



Evaluating of Pea Peels Meal as a Fishmeal alternative in Formulated Diet Ingredients of *Oreochromis niloticus*

Ahmed M. Desouky, Hossam M. Hwihy*, Walaa M. Shaban, Ahmad M. Azab

Marine Biology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

*Corresponding Author: Hossam.hwihy@azhar.edu.eg

ARTICLE INFO

Accepted: Nov. 29, 2023
Online: Dec. 12, 2023

Keywords:

Oreochromis niloticus,
Fishmeal replacement,
Plant protein,
Growth performance,
Hematology

ABSTRACT

In the present investigation, we endeavored to elucidate the ramifications of substituting fishmeal (FM) with fiber-devoid pea peels protein source with concentrations of 15 and 25% on various physiological parameters of the Nile tilapia, *Oreochromis niloticus*. A total of 120 *O. niloticus* fingerlings were meticulously categorized into three distinct quartile replicates, each encompassing 10 piscine subjects within a 50-liter aquarium setting. The dietary regimens administered during this experimental phase encompassed: A control group devoid of any FM substitution, T₁₅ featuring a 15% pea meal concentration and T₂₅ with a 25% concentration. The data presented here indicate that varying amounts of pea byproduct in the diet of *Oreochromis niloticus* had no noticeable effect on water quality variables. Treatment groups (T₁₅ and T₂₅, fed with 15 and 25% pea byproduct, respectively) had considerably better growth performance characteristics than the control group, including final weight and weight increase. However, there was no statistically significant difference between the values of DWG and SGR (daily weight gain). The values of food conversion ratio (FCR), feeding efficiency (FE) and protein efficiency ratio (PER) were significantly varied at partial replacement of fishmeal by 15% pea byproduct (T₁₅) compared to the control group. The hematological parameters and the proximate body composition showed no significant differences across all groups. These results intimated that the inclusion of pea meals as an alternative protein source can be seamlessly integrated into the dietary regimen of the Nile tilapia without engendering adverse physiological consequences. Such revelations offer invaluable academic insights into the potential incorporation of plant-derived protein sources in aquaculture, thereby proffering implications for sustainable pisciculture methodologies.

INTRODUCTION

Aquaculture significantly impacts addressing food insecurity, improving public health, and decreasing poverty levels in developing nations (FAO, 2014). To address the issue of protein deficiency in food, it is necessary to rapidly expand aquaculture. However, several environmental and economic limitations may hinder its development (Subasinghe, 2005; Gutierrez-Wing & Malone, 2006; Matos *et al.*, 2006).

As a result of fishmeal's great palatability and enhanced digestibility, it is widely used as a source of animal protein in the aquaculture feed business (Hodar *et al.*, 2020). Unluckily, using fishmeal in aquaculture diets raises production costs since wild fish stocks are reducing. On the other hand, the expansion of aquaculture in Egypt and around the world has led to a decline in fish stocks in response to the growing demands for fishmeal as protein source in aquaculture (Shaban, 2012; Ashour *et al.*, 2021). The current economic and

animal-formulated food crises in Egypt have made it even more important to look for cheaper and more sustainable alternatives to animal protein (Mugwanya *et al.*, 2023). Consequently, there has been considerable interest in introducing sustainable alternatives to fishmeal, which will reduce dependence on marine raw materials (Tacon, 2005). Fortunately, many plant-based protein sources can replace dietary fishmeal partially or completely (Kaushik *et al.*, 1995).

Pea (*Pisum sativum*) is a type of alternative plant protein source that shows promise for use in aquaculture species. Its protein and calorie content are so easily absorbed, and it also contains a high amount of digestible starch, ranging from 40- 50% (Cruz-Suarez *et al.*, 2001; Hickling, 2003). Studies have demonstrated that pea meal may be used as a total or partial replacement for fishmeal in the diets of farmed fish, without compromising the ability of the fish to thrive (Perez-Maldonado & Norton, 2010). Juvenile tilapia fed diets consisting of 25% pea meal as a substitute for fishmeal exhibited comparable growth and survived rates similar to those fed a fishmeal-based diet (Kumar *et al.*, 2020). Possible alternatives to fishmeal in the tilapia diets include pea meal, which may replace up to 50% of fishmeal in the diet, without compromising the growth performance (Chen *et al.* 2018). Pea meal's nutritional content may be increased by pre-treatment strategies viz. fermentation, according to recent studies (Liu *et al.*, 2022).

Pea peels are one kind of vegetable waste containing a lot of beneficial bioactive elements, including antioxidants and inflammatory fighters (Mejri *et al.*, 2019; Zhang *et al.*, 2020; Tassoni *et al.*, 2020). Due to their high protein and fiber content, pea peels may be used as both human food and animal feed (Hanan *et al.*, 2020; Abebe., 2022; Nasir *et al.*, 2022).

Pea meal is not a common or well researched ingredient in freshwater fish aquaculture. Pea meal's digestibility has been established for a variety of fish species beyond the Nile tilapia, including rainbow trout *Oncorhynchus mykiss* (Burel *et al.*, 2000; Thiessen *et al.*, 2003), milkfish (Borlongan *et al.*, 2003), Atlantic salmon *Salmo salar* (Overland *et al.*, 2009), and Asian sea bass *Lates calcarifer* (Ganzon-Naret, 2019).

In addition, the use of pea meal plant proteins as a fishmeal substitute in the diets of the Nile tilapia has only been the subject of a small number of research. Optimal inclusion levels for pea meal plant proteins have been reported differently by various researchers, adding to the data gap (Magbanua & Ragaza, 2023).

The purpose of this study was to examine the effects on *Oreochromis niloticus* growth performance, physiological responses, somatic indices, hematological parameters, and proximate composition when fishmeal was replaced by fiber-free pea byproduct derivatives at 15 and 25 percent of the total dietary intake, respectively.

MATERIALS AND METHODS

1. Experimental fish

Fingerlings of the Nile tilapia (*Oreochromis niloticus*) were obtained from the Animal House, Fish Breeding Lab at the Zoology Department in the Faculty of Science at Al-Azhar University, Cairo, Egypt. A total of 120 specimens of healthy fish (with total length ranging from 6.9 to 9.8cm and body weight ranging from 9.9 to 20.9g) were used in this work. The fish specimens were acclimatized for space at 50-liter plastic aquaria with continuous aeration for two weeks. Fish specimens were daily fed on a commercial diet.

2. Pea meal preparation

Pea byproduct was prepared from pea peels and empty peas as a by-product of pea processing during its harvested season (April 2022). A total of 20kg of fresh pea by-product was shade dried for 10 days, followed by further drying at 60°C until its weight reached a constant value, resulting in 3.74kg of dry weight, equivalent to 18.7%. After the drying process was completed, the dried pea peels were ground to fine powder by electric automatic mixer. Finally, fibers were physically isolated by pouring through a mesh screen that allows only finest particles to pass through; the produced powder was 2.7kg (72.2%). The produced powder was stored in a dry, dark container till used in diet formulations.

3. Formulated diet composition and construction

The main components of the experimental diet for fish were purchased from the Egyptian local market during the time of the experiment. The American official associated chemists protocols were used to determine the approximate compositions of the various formulations and a fiber-free pea meal for three examined diets, following the guidelines of AOAC, 2005. The process of overnight drying at 105°C revealed the sample moisture content. Moreover, the Kjeldahl method was used to calculate the protein content. The Soxhlet method was used to calculate the fat content using petroleum ether as a solvent, and 600°C was used to completely attain the ash of the substance. Table (1) displays the chemical composition (%) of the primary components in both the control and experimental diets. Furthermore, Table (2) shows the ingredient composition (%) of control and experimental diets, as well as their chemical analyses.

Table 1. Chemical composition (%) of the basic diet ingredients used in different diets

Ingredient	Dry matter %	Crude protein %	Ether extract %	Crude fiber %	Ash %
Fishmeal (62%)	93.2	62.1	5.7	1.5	21.8
Soybean meal (46-expel)	89.0	42	3.8	6.5	6
Pea by-product (Fiber-free)	87.0	24.5	1.5	3.5	6
Corn	87.7	8.3	4	2.4	1.2
Wheat bran	88.7	11.3	2.2	7.6	5.3
Fish oil	100	--	100	--	--

Table 2. Ingredients composition (%) and proximate analyses of the control and different experimental diets

Ingredient	Control (C)	Experimental diet	
		T ₁₅ (15% pea meal)	T ₂₅ (25% pea meal)
Fishmeal (62%)	20.0	15.0	10.0
Soybean meal (46-expel)	35.0	35.0	40.0
Fiber-free pea byproduct	0.0	15.0	25.0
Corn	25.0	10.0	8.0
Wheat bran	13.0	18.0	10.0
Fish oil	5.0	5.0	5.0
Vitamin and mineral premix ¹	2.0	2.0	2.0
Total	100.0	100.0	100.0
Chemical composition			
Dry matter %	90.7	89.6	89.5

Crude protein %	30.76	30.55	30.93
Lipid %	8.76	8.21	8.01
Fiber %	4.16	4.63	4.58
Ash %	7.45	7.34	6.71
GE (MJ/kg)	19.2	18.7	18.6
DE (MJ/kg)	13.4	13.3	13.4

¹Composition of vitamin mineral mixture of 1kg: Vitamin A - 50,00,000IU, Vitamin D3 - 10,00,000IU, Vitamin B2 - 2.0g, Vitamin E - 750 units, Vitamin K - 1.0g, Calcium pantothenate - 2.5g, Nicotinamide - 10.0g, Vitamin B12 - 6.0g, Choline Chloride - 150.0g, Calcium - 750.0g, Manganese - 27.5g, Iodine - 1.0g, Iron - 7.5g, Zinc - 15.0g, Copper - 2.0g, and Cobalt - 0.45g.

4. Experimental design

For evaluating alternative protein sources for the Nile tilapia fish feeds purposes, a 96-day trial was conducted using 120 fingerlings of the Nile tilapia. After acclimatization, the fish were divided into 3 groups for 50-liter glass aquaria with a stocking density of 10 fish/aquarium (4 replicated aquaria) for 96 days. The three groups, as shown in Table (2) were:

C: Fed on formulated 30% protein diet (20% fishmeal, and 0% pea meal).

T₁₅: Fed on formulated 30% protein diet (15% fishmeal and 15% pea meal).

T₂₅: Fed on formulated 30% protein diet (10% fishmeal and 25% pea meal).

5. Feeding and water regime

All fish in each experimental group were chosen and weighed every 15 days, and a consistent feeding rate of 3% of the fish's weight (dry feed/ day) was used to maintain weight. The typical fish weight was measured. Each group's weekly feed quantity was determined (feed grams/ fish/ week). Tanks had half their water capacity refreshed twice weekly with new, de-chlorinated tap water after the elimination of waste products (both dietary and otherwise).

6. Determination of water quality parameters

Each fish tank in all experimental groups had its temperature, pH, dissolved oxygen, and nitrogen-waste parameters monitored throughout the duration of the experiment. Adwa portable pH meter was used to measure temperature and pH daily. Total ammonia nitrogen (mg/ L), nitrite nitrogen (mg/ L), and nitrate nitrogen (mg/ L) were weekly measured by spectrophotometry, and dissolved oxygen (mg/ L) was measured twice weekly using the Winkler titration technique, following the guidelines recommended by the U.S. Environmental Protection Agency (APHA, 1995).

7. Determination of growth performance and feed utilization

Every 15 days, both the control and treatment fish groups were measured for weight. The following equations were used to get estimates for *Oreochromis niloticus* growth performance and feed utilization:

- Total length gain (cm/ fish) = final fish length (L_F) – initial fish length (L_I)
- Average daily length gain (mm / fish/ day) = total length gain (mm)/ duration period (days)
- Total weight gain (g/ fish) = final fish weight (W_F) – initial fish weight (W_I)
- Average daily weight gain (mg/ fish/ day) = total weight gain (mg)/ duration period (days)
- Growth in weight (%) = Total weight gain (g) – Initial fish weight (g) *100
- Specific growth rate (SGR, % / day) = ($\ln W_F - \ln W_I$) * 100/ experimental period (days)
- Feed intake (g/ fish) = $\sum \{Bi\}$ -weekly average fish weight*(daily feeding rate * 12 days)

- Food conversion ratio (FCR) = feed intake (g)/ total weight gain (g)
- Feed efficiency (FE) = Weight gain (g) / feed intake (g)
- Protein intake (PI) = feed intake (g) × Protein% in the diet/ 100
- Protein efficiency ratio (PER) = Total gain (g) / protein intake (g)

8. Determination of hematological parameters

Five fish from each group were chosen at random after the completion of the feeding trials. These fish were sedated with clove oil (0.5ml/ liter water) before their blood was sampled. Additionally, hematology samples were stored in EDTA-tubes.

Blood counts were taken to determine the total number of red blood cells (RBC's; cell 10^6 / mL), white blood cells (WBC's; cell 10^3 / ml), and platelets (10^3 / ml) in the blood (Dacie & Lewis, 2010). The cyano-methemoglobin technique (blood was diluted in a Drabkin solution) was used to spectrophotometrically estimate the hemoglobin concentration (HGB, g/ dL), with the use of a standard curve, following the method outlined by Noga (2010).

9. Proximate composition determination

Fish muscle proximate composition was analyzed, following the protocols outlined in AOAC (2005), as previously described for diet analysis.

10. Organ-somatic indices

Another five fish of each group were euthanized by sudden freezing. Each fish sample was dissected for liver, spleen, gonad, and viscera collection. Wet weighing to the closest 0.01g was used to determine the organ indices, and the following formula were used:

$$\text{Organ-somatic index} = \frac{\text{wet weight of organ (g)}}{\text{wet weight of fish (g)}} \times 100$$

11. Statistical analysis

At the 5% level of significance, we used the Shapiro-Wilk normality test and the Bartlett's homoscedasticity test. One-way analysis of variance was performed in SPSS (v-26) on the collected data. The Duncan Multiple range test was used to examine the possibility of a difference between the various treatments at the 5% level of significance.

RESULTS

Effect of fishmeal replacement by pea meal on water quality parameters

Water quality metrics of tanks containing *Oreochromis niloticus* fed with varying amounts of pea byproducts were compared to those of the control group (Table 3). The range of temperature was 20.1- 29.8°C; pH was 7.7- 8.4; DO was 4.6- 6.6mg/ dL; total ammonia nitrogen (TAN) was 0.04- 0.20mg/ L; nitrite-N was 0.10- 1.24mg/ L, and nitrate-N was 2.4- 71.8mg/ L. There were no significant differences among water quality parameters in the present study. These results indicate that the different levels of pea byproduct used in the Nile tilapia's diets did not significantly impact the measured water quality parameters (Table 3).

Table 3. Water quality with different levels of pea by-product on growth performance of *Oreochromis niloticus* for 96 days

Parameter	Control		T ₁₅		T ₂₅	
	0% pea byproduct		15% pea byproduct		30% pea byproduct	
	Average± SD	Range	Average ± SD	Range	Average ± SD	Range
Temperature (°C)	25.8± 2.7 ^a	20.2- 29.8	25.6± 2.7 ^a	20.1- 29.6	25.7± 2.7 ^a	20.1- 29.7
pH	8.1± 0.15 ^a	7.8- 8.4	7.9± 0.16 ^a	7.7- 8.4	8.0± 0.15 ^a	7.7- 8.4
DO (mg/ L)	6.2± 0.57 ^a	4.6- 6.6	6.3± 0.63 ^a	4.6- 6.6	6.4± 0.62 ^a	4.5- 6.6
TAN (mg/ L)	0.12± 0.06 ^a	0.04- 0.26	0.13± 0.02 ^a	0.17- 0.20	0.13± 0.03 ^a	0.11 - 0.20
Nitrite-N (mg/ L)	0.62± 0.11 ^a	0.10 - 0.21	0.65± 0.14 ^a	0.11- 1.23	0.65± 0.14 ^a	0.20 - 1.24
Nitrate-N (mg/ L)	46.8± 4.4 ^a	2.4- 62.6	51.3± 4.8 ^{a,b}	2.5- 67.7	55.2± 4.6 ^b	2.5- 71.8

DO: Dissolved oxygen, TAN: Total ammonia nitrogen, N: Nitrogen, SD: Standard deviation.

Values with different superscripts in the same row indicated significant differences ($P \leq 0.05$).

Effect of fishmeal replacement by pea meal on growth performance parameters

The results in Table (4) show the effect of feeding with different levels of pea byproduct (T₁₅ and T₂₅) on the growth performance of *Oreochromis niloticus*. These results revealed that the final weight was significantly higher in treatment groups T₁₅ and T₂₅ (42.88± 7.1g, and 47.42± 13.8g, respectively) compared to the control group (33.30± 1.8g). Similarly, the weight gain and growth in weight were significantly higher in T₁₅ and T₂₅ groups compared to the control group. The DWG and SGR values were non significantly varied in all cases.

Table 4. Effect of fishmeal replacement with different levels of pea by-product on growth performance parameters of *Oreochromis niloticus*

Growth performance item	Control	T ₁₅	T ₂₅
	0% pea byproduct	15% pea byproduct	30% pea byproduct
Initial weight (g)	14.71± 0.05 ^a	14.23± 0.27 ^a	14.52± 0.46 ^a
Final weight (g)	33.30± 1.8 ^b	42.88± 7.1 ^a	47.42± 13.8 ^a
Weight gain (g)	18.60± 1.8 ^b	28.65± 7.0 ^a	32.90± 13.4 ^a
Daily weight gain (mg/ fish/ day)	226.41± 11.6 ^a	301.04± 4.6 ^a	324.05± 84.9 ^a
Growth in weight (%)	144.15± 13.8 ^b	222.09± 54.4 ^a	255.07± 103.7 ^a
Specific growth rate (SGR)	0.63± 0.04 ^a	0.84± 0.13 ^a	0.88± 0.21 ^a

Values with different superscripts in the same row indicated significant differences ($P \leq 0.05$).

Effect of fishmeal replacement by pea meal on feed utilization parameters

Food conversion ratio (FCR) values were significantly varied at T₁₅ with the least value (1.52± 0.09), while T₂₅ was not significant with the highest value (2.09± 0.07) compared with the control group (1.96± 0.10). Feeding efficiency and protein efficiency ratio were significantly varied at T₁₅, with the highest values of 0.66± 0.04 and 2.20± 0.12, respectively. However, there was non-significant variation at T₂₅, where the lowest values were observed at 0.48±0.02 for feeding efficiency and 1.59±0.06 for protein efficiency ratio (Table 5).

Survival rate revealed non-significant differences among all groups, with the highest value at T₁₅ group and lowest survival rate at T₂₅ group, as shown in Table (5).

Table 5. Effect of fishmeal replacement with different levels of pea by-product on feed utilization parameters and survival rate of *Oreochromis niloticus*

Feed utility parameter	Control	T ₁₅	T ₂₅
	0% pea byproduct	15% pea byproduct	30% pea byproduct
Feed intake (g/ fish)	37.66± 9.3 ^b	44.32± 10.0 ^a	65.07± 10.2 ^a
Food conversion rate (FCR)	1.96± 0.10 ^a	1.52± 0.09 ^b	2.09± 0.07 ^a
Feeding efficiency (FE)	0.51± 0.03 ^a	0.66± 0.04 ^b	0.48± 0.02 ^a
Protein intake (g)	11.30± 9.3 ^b	13.30± 10.0 ^a	19.52± 10.2 ^a
Protein efficiency ratio (PER)	1.70± 0.09 ^a	2.20± 0.12 ^b	1.59± 0.06 ^a
Survival rate (%)	92.5± 4.3 ^a	95.0± 5.0 ^a	90± 7.1 ^a

Values with different superscripts in the same row indicated significant differences ($P \leq 0.05$).

Effect of fishmeal replacement by pea meal on hematological parameters

Table (6) displays the values of various hematological parameters in the Nile tilapia reared under different groups for 96 days. The results indicated that the white blood cells (WBCs), red blood cells (RBCs), hemoglobin concentrations (HGB), hematocrit value (HCT) and platelet counts (PLT) revealed non-significant differences in all groups of experiment.

Table 6. Effect of fishmeal replacement with different levels of pea byproduct on hematological parameters of *Oreochromis niloticus* reared for 96 days

Hematological parameter	C	T ₁₅	T ₂₅
	0% Pea byproduct	15% Pea byproduct	25% Pea byproduct
WBC's (cell×10 ³ / ml)	61.60± 11.49 ^a	74.65± 0.69 ^a	74.73± 10.66 ^a
RBC's (cell×10 ⁶ / ml)	1.74± 0.38 ^a	2.24± 0.24 ^a	2.21± 0.20 ^a
HGB(g/ dl)	10.20± 0.78 ^a	12.05± 1.59 ^a	12.03± 0.78 ^a
HCT (cm)	23.97± 6.07 ^a	30.70± 2.04 ^a	31.80± 4.90 ^a
PLT (cell×10 ³ / ml)	144.33± 1.13 ^a	198.00± 6.53 ^a	185.33± 41.06 ^a

Values with different superscripts in the same row indicated significant differences ($P \leq 0.05$).

Effect of fishmeal replacement by pea meal on body composition

Table (7) exhibits the percentages of proximate body composition of the Nile tilapia reared under different fishmeal replacements with pea meal for 96 days compared to the control group. Body composition (%) revealed non-significant differences between different levels of pea byproduct replacements or control groups at dry matter, protein, lipid, ash, carbohydrate, and gross energy.

Table 7. Effect of fishmeal replacement with different levels of pea by-product on body composition of *Oreochromis niloticus* reared for 96 days

Body composition	C	T ₁₅	T ₂₅
	0% pea byproduct	15% pea byproduct	25% pea byproduct
Dry matter (DM)%	28.45± 3.64 ^a	29.32± 0.81 ^a	27.28± 1.11 ^a
Protein %	38.92± 3.95 ^a	38.92± 2.90 ^a	38.02± 3.52 ^a
Lipid %	5.60± 1.16 ^a	5.52± 0.20 ^a	5.52± 0.03 ^a
Ash %	10.58± 1.67 ^a	9.69± 5.87 ^a	8.35± 0.51 ^a
Carbohydrate	10.58± 4.31 ^a	8.65± 6.91 ^a	9.47± 3.74 ^a
Gross Energy (Kcal/ Kg)	272.9± 19.24 ^a	272.5± 26.93 ^a	285.9± 6.53 ^a

Values with different superscripts in the same row indicated significant differences ($P \leq 0.05$).

Effect of fishmeal replacement with pea meal on somatic indices

Table (8) exhibits the values of the hepato-somatic index (HSI), spleen-somatic index (SSI), gonad-somatic index (GSI), and viscera-somatic index (VSI) in the Nile tilapia reared under different replacements of fishmeal with pea meal for 96 days compared to the control group. HSI, SSI, and VSI values revealed no significant difference among all groups in all cases, while GSI exhibited significant differences at T₁₅ and T₂₅ compared to the control group.

Table 8. Somatic indices of *Oreochromis niloticus* reared in different groups for 96 days

Indices	Control	T ₁₅	T ₂₅
	0% pea byproduct	15% pea byproduct	25% pea byproduct
Hepatosomatic index	2.49± 0.78 ^a	1.91± 1.03 ^a	2.33± 0.62 ^a
Spleen-somatic index	0.10± 0.07 ^a	0.72± 0.85 ^a	0.17± 0.05 ^a
Gonadosomatic index	5.32± 1.77 ^a	2.81± 0.52 ^b	3.36± 0.69 ^b
Viscerosomatic index	5.36± 5.03 ^a	1.67± 0.49 ^a	2.30± 0.99 ^a

Values with different superscripts in the same row indicated significant differences ($P \leq 0.05$).

DISCUSSION

Aquaculture continues to be a rapidly expanding sector in food production, with a growing need for alternative, sustainable, and cost-effective feed resources (FAO, 2018). Fishmeal, a high-quality protein source traditionally used in aqua feeds, is becoming increasingly expensive, and its use raises concerns over the sustainability of fisheries. Thus, plant-derived feed ingredients, such as pea meal, have been investigated as alternatives. Tilapia growth and feed consumption could be maintained if fishmeal was substituted 50% of the time, according to research by Zerai *et al.* (2008). In this study, researchers found that *Oreochromis niloticus* growth performance and protein consumption were not altered when fishmeal was replaced with pea byproduct (fiber-free). Although using pea by-products substantially reduced FCR in the T15 group (1.52 ± 0.09) compared to the control group's (1.96 ± 0.10), this difference may be attributable to the amino acid diversity provided by the pea meal. Pea protein concentrate may effectively replace fishmeal or soybean meal in diets for Atlantic salmon, *Salmo salar*, as shown by Overland *et al.* (2009). According to Grishma *et al.* (2019), common carp may benefit from having pea pod powder added to their food at a rate of 20%. According to Tibaldi *et al.* (2005), pea protein may be utilized to replace up to 60% of fishmeal protein in complete meals of sea bass. Hussin *et al.* (2010) found that fingerlings of the Nile tilapia may use pea meal as an alternative protein instead of 50% fishmeal without negative impacts on growth performance. Furthermore, pea plant protein sources are locally accessible at considerably cheaper costs than imported fishmeal.

The current results recorded the values of the hepatosomatic index (HSI), spleen somatic index (SSI), gonadosomatic index (GSI), and viscera somatic index (VSI) in the Nile tilapia reared under different levels of pea meal-based diets, and the present findings revealed that there were non-significant effects. HSI can reflect metabolic changes or liver health of fish gonado-somatic index (GSI). Stoneham *et al.* (2018) mentioned that the algae meal as a plant protein source had a lower HSI value of the tilapia compared with a fishmeal as an animal protein source due to the protective effect on the liver by reducing lipid accumulation and oxidative stress. Many authors mentioned that the level of crude protein did not affect the hepatosomatic index of the tilapia juveniles reared under biofloc condition that may be related to the higher metabolism of smaller fish (Abdel-Tawwab *et al.*, 2010; Kpundeh *et*

al., 2015; Da-Silva *et al.*, 2018; Hwihy *et al.*, 2021). Tacchi *et al.* (2012) in their study found a non-significant effect of somatic indices (cardiac somatic index, hepatosomatic index, and intestinal somatic index) for the Atlantic salmon fed on fishmeal or plant proteins.

Incorporating plant proteins in fish diets is common in aquaculture due to the high cost and sustainability concerns associated with fishmeal. However, this substitution can impact the health status of fish including their hematological parameters (Macusi *et al.*, 2023).

Hematological parameters (WBCs, RBCs, platelets, hemoglobin, and hematocrit value) of *Oreochromis niloticus* fed on different levels of pea byproduct (15, 25%) as a low economic protein source in the present work revealed non-significant differences compared to the control group that fed on fishmeal-based diet. The majority of the Nile tilapia blood parameters measured in the present work were within the species' normal range (Ayyat *et al.*, 2017; Mahmoud & El-Hais, 2017). Hemoglobin levels were shown to be significantly different when fishmeal was substituted with plant protein although hematocrit, white blood cell count, and red blood cell count were not significantly different (Jahanbakhshi *et al.*, 2013).

The current findings are consistent with those of Yue and Zhou (2008) and Zheng *et al.* (2012) who found that, hematological values were not statistically altered upon depending on cotton seed meal as a plant protein.

The present investigation found no statistically significant variations in the body composition percent of *Oreochromis niloticus* between the groups fed varying concentrations of pea byproduct replacements and the control group. The same finding was observed by Tibaldi *et al.* (2005). Crude protein and fat content did not change statistically in sea bass fed with pea protein concentrate based meal. Hussin *et al.* (2010) mentioned that juveniles of the Nile tilapia showed no negative effects on whole fish bodies' proximate composition of either incorporation of pea meal in the Nile tilapia diets, compared to the control group. Soltan *et al.* (2008) showed that dry matter and crude protein of the whole body increased with increasing the levels of plant protein meal (PPM) in diets, and the whole-body content of lipid and ash significantly ($P > 0.05$) increased with increasing plant protein meal content of tilapia diets.

CONCLUSION

The results suggest that replacing 50% of fishmeal and depending on fiber-free pea meal up to 25% of total diet formulations have no adverse effects on the growth performance of the Nile tilapia, as well as protein utilization, body indices, hematology, and proximal composition of the fish body. It is worthy to mention that, the dependence on 15% of fiber-free pea meal improves the feed conversion ratio and feeding efficiency of *Oreochromis niloticus* significantly.

REFERENCES

Abdel-Tawwab, M.; Ahmad, M.H.; Khattab, Y.A. and Shalaby, A.M. (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, **298**(3-4): 267-274.

- Abebe, B. (2022).** The dietary use of pigeon pea for human and animal diets. The scientific World Journal, **2022**: ID 4873008, 12 pages. <https://doi.org/10.1155/2022/4873008>
- AOAC, (2005):** Official Methods of Analysis of the Association of Official Analytical Chemists international. 1(16): 31-65.
- APHA (1995).** Standard Methods for Examination of Water and Wastewater. American Public Health Association, 18: 769. Washington D.C., USA.
- Ashour, M.; Abo-Taleb, H.A.; Hassan, A.K.M.; Abdelzaher, O.F.; Mabrouk, M.M.; Elokaby, M.A.; ... and Mansour, A.T. (2021).** Valorization use of amphipod meal, *Gammarus pulex*, as a fishmeal substitute on growth performance, feed utilization, histological and histometric indices of the gut, and economic revenue of grey mullet. Journal of Marine Science and Engineering, **9**(12):1336.
- Ayyat, M.S.; Mahmoud, H.K.; El-Hais, A.E. and Abd El-Latif, K.M. (2017).** The role of some feed additives in fish fed on diets contaminated with Cadmium. Environmental Science and Pollution Research, **24**(30): 23636-23645.
- Borlongan, I.G.; Eusebio, P.S. and Welsh, T. (2003).** Potential of feed pea (*Pisum sativum*) meal as a protein source in practical diets for *milkfish* (*Chanos Forsskal*). Aquaculture, **225**(1-4): 89-98.
- Burel, C.; Boujard, T.; Tulli, F. and Kaushik, S.J. (2000).** Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture, **188**(3-4): 285-298.
- Chen, M.; Li, P.; Wang, Q.; Yuan, Y.; Zhang, D.; Liang, X. and Huang, Z. (2018).** Dietary replacement of fishmeal by pea meal in diets for juvenile Nile tilapia, *Oreochromis niloticus*: Growth, feed utilization and digestibility. Aquaculture, **497**: 269-275.
- Cruz-Suarez, L.E.; Ricque-Marie, D.; Tapia-Salazar, M.; McCallum, I.M. and Hickling, D. (2001).** Assessment of differently processed feed pea (*Pisum sativum*) meals and canola meals (*Brassica sp.*) in diets for blue shrimp (*Litopenaeus stylirostris*). Aquaculture, **196**(1-2): 87-104.
- Dacie, J.V. and Lewis, S.M. (2010).** Preparation and Staining Methods for Blood and Bone-Marrow Films. In: Dacie JV, Lewis SM, eds. Practical Haematology. 8th ed. **Churchill Livingstone. Pp:** 83-96.
- Da-Silva, M.A.; De-Alvarenga, É.R.; Alves, G.; Manduca, L.G.; Turra, E.M.; De-Brito, T.S. and Teixeira, E. (2018).** Crude protein levels in diets for two growth stages of Nile tilapia (*Oreochromis niloticus*) in a biofloc system. Aquaculture Research. **49**(8): 2693-2703.
- FAO (2014).** The State of World Fisheries and Aquaculture. **Food and Agriculture Organization of the United Nations.** Opportunities and Challenges. 223.
- FAO (2018).** The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.
- Ganzon-Naret, E. (2019).** Utilization of raw, dehulled, autoclaved and soaked pea, *Pisum sativum* seed meals as replacement for fishmeal in practical diet formulation for juvenile sea bass in a recirculating system. World J. Agri. Soil Sci, **1**: 1-6.

- Grishma, T.; Abhed, P.; Shanthanagouda, A.H. and Hundal, J.S. (2019).** Effect of pea pod as feed ingredient on growth performance of common carp, *Cyprinus carpio*. Journal of Experimental Zoology, India, **22**(2): 795-799.
- Gutierrez-Wing, M.T. and Malone, R.F. (2006).** Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquaculture Engineering, **34**(3): 163-171.
- Hanan, E.;; Rudra, S.G.; Sagar, V.R. and Sharma, V. (2020).** Utilization of pea pod powder for formulation of instant pea soup powder. Journal of Food Processing and Preservation, **44**(11): e14888.
- Hickling, D. (2003).** Canadian feed peas industry guide. Pulse Canada, Winnipeg, Manitoba. www.pulsecanada.com/pdf/Feed%20Peas%20English.
- Hodar, A.R.; Vasava, R.J.; Mahavadiya, D.R. and Joshi, N.H. (2020).** Fishmeal and fish oil replacement for aqua feed formulation by using alternative sources: a review. Journal of Experimental Zoology India, **23**(1): 13-21.
- Hussin, M.S.; Al-Azab, E.S. and Taj Adeen, Y.M.H. (2010).** The effects of partial replacement of fishmeal by peas (*Pisum sativum*) and tomato pomace byproduct as non-conventional ingredients in diets fed to Nile Tilapia (*Oreochromis niloticus*) juvenile. Journal of Animal and Poultry Production, **1**(6): 225-240.
- Hwihiy, H.; Zeina, A.; Abu Husien, M. & El-Damhougy, K. (2021).** Impact of biofloc technology on growth performance and biochemical parameters of *Oreochromis niloticus*. Egyptian Journal of Aquatic Biology and Fisheries, **25**(1): 761-774.
- Jahanbakhshi, A.; Imanpoor, M.R.; Taghizadeh, V. and Shabani, A. (2013).** Hematological and serum biochemical indices changes induced by replacing fishmeal with plant protein (sesame oil cake and corn gluten) in the great sturgeon (*Huso huso*). Comparative Clinical Pathology, **22**: 1087-1092.
- Kaushik, S.J.; Cravedi, J.P.; Lalles, J.P.; Sumpter, J.; Fauconneau, B. and Laroche, M. (1995).** Partial or total replacement of fishmeal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture, **133**(3-4): 257-274.
- Kpundeh, M.D.; Qiang, J.; He, J.; Yang, H. and Xu, P. (2015).** Effects of dietary protein levels on growth performance and haemato-immunological parameters of juvenile genetically improved farmed tilapia (GIFT), *Oreochromis niloticus*. Aquaculture International, **23**: 1189– 1201.
- Kumar, V.; Sinha, A.K. and Romano, N. (2020).** Pea meal as a sustainable protein source in practical diets for juvenile tilapia, *Oreochromis niloticus*. Aquaculture, **526**: 735363.
- Liu, W.; Xie, J.; Ye, J.; Wu, X. and Qian, Y. (2022).** Fermented pea meal as a fishmeal replacer in the diet of tilapia: Effects on growth, immune response, gut microbiota, and disease resistance. Aquaculture, **541**: 736920.
- Magbanua, T.O. and Ragaza, J.A. (2023).** Growth and whole-body proximate composition of *Oreochromis niloticus* Nile tilapia fed pea meal: A systematic review and meta-analysis. Frontiers in Sustainable Food Systems, **7**: 1103263.
- Mahmoud, H.K. and El-Hais, A. (2017).** Effect of dietary garlic (*Allium sativum*) supplementation on Nile tilapia *Oreochromis niloticus* juveniles' performance under two stocking densities. Egyptian Journal of Nutrition and Feeds, **20**(1): 115-124.

- Matos, J.; Costa, S.; Rodrigues, A.; Pereira, R. and Pinto, I. S. (2006).** Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. *Aquaculture*, **252**(1): 31-42.
- Mejri, F.; Karmali, A.; Jaoued, N.; Casabianca, H. and Hosni, K. (2019).** Purification and partial characterization of peroxidases from three food waste by-products: broad bean pods, pea pods, and artichoke stems. *Applied biochemistry and biotechnology*, **189**(2): 576-588.
- Macusi, E. D., Cayacay, M. A., Borazon, E. Q., Sales, A. C., Habib, A., Fadli, N. and Santos, M. D. (2023).** Protein Fishmeal Replacement in Aquaculture: A Systematic Review and Implications on Growth and Adoption Viability in the Philippines. *Sustainability*, **15**(16): 12500. <https://doi.org/10.3390/su151612500>
- Mugwanya, M.; Dawood, M.A.; Kimera, F. and Sewilam, H. (2023).** Replacement of fishmeal with fermented plant proteins in the aquafeed industry: A systematic review and meta- analysis. *Reviews in Aquaculture*, **15**(1): 62-88.
- Nasir, G.; Zaidi, S.; Tabassum, N. and Asfaq. (2022).** A review on nutritional composition, health benefits and potential applications of by-products from pea processing. *Biomass Conversion and Biorefinery*: DOI: 10.1007/s13399-022-03324-0
- Noga, E.J. (2010).** *Fish Disease Diagnosis and Treatment*, 2nd ed. Wiley-Blackwell, Ames, Pp: 519.
- Overland, M.; Sorensen, M.; Storebakken, T.; Penn, M.; Krogdahl, Å. and Skrede, A. (2009).** Pea protein concentrate substituting fishmeal or soybean meal in diets for Atlantic salmon (*Salmo salar*). Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. *Aquaculture*, **288**(3-4): 305-311.
- Perez-Maldonado, R.A. and Norton, R.A. (2010).** The use of pea protein isolates as a replacement for fishmeal in the diets of juvenile barramundi (*Lates calcarifer*). *Aquaculture Research*, **41**(11): 1621-1630.
- Shaban, W.M. (2012).** Life history, reproduction and rearing of caprellid amphipods: Its possibility to use as live feed in mariculture. PhD thesis, Faculty of science (Boys), Al-Azhar University, Cairo, Egypt.
- Soltan, M.A., Hanafy, M.A. and Wafa, M.I.A. (2008).** Effect of replacing fishmeal by a mixture of different plant protein sources in Nile tilapia (*Oreochromis niloticus* L.) diets. *Global Veterinaria*, **2**(4): 157-164.
- Stoneham, T.R.; Kuhn, D.D.; Taylor, D.P.; Neilson, A.P.; Smith, S.A.; Gatlin, D.M.; ... and O'Keefe, S.F. (2018).** Production of omega-3 enriched tilapia through the dietary use of algae meal or fish oil: Improved nutrient value of fillet and offal. *PLoS One*, **13**(4): e0194241.
- Subasinghe, R. (2005).** Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. *Preventive Veterinary Medicine*, **67**(2, 3): 117-124.
- Tacchi, L.; Secombes, C.J.; Bickerdike, R.; Adler, M.A.; Venegas, C.; Takle, H. and Martin, S.A. (2012).** Transcriptomic and physiological responses to fishmeal substitution with plant proteins in formulated feed in farmed Atlantic salmon (*Salmo salar*). *BMC genomics*, **13**(1): 1-21.

- Tacon, A.G.J. (2005).** Salmon aquaculture dialogue: status of information on salmon aquaculture feed and the environment. *Int. Aquafeed*, **8**: 22-37.
- Tassoni, A.; Tedeschi, T.; Zurlini, C.; Cigognini, I.M.; Petrusan, J. I.; Rodríguez, Ó.; ... and Corvini, P.F. (2020).** State-of-the-art production chains for peas, beans, and chickpeas—valorization of agro-industrial residues and applications of derived extracts. *Molecules*, **25**(6): 1383.
- Thiessen, D.L.; Campbell, G.L. and Adelizi, P.D. (2003).** Digestibility and growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed with pea and canola products. *Aquaculture nutrition*, **9**(2): 67-75.
- Tibaldi, E.; Tulli, F.; Messina, M.; Franchin, C. and Badini, E. (2005).** Pea protein concentrated as a substitute for fishmeal protein in sea bass diet. *Italian Journal of animal science*, **4**(sup2): 597-599.
- Yue, Y.R. and Zhou, Q.C. (2008).** Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquaculture*, **284**(1-4): 185-189.
- Zerai, D.B.; Fitzsimmons, K.M.; Collier, R.J. and Duff, G.C. (2008).** Evaluation of brewer's waste as partial replacement of fishmeal protein in Nile tilapia, *Oreochromis niloticus*, diets. *Journal of the World Aquaculture Society*, **39**(4): 556-564.
- Zhang, S.J.; Hu, T.T.; Chen, Y.Y.; Wang, S. and Kang, Y.F. (2020).** Analysis of the polysaccharide fractions isolated from pea (*Pisum sativum* L.) at different levels of purification. *Journal of Food Biochemistry*, **44**(8): e13248.
- Zheng, Q.; Wen, X.; Han, C.; Li, H. and Xie, X. (2012).** Effect of replacing soybean meal with cottonseed meal on growth, hematology, antioxidant enzymes activity and expression for juvenile grass carp, *Ctenopharyngodon idellus*. *Fish Physiology and Biochemistry*, **38**: 1059-1069.