal | 26.00 | 22.75 | 19.50 | 16.25 | 13.00 | |
| Silage of Chicken Feather | 0.00 | 3.25 | 6.50 | 9.75 | 13.00 | |

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| Ti . | | | | | |
|--------------------------------------|--------|--------|--------|--------|--------|
| Flour | | | | | |
| Soy meal | 26.00 | 21.30 | 20.35 | 20.50 | 18.50 |
| Corn meal | 17.00 | 18.25 | 18.40 | 16.75 | 19.10 |
| Rice bran | 18.30 | 20.25 | 19.20 | 20.00 | 21.30 |
| Wheat flour | 5.20 | 7.20 | 11.30 | 13.00 | 14.00 |
| Fish oil | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Corn oil | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Mineral.Vitamin mix | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| $C_r 2O_3$ | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| CMC | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Total (g) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| The results proximate analysis | | | | | |
| Protein (%)* | 30.35 | 30.23 | 30.07 | 30.65 | 30.44 |
| Fat (%)* | 7.68 | 6.75 | 6.25 | 5.75 | 5.27 |
| BETN (%)* | 41.74 | 44.82 | 46.62 | 48.03 | 50.29 |
| Energy (kcal/g) ^a | 272.74 | 272.56 | 272.44 | 273.85 | 274.95 |
| Ratio E/F (kkal/g diet) ^b | 8.99 | 9.02 | 9.06 | 8.94 | 9.03 |

Notes:

- a. *Digestible Energy* was calculated using Wilson's method [19]. There was 1 g protein equivalent with 3,5 kcal/g, 1 g fat equivalent 8,1 kcal/g, and 1 g carbohydrate equivalent 2.5 kcal/g.
- b. According De Silva [20], the value of E/F for optimal growth was 8-12 kcal/g.
- * The results of proximate analysis at the Animal Diet Laboratory, Faculty of Animal Science, Diponegoro University (2018)

Water quality parameters included water temperature, dissolved oxygen, pH, and ammoniac. The measurement of temperature was conducted every day in the morning and afternoon, while the measurement of dissolved oxygen and pH was done every week. The measurement of fish weight was also scaled every week. The ammoniac was measured at the beginning and the end of the study. Variables of water quality that were tested were pH, temperature and Ammoniac.

The containers used in the study were made of plastic in box shape with the volume of 25 liter. There were 12 containers. Aerator to recirculate the water was placed in every container. Each of the box containers was sterilized using kalium permanganate to get rid of bacteria. After sterilizing, the box was washed using water and dried. In each box container was put sand filters and filled with the 20 ppt saline water.

The fish was raised in the box containers for 49 days. Fish was given dieting at satiation for 3 (three) times each day, at 06:00, 12:00, and 16:00. The measurement of weight gain was conducted every week. One hour after dieting, the waste was syphoned to maintain good water quality.

The observed parameters were specific growth rate (SGR), protein efficiency ratio (PER), diet conversion ratio (FCR), Efficiency diet utilization (EFU) [21], also protein digestibility (ADC_P) [22], and survival rate [17]. Content analysis of Cr_2O_3 in the diet and feces used modified calorimetric method [22]. The equations of the parameters were as follow:

SGR: ln Final weight – ln Initial weight x 100%

Time experiment

FCR: The amount of diet consumed x 100 %

(Final weight + Total weight fish dead) - Initial weight)

PER: Final weight – Initial weight x 100%

The amount of diet consumed x Protein content of diet

EFU: Final weight – Initial weight x 100%

The amount of diet consumed

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ADC_P:100 $\left\{ \frac{\% \text{ Cr}_2\text{O}_3 \text{ in the diet}}{\% \text{ Cr}_2\text{O}_3 \text{ in the feces}} \times \frac{\% \text{ protein in the feces}}{\% \text{ protein in the diet}} \right\}$

SR : (<u>Final count</u>) x 100% Initial count

To test normality, homogeneity, and additivity of the data was applied to identify the normality, homogeneity, and additivity of the data. After those tests, ANOVA analysis was implemented. If the results of ANOVA were highly significant (P<0.01) or significant (P<0.05), Duncan test was needed to determine the mean difference of the treatments [23]. Water quality was descriptively explained.

3. Results and Discussion

The results of the study on the effects of silage substitution of chicken feathers for fish meal in the diet on SGR, FCR, PER, EFU, ADC_P, and SR were shown in the Table 2, as follow:

Table 2. The Results of RGR, PER, FCR, EFU, ADC_P and SR of Saline Tilapia Fingerling

| | Treatments | | | | |
|---------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | A | В | С | D | E |
| SGR %/day) | 1.19±0.13 ^b | 2.61±0.22 ^a | 1.36±0.42 ^b | 1.25±0.35 ^b | 0.09±0.15 ^b |
| FCR | 2.37 ± 0.23^{b} | 1.48 ± 0.21^{a} | 2.12 ± 0.22^{b} | 2.24 ± 0.22^{b} | 2.30 ± 0.17^{b} |
| PER | 1.25±0.64 ^b | 3.29 ± 0.24^{a} | 2.26±0.31 ^b | 1.89±0.43 ^b | 1.13±0.23 ^b |
| EFU (%) | 55.57±0.36° | 78.35±0.45 ^a | 65.59±0.22 ^b | 60.15±0.12° | 53.36±0.27° |
| $ADC_{P}(\%)$ | 50.28±0.04 ^d | 76.68±0.37 ^a | 64.31±0.54 ^b | 55.02±0.34° | 53.43±0.29° |
| SR (%) | 98.60±1.04 ^a | 98.60±1.04 ^a | 96.78±0.43 ^a | 96.78±0.43 ^a | 90.98±0.51 ^a |

Note: The Values with the same superscripts in the same row show there was no significant different

ANOVA test showed that the substitution of chicken feather flour for fish meal was highly significant (P<0.01) on SGR of saline tilapia fingerlings (O. niloticus). The dose of the substitution of chicken feather flour for fish meal (12.5-37.5%) had the value of SGR ranged from 1.25 to 2.16%/day higher compared to without substitution (0%) with the value of 0.93%/day. The results were similar to the results of the studies of [11][14][24][25][26].

The treatment B (12.5% chicken feather silage) had the highest value of SGR, 2.61 %/day, followed by treatment C (1.36%/day), D (1.252%/day), E (1.16%/day) and A (0.09%/day). It was due to the right dose of the substitution of chicken feather flour for fish meal; therefore, the fish can utilize amino acids in the diet to improve the growth. Chicken feather contains a lot of essential amino acids, such as sistine, threonine, and arginine [2]. Treatment B also resulted in the highest values of ADC_P, EFU, PER and the lowest value of FCR with the values of 76.68%, 78.35%, 3.29 and 1.48 respectively. Treatment E (50% chicken feather silage) gave the lowest value of SGR; it was because of unfully degraded keratin. Others were also suspected that the diet was low in essential amino acids of metyonine, lysine and hystidine. In other study also reported that chicken feather silage has high protein, but has low in metyonine, lysine and histidine [8]. Meanwhile, the smell of diet in the treatment E caused the fish appetite to decrease. It was informed that diet with more than 50% made of chicken feather silage caused the fish to drop its palability [24].

Diet conversion ratio (FCR) was the measurement to quantify how the fish was able to gain weight from the diet intake [21]. The FCR was an indicator of diet conversion efficiency. Moreover, the less FCR the more efficient diet conversion was [27]. The low FCR indicated better quality of the diet [28]. The results of analysis variance proved that the effect of chicken feather silage substitution for fish meal on FCR of saline Tilapia was highly significant (P<0.01). Treatment B (12.5% chicken feather silage) has the lowest FCR with the value of 1.48, as shown in the Table 2. There is a s similar result on the study of lamb hair silage substitution in the diet in the *Labeo rohita* [29]. The study found that the FCR of *Labeo rohita* increased as lamb hair silage substitution in the diet increased. The best value of FCR was also followed by good values of ADC_P, EFU, PER and SGR in which they had the best values of 76.68%, 78.35%, 3.29 and 2.61 respectively. Therefore, it could be concluded

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that the chicken feather silage substitution in the treatment B was able to absorb, to digest, and to be utilized by saline (O. niloticus).

The FCR was used to identify the quality of the diet. One of the indicators of protein quality was the value of protein digestibility. Protein that was easily digested indicated better absorption of amino acids to the fish; in turn it increased growth [21]. The results of analysis variance verified that the effect of chicken feather silage substitution for fish meal on PER of saline Tilapia was highly significant (P<0.01). Treatment B (12.5% of chicken feather silage) had the highest PER value (3.29) and the treatment of E (50 % of chicken feather silage) had the lowest PER value (1.13). The high value of PER in the treatment B was due to the high values of ADC_P and EFU with the values of 76.68% and 78.35% respectively. These results are similar with other studies [11][14][25][26].

The efficiency of diet utilization (EFU) value of saline Tilapia was 12.5-37.5% higher for the fish given chicken feather silage substitution than that (0%) of not given chicken feather silage substitution. The treatment B (12.5% chicken feather silage) generated the highest EFU with the value of 78.35%. However, the more chicken feather silage substitution was given, the less EFU value was obtained. It was evidence that the dose in the treatment B was the right amount for saline Tilapia to produce the high value of EFU. These results are similar with other studies [11][14]. They studied in hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*) and *Oreochromis niloticus* respectively.

The treatment B (12.5% chicken feather silage) also resulted in the best ADCp with the value of 76.68%. The more chicken feather substitution was given, the lower ADCp was obtained. The treatment B indicated that the dose was the appropriate amount to be absorb by the fish. These results are similar with other studies in hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*) [11] and *Oreochromis niloticus* [14].

The results also show that chicken feather substitution for fish meal in the diet was insignificant (P>0.05) on survival rate (SR) of saline Tilapia. These results are similar with other studies [8][11][12][13][14][15]. The diet did not influence on survival rate of the fish, but initial treatment and media quality for aquaculture [31]. The parameters of water quality were presented in the Table 3.

Table 3. Parameters of Water Quality in the Saline Tilapia Aquaculture

| | Water Quality | | | | | |
|-------------|------------------|----------------|-------------|------------------------|--|--|
| Treatment | Temperature (°C) | pН | DO (mg/l) | NH ₃ (mg/l) | | |
| A | 27 – 32 | 6.75 - 8.02 | 4.45 - 4.87 | 0.0053 - 0.0053 | | |
| В | 27 - 32 | 6.83 - 8.00 | 4.58 - 4.68 | 0.0053 - 0.0053 | | |
| C | 27 - 32 | 6.50 - 8.00 | 4.60 - 4.75 | 0.0053 - 0.0053 | | |
| D | 27 - 32 | 6.75 - 8.03 | 4.62 - 4.82 | 0.0053 - 0.0053 | | |
| Feasibility | 14-38* | $6.50 - 8.5^*$ | >2* | <0.1* | | |

Note: * [32]

4. Conclusion

The results showed that the substitution of chicken feather silage for fish meal was very significant (P <0.01) at growth of Tilapia saline and a dose of 12.5% chicken feather silage (treatment B) was the best dose to increase the growth of Tilapia saline (O. niloticus).

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