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Graded levels of dietary pink oyster mushroom, *Pleurotus djamor* meal, affects growth, feed efficiency, lipase activity and fiber content in final whole body of Nile tilapia fingerlings, *Oreochromis niloticus*

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Article

Graded levels of dietary pink oyster mushroom, *Pleurotus djamor* meal, affects growth, feed efficiency, lipase activity and fiber content in final whole body of Nile tilapia fingerlings, *Oreochromis niloticus*.

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HIGHLIGHTS

- Diets formulated with up to 25% of POMM, did not compromised growth of tilapia.
- Diets formulated with graded levels of POMM did not affect survival of tilapia
- Diets formulated with 100% of POMM, increased crude fiber of whole of tilapia.
- Diets formulated with up to 25% of POMM did not compromise lipase activity of tilapia.

ABSTRACT:

Expansion of aquaculture industry is evidently accompanied by an urgent necessity of aquaculture feed production. Traditionally, fish meal (FM) and soybean meal (SBM) have been the primary protein source ingredient in aquaculture diets. However, over exploitation of these commodities has conducted to their unsustainability. Hence, research of unconventional protein alternatives has emerged. Mushroom meal is one of them. To date, mushroom meals have been investigated when supplemented in low levels in aquaculture diets. Furthermore, effects of diets supplemented with mushroom meals have assessed different parameters such as, haematology, immunity, anti–bacterial & anti–oxidant activities, and heat stress. Present study, is aimed to study 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*) fingerlings. Experimental design included a control diet (POMM0) formulated with soybean meal, as main protein source, and four diets designed with increasing levels of POMM: 25%(POMM25); 50%(POMM50); 75%(POMM75); and 100%(POMM100). Experimental diets and 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 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 fed POMM50, and POMM100 experimental diets. Crude fiber of final whole body of POMM100 resulted significantly higher (P<0.05) compared to that shown in fish fed the rest of 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 of 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 whole body of final fish.

Running title: Graded levels of pink oyster mushroom meal in Nile tilapia fingerlings diets.

Keywords: carcass, digestive physiology, fiber, growth, mushroom meal, tilapia.

INTRODUCTION

The expansion of the aquaculture industry is evidently accompanied by an urgent need of aquafeed production Gambelli et al. 2019, Botta et al. 2020, Chu et al. 2020). This condition leads to a necessity of a steady supply of protein. Traditionally, fish meal (FM) and soybean meal (SBM) have been the primary protein source ingredient in fish feeds (Wang et al. 2020). However, their over exploitation has conducted 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 the 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 mushroom as dietary inclusion in feeds for farmed aquatic organisms (Safari and Sarkheil 2018, Chelladurai and Venmathi–Maran 2019, Dawood et al. 2020a). Most of the studies using mushrooms have been mainly focused on the effects in 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 Agaricales order and Pleurotaceae family (Justo et al. 2013). *P. djamor*, is mainly produced with research and food purposes for human nutrition in Brazil and Mexico (Chintati et al. 2022). Although *P. djamor* has been widely studied as additive supplemented at low inclusion levels (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, Nattoh et al. 2022;), only few studies have been focused on the use of *P. djamor*, as dietary supplement, in fish feed formulations. Cruz–García et al. (2022), studied the effects of mushroom (*Pleurotus djamor* var. roseus) meal as feed supplement on the hematological responses and growth of Nile tilapia (*Oreochromis niloticus*) fingerlings when fed diets formulated with 0%, 15%, 20% and 25% of *P. djamor*. Therefore, present research is aimed 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

Animals were handled in compliance with the Norma Oficial Mexicana (NOM-062-ZOO-1999 2001). Masculinized Nile tilapia (*Oreochromis niloticus*, VAR gift) fingerlings (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 feeding experiment, 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.

Pink oyster mushroom (CH-240)

Pink oyster mushroom, strain CH–240, belonging to the herbarium of the DACBiol-UJAT, was reared in an edible mushroom greenhouse (28 ºC, using coconut paste as substrate), the harvest of the mushroom was done when there was a complete extension of the pileus. All farming process was carried out in an innocuous environment, in order to avoid contaminating *P. djamor culture*. Collected mushroom, were dried in an oven, pulverized with a hammer mill, and analyzed for proximate composition (AOAC 2020).

Experimental diets

Iso–nitrogenous and iso–lipidic diets were designed, including a control diet formulated with SBM (as main protein source) and four diets formulated with increasing levels of POMM. In each diet, protein level was adjusted by reducing SBM levels. Experimental diets were assigned as follows: 25% (POMM25), 50% (POMM50), 75% (POMM75) and 100% (POMM100) (Table 2). Diet formulation followed the method proposed by Álvarez–González et al. (2001). Experimental diets were designed with assistance of MIXITWIN V. 5.0 software. Diets were manufactured according to previously standardized methods at DACBiol–UJAT. Experimental diets were submitted to a proximate composition analysis AOAC (2020).

Feeding test and rearing system

Experimental diets were administered by triplicate for 45-day period. Each experimental tank was randomly assigned to each diet and the feeder (person in charge of feeding daily), was rotated in order to obtain a blinded feed delivery. Feeding test was conducted in a recirculating aquaculture system (RAS) maintaining a constant aeration. In order to avoid, 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 in order to avoid significant oscillation of temperature during all feeding experiment. Fish were fed three times per day (9:00, 13:00 and 17:00). Unconsumed feed and feces were siphoned 30 min after each feeding. RAS water was replaced (50%) every week. Water quality was monitored on daily basis: 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 0.01mg L^{-1}, California, USA. 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 and feed utilization samplings

All fish per tank were sampled for weight and total length every 15 days. At the end of the feeding test, additional to weight sampling, growth performance (weight gain–WG–, specific growth rate–SGR–, condition factor –K–), feed utilization parameters (feed conversion ratio –FCR–, daily feed intake–DFI–, daily protein intake –DPI–, protein efficiency ratio –PER–) and survival rate (SR) were calculated.

Proximate composition analysis

POMM, experimental diets and final whole body were submitted to proximate composition analysis (AOAC 2020) at Chemistry Laboratory of Norwest Biological Research Center (CIBNOR). Before sending to laboratory analysis, and in order to preserve biochemical profiles intact, whole body samples were lyophilized.

Digestive enzyme activity sampling and analysis

Upon completion of feeding test, three fish (per experimental tank) 9 fish (per experimental group), 45 fish (per all tested groups), were dissected in order to extract stomach and intestine for digestive enzyme activity analysis. The stomach samples were homogenized in buffer solution of glycine–HCl 0.1 M, pH 2 and the intestines were homogenized in solution of Tris–HCl 100 mM + $CaCl_2$ 10 mM pH 9. Both samples were centrifuged at 16000g for 30 min to extract the supernatant or enzymatic extract by separating in 400 µl aliquots and freezing at -20 ºC until their further use. The soluble protein concentration was evaluated using a bovine serum albumin calibration curve (600 mg/mL).

Alkaline proteases activity was determined according to (Walter 1984), using Hammerstein–grade casein 0.5% in buffer (100 mmol/L Tris-HCl; 10 mmol/L $CaCl_2$, pH 9); one unit of activity was defined as 1–µg of tyrosine released per min at Abs_{280}. The acid protease activity was determined using Anson (1938) technique, and hemoglobin (1%) in 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 citrate-phosphate; 50 mol/L NaCl, pH 7.5). Lipase activity was measured as previously described by Versaw et al. (1989) but using ß-naphthyl acetate 100 mmol/L 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 = [Δ abs x final reaction volume (mL) / CEM x time (min) x extract volume (mL)], and 2) Units x 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 x µg^{-1} x 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, Inc., Tulsa, OK) using a significance value of $P<0.05$. The results were presented as mean ± standard deviation, *SD*.

Results

Proximate composition of POMM

Proximate composition of POMM is shown in Table 1. 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.

Proximate composition of experimental diets

Experimental diets did not show relevant differences regarding to crude protein, crude lipid, ash and energy. However, crude fiber and ash increased as POMM level increased in experimental diets. While Nitrogen free extract decreased as POMM level increased in diets (Table 2).

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 fed 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 was not 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 POMM100 experimental group compared to that shown in the rest of 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 not significant (P>0.05) difference of lipase activity between POMM0 and POMM25% (Table 5).

DISCUSSION

Present study was designed to study the effects on growth, feed efficiency, protein utilization, survival, final whole body proximate composition, and digestive enzyme activities of Nile tilapia fingerlings, fed diets formulated with increasing levels of a locally available and unconventional protein meal, POMM. Levels of protein and lipid content of *P. djamor* in this study, are similar to those previously reported in Cruz–Solorio et al. (2014) and Salmones (2017). Mushroom species are characterized for their high fiber content. In this research, 20.05% of fiber was recorded in pink oyster mushroom meal. [11] reported 21.4% of crude fiber in edible *P. eryngii* powder. In contrast, lower crude fiber levels (9.29% - 8.60%) in two strains of *Pleurotus* spp were recorded by Cruz–Solorio et al. (2014).

Current study showed that a maximum 25% of POMM can be supplemented in Nile tilapia fingerlings without affecting WG and SGR of fish. Pink oyster mushroom has nutritional, nutraceutical, and biodegradable features Dulay et al. (2017). A limited inclusion of POMM may be attained to the presence of certain biochemical components naturally occurring in POMM that at high levels could produce certain growth depressing effects (Salmones 2017). Nutritional quality of *Pleurotus* has been widely studied and it has been robustly demonstrated. Studies have detected 16 components (2–pentanone, 3–pentanone, butyrate de methyl and 2–methyl–3 pentanone, 3–octanol, 3–octanone, among the main ones) influencing in *P. djamor* flavor, hence palatability (Zhang et al. 2022, Andrew 2023), affecting fish acceptance to feeds. This fact could explain why DFI of Nile tilapia fingerlings during a 45–day period, decrease as higher levels of POMM were supplemented in experimental diets. Other elements that are predominant in pink oyster mushroom 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 gastrointestinal tract Moumita and Das (2022).

In contrast, in present study, growth performance and feed utilization decreased as POMM level increased in experimental diet in Nile tilapia fingerlings. This can be explained by two factors. Firstly, amount of POMM supplemented in experimental diets, was remarkably higher (from 11 to 44% of 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 a 20% of crude fiber while in experimental diets, this nutrient consequently increased as POMM level increased. Results revealed that POMM25 experimental diet had 3.63% of crude fiber. This diet did not compromise 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 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). However, high levels of fiber can bind nutrients like lipids, proteins, and minerals (Obrero–Magbanua and Alano–Ragaza 2022), reducing their bioavailability. Fiber is the non-nutritive portion of feed ingredients. It is indigestible for carnivorous fish, while others such as channel catfish, has intestinal microflora capable of digesting small portion of dietary fiber (McLean 2023). Some herbivorous fish, such as grass carp, derive nutrients from fiber but some such as t*ilapia aurea*, do not Turchini et al. (2018). High fiber content often results in growth depression (Zhang et al. 2022), as seen in present 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). Present study, recorded a slight or significant ($P<0.05$) 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.

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 survival of experimental fish was not significantly affected by any experimental diet (Dawood et al. 2020a).

In present study, final whole body proximate composition (moisture, crude protein and crude lipid) were not affected in experimental fish after a 45–day feeding period. However, experimental fish fed POMM100 diet, recorded a significantly higher crude fiber, compared to that shown in the rest of experimental groups. These higher values are attained to the high level of fiber content (7.4%) in 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 consumes diets with high contents of fiber Adorian et al. (2016). This statement, correlates with present 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 most of fish species, including Nile tilapia (Hilton et al. 1983).

Present study analyzed digestive enzyme activities of Nile tilapia fingerlings fed diets formulated with increasing levels of POMM. Protease–acid, protease–alkaline, trypsin and chymotrypsin did not show significant differences among experimental groups. These enzymes have been proposed as indicators of the nutritional status in fish. Activity of these enzymes revealed the stomach 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). In other words, activities of enzymes digesting proteins in fish, revealed the effects of diets in physiological status of experimental fish Guerrero–Zarate et al. (2019). In our study, activity of leucine aminopeptidase of experimental Nile tilapia fingerlings showed no significant differences among experimental groups. This enzyme is considered as indicator of nutritional quality, since a 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 present research, lipase activity showed a significant ($P<0.05$) decrease in experimental groups fed POMM50, POMM75, 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 ANF´s (Thongprajukaew and Rodjaroen, 2020) In present 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). 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*) where this digestive enzyme is not efficiently expressed (Bjørgen et al. 2020). It is well demonstrated that omnivore species like Nile tilapia has a better starch digestion rate 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

Although diets formulated with increasing levels of POMM did not compromised FCR, PER, SR, Acid and alkaline proteases, trypsin, chymotrypsin, leucine aminopeptidase and amylase of Nile tilapia fingerlings, results obtained in present study, indicated that high levels of fiber naturally present in POMM, inevitably increase this nutrient in experimental diets, hence significantly affecting other parameters such as, WG, SGR, DFI, DPI, and lipase activity when POMM is supplemented in levels above 25%. While a 100% supplementation, triggered an accumulation of fiber in final whole body of Nile tilapia fingerlings. This may be attained 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 final whole body of Nile tilapia fingerlings.

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Table 1. Proximate composition of POMM.

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 (kcal kg^{-1})	3899

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Table 2. Dietary ingredients and proximate composition of experimental diets for Nile tilapia fingerlings, formulated with increasing levels of POMM.

Ingredients (%)	**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	13	8	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	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 (g 100 g $^{-1}$ 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 (%)[1]	38.78	37.84	35.46	34.59	32.56
energy (kcal kg^{-1})[2]	4776	4704	4638	4566	4499

[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.

[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.

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

[1]Calculated manually: Nitrogen free extract and gross energy were manually calculated as follows: Nitrogen free extract (%) = [100 – (Protein + Lipid + Ash + Crude fiber)].

[2]Calculated manually: gross energy (kcal Kg^{-1}) = [(crude protein X 5.65) + (lipid X 9.4) + (NNE X 4.15)] x 10 (Gatlin III 2010).

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Table 3. Growth, feed performance, protein utilization and survival of Nile tilapia fingerlings fed formulated diets with increasing levels of POMM for 45 days.

Growth	POMM0	POMM25	POMM50	POMM75	POMM100
WG (%)[8]	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 (%)[3]	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[4]	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[1]	2.54 ± 0.35	2.34 ± 0.10	2.84 ± 0.24	2.95 ± 0.51	3.22 ± 0.21
DFI (g day^{-1})[5]	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})[6]	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[7]	1.20 ± 0.18	1.32 ± 0.06	1.09 ± 0.09	1.08 ± 0.20	0.97 ± 0.06
SR (%)[2]	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

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

[1] Weight gain: [(final average weight – initial average weight) / (final average weight)] x 100.
[2] Specific Growth Rate: {[(*ln* final weight) – (*ln* initial weight)] / days x100}.
[3] Condition Factor: [(final average weight / final total length^3) x 100].
[4] Feed Conversion ratio: [(Feed consumed, g) / (gain in weight, g)].
[5]Daily Food intake: {(consumed protein, g) / [time, day x N (final fish number)]}.
[6] Daily Protein intake: [(food consumption, g day base) / (number of fish / day)].
[7] Protein efficiency ratio: [(weight gain, g) / (protein intake in Dry Matter, g)].
[8] Survival rate: {(Initial fish number) – [(Final fish number) / (Final fish number)] x 100}.
Different letters mean significant differences (P<0.05).

Table 4. Whole body proximate composition of Nile tilapia fingerlings, fed formulated diets with increasing levels of POMM, for 45 days

Proximate composition (g 100 g^{-1} DM)	Experimental groups				
	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 ± 2.8	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

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

Table 5. Digestive enzyme activities of Nile tilapia fingerlings, fed formulated diets with increasing levels of POMM, for 45 days

Enzyme activity (U mg protein^{-1})	**Experimental groups**				
	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	6.47x10^{-03} ± 2.15x10^{-03}	7.83x10^{-03} ± 1.78x10^{-03}	5.99x10^{-03} ± 2.71x10^{-03}	5.94x10^{-03} ± 1.13x10^{-03}	7.15x10^{-03} ± 6.02x10^{-04}
Chymotrypsin	2.35x10^{-02} ± 4.87x10^{-04}	2.41x10^{-02} ± 1.47x10^{-03}	2.22x10^{-02} ± 2.98x10^{-03}	2.25x10^{-02} ± 2.92x10^{-03}	2.19x10^{-02} ± 2.55x10^{-03}
Leucine aminopeptidase	8.66x10^{-04} ± 2.96x10^{-04}	1.11x10^{-03} ± 1.38x10^{-04}	1.13x10^{-03} ± 3.37x10^{-04}	8.96x10^{-04} ± 2.09x10^{-04}	1.15x10^{-03} ± 4.34x10^{-04}
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

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