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Effects of replacing various combinations of mixed plant meals (lupine, sesame, and jojoba) for the fishmeal, on *Tilapia zilli* growth, immunity, and histological changes

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ABSTRACT

The current study was conducted to evaluate the effects of different combinations of four dietary plant meal protein treatments {Mix (lupine, sesame, and jojoba meal), Mix+ (Methionine and Lysine), Soy con. (concentrate)+ and Soy+)} for *Tilapia zillii* on growth performance, biochemical indicators, and histological changes for 60 days. The results indicated that the highest significant final weight was recorded in the (Mix +), followed by the control group. The food conversion ratio and survival rate were better in these groups. The results of the study revealed that haemoglobin levels (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean cell volume (MCV) were all significantly increased (except for the soy con+ group) between the initial and final blood samples from fish fed the commercial diet and various diets used in the experiment. Blood samples from fish given both commercial food and different diets showed considerably elevated biochemical markers, except glucose and cholesterol. The histological examination revealed that an increase in the length of villi and the number of goblet cells were recorded in the intestine of *Tilapia zillii* groups fed on Mix and Mix+ groups, compared to the control group. Finally, when compared to fish meal, plant protein is the only simple, affordable, and easily accessible substitute for fishmeal.

INTRODUCTION

FAO reports that aquaculture has expanded more quickly than other significant food production sectors, with the goal of fulfilling rising global demand for fish while protecting natural fish populations **(Hua *et al.*, 2019; Afridi *et al.*, 2019; Cooney *et al.*, 2021; Khalil *et al.*, 2023)**, it gives hundreds of millions of people around the world a significant amount of high-quality protein **(Yuan *et al.*, 2017; Chumpol *et al.*, 2018)**. *Tilapia zillii* (Gervais, 1848) along with the other tilapias have been the object of considerable research because of its suitability for aquaculture. It can tolerate a wide range of temperature and salinity and can utilize aquatic vegetation **(Guezi *et al.*, 2021)**. Fish feed technology is one of the aquaculture industry's least developed sectors, especially in Africa and other poor nations **(Gabriel *et al.*, 2007; Soliman *et al.*, 2022)**. According to **(Gabriel *et al.*, 2007)** fish feed makes up at least 60%–70% of the overall cost of production. The high price of fish feed is one issue impeding Egypt's aquaculture growth **(Badrey *et al.*, 2019)**. Fish feed production has experimented with both animal- and plant-origin protein sources, with varied degrees of success **(Mursy *et***

***al*., 2022)**. Until recently, for a variety of reasons, including its high protein content, superior essential amino acid profile, improved nutrient digestibility, low cost, and convenience of availability, fish meal was the main source of protein in the fish feed.

Vegetable products have drawn researchers' attention as ingredients for fish feed production since they are available in high natural abundance **(Mursy *et al*., 2022)**. Due to its high protein content, omega-3 polyunsaturated fatty acids, balanced amino acid composition, and ease of digestion, fishmeal (FM) is an ideal source of dietary protein for aquaculture species **(Hamidoghli *et al*., 2018; Biswas *et al*., 2020; Zamani *et al*., 2020)**. However, the dependence of aquaculture on fishmeal as a source of protein is a growing worry for this business in terms of its sustainability and profitability. Trials have been conducted by aquaculturists all over the world to find substitute fishmeal ingredients that can be affordable, accessible, and still give the same nutritional value for the target species **(Camacho-Rodrnguez *et al*., 2015; Henry *et al*., 2015; Wang, *et al*., 2020)**. Many fish species' diets have been studied for partial and complete substitution of fishmeal using other protein sources **(Lazzarotto *et al*., 2018; Wang, *et al*., 2020)**. For many species of aquafeeds, plant protein sources are therefore the most promising substitutes for fishmeal **(Havasi *et al*., 2015; Gao *et al*., 2018)**. Fishmeal replacement solely by plant-based alternatives is difficult due to the presence of anti-nutritional factors (ANFs) **(National Research Council, 2011; Lei *et al*., 2021)**, including alkaloids, lectins, protease inhibitors, phytates, saponins, and tannins, deficiency of lysine and methionine and concerns about digestibility and palatability **(Gemrde and Ratta, 2014)**. Researchers are trying to decrease these concerns through exogenous supplementation of essential amino acids or biochemical modification of the component **(Lewis *et al*., 2019)**. In this context, plant-derived raw materials have taken on the role of fish meal in tilapia meals, with soybean meal reaching a 100% substitution or at lower levels when employing novel sources like lupine, sesame, and jojoba. The benefits come not only from the availability and economic advantages but also from the fact that these plant proteins are lower in phosphate and nitrogen than animal proteins, lessening the likelihood of pond eutrophication.

Many studies have been conducted to determine whether various agricultural-based products, such as corn/wheat gluten meals **(Giannenas *et al*., 2017)**, fermented lupin (*Lupinus angustifolius*) **(Van Vo *et al*., 2015)**, jojoba meal (JM), and sesame meal (SSM), are suitable for commercial application in aquafeeds **(Bilgin *et al*., 2007; Dadgar *et al*., 2009; Hu *et al*., 2015)**, pomegranate peel **(Badrey *et al*., 2019)**, and alfalfa, peanut leaves **(Mursy *et al*., 2022)**. Therefore, in order to make the aquaculture sector successful, it is essential to use fish food that has basic ingredients at reasonable prices **(Naiel *et al*., 2020)**. However, in order to achieve an environmentally friendly and more productive fish farming system, this trend must be replaced with cheap components **(Wang *et al*., 2020; Monica and Jayaraj, 2021)**. The combination of several protein sources might be able to satisfy FM-like dietary needs. As a result, aqua diets and gut health have a significant interaction that affects physiological elements like immunity and digestion **(Butt and Volkoff, 2019)**. The goal of the current study was to determine how different combinations of plant meals, including lupine, sesame, and jojoba meal, would affect growth performance, biochemical indicators, and intestinal histology.

MATERIALS AND METHODS

This experiment was carried out in the Department of Zoology, Faculty of Science, Al-Azhar University (Assiut branch). Fish were obtained from a private fish farm in Assiut Governorate, Egypt. The fish were allowed to acclimate to the laboratory conditions for three weeks before the start of the experiment and fed a diet of 25% crude protein before the start of the experiments. As advised by the **NRC (1993)**, the diet utilized in the experiment was designed to provide all the nutrients needed for tilapia.

There were five diet groups in the experimental design. **Table (1)** illustrated that the first group was fed with a 25% CP as a control; the other groups diets were formulated to represent four dietary treatments {Mix (lupine, sesame, and jojoba meal), Mix + (Methionine and Lysine), Soy con. (concentrate)+ and Soy+). The diet was formed into tiny balls and ground into spaghetti-like texture using an industrial meat grinder. After spreading the diet over a metal plate, it was baked for 36 hours at 60°C. To separate the pellets into a size acceptable for the experimental fish size of 1 mm diameter, chopped dried meals were sieved via conventional sieves. Diets were kept in plastic bags with labels and kept at -20°C in the freezer until needed. For ninety days, the feed was manually dispersed in two equal amounts on one side of the aquariums at nine in the morning and three in the afternoon (**Table 1**).

Chemical analysis

At the end of each experiment, samples of fish and feed were taken. Fish were dissected to take a piece of fish body in closed containers and stored in the deep freezer for chemical analysis to determine the proximate composition analysis of fish and diets, including dry matter (DM), for crude protein by the Kjeldahl method using a Kjeltech auto-analyzer (Model 1030, Tecator, Hoganas, Sweden) **(Bligh and Dyer, 1959)**. Ether extracts (EE) and ash contents were determined according to **AOAC (1995)**.

Measurement of Growth Performance

Fish samples were taken every month day to determine total body weight (g) and total body length (cm). Feed quantity was always changed according to the increase in the body weight of the fish. Total weight gain (TWG) (g), average daily body weight gain (ADG), specific growth rate (SGR), feed conversion ratio protein (FCR), and survival rate (SR) were determined according to **Castell and Tiewes (1980)** as follows: TWG = [FBW - IBW], Servival rate (SR%) = (No. of fish at the end/No. of fish at start) x 100, Specific growth rate (SGR%)= log FW - log IW/t * 100, FCR = FI/WG

Haematological analysis

Whole blood was assessed for haemoglobin concentration (Hb), haematocrit (Hct), and red blood cell (RBC) count by using an automated technical analyser (Celltac α MEK-6400J/K). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cell (WBC) count were calculated according to the methods of **Dacie and Lewis (2002)**.

Table (1): The composition and chemical analyses (% on dry matter basis) of the trial diets

Ingredients composition	Composition (%) experimental diets.				
	CTR	Mix	Mix +	Soy +	Soy con +
Fish meal (68%)	10	0	0	0	0
Soybean meal (46%)	30	0	0	39	0
Soybean meal concentrates (48%)	30	0	0	0	38
Sesame meal (36%)	0	25	25	0	0
Lupine meal (36%)	0	25	25	0	0
Jojoba meal (26%)	0	10	10	0	0
Rice bran	25	10	10	22	22
Wheat bran	22	20	20	25	25
Yellow corn	9.7	6.7	6.2	10.2	11.2
Fish oil	3	3	3	3	3
Premix [1]	0.3	0.3	0.3	0.3	0.3
Methionine	-	-	0.25	0.25	0.25
Lysine	-	-	0.25	0.25	0.25
Chemical composition %					
Dry matter (D.M.)	95.3	95.6	95.5	95.7	95.4
Crud protein (CP)	25.4	25.3	25.3	25.2	25.3
Ether extract	11.2	11.6	11.5	11.5	11.4
Ash	12.3	11.7	11.2	12.1	12.4
Crude fiber	4.86	4.88	4.90	4.92	4.96
Nitrogen-free extract (N.F.E.)	46.24	46.52	47.1	46.28	45.94
Gross energy (kcal/100g D.M.)[3]	439.1	440.9	443.9	443.4	439.0

1 Premix Composition: - Each 1 kg contains: Vit A (400000 i.u.); Vit D3 (100000 i.u.); Vit E (230 mg); Vit K3 (165mg); Vit B1 (300 mg); Vit B2 (80mg); Vit B6 (200mg); Vit B12 (1mg); Vit C (650mg); Niacin (1000mg); Methionine(3000mg); Choline chloride (10000mg); Folic acid (100mg); Biotin (2mg); Pantothenic acid (220mg); Magnesium sulfate (1000mg); Copper sulfate (1000mg); Iron sulfate (330mg); Zinc sulfate (600mg); Cobalt sulfate (100mg); Calcium carbonate up to (1000g).

2 Plant oil (mix of 1 linseed oil: 1 soy oil).

3 Gross energy (G.E.) was calculated as 5.64, 9.44, and 4.11 kcal/100g for protein, lipid, and N.F.E., respectively **(NRC, 1993)**.

Biochemical analysis

The concentrations of glucose was determined according to Trinder (1969); total protein (TP) based on biuret method **(Henry *et al*., 1974)**; cholesterol **(Atamanalp *et al*., 2003)**; triglycerides **(Chawla *et al*., 2003)**; kidney function (urea and creatinine) according to **Reitman and Frankel (1957)**; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were determined using the techniques developed by **Reitman and Frankel (1957); Henry (1964); Trinder (1969); Friedewald *et al*. (1972)** and **Thomas (1992)**.

Histological examinations

Samples for histopathological examination tacked from fish intestine of different diets groups. Intestinal samples were collected from different groups, fixed in 10% neutral buffered formalin. After dehydration and clearance, the tissues were embedded in paraffin and sectioned in 5 µm thickness. The serial sections were subjected to staining with Hematoxylin and Eosin **(Bancroft and Layton, 2012)**. The Histomorphometric analysis was performed using ImageJ analysis software (National Institutes of Health, MD, USA).

Statistical analysis

Data were presented as mean±SD. The results were subjected to one-way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance. Data were

analyzed using **SPSS (1997)** program, Version 16. Differences between means were compared using Duncan's multiple range tests at $p < 0.0^{\circ}$ level.

RESULTS

Growth performance

The growth performance parameters of fish fed with the different experimental diets during the experiment are summarized in **Table (2)**. The higher FW, WG, and SR and the best FCR were recorded in (Mix +). The obtained data showed that growth performance was the best in the (Mix +) group. Survival rate (SR) exhibited the highest values for Mix +, Soy con +, and Control, respectively, which were significantly ($p < 0.05$) higher than those of the other treatments. The highest significant final weight was recorded in the control and (Mix +) groups, which was comparable with that of all treated groups. Fish reared at Mix level showed the lowest significant weight gains, but the other treatments had no significant differences from each other. Statistically significant ($p \leq 0.05$) differences in SGR were found among the control, Mix, and Mix + groups compared to the Soy con+ and Soy+ groups. It is obvious that the lowest (best) significant FCR was recorded at Mix + Treatment, and all the other treatments have no significant differences from each other.

Table (2): Growth performance and feed utilization indices of fish from *O. Zilli* during the experiment

Treatment	Experimental diets				
Parameters	Control	Mix	Mix +	Soy +	+Soy con
IW (g/fish)	3.2±0.1	3.2±0.1	3.1±0.3	3.1±0.3	3.2±0.3
FW (g/fish)	23.1±0.1^{a}	21.5±0.2^{ab}	23.1±0.1^{a}	22.6±0.2^{b}	22.5±0.2^{b}
TWG (g/fish)	19.9±0.05^{a}	18.3±0.4^{b}	20.0±0.3^{a}	19.5±0.1^{a}	19.3±0.4^{a}
SGR (%/d)	2.5±0.2^{a}	2.4±0.2^{a}	2.3±0.2^{a}	2.1±0.3^{b}	2.1±0.3^{b}
FCR	1.7±0.2^{a}	1.9±0.1^{a}	1.4±0.3^{b}	1.7±0.4^{a}	1.7±0.2^{a}
SR (%)	95.3±1.2^{a}	94.4±2.3^{b}	96.4±1.2^{a}	88.9±1.2^{c}	95.4±1.0^{a}

Means in the same row with different superscript letters are significantly different at ($p < 0.05$)

Biochemical composition of whole fish

The effect of different combinations four dietary treatments of plant meals for *Tilapia zillii* on biochemical composition of whole fish in present experiment is illustrated in **Table (3)**. Moisture contents were the best when fish fed Mix+ also ether extract. The highest significant ($p \leq 0.05$) ether extract (37.01%) and the lowest significant ($p \leq 0.05$) content of crude protein of body composition. Ash content showed significant ($p \leq 0.05$) differences among the tested plant protein meals.

Haematological parameters

The mean values for Hb, RBCs, WBCs, MCH, MCHC, Hct, and MCV of *Tilapia zilli* fed different diets are presented in **Table (4).** Statistical analysis of these variables revealed significant differences between the different groups and the control diet (except for the RBCs at 90 days). Hb, MCH, MCHC, and MCV were all significantly increased in these parameters, with the exception of the soy con+ group, between the initial and final blood

samples from fish fed the commercial diet and various diets used in the experiment. Non-significant (P>0.05) differences were observed in the final RBCs. However, there was erratic fluctuation between the three parameters (RBCs, WBCs, and Hct) in all groups. All scored haematological parameters were diet-dependent **(Table 4)**.

Table (3). Biochemical composition of whole fish feeding trial (dry weight basis)

Treatment	Experimental diets					
Parameters	Initial	Control	Mix	Mix +	Soy +	Soy con +
Moisture	70.57±0.26	69.13±1.57^{a}	66.74±1.56^{b}	64.87±1.35^{c}	70.47±0.96^{a}	65.62±1.21^{b}
CP	64.45±0.36	58.55±0.55^{a}	53.65±0.25^{b}	53.42±1.00^{b}	59.45±0.35^{a}	54.55±0.55^{b}
EE	18.34±0.68	24.74±0.23^{d}	37.25±0.25^{a}	37.01±057^{a}	28.85±0.35^{c}	33.15±0.57^{b}
Ash	17.10±0.43	16.74±0.75^{a}	9.15±0.45^{c}	9.60±0.40^{c}	11.65±0.06^{b}	12.30±0.23^{b}

Means in the same row with different superscript letters are significantly different at ($p < 0.05$)

Table (4): Haematological parameters (mean ±SD) of *T. zilli* for 45 and 90 days

Treatment		Experimental diets				
Parameters	Period	Control	Mix	Mix +	Soy +	Soy con +
Hb (g/dl)	45 days	9.18±0.3^{ab}	8.3±0.3^{a}	9.5±0.4^{ab}	9.2±0.4^{ab}	10.5±0.4^{b}
	90 days	10.2±0.4^{ab}	9.1±0.2^{b}	10.2±0.2^{ab}	8.2±0.1^{a}	11±0.3^{c}
RBCs ($\times 10^{6}$ µl)	45 days	2.1±0.1^{ab}	1.8±0.1^{ab}	2.2±0.2^{ab}	1.7±0.1^{a}	2.4±0.1^{b}
	90 days	2.2±0.2^{a}	1.7±0.1^{a}	2.3±0.1^{a}	2.1±0.1^{a}	2.3±0.1^{a}
WBCs ($\times 10^{3}$ µl)	45 days	27.9±0.7^{a}	28.1±1.3^{a}	33.4±0.6^{b}	35.6±0.4^{b}	33.1±1.3^{b}
	90 days	27.9±0.7^{a}	28.5±0.7^{a}	34.1±0.4^{b}	35.8±0.7^{b}	32.9±1.5^{b}
MCH (pg)	45 days	43.7±3.5^{a}	45.6±5.0^{b}	43.4±3.2^{a}	53.4±2.9^{ab}	43.6±3.2^{a}
	90 days	46.5±4.4^{ab}	53.3±2.1^{c}	44.8±4.6^{b}	38.8±1.4^{a}	48.8±5.2^{ab}
MCHC (g/dL)	45 days	27.7±0.1^{a}	29.2±2.0^{ab}	33.3±1.7^{b}	35.3±1.2^{c}	34.4±1.2^{bc}
	90 days	29.2±1.2^{a}	34.1±1.1^{bc}	37.2±0.6^{c}	31.0±0.6^{b}	36.4±1.6^{c}
Hct (%)	45 days	33.0±0.9^{c}	28.7±0.9^{ab}	28.5±0.4^{b}	26.0±0.4^{a}	30.6±1.5b^{c}
	90 days	35.0±0.1^{c}	26.9±0.6^{a}	27.5±0.3^{a}	26.5±0.8^{a}	30.2±0.4^{b}
MCV (fL)	45 days	157.5±13.2^{ab}	155.1±6.0^{ab}	132.5±16.9^{b}	151.6±8.0^{ab}	127.4±12.5^{a}
	90 days	160.2±19.8^{b}	156.9±10.9^{b}	120.5±12.4^{a}	125.2±5.4^{a}	133.4±9.9^{a}

Blood biochemistry

The mean concentrations of glucose, total protein, triglyceride, cholesterol, urea, creatinine, ALT, and AST in the blood serum of *Tilapia zilli* fed different diets are presented in **(Table 5)**. Total protein, triglycerides, urea, ALT, and AST end levels were significantly (P<0.05) higher than starting values. In contrast, the decreased levels of glucose and cholesterol in the blood samples from fish fed both the commercial diet and various diets were significantly (P<0.05) lower. While blood samples from fish given the commercial food and the other diets utilized in the current investigation showed non-significant variations in the blood creatinine levels **(Table 5)**.

Table (5): Biochemical parameters (mean ±SD) of *O. zilli* for 45 and 90 days

Treatment	Experimental diets					
Parameters	Period	Control	Mix	Mix +	Soy +	Soy con+
GL(mg/dl)	45 days	55.9±2.0^{c}	51.3±0.1^{ab}	53.5±1.9^{bc}	43.0±0.5^{b}	40.2±0.1^{a}
	90 days	40.8±0.1^{ab}	42.1±2.7^{ab}	46.3±2.3^{b}	38.4±1.8^{a}	40.1±1.3^{ab}
T.P (mg/dl)	45 days	2.9±0.1^{a}	3.0±0.5^{a}	2.3±0.1^{a}	2.8±0.1a	3.1±0.1^{a}
	90 days	3.1±0.2^{ab}	3.8±0.09^{b}	2.9±0.3^{a}	3.1±0.03^{ab}	3.2±0.1^{ab}
Tg(mg/dl)	45 days	189.3±4.6^{b}	171.6±8.5^{a}	188.1±2.4^{b}	192.5±1.3^{b}	192.4±5.5^{b}
	90 days	200.5±0.7^{a}	196.3±1.9^{a}	243.2±13.8^{b}	192.7±1.0^{a}	254.4±9.8^{b}
Chlo (mg/dl)	45 days	80.4±1.9^{a}	80.4±1.1^{a}	71.5±2.8^{a}	77.9±1.0^{a}	77.7±6.0^{a}
	90 days	88.5±0.7^{b}	78.9±4.2^{ab}	70.5±0.3^{a}	77.3±1.4^{ab}	77.4±5.7^{ab}
Ur (mg/dl)	45 days	4.2±0.6^{a}	3.8±0.1^{a}	3.5±0.3^{a}	4.1±0.7^{a}	3.1±0.3^{a}
	90 days	4.3±0.6^{ab}	5.3±0.3^{b}	5.2±0.1^{ab}	4.7±0.4^{ab}	3.7±0.0^{a}
Crt (mg/dl)	45 days	0.4±0.0^{a}	0.3±0.0^{a}	0.7±0.0^{b}	0.4±0.0^{a}	0.5±0.0^{ab}
	90 days	0.5±0.1^{a}	0.3±0.0^{a}	0.6±0.0^{a}	0.4±0.0^{a}	0.5±0.0^{a}
ALT(U/L)	45 days	24.8±0.8^{b}	23.2±0.8^{ab}	23.2±0.5^{ab}	20.6±0.8^{a}	24.7±0.6^{b}
	90 days	23.9±0.7^{a}	25.3±0.4^{a}	24.7±0.7^{a}	22.9±0.5^{a}	23.5±1.0^{a}
AST(U/L)	45 days	25.7±0.3^{b}	24.0±0.6^{b}	24.4±0.4^{b}	21.6±0.6^{a}	25.2±0.4^{b}
	90 days	30.5±0.3^{c}	26.5±0.5^{ab}	27.9±0.6^{bc}	23.8±1.7^{b}	29.9±0.2^{c}

Intestinal histology:

At the end of this study, the number of their goblet cells, the width and height of villi, and the muscular epithelial width were measured in the intestine for each of the control and other groups. Findings from the present study showed remarkable variations among control and other groups **(Table 6** and **Fig. 1)**. The highest number of goblet cells was recorded in the Mix treatment (17.6) followed by Mix+ (16.2). While the lowest number of goblet cells was in Soy+ (11). Significant increments in villi width were recorded in fish fed Soy con+ and Soy+ (40.6, 40.3), respectively, compared to the control group and other groups. while the length of villi in the Mix group was increased by 142.6, followed by the control group at 111.6. Significant increment in muscular epithelial width in the Soy+ and Soy con+ (30.2, 30), respectively, while significant reduction in muscular epithelial width in fish fed Mix and Mix+ (27, 18).

Table (6): Measurements of the intestinal morphology of *Tilapia zilli* fish at the end of the feeding trial

Treatment	Experimental diets				
Parameters	Control	Mix	Mix +	Soy +	Soy con +
Goblet cells (numbers)	13.3±0.29^{b}	16.2±0.78^{a}	17.6±0.78^{a}	13.1. ±0.39^{b}	11±0.51^{ab}
Villus width (µm)	25.6±1.0^{c}	31±1.3^{b}	37.6±1.2^{a}	40.6±1.2^{a}	40.3±0.2^{a}
Villi length (µm)	111.6±3.9^{b}	124.6±2.7^{a}	95.7±2.3^{ab}	87.1±2.9^{ab}	88.9±0.6^{ab}
Muscular epithelial of width (µm)	20.6. ±0.7^{b}	27±1.3^{a}	18±1.0^{ab}	30±1.0a	30.2±0.5^{a}

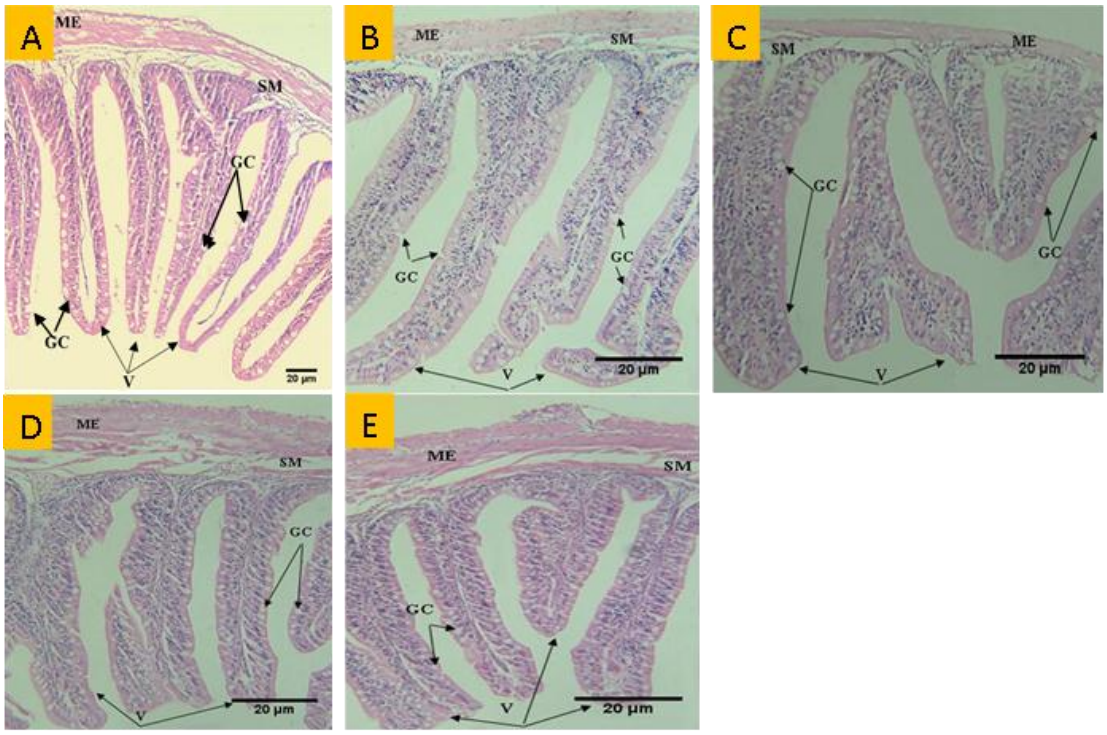

Fig. (1). Photomicrographs of T.S. of the intestines of Tilapia zilli reared under fed different diets. (A) commercial diet control; (B) mix; (C) Mix+; (D) Soy con+ and (E) Soy+, respectively, showing intestinal branched villi (V), muscular epithelial (ME), mucosal layer (SM) and goblet cells (GC). HX &E stain; bar = 40 µm.

DISCUSSION

The investigation of the effects of alternative protein sources on growth performance, feed utilization, or body composition is the general approach in nutritional studies **(Ergun *et al.*, 2008; Yi-git *et al.*, 2010)**. However, it's crucial to consider the health and welfare of the fish in addition to the protein source itself while looking for fishmeal substitutes **(Acar *et al.*, 2018)**. So, future aquaculture development should prioritize the creation of sustainable and viable alternative proteins, as noted by **Weiss *et al.* (2020)**. The current study has shown that *Tilapia zilli* fed various diets were able to grow normally, with the best growth performance going to the (Mix +) treatment. The current study's findings were similar to those of **Chien & Chiu (2003)** for Tilapia, who found that fish growth performance variables remained unaffected when up to 67% of blue lupine was substituted for soybean meal in part. Similarly, replacing soymeal meal with up to 30% lupine meal did not affect gilthead seabream growth performance **(Robaina *et al.*, 1995)**. Furthermore, adding lupin meal to the diet did not significantly alter the common carp's growth performance **(Li *et al.*, 2017; Anwar *et al.*, 2020; Weiss *et al.*, 2020)**. Similar outcomes were obtained by feeding black tiger shrimps with lupine seed meal **(Smith *et al.*, 2007)**. Furthermore, a study by **Guo *et al.* (2011)** suggested that sesame meal might be given to Nile tilapia (*Oreochromis niloticus*) diets in place of soybean meal without having negative impacts. Additionally, there were no discernible changes between the experimental group and the control group in terms of the survival rate, weight gain, or specific growth rate of shrimp fed soybean meal, sesame meal, or fermented soybean meal in place of fishmeal **(Bae *et al.*, 2020)**. Because of its high protein

content, balanced amino acid composition, and acceptable price, soybean meal is a suitable substitute for fishmeal in aquafeed **(Azarm & Lee, 2014)**.

Whole-body composition can indicate directly and indirectly an animal's growth **(*Li et al*., 2020; Liang *et al*., 2022)**. The results of this study showed that the moisture, crude lipids, protein, and ash contents of *Tilapia zilli* were significantly affected by any replacement levels. Variety results for moisture, crude protein, and ash contents after replacing Fish meal with plant protein were also found in the hybrid of *Carassius auratus gibelio* ♀ × *Cyprinus carpio* ♂ **(Liu *et al*., 2020)**. Furthermore, **Rahimnejad *et al*. (2003)** suggested that replacement of FM with fermented soybean meal had no significant impact on the crude protein and ash contents of Japanese seabass (*Lateolabrax japonicus*). However, previous studies have stated that soybean can reduce crude lipid content in rainbow trout fry. Additionally, the study of **Duan *et al*. (2022)** confirmed that the replacement of FM with fermented soybean lessens the level of crude lipid in the fish body of hybrid snakehead (*Channa argus* × *Channa maculate*). These dissimilarities could be caused by differences in fish species, experimental setups, and methods of soybean processing. In the present study, *Tilapia zilli* fed the Soy+ diet had highest crude protein content, followed by the control, and the crude protein content showed no marked discrepancies among other three groups. Similarly, studies suggested that dietary plant protein sources addition reduced body protein contents in many fish species. We also found that the crude lipid content was highest for *Tilapia zilli* fed the Mix diet and lowest for fed the control. **Alam *et al*. (2018)** found a higher lipid content in the whole body in flounder fed 75% and 100% cottonseed meal diets compared with fish fed a fish meal-based diet. Potential toxicological effects of dietary ANFs in the plant proteins may impair protein and lipid deposition of fish.

Hematological parameters are useful tools for tracking the health of fish that are impacted by their surroundings and diet **(Clauss *et al*., 2008)**. Blood biochemistry analyses are not commonly used as diagnostic tools, despite previous studies showing that stress, infection, impaired feeding, and specific nutritional deficiencies all cause changes in fish blood constituents. These data are still missing for the majority of fish species **(Peres *et al*., 2014)**. Numerous immunostimulants, such as marine and terrestrial plants, have already undergone testing and been given the all-clear to boost immunological resistance and activity **(Huang *et al*., 2006)**. In the current study, Hb and RBCs showed significant differences between the different groups and the control diet (except for the RBCs at 90 days). Hb, MCH, MCHC, and MCV were all significantly increased in these parameters, with the exception of the soy con+ group, between the initial and final blood samples from fish fed the commercial diet and various diets used in the experiment. Which may be attributed to the combination of different plant components that may decrease the harmful effects of each other and improve fish health. According to **Acar *et al*. (2018)**, there were no variations in Hb and RBC levels across all groups; however, there was a substantial drop in Hct values in those fed a high-lupine diet. According to **Acar *et al*. (2018)**, there was no discernible impact of including white lupin meal in experimental meals on the fundamental blood parameters or overall health of carp. **Saleh (2020)** reported a gradual decrease in sea bass RBC count, Hb and Hct coincided with a gradual increase in the WBC, lymphocyte, and monocyte counts as the dietary SM increased. In general, the fish fed higher SM-level diets showed some

pathological signs. The WBC counts and MCH values were within the average normal reference range for aquacultured sea bass (*Dicentrarchus labrax*) reported by **Filiciotto *et al.* (2012)** and the results of **Wassef *et al.* (2017)**. In the same line with **Rinchard *et al.* (2003)** when fed rainbow trout for 9 months with feeds using cottonseed pulp as a replacement for fishmeal with different ratios, found that the levels of Hb and Hct significantly decreased with an increase in plant protein in the diet.

New ingredients for aquafeeds, especially plant-derived products, can have impacts on the metabolism of the animal that might not be expressed on the growth level but in metabolic parameters **(Weiss *et al.*, 2020)**. The food type effect on shrimp metabolic parameters might evaluate the physiological status of *L. vannamei* **(Pascual *et al.*, 2003)**. The metabolic data of shrimp-fed diets with the inclusion of 10% lupin meal has no negative influence, while supplementation with lupin meal (20% and 30%) causes lower metabolite content in the total hemolymph **(Weiss *et al.*, 2020)**. In general, hematological parameters of fish can often reveal the overall health condition, physiological stress level, and nutritional metabolism in response to experimental diets **(Sun *et al.*, 2019)**. One rather quick-reacting metric for assessing acute reactions during stress management is glucose level according to **Rodrıguez & Le Moullac (2000)**. handling, temperature and salinity changes, and exposure to ammonia can all cause stress **(Aparicio-Simón *et al.*, 2010)**. The present findings revealed a reduction in the glucose levels of *Tilapia zilli* fed various diets, which is comparable to the findings of studies on shrimp **(Dayal *et al.*, 2020; Weiss *et al.*, 2020)**. Rainbow trout's blood glucose level was not adversely affected by lupin meal in diets **(Acar *et al.*, 2018)**. Another serum parameter, such as total protein, is used as an indicator for protein metabolism and the health status of the fish **(Maita, 2007; Maulu *et al.*, 2021a)**. In the present research, total protein levels were significantly ($P<0.05$) higher than starting values, so it did not influence fish health or immunity, This may be due to the presence of more different types of proteins that cause useful effects and resist the harmful effects of antinutritional factors. The increase of TP in serum indicated that the absorption and metabolism levels of animals are improved, which can promote protein synthesis and nitrogen deposition **(Tavakoli *et al.*, 2019)**, which was consistent with the previous study of **Zhu *et al.* (2022).** their results displayed that the inclusion of *Clostridium autoethanogenum* protein (CAP) prominently enhanced the serum TP in the diets of juvenile largemouth bass and **Macias-sancho *et al.* (2014)** for Pacific white shrimp. **Saleh (2020)** found serum protein levels were increased with elevated SM amounts in the European sea bass diet. Conversely, **Chen *et al.* (2019)** reported that there was no effect on juvenile black sea bream's serum TP. In addition, **Li *et al.* (2021)** observed that no significant changes were found in serum TP in Jian carp (*Cyprinus carpio* var. Jian) fed with a diet including CAP for ten weeks. Also, **Acar *et al.* (2018)** reported that the addition of more than 15% lupine meal to the rainbow trout diet caused a decrease in serum total protein values compared with the control group. According to previous studies **(Mikołajczak *et al.*, 2020; Li *et al.*, 2021; Maulu *et al.*, 2021a)**, blood cholesterol and triglycerides are significant markers of fish health state, lipid metabolism, and immunity. It is known that plant protein sources influence the metabolism of cholesterol **(Forsythe, 1995)**. The current study found that cholesterol levels were significantly lower in the blood samples of *Tilapia zilli* fed different diets, which is consistent with findings from **Saleh (2020)** when increasing the SM3

level in European sea bass diet and when using plant protein as a substitute for fishmeal in feed for rainbow **(Romarheim *et al*., 2006; Yamamoto *et al*., 2007)**.

According to the current study, *Tilapia zilli* given to different food groups had considerably higher blood triglyceride levels. Similar results were found in juvenile Jian carp **(Li *et al*., 2021)**; rainbow trout **(Saleh, 2020)** with increasing the SM3 level in the European sea bass diet. The liver function of fish is frequently diagnosed by the hepatic enzymes, namely ALT and AST, which are two significant aminotransferases that can cross the plasma membrane when liver cells are injured or damaged **(Kalhoro *et al*., 2018; Biswas *et al*., 2020; Mikołajczak *et al*., 2020)**. In the present study, it was found that the end levels of ALT and AST were substantially higher than the initial values. In gibel carp, comparable outcomes with the addition of 60% yeast culture were documented to raise plasma AST and ALT **(Zhang *et al*., 2018)**. This is consistent with the findings of **Sanden *et al*. (2006).** They found that feeding rainbow trout diet containing greater inclusion concentrations of soybean meal caused an increase in the activity of their liver enzymes. On the other hand, **Acar *et al*. (2018)** discovered that hepatic enzyme levels drop when lupin meal is added to the diet. This finding may be related to lupin meal's ability to preserve organs and tissues. According to **Chen *et al*. (2019)**, there was no discernible difference between the control group's serum ALT and AST activity and that of the fish fed a diet containing CAP 58.2%. According to **Lee *et al*. (2020)**, the addition of single cell protein (SCP) treatments to the food had no effect on the blood ALT and AST levels in rainbow trout. Additionally, **Zhu *et al*. (2022)** found that the liver function of largemouth bass is unaffected when fish meal is substituted with CAP within 63% of the total.

Many studies have shown that feed composition affects fish intestine health, and intestinal histological examinations are regarded as essential measurements for evaluating tissue alterations induced by different dietary supplements **(Pereira *et al*., 2019)**. Histopathological changes in the intestine in fish-fed plant feedstuffs may vary depending on the species and size, quality and processing of the ingredients, diet formulation, and culture system used in the experiments **(Bonaldo *et al*., 2008; Dayal *et al*., 2013)**. The digestibility coefficients also confirmed that Nile tilapia are capable of effectively utilizing plant protein sources in their diets and still achieve good growth during their early life stages **(Obirikorang *et al*., 2020)**. Intestine is crucial to guaranteeing the nutritional efficiency of the diet as well as animal health. For this reason, this work studied different variations in the intestine morphology of fish fed experimental diets. In the present study, the intestines of fish fed the Mix diet treatment exhibited a remarkable increase in the height and width of villi. The length of villi is a useful histological parameter that could be followed in evaluating different types of commercial feed **(Toutou *et al*., 2019)**. An increase in the length of villi is associated with an increase in the surface area for the absorption of nutrients **(Aanyu *et al*., 2014)**. The longer villi found in fish fed on Mix diet treatment indicated higher efficiency in the absorptive process and are good for the growth and welfare of animals **(Da Silva *et al*., 2012; Amoah *et al*., 2020)**. It is thus apparent that the inclusion of plant ingredients in aquafeeds generally elicits deleterious histopathological responses in the gut of fish. Besides the finding of **Obirikorang *et al*. (2020)**, the replacement of fishmeal with other plant protein ingredients like jojoba meal **(Shamma *et al*., 2014; Saleh & Toutou, 2015; Elsanhoty *et al*.,**

2017) resulted in histopathological changes in the sea bream. Similar to our findings, the mucosal height and thickness of the intestinal villi increased in the tilapia fed with 5% and 10% dietary *Clostridium autoethanogenum* protein (CAP) **(Maulu *et al*., 2021b; Zhu *et al*., 2022)**. Likewise, data from studies on Pacific white shrimp also showed that supplemental levels of *Clostridium butyricum* (less than 45% bacterial protein meal) **(Duan *et al*., 2017)**, Methanotroph (*Methylococcus capsulatus*, Bath) **(Chen *et al*., 2021)** and *Bacillus licheniformis* **(Amoah *et al*., 2020)** increased the intestinal villus length, villus width, muscular layer thickness, and epithelium height. Contrarily, in largemouth bass, the inclusion of CAP showed strikingly reduced intestinal muscle thickness and villus height **(Yang *et al*., 2021)**. Another study has also illustrated that dietary supplementation of CAP in feed has no remarkable effect on gut histology in Jian carp **(Li *et al*., 2021)**.

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