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Graded levels of dietary pink oyster mushroom, *Pleurotus djamor* meal, affect growth, feed efficiency, lipase activity, and fiber content in final whole body of fingerlings of the Nile tilapia, *Oreochromis niloticus* (Actinopterygii: Cichliformes: Cichlidae)

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Abstract

The presently reported study was aimed to determine the effects of graded levels of dietary pink oyster mushroom (*Pleurotus djamor*) meal (POMM), in growth, feed efficiency, protein utilization, digestive enzymes activities, and whole-body proximate composition of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), fingerlings (0.3 ± 0.01 g). The experimental design included a control diet (POMM0) formulated with soybean meal, as the main protein source, and four diets designed with increasing levels of POMM: 25% (POMM25); 50% (POMM50); 75% (POMM75); and 100% (POMM100). Experimental diets were administered to 420 fish, randomly distributed in 15, 100-L tanks. The feeding experiment lasted 45 days. Diets and the final whole body were submitted to a proximate composition analysis. Growth, feed efficiency, protein utilization, and digestive enzyme activities were assessed. Compared to POMM0 and POMM25, weight gain (WG), and specific growth rate (SGR), significantly ($P < 0.05$) decreased in fish that were fed POMM50, POMM75, and POMM100%. Feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR) were not significantly affected by experimental diets. Daily feed intake (DFI), and daily protein intake (DPI), decreased as POMM increased in diets. Compared to POMM0 experimental group, condition factor (*K*), showed a significantly higher value in fish that were fed POMM50, and POMM100 experimental diets. Crude fiber of the final whole body of POMM100 resulted in significantly higher ($P < 0.05$) compared to that shown in fish fed the rest of the experimental diets. Acid and alkaline proteases, trypsin, chymotrypsin, leucine aminopeptidase, and amylase of Nile tilapia fingerlings, were not significantly affected by experimental diets. Compared to fish fed POMM0 and POMM25 diets, experimental fish fed POMM50, POMM75, and POMM100 showed a reduction in lipase activity. In conclusion, a POMM level higher than 25% affects growth and lipase activity. While a POMM level higher than 50% affects fiber content in a whole body of the final fish.

* Both authors contributed equally to this study.

Keywords

carcass, digestive physiology, fiber, growth, mushroom meal, tilapia

Introduction

The expansion of the aquaculture industry is evidently accompanied by an urgent need for aquafeed production (Gambelli et al. 2019; Botta et al. 2020; Chu et al. 2020). This condition leads to the necessity of a steady supply of protein. Traditionally, fishmeal (FM) and soybean meal (SBM) have been the primary protein source ingredient in fish feeds (Wang et al. 2020). However, their over-exploitation has led to a shortage of these commodities (Galkanda-Arachchige and Davis 2019; Ye et al. 2019; Li et al. 2023; Nunes et al. 2022; Soltan et al. 2023). Therefore, several studies have been conducted to investigate alternative and unconventional protein meals for aquaculture diets. One of these, are mushrooms (Chelladurai and Venmathi-Maran 2019).

Edible mushrooms are a rich source of caloric value, essential fatty acids, amino acids, protein levels, vitamins, and minerals. To date, there are several studies focusing on the research of products derived from mushrooms as dietary inclusion in feeds for farmed aquatic organisms (Safari and Sarkheil 2018; Chelladurai and Venmathi Maran 2019; Dawood et al. 2020a). The majority of the studies using mushrooms have been mainly focused on the effects on aquatic organism immunity, hematological profiles, disease resistance, and growth (Katya et al. 2016; Chelladurai and Venmathi Maran 2019; Dawood et al. 2020a).

Pleurotus spp. is an edible mushroom that belongs to the order Agaricales and the family Pleurotaceae (see Justo et al. 2011). Pink oyster mushroom (POM), *P. djamor*, is mainly produced for research and food purposes for human nutrition in Brazil and Mexico (Chintati et al. 2022). Previous studies have shown that this species contains 31.48–35.50 g 100 g^{-1} of protein, while crude fiber content, ranges from 8.00 to 14.60 g 100 g^{-1}. (Jegadeesh et al. 2020). Although *P. djamor* has been widely studied as an additive supplemented at low inclusion levels (Nattoh et al. 2016; Zhang et al. 2016; Hu et al. 2017; Jiao et al. 2017; Maity et al. 2019; Pereira de Oliveira and Naozuka 2019; Vasconez-Velez 2019), only a few studies have focused on the use of *P. djamor* as a dietary supplement in fish feed formulations. Cruz-García et al. (2022) studied the effects of mushroom (*Pleurotus djamor* var. *roseus*) meal as a feed supplement on the hematological responses and growth of the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), fingerlings when fed diets formulated with 0%, 15%, 20%, and 25% of *P. djamor*. Therefore, the presently reported research aims to study the effects (growth, feed efficiency, protein utilization, whole body proximate composition, and digestive enzyme activities) of increasing levels (0%, 25%, 50%, 75%, and 100%) of *P. djamor* meal, in diets for Nile tilapia fingerlings (used as model fish species).

Material and methods

Experimental Nile tilapia fingerlings. Masculinized, Nile tilapia (*Oreochromis niloticus*) fingerlings (genetically improved farmed tilapia = GIFT strain; Ponzoni et al. 2011) (0.3 ± 0.01 g) were obtained from the brood stock in the Tropical Aquaculture Laboratory of the Academic Division of Biological Sciences (DACBiol), Juarez Autonomous University of Tabasco (UJAT). Before the feeding experiment, the health status of Nile tilapia fingerlings was checked by visual observation, according to indications proposed by Johansen et al. (2006). 420 fish were randomly distributed in 15 (100 L) plastic tanks.

POM (CH-240). POM, strain CH-240, belonging to the herbarium of the DACBiol-UJAT, was reared in an edible mushroom greenhouse (28°C, using coconut paste as substrate), the harvest of the mushroom was carried out when there was a complete extension of the pileus. All farming processes were carried out in an innocuous environment, to avoid contaminating *P. djamor* culture. Collected mushrooms were dried in an oven, pulverized with a hammer mill, and analyzed for proximate composition (AOAC 2019).

Experimental diets. Iso-nitrogenous and iso-lipidic diets were designed, including a control diet formulated with SBM (as the main protein source) and four diets formulated with increasing levels of POMM. In each diet, the protein level was adjusted by reducing SBM levels. Experimental diets were assigned as follows: 25% (POMM25), 50% (POMM50), 75% (POMM75), and 100% (POMM100) (Table 1). Diet formulation followed the method proposed by Álvarez-González et al. (2001). Experimental diets were designed with the assistance of MIXITWIN V. 5.0 (Agricultural Software Consultants, San Diego, CA, USA) software. Diets were manufactured according to previously standardized methods at DACBiol-UJAT. Experimental diets were submitted to a proximate composition analysis AOAC (2019).

Feeding test and rearing system. Experimental diets were administered in triplicate during a 45-day period, at satiation level. Each experimental tank was randomly assigned to each diet and the feeder (person in charge of

Table 1. Dietary ingredients and proximate composition of experimental diets for fingerlings of Nile tilapia (*Oreochromis niloticus*), formulated with increasing levels of POMM (pink oyster mushroom meal).

Ingredient [%]	Treatment (substitution level)				
	POMM0	POMM25	POMM50	POMM75	POMM100
Soybean meal 44%[c]	21	15	11	5	0
Pink oyster mushroom meal[b]	0	11	22	33	44
Sorghum meal 9%[c]	26	21	12	7	1
Pork meal 50%[a]	25	25	26	26	26
Fish meal 65%[a]	14	14	15	15	15
Sardine Oil[a]	6	6	6	6	6
Soybean Oil[d]	3	3	3	3	3
Grenetine[e]	2	2	2	2	2
Previt[f]	1.5	1.5	1.5	1.5	1.5
Premin[f]	1	1	1	1	1
Vitamin C[g]	0.5	0.5	0.5	0.5	0.5
Proximate composition (dry matter)					
Crude Protein [%]	33.13	32.42	32.76	31.98	32.03
Crude lipid [%]	13.77	13.85	13.99	14.08	14.23
Ash [%]	11.94	12.26	12.88	13.20	13.78
Crude fiber [%]	2.39	3.63	4.91	6.15	7.40
Nitrogen-free extract [%]	38.78	37.84	35.46	34.59	32.56
Energy [kJ kg^{-1}]	19.93	19.63	19.36	19.06	18.78

Numbers following POMM denote percentage of diet substitution with pink oyster mushroom meal.

[a] Marine and Agricultural Protein (Proteínas marinas y agropecuarias S.A. de C.V., Guadalajara, Jalisco.

[b] Edible mushroom greenhouse, Academic Division of Biological Science (DACBiol), Juarez Autonomous University of Tabasco (UJAT), Villahermosa, Tabasco.

[c] GALMEX Comercializadora de Insumos Agrícolas S.A. de C.V., Villahermosa, Tabasco (19800-20100 IU g^{-1}).

[d] Pronat Ultra, Mérida, Yucatán.

[e] D'gari, Productos alimenticios y dietéticos relámpago, S.A. de C.V., Tlalpan, D.F.

[f] Consorcio Súper S.A. de C.V., Guadalajara, Jalisco.

[g] DSM® C-EC (Roche) active agent 35%.

feeding daily), was rotated to obtain a blinded feed delivery. The feeding test was conducted in a recirculating aquaculture system (RAS) (2 L min^{-1}) maintaining a constant aeration. To decrease the effects of the natural high temperature *per se* existing in Villahermosa city (tropical weather), the RAS was designed and built under a controlled air conditioner environment, hence decreasing significant oscillation of temperature during all feeding experiments. Fish were fed three times per day (09:00, 13:00, and 17:00 h). Unconsumed feed and feces were siphoned 30 min after each feeding. RAS water was replaced (50%) every week. Water quality was monitored daily: Dissolved oxygen (DO) (5.19 ± 0.3 mg L^{-1}) and temperature (28 ± 0.1°C) were measured with a YSI 55 oximeter, with an accuracy of 0.1°C and 0.01 mg L^{-1}, respectively. While pH (7.2 ± 0.1) was assessed with a potentiometer (Hanna Instruments, HI 98311, Rhode Island, USA). These parameters were measured in both experimental tanks and in the 4 m^3 main reservoir of RAS.

Growth, feed efficiency, protein utilization, and survival. All fish per tank were sampled (benzocaine 20% w/v. SIGMA–Aldrich, USA), for weight and total length every 15 days. Animals used for organ sampling were carried out following the guidelines of the Mexican Norm NOM-062-ZOO-1999 (NOM 2001) for the management of animals in a laboratory for experimental use. At the end of the feeding test, in addition to weight sampling, growth performance (weight gain, W_G [%]; specific growth rate, SGR [% day^{-1}]; condition factor, K), feed utilization parameters (feed conversion ratio, FCR; daily feed intake, DFI [g day^{-1}]; daily protein intake, DPI [g day^{-1}]; protein efficiency ratio, PER, and survival rate SR [%]) were calculated as follows:

$$W_G = 100\ W_{FF} \times W_{IF}^{-1}$$

$$\mathrm{SGR} = 100\ [\ln W_{FF} - \ln W_{IF}] \times t^{-1}$$

$$K = 100[W_{FF} \times L_{FF}^3]$$

$$\mathrm{FCR} = W_{FC} \times W_G^{-1}$$

$$\mathrm{DFI} = (W_{CD} \times t^{-1}) \times N_F$$

$$\mathrm{DPI} = (W_{PCD} \times t^{-1}) \times N_F$$

$$\mathrm{PER} = W_G \times W_{PI}^{-1}$$

$$\mathrm{SR} = 100(N_{IF} - N_{FF}) \times N_{FF-1}$$

where W_{FF} is the final mean weight of fish [g], W_{IF} is the initial mean weight [g], t is the time of rearing [day], L_{FF} is the final mean length of fish [cm], W_{FC} is the weight

of the total feed consumed [g], W_{CD} is the weight of feed consumed per day [g], N_F is the number of fish, W_{PCD} is the weight of protein consumed per day [g], W_{PI} is protein intake [g], N_{IF} is initial number of fish. N_{FF} is the final number of fish.

Proximate composition analysis. POMM, experimental diets and final whole body were submitted to proximate composition analysis (AOAC 2019) at the Chemistry Laboratory of Norwest Biological Research Center (CIBNOR), La Paz, BCS, Mexico. Before sending to laboratory analysis, and to preserve biochemical profiles intact, whole body samples were lyophilized. Nitrogen free extract (NFE) [g kg^{-1}] and gross energy (E_G) [kJ kg^{-1}], were manually calculated as follows:

$$NFE = 100 - (CP + CL + CF + A)$$

$$E_G = [100\ (CP \times 23.6) + (CL \times 39.5) + (NFE \times 17.2)]$$

where CP is crude protein content [%], CL is crude lipid content [%], CF is crude fiber content [%], and A is the ash content [%], NFE is the nitrogen free extract [%]. The numerical values in the gross energy formula represent respective energy conversion factors.

Digestive enzyme activity sampling and analysis. Upon completion of the feeding test, three fish (per experimental tank), 9 fish (per experimental group), and 45 fish (per all tested groups), were starved for 24 h and then the final sampling was carried out. Fish were anesthetized with 20% w/v benzocaine (SIGMA-Aldrich, USA). Fish were slaughtered under freezing conditions to avoid organisms suffering. Then, fish were dissected to extract the stomach and intestine for digestive enzyme activity analysis. The stomach samples were homogenized in a buffer solution of glycine-HCl 0.1 M, pH 2 and the intestines were homogenized in the solution of Tris-HCl 100 mM + $CaCl_2$ 10 mM pH 9. Both samples were centrifuged at 16 000 *g* for 30 min to extract the supernatant or enzymatic extract by separating in 400 µL aliquots and freezing at –20°C until their further use. The soluble protein concentration was evaluated using a bovine serum albumin calibration curve (600 mg mL^{-1}).

Alkaline protease activity was determined according to (Walter 1984), using Hammerstein-grade casein 0.5% in buffer (100 mmol L^{-1} Tris-HCl; 10 mmol L^{-1} $CaCl_2$, pH 9); one unit of activity was defined as 1-µg of tyrosine released per min at absorbance 280 nm (abs_{280}) The acid protease activity was determined using Anson (1938) technique, and hemoglobin (1%) in a buffer solution of glycine-HCl 0.1 M, pH 2. The released peptide levels were determined through a quartz cell (700 µL) at 280 nm in the spectrophotometer. Trypsin activity determination used the Erlanger et al. (1961) technique with the substrate BAPNA (N-α-benzoyl-DL-arginine p-nitroanilide) with dimethyl sulfoxide (DMSO). Sample reading was conducted with a spectrophotometer at 410 nm. Chymotrypsin activity was determined following the method proposed by Del Mar et al. (1979). Absorbance was measured at 405 nm. Leucine aminopeptidase activity was evaluated following the methodology proposed by Maraux et al. (1973). Absorbance was measured at 410 nm. The α-amylase activity was determined by the method of Robyt and Whelan (1968), using soluble starch (2%) in a buffer (100 mmol L^{-1} citrate-phosphate; 50 mol L^{-1} NaCl, pH 7.5). Lipase activity was measured as previously described by Versaw et al. (1989) but using ß-naphthyl acetate 100 mmol L^{-1} as substrate; one unit of activity was defined as 1 µg de naphthol released per min at 540 nm.

The enzymatic activity of the extracts was determined with the following equations:

1. Units per mL = [Δabs × final reaction volume (mL) CEM^{-1} × time (min) × extract volume (mL)], and
2. Units × mg of protein^{-1} = Units per mL mg of soluble protein^{-1}. Δabs is determined by the length of the wave of each technique and the CEM is the molar extinction coefficient for the reaction product (mL × µg^{-1} × cm^{-1}). All enzyme activities were expressed per mg of protein. Protein concentration was determined according to Bradford (1976), using a standard curve with bovine serum albumin (BSA). All assays were performed in triplicate.

Statistical analysis. Data was statistically analyzed by one-way ANOVA, previously verified the assumptions of normality (Kolmogorov–Smirnov test) and homoscedasticity (Levine test). Where significant differences were assessed, applying a Tukey test. Analyses were performed with the statistical software Statistica TM v.8.0 (StatSoft, Tulsa, OK, USA) using a significance value of $P < 0.05$. The results were presented as mean ± standard deviation, SD.

Results

Proximate composition of POMM. The proximate composition of POMM is shown in Table 2. Crude protein and crude fiber showed similar values. As expected, crude lipid recorded a remarkably lower value (0.50%). While the nitrogen-free extract recorded the highest content (45.96%), compared to other nutrients.

Table 2. Proximate composition of POMM (pink oyster mushroom meal) in diets for fingerlings of Nile tilapia (*Oreochromis niloticus*).

Component	Value
Moisture [%]	4.61
Crude protein [%]	21.37
Crude lipid [%]	0.50
Crude fiber [%]	20.05
Ash [%]	7.51
Nitrogen-free extract [%]	45.96
Gross energy [kJ kg^{-1}]	13.15

Proximate composition of experimental diets. Experimental diets did not show relevant differences regarding crude protein, crude lipid, ash, and energy. However, crude fiber and ash increased as the POMM level increased in experimental diets. While nitrogen-free extract decreased as POMM level increased in diets (Table 1).

Growth performance, feed utilization, and survival. All experimental diets were well accepted by the fish during the feeding test. Experimental diets did not affect feed conversion rate (FCR), protein efficiency ratio (PER), and survival rate (SR). In contrast, fish that were fed the POMM25 diet did not show significant ($P > 0.05$) differences in weight gain (WG), and specific growth rate (SGR), compared to those shown in experimental group POMM0. While POMM50, POMM75, and POMM100 experimental groups, showed significantly ($P < 0.05$) lower WG and SGR compared to those shown in POMM0 experimental group. Although *K* did not show significant ($P > 0.05$) differences among POMM0, POMM25, and POMM75 experimental groups, there was a significantly higher ($P < 0.05$) *K* value in POMM50 and POMM100 experimental groups, compared to that recorded in POMM0 experimental group. DFI and DPI significantly ($P < 0.05$) decreased as levels of POMM increased in experimental diets (Table 3).

Whole body proximate composition. There were no significant ($P > 0.05$) differences, among experimental groups, in terms of moisture, crude protein, and crude lipid contents. In contrast, crude fiber resulted significantly ($P < 0.05$) higher in the POMM100 experimental group compared to that shown in the rest of the experimental groups (Table 4).

Digestive enzyme activities. Acid protease, alkaline protease, trypsin, chymotrypsin, leucine aminopeptidase, and amylase activities were not significantly ($P > 0.05$) affected by consumed experimental diets. However, lipase activity resulted significantly ($P < 0.05$) lower in POMM50, POMM75, and POMM100 experimental groups compared to that observed in POMM0 and POMM25%. There was no significant ($P > 0.05$) difference in lipase activity between POMM0 and POMM25% (Table 5).

Discussion

The presently reported study was designed to determine the effects on growth, feed efficiency, protein utilization, survival, final whole body proximate composition, and digestive enzyme activities of Nile tilapia fingerlings, which were fed diets formulated with increasing levels of a locally available and unconventional protein meal, POMM. Levels of protein and lipid content of POM in this study are similar to those previously reported in Cruz-Solorio et al. (2014) and Salmones (2017). Mushroom species are characterized by their high fiber content.

Table 3. Growth, feed performance, protein utilization and survival of fingerlings of Nile tilapia (*Oreochromis niloticus*) fed formulated diets with increasing levels of POMM (pink oyster mushroom meal) for 45 days.

Parameter	Treatment (substitution level)				
	POMM0	POMM25	POMM50	POMM75	POMM100
Initial weight [g]	0.30 ± 0.05	0.31 ± 0.02	0.29 ± 0.06	0.30 ± 0.03	0.31 ± 0.02
Final weight [g]	1.98 ± 0.10^{a}	1.71 ± 0.19^{ab}	1.34 ± 0.11^{bc}	1.30 ± 0.10^{c}	1.20 ± 0.12^{c}
WG [%]	659.2 ± 97.7^{a}	553.1 ± 23.4^{ab}	462.4 ± 35.9^{bc}	433.7 ± 80.4^{c}	388.1 ± 22.9^{c}
SGR [% day^{-1}]	4.49 ± 0.28^{a}	4.16 ± 0.08^{ab}	3.83 ± 0.15^{bc}	3.70 ± 0.32^{c}	3.52 ± 0.11^{c}
K	1.64 ± 0.04^{b}	1.66 ± 0.03^{ab}	1.72 ± 0.02^{a}	1.69 ± 0.02^{ab}	1.71 ± 0.03^{a}
FCR^{Q}	2.54 ± 0.35	2.34 ± 0.10	2.84 ± 0.24	2.95 ± 0.51	3.22 ± 0.21
DFI [g day^{-1}]	0.110 ± 0.00^{a}	0.086 ± 0.00^{b}	0.086 ± 0.00^{b}	0.083 ± 0.00^{c}	0.082 ± 0.00^{d}
DPI [g day^{-1}]	0.037 ± 0.01^{a}	0.029 ± 0.01^{b}	0.028 ± 0.00^{c}	0.027 ± 0.00^{d}	0.026 ± 0.01^{e}
PER	1.20 ± 0.18	1.32 ± 0.06	1.09 ± 0.09	1.08 ± 0.20	0.97 ± 0.06
SR [g]	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

Numbers following POMM denote percentage of diet substitution with pink oyster mushroom meal. Values in each row superscript with different lower-case letters indicate significant differences between groups ($P < 0.05$). WG = weight gain; SGR = specific growth rate; *K* = condition factor; FCR = feed conversion ratio; DFI = daily food intake; DPI = daily protein intake; PER = protein efficiency ratio; SR = survival rate.

Table 4. Whole body proximate composition of fingerlings of Nile tilapia (*Oreochromis niloticus*), fed formulated diets with increasing levels of POMM (pink oyster mushroom meal), for 45 days.

Proximate composition [%]	Treatment (substitution level)				
	POMM0	POMM25	POMM50	POMM75	POMM100
Moisture	6.54 ± 1.53	5.76 ± 1.74	6.19 ± 1.54	7.82 ± 0.69	5.21 ± 1.03
Crude protein	57.35 ± 2.80	56.66 ± 1.30	55.13 ± 1.40	53.13 ± 0.80	52.77 ± 1.20
Crude lipid	22.97 ± 1.14	24.50 ± 1.45	23.68 ± 2.08	26.64 ± 2.78	26.49 ± 1.50
Crude Fiber	0.14 ± 0.00^{b}	0.00 ± 0.00^{bc}	0.00 ± 0.00^{bc}	0.16 ± 0.02^{b}	0.24 ± 0.03^{a}

Numbers following POMM denote percentage of diet substitution with pink oyster mushroom meal.

Values in each row superscript with different lower-case letters indicate significant differences between groups ($P < 0.05$).

Table 5. Digestive enzyme activities of fingerlings of Nile tilapia (*Oreochromis niloticus*), fed formulated diets with increasing levels of POMM (pink oyster mushroom meal), for 45 days.

Enzyme activity [U mg protein^{-1}]	Treatment (substitution level)				
	POMM0	POMM25	POMM50	POMM75	POMM100
Acid protease	4.72 ± 3.52	5.68 ± 1.58	6.57 ± 1.62	6.07 ± 0.78	6.30 ± 1.36
Alkaline protease	9.93 ± 3.28	8.36 ± 3.30	9.17 ± 3.10	10.20 ± 1.48	10.29 ± 0.84
Trypsin	$6.47 \times 10^{-3} \pm 2.15 \times 10^{-3}$	$7.83 \times 10^{-3} \pm 1.78 \times 10^{-3}$	$5.99 \times 10^{-3} \pm 2.71 \times 10^{-3}$	$5.94 \times 10^{-3} \pm 1.13 \times 10^{-3}$	$7.15 \times 10^{-3} \pm 6.02 \times 10^{-4}$
Chymotrypsin	$2.35 \times 10^{-2} \pm 4.87 \times 10^{-4}$	$2.41 \times 10^{-2} \pm 1.47 \times 10^{-3}$	$2.22 \times 10^{-2} \pm 2.98 \times 10^{-3}$	$2.25 \times 10^{-2} \pm 2.92 \times 10^{-3}$	$2.19 \times 10^{-2} \pm 2.55 \times 10^{-3}$
Leucine aminopeptidase	$8.66 \times 10^{-4} \pm 2.96 \times 10^{-4}$	$1.11 \times 10^{-3} \pm 1.38 \times 10^{-4}$	$1.13 \times 10^{-3} \pm 3.37 \times 10^{-4}$	$8.96 \times 10^{-4} \pm 2.09 \times 10^{-4}$	$1.15 \times 10^{-3} \pm 4.34 \times 10^{-4}$
Lipase	130.09 ± 13.24^{a}	130.47 ± 12.31^{a}	96.05 ± 17.56^{b}	84.51 ± 7.27^{b}	90.29 ± 23.09^{b}
Amylase	141.63 ± 41.78	176.20 ± 7.69	168.51 ± 22.36	154.45 ± 27.63	147.14 ± 20.44

Numbers following POMM denote percentage of diet substitution with pink oyster mushroom meal. Values in each row with different superscript letters indicate significant differences between groups ($P < 0.05$).

In this research, 20.05% of fiber was recorded in POMM. In contrast, lower crude fiber levels (8.60%–9.29%) in two strains of *Pleurotus* spp. were recorded by Cruz-Solorio et al. (2014).

The presently reported study showed that a maximum of 25% of POMM can be supplemented in Nile tilapia fingerlings without affecting the WG and SGR of fish. POM has nutritional, nutraceutical, and biodegradable features (Dulay et al. 2017). Limited inclusion of POMM may be attributed to the presence of certain biochemical components naturally occurring in POMM that at high levels could produce certain growth depressing effects (Salmones 2017). The nutritional quality of *Pleurotus* has been widely studied and it has been robustly demonstrated. Previous studies have detected 16 components (2-pentanone, 3-pentanone, methyl butyrate, and 2-methyl-3 pentanone, 3-octanol, 3-octanone, among the main ones) influencing *P. djamor* flavor, hence palatability (Zhang et al. 2022a; Andrew 2023), affecting fish acceptance to feeds. This fact could explain why the DFI of Nile tilapia fingerlings during a 45-day period decreased as higher levels of POMM were supplemented in experimental diets. Previous studies have demonstrated that high-fiber content diets decrease feed intake in species such as rainbow trout (Hilton et al. 1983). Other elements that are predominant in POM are bioactive components with anti-carcinogenic, immune stimulants, antibiotic, anti-inflammatory, immune stimulant, and antioxidant properties (Salmones 2017). These components confer certain benefits (when present at certain levels) to fish physiology, growth performance, feed efficiency, and nutrient utilization, as evidenced in Dawood et al. (2020a), who found that Nile tilapia fingerlings fed 2% and 4% supplementation levels of dietary white bottom mushroom powder, improved growth performance, digestibility, and feed intake. Dawood et al. (2020b) suggested that these benefits may be due to the content of non-digestible polysaccharides (acting as prebiotics), that can modulate the intestinal microbiota to secrete digestive enzymes in the fish's gastrointestinal tract Moumita and Das (2022). In contrast, in the presently reported study, growth performance and feed utilization decreased as the POMM level increased in the experimental diet in Nile tilapia fingerlings. This can be explained by two factors. Firstly, the amount of POMM supplemented in experimental diets was remarkably higher (from 11% to 44% of the total content of each diet) (Table 2), so bioactive components (such as antimicrobial, antioxidant, immune stimulant) naturally existing in *P. djamor*, were considerably higher. This abundant presence of bioactive components may cause a depressed growth rather than stimulating it. Secondly, a gradual increase of POMM in experimental diets, inevitably added higher fiber amounts to the feed. POMM showed 20% of crude fiber while in experimental diets this nutrient consequently increased as POMM level increased. Results revealed that the POMM25 experimental diet had 3.63% of crude fiber. This diet did not compromise the growth of Nile tilapia fingerlings, while diets with a higher inclusion level of POMM showed a higher fiber content (4.91%, 6.15%, and 7.14%; POMM50, POMM75, and POMM100 diets, respectively) and a significantly lower growth of experimental fish. Hilton et al. (1983), reported a reduction in the growth of rainbow trout when fed high-fiber diet. At certain levels, dietary fiber apparently influences the movement of nutrients along the gastrointestinal tract and significantly increases nutrient absorption (Lin et al. 2020). Fiber is the non-nutritive portion of feed ingredients. This nutrient is indigestible for carnivorous fish, while others such as channel catfish, have intestinal microflora capable of digesting small portions of dietary fiber (McLean 2023). Some herbivorous fish, such as grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), derive nutrients from fiber but some such as blue tilapia, *Oreochromis aureus* (Steindachner, 1864), do not (Turchini et al. 2018). High fiber content often results in growth depression (Zhang et al. 2022b), as seen in the presently reported study.

In aquaculture, condition factor (*K*) is a numerical value given to aquatic organisms that reflects this condition. A low *K* value could be determined by several factors such as stress, disease, starvation, and deficient nutrient composition in diets among the main ones. A high *K* value indicates a healthy fish and an optimal nutrient balance in diet (Kim and Cho 2019). The presently reported study recorded a slight or significant increased *K* value in fish fed diets supplemented with increasing levels of POMM, compared to fish fed POMM0 diet. This result suggests that experimental diets cover the necessary nutritional requirements

for Nile tilapia fingerlings and even higher inclusion levels of POMM did not compromise the condition of the fish.

The results of SR indicated that increased levels of POMM did not affect Nile tilapia fingerlings' health for a 45-day period. A previous study testing dietary white button mushroom supplemented at 0, 0.5, 1, 2, and 4% in Nile tilapia, demonstrated that the survival of experimental fish was not significantly affected by any experimental diet (Dawood et al. 2020a).

In our presently reported study, the final whole-body proximate composition (moisture, crude protein, and crude lipid) was not affected in experimental fish after a 45-day feeding period. However, experimental fish that were fed the POMM100 diet, recorded a significantly higher crude fiber, compared to that shown in the remaining experimental groups. These higher values are attained due to the high level of fiber content (7.4%) in the POMM100 experimental diet. During all the history of fish nutrition science, fiber has been considered as an energy depletion agent, with undesirable effects when fish consume diets with high contents of fiber (Adorian et al. 2016). This statement correlates with presently reported research where high levels of crude fiber in experimental diets, mainly produced two effects: a decreased growth in experimental Nile tilapia fingerlings and an accumulation of this nutrient in final whole body proximate composition. Fiber accumulation in whole body composition is explained because this nutrient is poorly digested by the majority of fish species, including Nile tilapia (Hilton et al. 1983).

The presently reported study analyzed digestive enzyme activities of Nile tilapia fingerlings that were fed with diets formulated with increasing levels of POMM. Acid proteases, alkaline proteases, trypsin, and chymotrypsin did not show significant differences among experimental groups. These enzymes have been proposed as indicators of the nutritional status of fish. The activity of these enzymes indicates the digestive system functionality and ability of nutrient assimilation in the intestine (Wang et al. 2022). This enzymatic unaffected status can be correlated with the presence of sufficient protein in experimental diets, whereas a low value shall be correlated with starvation or feed deficiency (Xavier et al. 2023). Additionally, the activities of enzymes digesting proteins in fish revealed the effects of diets on the physiological status of experimental fish (Guerrero-Zarate et al. 2019). In our study, the activity of leucine aminopeptidase of experimental Nile tilapia fingerlings showed no significant differences among experimental groups. This enzyme is considered an indicator of nutritional quality, since greater digestion, at a parietal level from luminal digestion by endoproteases, hydrolyzes peptides to release amino acids and to promote their absorption (Wang et al. 2022). This enzyme is a proteolytic enzyme that hydrolyses the peptide bond adjacent to a free amino group. Hence, it can be inferred that leucine aminopeptidase can hydrolyze ingested proteins of mushroom meal (Solovyev et al. 2023).

In presently reported research, lipase activity showed a significant decrease in experimental groups fed POMM50, POMM75, and POMM100 diets, which is correlated with a lower growth performance. There are several factors impacting lipid enzyme secretions including feeding habits, feed preferences, formulation of diets, and ANFs (Thongprajukaew and Rodjaroen 2020). In the presently reported study, fiber could have reduced the activity of lipase in experimental fish (Mirghaed et al. 2018). This can be explained by the interference of fiber in not only the hydrolysis of lipids but also in the absorption of fatty acids (Dawood et al. 2020b).

In this research, α-amylase activities did not show significant differences among experimental groups. α-amylase activity is modified according to the ingredients of diet formulation (Mohtashemipour et al. 2023). In this regard, α-amylase is positively correlated with dietary carbohydrate level (Qu et al. 2022). The ability to secrete more α-amylase for dietary polysaccharides hydrolysis seems to be more efficient in herbivorous and omnivorous species (e.g., Nile tilapia) than in carnivorous fish such as rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), where this digestive enzyme is not efficiently expressed (Bjørgen et al. 2020). It is well demonstrated that omnivore species like the Nile tilapia has a better starch digestion rather than opportunistic carnivore species (Ferreira et al. 2022). This fact can explain that even at high nitrogen-free extract in all experimental diets in this study, no differences in α-amylase activity among experimental groups were shown.

Conclusions

Diets formulated with increasing levels of POMM did not compromise feed efficiency, protein utilization, survival, and digestive enzyme activities (except that of lipase), of Nile tilapia fingerlings. In contrast, levels above 25% affected growth, DFI, and DPI. While an accumulation of fiber in the final whole body of fish that were fed diets formulated with 100% of POMM, was promoted. This may be attained due to two factors: firstly, the interference of fiber in the hydrolysis of lipids and in the absorption of fatty acids, and, secondly, fiber is poorly digested, therefore it is accumulated in the final whole body of Nile tilapia fingerlings. Further studies are suggested to assess the metabolic pathways through which fiber naturally occurring in POMM interferes with the lipid metabolism of Nile tilapia.

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