Utilization of tuna by-product and blood meal as a protein ingredient from animal waste product as a diet of Nile tilapia, *Oreochromis niloticus*

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Abstract. A feeding trial was conducted to evaluate the nutritive value of mixture of tuna by-products meal (TBM) and blood meal (BM) as a possible protein source in diets for juvenile Nile tilapia *Oreochromis niloticus* with mean initial body weight 2.6±0.1 g. TBM was replaced with BM 0% (control diet), 10% (diet I), 20% (diet II), 30% (diet III) and 40% (diet IV). Ten fish were placed in each of 15 rectangular tanks (60 x 40 x 40 cm) filled with 50-L aerated dechlorinated freshwater. Three tanks then formed each diet group. In the tanks, fish were provided with diets ad libitum twice a day (morning and afternoon) for 50 days. At the end of the feeding test, the feed intake (FI) and weight gain (WG) were determined. Subsequently, feed conversion ratio (FCR), specific growth rate (SGR), and survival rate (SR) were estimated. There were significant differences ($p < 0.05$) in growth performance among fish fed diet II (20% TBM and 20% BM). These results showed that combination TBM and BM (diet II) increased WG and SGR but decreased FCR. Diet II showed the proportion of dietary protein resulted in a significant improvement in FCR. Based on overall performances of fish, BM is suitable protein source as TBM replacement for juvenile Nile tilapia. Growth and nutrient utilization were significantly influenced by replacing TBM with BM.
Key Words: blood meal, FCR, Nile tilapia, protein source, SGR.

Introduction. Fish nutrition plays a very important role in the aquaculture industry for a better product quality. The growth of the aquaculture industry can be achieved with the use of appropriate feed. One of the important ingredients used in commercial aquaculture feed formulations is fish meal which has high protein quality and palatability. Replacing expensive fish meal in aquafeed with a cheaper protein source is one way to reduce production costs in fish farming (Hardy 1996; Jamil et al 2007; Trosvik et al 2013). In order to fulfill the fish feed need, alternative feed formulations were sought from the tuna industry and from slaughtered animals to be used as a protein source of animal origin (Aladetohun & Sogbesan 2013; Hernández et al 2013; Hamed et al 2017; Kim et al 2019).

Fish play an important role in fighting hunger and nutrition. Fish is not only a source of proteins and healthy fats, but also a unique source of essential nutrients, including long-chain fatty acids, iodine, vitamins, minerals and calcium. The multiple benefits of fatty fish high in Omega-3 fatty acids and small fish eaten whole, containing nutrients in the skin and bones, clearly illustrates seafood's irreplaceable nutritional value. An increased focus on fish and nutrition aids both developing countries and the developed world. In many developing countries, fish is the main or the only source of animal protein, and is essential for providing micronutrients to food insecure populations. Dietary patterns are also shifting in developed and middle-income countries, and an increasing emphasis on both coronary and overall health has led to an increased demand for fish among those populations (Hasan & Malwart 2009; Hernández et al 2013; FAO 2018; Perez-Velazquez et al 2018).

In aquaculture, the provision of high-quality aquafeeds that satisfy the nutritional requirements of cultured species and optimize growth is a prerequisite to increasing yields, lowering production costs, and improving economic returns for the farmers. Good management and control of feed quality and feed management strategy in fish farming is of critical importance in maintaining a cost-effective and environmentally sustainable industry (White et al 2018).

Concerning the objective of the present study, it is urgently needed either to develop a feasible method for the treatment of tuna by-product meal (TBM) and blood meal (BM) to seek greater utilization in aquaculture. For the latter purpose, it is necessary to determine the nutritive value of the TBM and BM to examine the possible effects on the growth of Nile tilapia, *Oreochromis niloticus*. This study evaluated the use of TBM and BM as a diet ingredient for the Nile tilapia and its possible effects on the growth performance of the fish were determined.

Material and Method. Juvenile Nile tilapia were taken from the Tatelu Fresh Water Cultivation Fishery Center (BPBAT, Tatelu). The juvenile Nile tilapia were randomly selected and placed at the tank (500 L) for two weeks acclimation period. During this period, the tank was supplied with aerated dechlorinated freshwater at a stocking density of 300 fish. The formulation and proximate composition of the test diets are shown in Table 1. All ingredients were ground using a laboratory mill and mixed in a Hobart food mixer for 15 minute. Hot water (approximately 50°C) was added to get the right consistency for pelleting, and mixing continued for 15 additional minute. The resulting mash was passed through a meat grinder to produce pellets of 2 mm, which were dried in oven for 2 h at 80°C. A sample of each diet was retained and stored, in plastic bags at -20°C, until analyses for proximate compositions. In acclimation, fish were fed with 2% wet body weight daily ration. Feeding was discontinued 48 h before transfer into the experimental tanks. This research was carried out on March-August 2020 at Aquaculture Laboratory, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado.

Ten fish were placed in each of 15 rectangular tanks (60 x 40 x 40 cm) filled with 50-L aerated dechlorinated freshwater. Three tanks then formed each diet group. In the tanks, fish were provided with diets ad libitum twice a day (morning and afternoon) for 50 days. The test fish achieved apparent satiation one hour after feeding since, as visually observed, most of them ceased ingesting the supplied pellets. The amounts of feeds consumed by fish in each tank were recorded daily by noting the number of pellets ingested. One-third of the water in tanks was replaced every morning throughout the study period. Four diets with different levels of TBM and BM were formulated (Table 1). Before the feeding experiment, all fish were conditioned for one week, wherein they were adjusted to the pelleted diets and standardized environmental conditions. At the end of acclimation period, the fish were weighed and then started on the respective experimental diets for 50 days to evaluate the effects of dietary on the growth and feed utilization of Nile tilapia. Each tank was randomly assigned to dietary treatments. Then, fish were transferred to each experimental tank. The average weight of fish (mean±SD) was mean initial body weight 2.6±0.1 g. All dietary treatments were tested in three replicate groups of fish with uniform size.

At the end of the feeding test, the feed intake (FI) = total amount of diet consumed and weight gain (WG) = (final body weight - initial body weight) were determined. Subsequently, feed conversion ratio (FCR) = feed intake/weight gain and specific growth rate (SGR) = (Ln final body weight – Ln initial body weight)/d x 100; survival rate (SR) = number of fish at the end/number of fish at the beginning x 100 were estimated.

Proximate compositions of the test diets are shown in Table 1. Moisture and crude ash contents (AOAC 1990) were determined by heating in a muffle furnace at 105^{o}C and 550^{o}C for 12 hours, respectively, crude lipids by a Soxhlet extraction method (Bligh & Dyer 1959), and crude proteins (N X 6.25) by a semi-micro Kjeldahl method (Bligh & Dyer 1959).

Statistical evaluation of the data was conducted using SPSS 22.0 software package (SPSS; Chicago, IL). ANOVA was used to identify any statistical differences (p <

0.05) in weight resulting from each test diet formulation. A Tuckey test was subsequently used to determine any significant difference among variable means for selected parameters.

Water temperature (°C), pH and dissolved oxygen (DO; mg L^{-1}) were recorded daily at 07:00 am and 16:00 pm using YSI Pro20 digital probes. Un-ionized ammonia fraction of the total ammonia concentration was recorded daily using YSI 9500.

Table 1

Formulation of composition the experimental diets used for feeding experiment

Ingredient	*Diet (g/100 g))*				
	Control	*I*	*II*	*III*	*IV*
Fishmeal tuna by-product	40	30	20	10	0
Blood meal	0	10	20	30	40
Rice bran flour	10	10	10	10	10
Corn meal	10	10	10	10	10
Coconut by-product	15	15	15	15	15
Tapioca flour	5	5	5	5	5
Coconut oil	7	7	7	7	7
Vitamin mix	8	8	8	8	8
Mineral mix	5	5	5	5	5
Total	100	100	100	100	100
Proximate composition					
Crude protein	27.6	27.7	28.6	30.0	31.3
Crude lipid	9.4	9.5	9.1	11.5	11.8
Moisture	4.4	9.0	5.7	6.4	4.1
Ash	22.2	18.3	18.6	20.5	17.4
Fiber	7.3	8.2	6.9	6.7	7.9
Nitrogen free extract	29.1	27.8	31.2	24.9	27.6

Results. All fish survived during the feeding experiment. Diets I-IV appeared palatable to the fish tested. The significant difference from the control was neither found in final weight for diets II and IV, nor in feed intake (FI) for diets I - III. The results shown that control diet, diets I, III and IV, did not give any significant effects on FCR, SGR. Diet II gave the lowest values for FCR (Table 2). However, growth performance was noted significantly low in control and diet IV with 0% and 40% of BM. All fish groups showed different FI values, which might be caused by different palatability and acceptance of fish for each diet. FCR values also differed significantly among the groups of fish. The fish fed with diet I and II showed the best FCR values. Furthermore, SGR in diet II showed the best value, being significant different ($p < 0.05$) among all diets. These parameters were significantly different between the treatments. Survival rate were 100% in fish fed the test diets and there were no differences among treatments at the end of the feeding trial. Throughout the experiment, water temperature ranged between 25.2 and 27.1°C, pH 7.0-7.2, dissolved oxygen 6.3-7.1 mg L^{-1}, and unionized ammonia 7.5-8.5 µg N L^{-1}.

Table 2

Growth performance of Nile tilapia fed different levels of feedstuff for 50 days

Growth performance	*Diet*				
	Control	*I*	*II*	*III*	*IV*
Initial weight (g)	2.6 ± 0.1^{a}	2.6 ± 0.1^{a}	2.6 ± 0.2^{a}	2.6 ± 0.1^{a}	2.6 ± 0.1^{a}
Final weight (g)	$10.8\pm1.2^{a,b,d}$	$10.6\pm1.2^{a,b,d}$	13.2 ± 1.1^{c}	$9.7\pm1.4^{a,b,d}$	7.4 ± 0.1^{e}
Weight gain (g)	$8.2\pm1.2^{a,b,c,d}$	$8.0\pm0.3^{a,b}$	10.6 ± 1.1^{c}	$7.0\pm1.3^{a,b,d}$	4.8 ± 0.1^{e}
Feed intake (g)	18.6 ± 1.4^{a}	$14.5\pm0.6^{b,c,d}$	$15.7\pm1.2^{b,c,d}$	$16.1\pm1.2^{b,c,d}$	13.3 ± 0.8^{e}
Feed conversion ratio	$2.3\pm0.2^{a,b,d}$	$1.8\pm0.2^{a,b}$	1.5 ± 0.3^{c}	$2.4\pm0.3^{a,b,d}$	2.8 ± 0.2^{e}
Specific growth rate (%)	$2.8\pm0.2^{a,b,d}$	$2.8\pm0.2^{a,b,d}$	3.2 ± 0.2^{c}	$2.6\pm0.3^{a,b,d}$	2.1 ± 0.0^{e}
Survival rate (%)	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}

Note: Values are mean±S.E. of triplicate treatments. Means indicated by the same superscripts did not differ at the 5% level as determined by the Duncan's multiple range test.

Discussion. Development of an alternative protein source for the partial or direct replacement of fishmeal for fish species has been studied, with marginal success (Hernández et al 2013; Wong et al 2016; Afreen & Ucak 2020). In this study, combination of TBM and BM seemed useful for the growth of Nile tilapia from the nutritive point of view. The protein contents of test diets agreed well with those recommended for Nile tilapia (El-Saidy & Gaber 2005; Kim et al 2014; Nhi et al 2018; Kim et al 2019). This suggests that a partial replacement of TBM and BM might be possible to achieve a similar growth performance in fish. As such, the relatively lower cost and greater accessibility of that combination could reduce feeding costs, thus improving the economic efficiency of fish production.

In addition to being an excellent source of energy, the combination of TBM and BM has an advantage in maximizing the growth of fish, since higher growth rates were obtained with that combination in their diets. This study also demonstrated that the BM could replace the TBM up to 20% on dry weight basis without compromising the growth performance. This inclusion level was the highest tested, so it is possible that could be used without deleterious effects.

Combination of TBM and BM in diet II increased WG and SGR but decreased FCR (Table 2). Diet II showed the proportion of dietary protein resulted in a significant improvement in FCR. In spite of this, FCR was noted to decrease with an increased dietary protein content (El-Saidy & Gaber 2002; Jeon et al 2014; Kim et al 2014, 2019; Saidi et al 2014; Twahirwa et al 2020). The fish fed with diet II gave a relatively high WG and SGR, suggesting a growth-promoting effect of TBM and BM indicating that this percentage may be used in Nile tilapia practical diets.

Since, in this study, diet II increased WG and SGR, it could be utilized as an alternative ingredient in fish diets. However, as in formulated diets it might vary according to species. As a by-product from tuna factory and results of slaughterhouse waste, the low cost and high accessibility of this diet may potentially increase the economic benefits to be derived from fish production. Hence, the use of TBM and BM in aquaculture as a replacement for commercial feed appears to be viable and important, but the digestibility of the protein, amino acids, and lipid and energy components in relation to the different size classes of fish must be further determined to fully evaluate the dietary potential of TBM and BM. From the economic standpoint, replacement of fish meal by cheaper animal by-product meals in a practical diet for rainbow trout (*Oncorhynchus mykiss*) can alleviate the problem of low fish meal availability and high cost. Fish survival was not affected by experimental diets and similar results were obtained by Millamena (2002).

TBM is composed of body parts that are not suitable for human consumption and include heads, bones, viscera and skins. Because its multi specific origin highly varies in its physical characteristics and chemical composition, and it is highly unstable, it requires special preservation methods to maintain its nutritional quality, before it is processed as a meal, or stabilized using other methods including the chemical or microbial silage, resulting in a liquid or semiliquid product that generally contains 31-56% crude protein and 6-8% crude fat. Approximately half of the processed seafood is discarded as a waste product, most of the time with negative environmental effects (Hardy & Gatlin III 2002; Garrido Gamarro et al 2013; Kokou & Fountoulaki 2018; Mo et al 2018). TBM and BM were confirmed safe for use as formulation diet in tilapia aquaculture, because they did not affect the overall growth performance.

Conclusions. Overall, the use of TBM and BM as a protein source in Nile tilapia aquaculture is expected to reduce the cost of feed products with other fishmeal substitutes. Based on overall performances of fish, BM is suitable protein source as fish meal replacement for juvenile Nile tilapia. Growth and nutrient utilization were significantly influenced by replacing TBM with BM. Further studies to determine the long-term effect on the performance of fish fed the fish meal replacement diet under on-farm conditions is suggested.

References

Afreen M., Ucak I., 2020 Fish processing wastes used as feed ingredient for animal feed and aquaculture feed. Journal of Survey in Fisheries Sciences 6(2):55-64.

Aladetohun N. F., Sogbesan O. A., 2013 Utilization of blood meal as a protein ingredient from animal waste product in the diet of *Oreochromis niloticus*. International Journal of Fisheries and Aquaculture 5(9):234-237.

Association of Official Analytical Chemists, 1990 Official methods of analysis. 15th edition, AOAC Press, Washington, 1298 pp.

Bligh E. G., Dyer W. J., 1959 A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37(8):911-917.

El-Saidy D. M. S. D., Gaber M. M. A., 2002 Complete replacement of fish meal by soybean meal with dietary L-lysine supplementation for Nile tilapia *Oreochromis niloticus* (L.) fingerlings. Journal of the World Aquaculture Society 33(3):297-306.

El-Saidy D. M. S. D., Gaber M. M. A., 2005 Effect of dietary protein levels and feeding rates on growth performance, production traits and body composition of Nile tilapia, *Oreochromis niloticus* (L.) cultured in concrete tanks. Aquaculture Research 36(2): 163-171.

FAO, 2018 Achieving blue growth: building vibrant fisheries and aquaculture communities. FAO, Rome, 28 pp.

Garrido Gamarro E., Orawattanamateekul W., Sentina J., Gopal T. K. S., 2013 By-products of tuna processing. FAO, Rome, GLOBEFISH Research Programme 112, 48 pp.

Hamed S. S., Jiddawi N. S., Bwathondi P. O., 2017 Effects of blood meal as a substitute for fish meal in the culture of juvenile silver pompano *Trachinotus blochii* (Lacepède, 1801) in a circulating aquaculture system. Western Indian Ocean Journal of Marine Science 16(1):1-11.

Hardy R. W., 1996 Alternate protein sources for salmon and trout diets. Animal Feed Science and Technology 59(1-3):71-80.

Hardy R. W., Gatlin III D. M., 2002 Nutritional strategies to reduce nutrient losses in intensive aquaculture. In: Avances en nutrición acuícola VI. Cruz-Suarez I. E., Ricque-Marie D., Tapia-Salazar M., Gaxiola-Cortes M. G., Simoes N. (eds), Memorias del VI Simposium Internacional de Nutrición Acuícola, 3 al 6 de Septiembre del 2002, pp. 23-34.

Hasan M. R., Halwart M., 2009 Fish as feed inputs for aquaculture: practices, sustainability and implications. FAO, Rome, Fisheries and Aquaculture Technical Paper 518, 407 pp.

Hernández C., Olvera-Novoa M. A., Voltolina D., Hardy R. W., González-Rodriguez B., Dominguez-Jimenez P., Valverde-Romero M., Agramon-Romero S., 2013 Use of tuna industry waste in diets for Nile tilapia, *Oreochromis niloticus*, fingerlings: effect on digestibility and growth performance. Latin American Journal of Aquatic Research 41(3):468-478.

Jamil K., Abbas G., Akhtar R., Lin H., Li Z., 2007 Effects of replacing fishmeal with animal by-products meal supplementation in diets on the growth and nutrient utilization of mangrove red snapper. Journal of Ocean University of China 6(3):292-298.

Jeon G. H., Kim H. S., Myung S. H., Cho S. H., 2014 The effect of the dietary substitution of fishmeal with tuna by-product meal on growth, body composition, plasma chemistry and amino acid profiles of juvenile Korean rockfish (*Sebastes schlegeli*). Aquaculture Nutrition 20(6):753-761.

Kim H. S., Jung W. G., Myung S. H., Cho S. H., Kim D. S., 2014 Substitution effects of fishmeal with tuna byproduct meal in the diet on growth, body composition, plasma chemistry and amino acid profiles of juvenile olive flounder (*Paralichthys olivaceus*). Aquaculture 431:92-98.

Kim K., Park Y., Je H. W., Seong M., Damusaru J. H., Kim S., Jung J. Y., Bai S. C., 2019 Tuna byproducts as a fish-meal in tilapia aquaculture. Ecotoxicology and Environmental Safety 172:364-372.

Kokou F., Fountoulaki E., 2018 Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. Aquaculture 495:295-310.
Millamena O. M., 2002 Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper *Epinephelus coioides*. Aquaculture 204(1-2):75-84.
Mo W. Y., Man Y. B., Wong M. H., 2018 Use of food waste, fish waste and food processing waste for China's aquaculture industry: needs and challenge. Science of the Total Environment 613-614:635-643.
Nhi N. H. Y., Da C. T., Lundh T., Lan T. T., Kiessling A., 2018 Comparative evaluation of brewer's yeast as a replacement for fishmeal in diets for tilapia (*Oreochromis niloticus*), reared in clear water or biofloc environments. Aquaculture 495:654-660.
Perez-Velazquez M., Gatlin III D. M., González-Félix M. L., García-Ortega A., 2018 Partial replacement of fishmeal and fish oil by algal meals in diets of red drum *Sciaenops ocellatus*. Aquaculture 487:41–50.
Saidi S., Deratani A., Belleville M. P., Amar B. R., 2014 Production and fractionation of tuna by-product protein hydrolysate by ultrafiltration and nanofiltration: impact on interesting peptides fractions and nutritional properties. Food Research International 65(C):453-461.
Trosvik K. A., Webster C. D., Thompson K. R., Metts L. A., Gannam A., Twibell R., 2013 Effects on growth performance and body composition in Nile tilapia, *Oreochromis niloticus*, fry fed organic diets containing yeast extract and soyabean meal as a total replacement of fish meal without amino acid supplementation. Biological Agriculture and Horticulture 29(3):173-185.
Twahirwa I., Wu C., Ye J., Zhou Q., 2020 The effect of dietary fish meal replacement with blood meal on growth performance, metabolic activities, antioxidant and innate immune responses of fingerlings black carp, *Mylopharyngodon piceus*. Aquaculture Research 2020:1-13.
White P. G., Shipton T. A., Bueno P. B., Hasan M. R., 2018 Better management practices for feed production and management of Nile tilapia and milkfish in the Philippines. FAO, Rome, Italy, 95 pp.
Wong M. H., Mo W. Y., Choi W. M., Cheng Z., Man Y. B., 2016 Recycle food wastes into high quality fish feeds for safe and quality fish production. Environmental Pollution 219:631-638.

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