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Hydrolyzed Chicken Feather Meal as Protein Source for Red Tilapia (*Oreochromis* sp.) Aquafeeds

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ABSTRACT

An 84-day feeding trial was conducted to determine the effects of Hydrolyzed Chicken Feather Meal (HCFM) supplementation on the growth, feed efficiency, survival rate and carcass composition of red tilapia (*Oreochromis* sp). A group of red tilapias (initial weight 24.09 ± 0.05 g) were fed 3, 6, 9 and 12% HCFM as a supplement and compared to a control group without supplementation. After 84 days, growth parameters such as body weight gain (BWG), average weekly gain (AWG), daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER), survival rate (SR) and carcass proximate of red tilapia were measured. The results showed that HCFM up to 12% could be used as supplementation and showed positive effects on all growth parameters. Supplementation of HCFM above 6% in the red tilapia diet increased the protein and fat content of red tilapia's carcass. The highest survival was found on red tilapia fed 9% HCFM supplementation in the diet. The current study also found that supplementation of HCFM in the diet is possible for red tilapia without impairing growth, feed intake, survival rate or carcass composition. Altogether, the results suggest that HCFM is an optional ingredient which can be used as an alternative for fish meal in aquafeed for red tilapia.

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N surveyed the location and collected the data. N and RAN analyzed the data and wrote the manuscript.

Key words
Hydrolyzed chicken feather meal, Growth parameters, *Oreochromis* sp., Carcass composition, Survival rate.

INTRODUCTION

Fish meal (FM) plays an important role as an ingredient for fish feed (Caruso, 2015) and is known as a prime protein source of high quality and is a very digestible feed ingredient (Miles and Chapman, 2006; Tantikitti *et al*., 2016). Approximately 30% of total fish caught are converted to FM and fish oil (Barroso *et al*., 2014). However, there is a limitation in the production of FM due to an increasing price of this source (FAO, 2010; García-Romero *et al*., 2014; Kurbanov *et al*., 2015). Although FM is still a favourite among fish farmers, the search for potential alternative ingredients has become a well-recognized priority for sustainable development of the aquaculture industry. The use of poultry to replace FM that is often scarce, expensive, of limited availability, and which leads to high fish production costs provides an alternative approach and has been gaining momentum.

Replacing FM with cheaper ingredients such as chicken feather meal is a priority for nutrition research. Chicken feather is waste from slaughterhouses that has been found to have considerable economic potential to replace FM. Chicken feathers can be used for animal feed because they have high protein content, which comprises 80–90% dry matter and exceeds the raw protein content of soybean meal (42.5%) and fish meal (66.5%). Moreover, proximate analysis of chicken feathers found the following compositions: crude lipid (0.83%), crude fiber (2.15%), crude protein (82.36%), ash (1.49%), NFE (1.02%) and moisture content (12.33%) whereas ultimate analyses resulted in carbon (64.47%), nitrogen (10.41%), oxygen (22.34%) and sulphur (2.64%) (Tesfaye *et al*., 2017). However, chicken feather meal only has the digestibility of dry and organic matter *in vitro,* respectively at 5.8% and 0.7% because it has a fiber-shaped protein type (fibrous protein). Thus, chicken feathers must be hydrolyzed to improve digestibility (Bureau *et al*., 1999) and can be used as a protein source in fish diets such as red tilapia (*Oreochromis* sp.). Previous research noted that the used of poultry by product has been conducted in some fish research such as Koi fish, *Anabas testudines* (Bloch) (Bhaskar *et al*., 2015), golden pompano (*Trachinotus ovatus*) (Wang *et al*., 2017) and juveniles of European seabass (*Dicentrarchus labrax*) (Campos *et al*., 2017). However, there has been limited research on the effects of dietary HCFM as a protein source on the growth, survival and feed efficiency of red tilapia.

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Tilapia are generally placed as the second most commonly used aquaculture fish in the world (Fitzsimmons, 2010) and reached more than 5,000,000 metric tons of global production in 2016 (Fitzsimmons, 2016). As one of the important species in Indonesia aquaculture, the production of tilapia has also increased gradually in some Indonesian regions such as South Sumatera, West Java and East Kalimantan (Widiarti, 2015). Meanwhile, red tilapia are one of the most popular freshwater and economically important food fish, and have high protein levels (Nakphet *et al*., 2017; Pauly and Zeller, 2017; Roslan *et al*., 2014). Red tilapia can be cultivated with several advantages such as rapid growth, ease of breeding and resistance to pests and diseases as well as being able to adapt to environmental changes; they are popular for both local and export markets. The production of red tilapia can be accelerated by providing feed containing high quality nutrients in accordance with the requirements of fish to increase growth in shorter time periods.

To evaluate growth performance and health of fish, various physiological tools such as body weight gain (BWG), average weekly gain (AWG), daily weight gain (DWG), specific growth rate (SGR) (Nur *et al*., 2017; Yusup and Nugroho, 2017), feed conversion ratio (FCR) (Fum *et al*., 2017), feed efficiency (FE) (Moutinho *et al*., 2017), protein efficiency ratio (PER) (Azaza *et al*., 2015), survival rate (Cai *et al*., 2015) and carcass proximate (Aryani *et al*., 2017) have been successfully used. Thus, the current study was designed to evaluate the effects of dietary HCFM as a protein source on the growth, feed efficiency, survival rate and carcass composition of red tilapia.

MATERIALS AND METHODS

Animals and experimental setup

Three-month-old red tilapia (n=900) (average initial weight 24.09 ± 0.05 g) were provided from local fish breeding sources in Cirata, Cianjur West Java (Indonesia) and acclimated at PT Suri Tani Pemuka, Cianjur West Java, Indonesia for one week. The fish were then randomly distributed into five experimental groups, namely control and four diets supplied with 3, 6, 9 and 12% of HCFM. Each experimental group was in triplicate with 50 fish per replicate being 15 experimental pond cages. These cages were randomly assigned to each group dietary treatment. The daily feed was divided into five parts (8, 10, 12 am, 2 and 4 pm) by using at satiation method. The fish were fed until apparent satiation and the feeding experiment lasted for 84 days. To quantify the exact feed intake, refused feed was siphoned out immediately, dried and weighed. Temperature, pH and dissolved oxygen (DO) were measured daily with a routine thermometer, an HM-7 pH meter (pH 110, Eutech Instrument Cyberscan, Thermo Scientific, Illinois, USA) and a DO meter (YSI 550A Clandon, Ohio, USA). Nitrate, nitrite and ammonium were measured and recorded weekly using chemical test kits (Rochelle Salt Solution and Nessler Reagent, HACH, Loveland, Colorado, USA).

Diet preparation

All raw materials for control and test diets were prepared, weighed and mixed with a Getra mixer (B20-F volume 20 L Series R10240, China). A pelleting machine was used to obtain wet strands that were of 3 mm diameter. Both control and test diets were dried in an oven (30ºC), allowed to cool at room temperature, and stored at 27ºC throughout the experimental period. Determination of feed composition proximate analysis including dry matter, crude protein, crude fat and ash was performed. The compositions of the control and test diets are shown in Table I.

Table I.- Ingredient and proximate composition of control and trial diets.

Ingredients	Types of diet with hydrolyzed feathers meal inclusion				
	Control	3%	6%	9%	12%
Corn	26.00	23.00	20.00	17.00	14.00
Wheat by product	18.01	18.00	18.00	18.00	18.00
Soybean meal	17.64	18.31	18.65	19.00	20.20
Sunflower meal	5.30	5.30	5.30	5.30	5.30
Cassava starch	7.20	7.20	7.20	7.20	7.20
HCFM	0.00	3.00	6.00	9.00	12.00
Animal protein*	18.00	17.34	17.03	16.71	15.41
Fish meal	3.00	3.00	3.00	3.00	3.00
Mix oil	1.75	1.72	1.66	1.60	1.66
Vitamin mix	0.25	0.25	0.25	0.25	0.25
Proximate composition (%)					
Dry matter	90.8	91.6	91.4	91.8	90.9
Crude protein	35.5	36.0	36.7	37.4	37.4
Crude fat	5.1	5.2	5.0	4.8	4.6
Ash	11.1	9.5	9.3	9.3	9.4

*Animal protein: Bone meal and blood meal.

Sampling and analytical procedure

Body weights (BW) of fish from each tank were recorded at weeks 4, 8 and 12 of the experiment. At the end of the experiment, BWG, AWG, DWG, SGR (Nur *et al*., 2017; Yusup and Nugroho, 2017), FCR (Fum *et al*., 2017), FE (Moutinho *et al*., 2017), PER (Azaza *et al*., 2015) and SR (Cai *et al*., 2015) were measured.

Proximate analysis

To calculated carcass composition, fish was transformed into meal for a proximate standard test (AOAC, 2005) by using a chemical test and NIRS automatic machine. Fish were measured for protein and fat contents.

Statistical analysis

Results were expressed as mean ± standard error (SE). Proximate analysis and growth data were objected to analysis of variance (ANOVA) using IBM SPSS Statistics 22 (SPSS, Inc., USA). Comparisons among treatment means were carried out by one-way analysis of variance followed by Duncan's test. Standard Error (±SE) was calculated to identify the range of means. Percentage data were transformed by arc-sine transformation prior to ANOVA and reversed afterwards. All significant tests were at $P<0.05$ levels.

RESULTS

Growth, feed efficiency and survival rate

After 84 days of feeding trial, the BWG and AWG of red tilapia fed dietary 12% of HCFM supplementation were significantly higher (P<0.05) than red tilapia fed other diets. The red tilapia fed dietary HCFM inclusion above 3% also had significantly higher DWG than control diet. Meanwhile, SGR, FCR, FE and PER were not affected by the supplementation of HCFM in the diet of red tilapia (Table II). At the end of feeding trial, the lowest survival rate was found for red tilapia fed with 12% of HCFM inclusion whereas the highest survival rate was shown for the red tilapia fed control and 9% HCFM supplementation in the diet (Fig. 1).

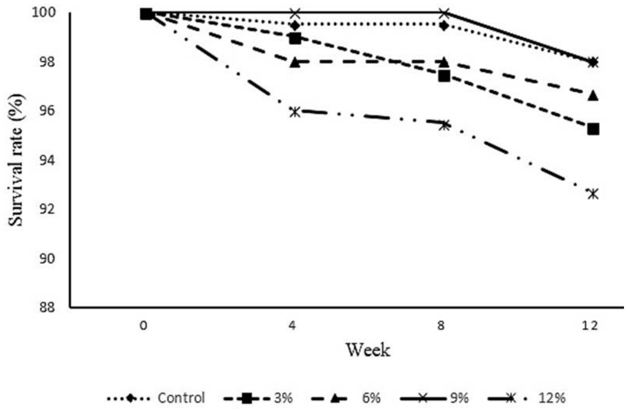

Fig. 1. Survival rate of red tilapia (*Oreochromis* sp.) with different concentrations of hydrolyzed chicken feather meal inclusion (HCFM) for 12 weeks.

Table II.- Growth performance of red tilapia (*Oreochromis* sp.) with different concentration of hydrolyzed feathers meal.

Parameters	Hydrolyzed feathers meal inclusion				
	Control	3%	6%	9%	12%
BWG (g/fish)	77.56 ± 0.52[a]	64.43 ± 0.17[b]	80.93 ± 0.20[c]	83.96 ± 0.87[c]	84.20 ± 0.73[d]
AWG (g/fish)	6.46 ± 0.44[a]	5.36 ± 0.89[b]	6.74 ± 0.16[a]	6.99 ± 0.72[a]	7.01 ± 0.60[c]
DWG (g/fish)	0.92 ± 0.06[a]	0.76 ± 0.12[b]	0.96 ± 0.02[c]	0.99 ± 0.10[c]	1.00 ± 0.08[c]
SGR (%/day)	1.70 ± 0.13	1.53 ± 0.31	1.71 ± 0.04	1.78 ± 0.20	1.78 ± 0.17
FCR	1.74 ± 0.04	2.07 ± 0.15	1.86 ± 0.09	1.91 ± 0.16	1.75 ± 0.08
FE (%)	57.64 ± 1.54	48.43 ± 3.44	53.90 ± 2.83	53.24 ± 4.96	57.30 ± 2.86
PER	1.62 ± 0.04	1.35 ± 0.09	1.47 ± 0.07	1.42 ± 0.12	1.53 ± 0.07

Values are Means ± Standar error. Means in the same row having different superscript letters (a, b, c) indicated significant differences (*P<0.05*). BWG, body weight gain; AWG, average weight gain; DWG, daily weight gain; SGR, specific growth rate; FCR, feed conversion ratio; FE, feed efficiency, PER, protein efficiency ratio; SR, survival rate.

Table III.- Proximate analysis of hydrolyzed feathers meal of fish.

Parameters (%)	Hydrolyzed feathers meal inclusion				
	Control	3%	6%	9%	12%
Crude protein	48.50 ± 0.25[a]	49.59 ± 0.05[a]	51.32 ± 0.12[b]	51.40 ± 0.03[b]	51.93 ± 0.27[c]
Crude fat	20.84 ± 0.15[a]	19.67 ± 0.18[a]	21.40 ± 0.22[b]	21.56 ± 0.21[b]	23.00 ± 0.11[c]

Fish carcass composition

Table III shows carcass compositions (%/100 g wet weight basis) of the red tilapia at the five levels of HCFM supplementation after the 84-day experimental period. Crude protein and crude fat of red tilapia fed 12% HCFM supplementation showed a high percentage after this 84-day period.

Water quality parameter

Water quality parameters were recorded during the experiments along with their tolerable limits (Table IV). Average temperature, pH, DO, nitrite and TAN for this research were found to be at the tolerable limit.

Table IV.- Water quality parameters.

Parameters	Mean± SE	Tolerable limits
Temperature (°C)	22.13 ± 0.17	20.9 – 24.3
pH	7.35 ± 0.25	6.5 – 9.0
DO (ppm)	5.70 ± 0.31	3-12
Nitrite (ppm)	0.17 ± 0.07	0.5
TAN (ppm)	0.14 ± 0.02	0.5

DISCUSSION

Growth performance

Positive effects of growth of red tilapia were observed when fish were fed 12% HCFM in the diet. The body weight of red tilapia also increased with the increasing level of HCFM. According to Grazziotin *et al.* (2008), Moritz and Latshaw (2001) and Wiradimadja *et al.* (2014), chicken feathers contain keratin proteins that have low digestibility. However, hydrolysis can increase digestibility to 54–76%. Further, the absorption of nutrients becomes more optimal when consumed at high concentrations. This suggests that the use of HCFM as a protein source in aquafeeds could replace some other animal protein sources with the same result. This study also found that the 12% of HCFM supply in the diet of red tilapia for 84 days resulted in higher BWG (84.20 ± 0.73 g/fish), AWG (7.01 ± 0.60 g/fish) and DWG (1.00 ± 0.08 g/fish) than the control group. This finding is similar to previous research revealing that replacement of fish meal by using poultry also increased fish growth parameters such as in juvenile Nile tilapia (*Oreochromis niloticus*) (Yones and Metwalli, 2016), juveniles of rainbow trout (*Oncorhynchus mykiss*) (Barreto-Curiel *et al*., 2016) and Japanese sea bass (*Lateolabrax japonicus*) (Wang *et al*., 2015). Another current study, however, mentioned that poultry used as an FM replacement in the diet of juvenile Black Sea Bass *Centropristis striata* from 40–90% led to no significant differences in BWG (Dawson *et al*., 2018). Similarly, no effects on growth performance were found in Atlantic salmon (*Salmo salar*) fed diets with poultry meal and blood meal in combination with poultry oil (Hatlen *et al*., 2015; Aryani *et al*., 2017).

It is assumed that the amount of protein contained in the feed that can be digested was sufficient to support fish growth. Growth may stop and a decrease in body weight can occur if protein is deficient. Further, protein in the body system can be broken down to maintain more critical functions such as the immune system (Magnadóttir, 2006; Uribe *et al*., 2011). In addition to protein metabolism, low fish growth rates can occur due to the amount and characteristics of keratin protein contained in feather meals, and only a small amount of protein can be absorbed by the body. Moreover, keratin proteins have a strong double bond that inhibits breakdown by proteolytic enzymes in the digestive tract (Montes-Zarazúa *et al*., 2015; Sinkiewicz *et al*., 2017). Growth values exceeding 1% indicate that the amount of protein contained in the feed and digested by the fish is sufficient, providing a balance of essential amino acids in the diet. In addition, specific growth rate is also affected by the balance of nutrients in the diet that can be digested by fish, particularly the amino acid content. These results also indicate that the use of HCFM up to 12% is still acceptable by red tilapia.

Current findings also found that SGR, FCR, FE and PER of red tilapia fed any level of HCFM were not significantly different to the control. These findings showed that a supply with 3–12% of HCFM in the diet of red tilapia can be applied with no adverse effects on fish growth. This result is in accordance with previous research noting that replacing FM with other soy protein (33–100%) led to no significant difference in growth performance or nutrient utilization of rainbow trout, *Oncorhynchus mykiss* (Kaushik *et al*., 1995). Moreover, protein efficiency is influenced by several factors including fish size, physiological functions of fish, feed quality and feeding rate. High PER values also indicate a good feed quality (Cho, 1992; Council, 2011; De Silva and Anderson, 1994). In addition, HCFM inclusion in the diet up to 12% reflects that the quality of this ingredient can match the value of the feed using animal protein meal, which is beneficial because animal protein generally has high prices and materials that compete with human needs.

Survival

Although 12% HCFM inclusion reduced survival of red tilapia to 92%, this survival rate was at an acceptable level. This result is similar to past research finding that substituted fish meal using another source of protein in the diet of *Clarias gariepinus* fingerlings (Djissou *et al*., 2016) led to a high survival rate above 90% or similar survival rate with control (100%) with respect to juvenile Ussuri catfish *Pseudobagrus ussuriensis* (Bu *et al*., 2017). In contrast, study replacement fish meal with soya protein concentrate on snakehead (*Channa striata*) showed lower survival rates compared to control (Hien *et al*., 2017). In addition, a previous study on the use of fermented feather meal as a fish meal replacement on the survival of *Oreochromis niloticus* revealed that the survival of the fish was not affected by any concentration of fermented feather meal substitution (Arunlertaree and Moolthongnoi, 2008). Hence, complete nutrition in the feed provides energy for the activity and survival of fish. The quality of feed is determined by its nutrient content because fish utilize the feed to obtain energy in accordance with their needs.

Diet composition

Diets were formulated to be optimal for growth and survival of red tilapia by inclusion of HCFM up to 12%, with no significant negative effects on fish growth performance and survival rate. The inclusion of HCFM might decrease diet costs, which explains the lower values of cost for per-kg fish production. The present study revealed that diet composition with HCFM inclusion had higher protein, low fat and ash content than a diet without HCFM substitution. The higher protein in the diet supports and affects fish weight. Meanwhile, freshwater fish such as red tilapia often require fatty acids such as 18 carbon n-3 fatty acid and linolenic acid (18:3-n-3), in quantities ranging from 0.5 to 1.5% of dry diet (Craig and Helfrich, 2009). As a comparison, previous research suggests that hybrid tilapia needs 5% crude fat for minimum growth and can be increased until 12% of crude fat for maximum growth (Chou and Shiau, 1996). Further, ash is the result of combustion of organic materials in the form of minerals and the lower ash content indicates that the feed is more easily digested by the fish.

Fish carcass composition

Generally, tilapia has a protein content of approximately 19.04% and a fat content of 19.67–23.00% (Foh *et al*., 2011). The current results determined that red tilapia fed 12% HCFM supplementation in the diet had the highest protein (51.93%) and fat (23.00%) contents. This finding is similar to previous research stating that tilapia fed with 20% fish meal substitution had significantly higher total protein in their muscle compared to tilapia fed commercial feeds. In addition, *Oreochromis niloticus* fed 20% fish meal substitution had also minimum lipid content (Al-Ghanim *et al*., 2017). In contrast, another study revealed no significant effects on crude protein and fat of Juvenile tilapia fed poultry instead of fish meal (Yones and Metwalli, 2016). The present results also suggested that the protein content in the HCFM can be applied to increase the protein content in fish carcass, in comparison with red tilapia fed without inclusion of HCFM.

Water quality

The quality of water plays a pivotal role in the growth of fish. These water parameters (temperature, pH, DO, nitrite and TAN) remained within the suitable range required for red tilapia (Alabaster and Lloyd, 1980; Hepher *et al*., 1983), and there were only minor differences among the treatments. The average water temperature recorded during the experiment was 22.13± 0.17 °C, which is in a tolerable limit for red tilapia (20.9–24.3°C); pH was in a tolerable range (7.35± 0.25), the DO levels recorded during the experiment were 5.70± 0.31 ppm and TAN was at an acceptable level (0.14± 0.02 ppm) that supports the life of the red tilapia. Overall, water quality parameters such as temperature, pH, DO, nitrite and TAN were found to be favourable for fish growth and survival during the trial.

CONCLUSION

Based on growth, feed efficiency, survival rate and carcass composition, the use of hydrolyzed chicken feather meal up to 12 % is possible for red tilapia aquafeed without impairing growth and feed intake. The carcass composition (crude protein and crude lipid) of red tilapia was recorded to be highest at 6% of HCFM inclusion. Further research should be conducted to determine the effects of prolonged feeding with current ingredients at high concentration.

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Statement of conflict of interest

The authors declare no conflicts of interest regarding the publication of this article.

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