Effects of Phyprozyme HP Supplementation in Diets on Body Weight Composition in Nile Tilapia (*Oreochromis niloticus*)

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***Abstract* - This study was designed to evaluate the body weight composition of Nile tilapia, (*Oreochromis niloticus*) fed diets with various inclusion levels of Phyprozyme HP. Phyprozyme HP enzymes are standardized to contain a combination of Protease, Xylanase, Hemicellulase, and Phytase. The body weight composition was presented as a percentage of moisture, fat, ash, and crude protein. The fish samples in this study used Nile tilapia with an average weight of (11.8 ± 8.6) g/fish and the stocking density of 20 fish/ hapa (70×45×90 cm^3). Eight treatments with two replicates were used: T1 control (0,0), T2 (0,05), T3 (0,12), T4 (0,19), T5(0,26), T6 (0,33), T7 (0,40), and T8 (0,47) g phyprozyme HP/ kg^{-1} feed and were fed three times daily (7:00 AM, 12:00, and 16:00 PM) at feeding rate of 5 % of the total body weight. After 56 days, there were no significant differences between the treatments in terms of moisture and protein contents of body composition.The highest value (81.52 ± 2.96) of moisture was observed with fish fed (T8) and the lowest value (77.65 ± 3.32) was obtained with fish fed (control). The highest value of protein (11.68 ± 0.72) was observed with (initial weight) and the lowest value (7.43 ± 0.12) was obtained with fish fed (control). There was a significant difference (P<0.05) in crude fat and ash contents between initial weight and other dietary treatments. Crude fat content was highest in initial weight (2.45 ± 0.49) and lowest in fish fed (T2) (0.39 ± 0.02). The ash content was highest in T7 (2.67 ± 0.28) and lowest in fish fed (T6) (1.47 ± 0.06).**

***Keywords:*Nile tilapia (Oreochromis *niloticus*), Phyprozyme HP, Carcass composition**

I. INTRODUCTION

Nile tilapia, (*Oreochromis niloticus*) is an extensively cultured fish because it grows and multiplies under a variety of environmental conditions and tolerates handling stress. Tilapias do well in extensive, semi-intensive and intensive culture systems[1]. Nile tilapia has become the predominant culture species due to its proven superior growth compared to the other species [2]. Tilapias are now the second most commonly farmed fish next to carps [3]. Currently, tilapias are cultured in about 100 countries in tropical and subtropical regions.

Recently, demandfor Tilapia (*Oreochromis niloticus*) consumption has steadily increased as *O. niloticus* is low priced and has a high nutritional value. The whole fish and the filet are admirable for the consumers. As a result, it affects the trend of both domestic and export consumption. Moreover, Nile tilapia has many excellent advantages such as fast-growing, high growth rate, easy breeding, high fibrillate protein, good taste, white cotton meat, with more Omega-3 fatty acids than other wild freshwater fish and wild estuarine fish. The fish filet consists of several ingredients, such as moisture, proteins, lipids, vitamins, and minerals, all of which contribute to the overall composition of the meat.

The composition of the fish body is influenced by exogenous and endogenous influences [4]. Exogenous factors affecting the composition of the fish body include fish diet, e.g. composition and frequency as well as the environment in which it is found e.g. salinity and temperature. Diet is the largest exogenous factor affecting the body composition of fish.

The study of the chemical composition of fish is an important aspect of fish flesh quality since it influences both keeping quality and the technological characteristics of the fish (Huss, 1988) [5]. Fish and fishery products, as with many animal products, contain water, proteins and other nitrogen compounds, lipids, carbohydrates, minerals, and vitamins. Nevertheless, depending on age, sex, climate and season the chemical composition of fish varies greatly from one species and from one individual fish to another [4].

Phyprozyme HP is a multi-enzyme system that is derived from dried extracts from Fungal (*Aspergillus niger* and *Aspergillus oryzae*), Bacterial (*Bacillus Subttilis*), and Escherichia coli fermentation. Phyprozyme HP enzymes are standardized to contain a combination of Protease, Xylanase, Hemicellulase, and Phytase. These Enzymes are specially designed for improving the digestibility of feed ingredients to enhance growth and improve efficient utilization of feed in swine, poultry, but have not been studied in fish diets.

In contrast, little information is available concerning the effect of supplementation of enzymes on the body weight composition of Nile tilapia. To fill this gap, the present study was conducted to know the effect of different levels of phyprozyme HP incorporated in the diets on body weight composition Nile tilapia (*Oreochromis niloticus*).

II. LITERATURE REVIEW

Fish is consumed by a large percentage of the world's population due to its high-quality protein. It contains the most important components of nutrients and serves as a source of energy for humans [6]. The majority of the nutrition lists recommend that human beings should consume fish every day [7]. Regular fish consumption may reduce the risk of cancer, colon, breast, and prostate cancer

[8], decrease the risk of dementia and Alzheimer's disease [9].

Knowledge of the proximate composition of fish is essential for estimating their energy value and for planning the most suitable industrial and commercial processing [10]. Generally, the composition of live-weight, whole fish is 70 to 80% water, 20 to 30% protein, and 2 to 12% lipid [11]. However, in different environmental conditions, the composition of the fish may differ in relation to the differences in water quality, feeding conditions, sex, and state of maturity and capture condition (Javaid MY, Salam A, Khan MN, Naeem M 1992) [12].

Fish having energy depots in the forms of lipids that indicate the quality of fish, the fish oil contains a high amount of polyunsaturated fatty acid which reduces serum cholesterol to prevent a number of heart diseases. Fish meat is also a rich source of minerals and the most important micro-elements are Zinc (Zn), Iron (Fe), and Copper (Cu) [13].

Due to the enormous change in climate, seasonal and industrial growth, there could be wide differences in the biochemical constituents of the fish. It is therefore essential to document the proximate composition of the fish periodically in the region.

Fish fillet consists of several ingredients, such as moisture, proteins, lipids, vitamins, and minerals, all contributing to the meat composition as a whole. Fish body composition is affected by both exogenous and endogenous factors [4].

Various studies have examined the effects of the concentration of temperature, light, salinity, pH, and oxygen on the proximate composition of fish, but these factors appear to have very limited effects. On the other hand, endogenous factors are genetic and are linked to the life stage, age, size, sex and anatomical position of the fish. These endogenous factors govern most of the principles which govern fish composition (Huss, 1995) [4].

The nutritional value of fish meat comprises the contents of moisture, dry matter, protein, lipids, vitamins, and minerals plus the caloric value of the fish[14]. There are, therefore, a number of variables that may affect the overall chemical composition of fish meat. However, there is little information on the effects of sex and size (age) on the individual chemical components of Nile tilapia meat.

III. RESEARCH METHODS

The present study was carried out at the ndayu park, Sragen, Central Java, Indonesia from December 2019 to January 2020 for a period of 56 days.

2.1 Experimental design and conditions

Nile tilapia fingerlings with initial weight ranging from (11.9 ± 8.6 g)grams were used in the study. The fingerlings were obtained from the ndayu park farm, Sragen, Central Java, Indonesia. Before the start of the experiment, the fingerlings were acclimatized for one week. The experiment was conducted in16 hapa nets with a size 70×45×90 cm3 (inside aquaculture fiberglass tank) under eight treatments with two replicates. The fish have randomly distributed into the hapa nets a stocking density of 20 fingerlings per hapa. Feeding frequencies was three times a day (8:00 AM, 12:00, and 16:00 PM), The feeding rate was adjusted according to the size of fish 5% according to body weight throughout 56 days in the experimental period. The aquaculture fiberglass tanks were cleaned two times every week and a third of the water replaced before feeding.

2.2 Formulation of experimental diets

Eight diets were formulated (Approximately 26,7% Crude Protein), one diet without enzyme (to control diets) and seven diets contain different levels of phyprozyme HP (quantity) (Table 1). Phyprozyme HP was added to the formulated experimental diets at T1 control (0,0), T2 (0,05), T3 (0,12), T4 (0,19), T5(0,26), T6 (0,33), T7 (0,40),

and T8 (0,47) g phyprozyme HP/ kg-1 feed. The Phyprozyme was used is a multi-enzyme produced by Diasham Resources PTE LTD, Singapore. The ingredients that were used in the experiment were obtained from the local market. All the ingredients were powder. The enzyme at predetermined levels was added to the ingredients. All ingredients were mixed in a mixer, then fish oil and 20.5% distilled water were added, and pellets were made with a laboratory pelletizer and were dried for about 14 h at 32°C. The particle size of the diets was determined by the age of fish.

Table 1 The Composition of ingredients (g/100g dry matter)

Ingredients	T1	T2	T3	T4	T5	T6	T7	T8
Phyprozyme HP[1] (g)	0	0.05	0.12	0.19	0.26	0.33	0.40	0.47
Soybean meal	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0
Fish meal	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
Azolla	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Rice bran	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Corn meal	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Wheat flour	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Fish oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Aquavit premix[2]	3	3	3	3	3	3	3	3
Total	100	100	100	100	100	100	100	100

[1] 1 g phyprozyme HP contain (phytase 2,000 FTU, Xylanase 20,000 BXU, Protease 12,000 PCU, Hemicellulase 400 HMCU.

[2] Aquavit premix (Multivitamins and minerals) supplied per 100 g of dry feed (3 g) Aquavit premix contain: Vit. A 9000 IU; Vit. D3 3000 IU; Vit. E 22.5 mg; Vit. K3 3.6 mg; Vit. B1 9 mg; Vit. B2 13.5 mg; Vit. C 24 mg; Ca pantothenate 12 mg; Folic acid 4.5 mg; Nicotinamide 60 mg; Amino acids 30 mg; Inositol 37.5 mg; Manganese sulphate 0.08 mg; Zinc sulphate 0.08 mg; Copper sulphate 0.006 mg; Cobalt chloride 0.02 mg; Sodium selenite 0.15 mg

Table 2 Proximate analysis of ingredients

Treatments	T1	T2	T3	T4	T5	T6	T7	T8
Moisture	8.5	8.1	9	9.1	8.7	8.5	9.4	8.6
Crude protein	25.3	24.5	26	25.9	26.7	26.6	26	26.2
Crude lipid	8.9	9	8.2	8.3	8.4	8.3	8.6	8.8
Ash	9.4	10.1	9.2	8.8	8.5	8.5	8.4	8.4

2.3 Proximate chemical analysis

Proximate analysis of the experimental diets and fish were conducted at the Food and Nutrition Laboratory, Department of Agricultural Technology, Faculty of Agriculture, Sebelas Maret University.

The proximate analysis was carried out according to the standard methods by the Association of Official Analytical Chemists [15]. Samples of eight fish were taken at the times of stocking and harvest for the initial and final proximate carcass analyses respectively. The following nutrients were analysed: Moisture, Crude Protein (CP), crude fat (CF), and ash.

2.3.1. Moisture Content Determination

Samples of eight fish and diets were taken for the initial and final proximate carcass analyses respectively. then were dried in an electric oven at 105 C^0 for 24-30 hours to obtain a constant weight. The moisture content was calculated as follows: -

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Dry weight}}{\text{Initial weight}} \times 100$$

2.3.2 Crude Protein Determination

The Kjeldahl method for the estimation of nitrogen was applied. Nitrogen content was converted to protein percentage by multiplying by 6.25 as follows:

$$\text{Protein \%} = \frac{(Va - Vb) \times N \times 14 \times 6.25}{1000 \times Wt} \times 100$$

Whereas:

Va = volume of HCL will be used in titration

Vb = volume of sodium hydroxide of known normality will be used in back titration

14 = conversion factor of ammonium sulfate to nitrogen

6.25= conversion factor of nitrogen to protein

Wt= weight of the sample

N= normality of NaOH

2.3.3 Crude Fat Determination

The fat content of eight samples was determined according to the Soxhlet method by ether extract using 2 gm of fish samples. The extraction was continued for 5 hours at 100 C0 before finding the weight of the extracted fat. The fat percentage was calculated as follows:

Fat % = Extracted fat weight / Sample weight x 100

2.3.4 Ash Content Determination

Ash was determined by heating 1 gm at 550 C^0 in muffle furnace until a constant weight was obtained. Ash content percentage was given by the following formula:

Ash % = Ash weight / Sample weightx 100

2.4 Statistical Analysis

The collected data were subjected to one-way ANOVA to determine if significant differences occur among the dietary treatments. and Duncan's multiple range test was used to compare the differences between means (Duncan, 1955). Effects with a probability of $P< 0.05$ was considered significant. Statistical analyses were performed using SPSS (Statistical Package for Social Sciences, Version 21, IBM Corporation, New York, USA).

IV. RESULTS AND DISCUSSION

The initial and final mean body compositions of Nile tilapia fed on various supplementation of phyprozyme HP diets are presented in table 4. A slight difference between treatments was observed in terms of moisture and protein contents of body composition (%) but non-significant. The highest value (81.52 ± 2.96) of moisture was observed with fish fed (T8) and the lowest value (77.65 ± 3.32) was obtained with fish fed (control). Also, the same trend was observed with the protein content. The highest value (11.68 ± 0.72) was observed with fish fed (initial weight) and the lowest value (7.43 ± 0.12) was obtained with fish fed (control). Crude fat content was highest in initial weight (2.45 ± 0.49) and lowest in fish fed (T2) (0.39 ± 0.02). There was a significant difference ($P<0.05$) in crude fat content between initial weight and other dietary treatments. On the other hand, the final ash contents of fish fed T7 were higher than initial weight and other dietary treatments. The ash content was highest in T7 (2.67 ± 0.28) and lowest in fish fed (T6) (1.47 ± 0.06). However, there were ash contents not affected ($P>0.05$) by the supplementation of phyprozyme HP in diets fed.

Table 3 The Carcass characteristics of whole-body Nile tilapia fed diets with various levels of phyprozyme HP for 56 days

Treatments	Protein	Lipid	Moisture	Ash
Initial	11.68 ± 0.72	2.45^{a} ± 0.49	77.65 ± 3.32	2.57^{a} ± 0.60
T1	7.43 ± 0.12	0.40^{b} ± 0.14	81.31 ± 2.99	1.56^{b} ± 0.40
T2	9.25 ± 0.03	0.39^{b} ± 0.02	80.11 ± 2.74	2.24^{ab} ± 0.09
T3	8.64 ± 2.14	0.42^{b} ± 0.01	79.98 ± 5.30	2.11^{ab} ± 0.21
T4	8.73 ± 2.22	0.44^{b} ± 0.04	80.02 ± 1.42	2.20^{ab} ± 0.06
T5	10.67 ± 1.52	0.79^{b} ± 0.14	79.68 ± 6.26	2.60^{a} ± 0.50
T6	10.54 ± 2.91	0.41^{b} ± 0.02	80.41 ± 1.35	1.47^{b} ± 0.06
T7	9.49 ± 0.07	0.44^{b} 0.04	81.28 ± 3.22	2.67^{a} ± 0.28
T8	10.68 ± 1.42	0.55^{b} ± 0.12	81.52 ± 2.96	1.70^{b} ± 0.36

*Values are expressed as mean± SD. Values in the same row having different superscript letters are significantly different (P<0.05). Comparisons were made between dietary treatments and excluded the initial values.

The effect of supplemental phyprozyme HP on carcass characteristics of the whole-body Nile tilapia (*Oreochromis niloticus*) are given in table 3. Proximate body composition means the determination of the water, protein, fat and ash content of the fish [11]. and this is considered as a good indicator of its physiological condition and health [16], moreover, it is essential in order to maximize their utilization [17]. The present study has shown changes in the chemical composition of Nile tilapia which appear to be not related to the variation of the diets that's mean these changes are not due to the supplementation of phyprozyme Hp to diets.

No significant differences were detected in the protein and moisture contents of the whole carcass composition of Nile tilapia when the diet was supplemented with Phyprozyme HP. There was a significant difference (P<0.05) in crude fat and ash contents between initial weight and other dietary treatments. This body composition finding concurs with [18] who report that there were no differences in the dry matter, crude lipid or crude ash contents of shrimp fed diets supplemented with a protease complex; however, the crude protein content of whole shrimp was higher than that in shrimp fed diets not supplemented with the protease complex. also, [19]observed that, there was no significant differences were noted in any of the proximate composition parameters of Nile tilapia fed diets containing pineapple (*Ananascomosus*) [19]. Furthermore, the present study showed There was no significant difference was observed in moisture and protein contents. A significant difference was observed in lipid and ash content. To date, no reports have indicated the effect of phyprozyme HP supplementation on the carcass characteristic of Nile tilapia. The results from the current study provide a good starting point for studying the effect of phyprozyme HP supplementation diets on the body weight composition of Nile tilapia.

V. CLOSING

A. Conclusions

The results suggested that phyprozyme HP could be a supplement in tilapia diets without any negative effect of the carcass composition of Nile tilapia. It is likely that more than 56 days is required to observe the impact of phyprozyme HP addition on carcass composition. Thus, more experiments are needed to validate our results.

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