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Effects of Mushroom Fortified Basal Diet on Fish and Its Effluent Nutrients on Oyster Mushroom (*Pleurotus ostreatus*) Productivity

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Abstract

This study evaluates the growth performance of *Clarias gariepinus* fed Oyster mushroom supplemented diet at 0% (T1 control, Basal diet without mushroom), 1.0% (T2), 1.5% (T3), 2.0% (T4) and 2.5% (T5) respectively in triplicate laid out in a completely randomized design (CRD). Each tank was stocked with 20 post juveniles, fed twice daily while water quality was monitored for pH, ammonia and temperature. The productivity of oyster mushroom fed effluents with different levels of nutrient from the study fish tank (T2 -T6) but with an additional control without fish effluent (T1) respectively. The substrates were prepared using sawdust, rice bran and chalk ($CaCO_3$), water and fish effluents before being bagged, sterilized, inoculated with mushroom spawn and observed for mycelium colonization at 25ºC-30ºC. They were then moved to the growth chamber for watering, sprouting and fructification, before data collection. There were significant differences ($p<0.05$) between all growth parameters (fish and mushroom), T2 in fish production had the highest mean weight gain and growth rate, protein efficiency ratio and lowest feed conversion ratio, indicative of better utilization of the experimental diets. T5 had the highest survival rate; which shows that high inclusion levels in formulated feed can improve the fish immune system. T4 in *Pleurotus ostreatus* production had the highest percentage biological efficiency value, T4 is therefore recommended for mushroom production in a fish-cum-mushroom culture, since it increased its growth performance. Mushroom integration in fish production and the inclusion of mushroom in fish feed formulation will not only open up channels of producing high quality fish feeds that guarantees increased fish productivity at low inclusion levels, maximize resources recycling, but can also create a diversified income stream for farmers and minimize dependence on a single income source.

Keywords:
Biological efficiency, Mushroom, Dry matter, Productivity, Pleurotus ostreat

Introduction

Mushroom consumption can be traced as far back to the Egyptians, who considered them a delicacy reserved for Pharaohs (Jahan *et al.*, 2010). The oyster mushroom (*Pleurotus ostreatus*), is one of the more commonly sought wild mushrooms grown for their delicious taste and high nutritional content (Tesfaw *et al.*, 2015). They belong to the class Basiimycetes, subclass Hollobasidomycetidae and order Agricales. They grow wild in the forest and are benzaldehyde (Beltran *et al.*, 1997*)*. Nigeria with its unique climatic conditions of tropical rain forest in the south and sub-Saharan condition in the north is home to diverse species of mushrooms. The common mushrooms in Nigeria include: *Termitomyces*, *Pleurotus*, *Lentinus*, *Lenzites*, *Trametes*, *Ganoderma*, *Pycnoporus*, and *Coriolopsis* (Ola and Oboh,

2001; Orngu *et al*., 2021). Mushrooms have high nutritional, organoleptic, functional and medicinal properties (Osemwegie *et al.*, 2002, Ergonul *et al.*, 2013). It is considered a very rich source of non-starchy carbohydrates, dietary fiber and protein which can also be used as substitute for meat in vegetarian diets. Oyster mushroom constitutes about 25-30% protein, 2.50% fat, 17-44% sugar content, 17-38% mycocellulose and approximately 8-12% mineral content such as potassium, phosphorus, calcium and sodium (Stanley and Odu, 2012).

Oyster mushroom can naturally grow on dead and decaying woods and organic matter. But changes in climatic patterns and the fear of harvesting wild poisonous mushrooms growing on soft wood has made wild harvesting difficult (Zhao *et al*., 2022). Therefore, there is the need to artificially grow them in commercial quantity at a reasonable cost with improved growth and nutritional characteristics. Badu *et al.* (2011) and Patil *et al*. (2010) reported that the quality and yield of oyster mushroom depended on the nutritional and chemical contents of the substrates. Oyster mushrooms can be grown in a variety of substrates, including agricultural waste. Significant quantities of agricultural wastes are generated annually in Nigeria and other parts of Africa which are usually left to decay or are disposed through burning (Tesfaw *et al*., 2015). By integrating oyster mushroom cultivation with fish farming, fish effluent can be used for mushroom cultivation, thereby providing a sustainable source of nutrients for mushroom growth which is a value-addition.

Mushrooms are grown artificially by inoculating mushroom mycelia or spawn into substrate which are mostly lignocellulose material derived from agricultural waste, readily available in Nigeria. Which supports the growth, development and fruiting of mushroom (Adedokun, 2014, Onyeka *et al.*, 2017). Although mushrooms are cultivated on substrates, but different supplements can be used to enrich the medium. These supplements help to improve mushroom yield, usually evaluated by the biological efficiency of the substrate in mushroom production (Jeznabadi *et al*., 2016).

Integrated aquaculture allows for efficient utilization and recycling of resources in order to achieve higher productivity. Little interest has been shown in the cultivation of Oyster mushrooms in Nigeria until recently (Igile *et al*., 2015). The different *Pleurotus* species are *Pleurotus ostreatus, Pleurotus florida, Pleurotus sajor-caju, Pleurotus sapidus, Pleurotus eous, pleurotus membranaceous and Pleurotus flabellatus* (Ladli, 2020). *Pleurotus ostreatus* is characterized by high water content and low calorific value (1510 kJ kg1 edible parts), making it suitable for inclusion into calorie-controlled diets (Jaworska *et al.*, 2009).

This study was therefore undertaken to evaluate the impact of supplementing fish feed with Oyster mushroom to grow *Clarias gariepinu*s and using the same fish culture effluent to grow *Pleurotus ostreatus* so as to evaluate its growth performance.

Materials and Methods

Study Area: This study was carried out in the experimental fish farm of the Department of Aquaculture and Fisheries Management, Faculty of Agriculture, and in the Department of Plant Biology and Biotechnology, University of Benin, Edo State with a Latitude N 60 20'.3.2' and Longitude E 50 36'.2.5', Ovia North East LGA of Edo State, Nigeria.

Experimental Fish Culture and Management:

Experimental Design

This experiment was designed as a Completely Randomized Design (CRD), with 15 experimental units having 5 treatments and 3 replicates.

Fish culture

Before the commencement of this experiment, 300 post juveniles of *Clarias gariepinus* was procured from a reputable hatchery in Benin City and then acclimated for a week before being randomly allocated into 5 treatment tanks labelled appropriately with 3 replicates each, making a total of 15 tanks. Prior to this, each tank was properly washed and disinfected, before water was impounded. Each tank was stocked with 20 post juveniles of *C. gariepinus*, the experiment lasted for a period of 9 weeks.

Growth parameters of *Clarias gariepinus* were recorded every 3 weeks for computation according to standard methods. Protein Efficiency Ratio (PER), feed intake, weight gain, absolute growth rate (g/day) and relative growth rate (%) (Rahman and Arifuzzaman, 2021), food conversion ratio (FCR) (Morris *et al*.,2020) and survival rate (%).

Freshly harvested oyster mushroom (*Pleurotus ostreatus*) was purchased from Mycofarm and Allied Synergy Limited (MASL), Benin-City. At the laboratory, it was rinsed severally with clean water before being oven-dried at 60°C for 48 hours. Subsequently grounded into a smooth mushroom powder (MP), which was used to supplement the five Iso-nitrogenous basal diet of 40% crude protein at different inclusion levels on a dry matter basis (0%, 1.0%, 1.5%, 2.0%, and 2.5%) using Pearson Square Method, the proximate composition of each feed was determined before the commencement of the study. Fish were fed to apparent satiation twice daily (8.00 a.m, and 5.00 p.m).

Experimental Mushroom Culture and Management

Effluents from the 15 experimental tanks was used to establish the substrates for the mushroom spawns obtained from MASL for the mushroom culture.

Substrate Preparation: The substrate which was made up of weighed saw dust (1.56kg) as the bulk substrate, rice bran (0.4kg) as the supplement and Chalk ($CaCO_3$) (0.04kg) as the conditioner, were mixed together with the fish effluents. At different intervals, hand-fit test was carried out to avoid waterlogging and covered for 24hrs. After this period, it was mixed again and the wet substrates bagged in polyethylene bags and tied in triplicates.

Sterilization and Substrate Inoculation: The bagged substrates were autoclaved at 121°C for 6 hours after which they were allowed to cool for 24hrs prior to inoculation. Mother spawns of oyster mushroom collected from the culture house of MASL were inoculated aseptically into the sterilized substrate bags. Grams of spawn was weighed and inoculated to each substrate, tied up with a piece of foil paper, held with a rubber band around the neck and taken into a dark room for mycelium colonization at 25°C - 30°C and 90% relative humidity. The spawn run time which although is dependent on the size of the bag, amount of spawn, strain and atmospheric temperature available; during this study was observed to be 3 weeks for mycelium colonization.

Fructification and Treatment: After 21 days, substrate bags were moved to mushroom growth chamber for photosynthesis to enable sprouting and fructification. Tiny holes were punctured on the polyethylene bags using a wire. The polyethylene bags were opened and watered with 150ml of water/effluent, using different spray bottles for each treatment daily to avoid cross-contamination. Environmental conditions in the growth chamber necessary for fructification were closely monitored. Fructification started after two weeks of watering for some of the treatments and gradually increased across all treatment bags.

Mushroom Growth Parameters Analysis:

The growth parameters of the mushroom on different substrates measured included the height of the fruiting bodies: measured in centimeters using a meter rule from the base of the stripe to the pileus. Mean number of fruiting bodies; counted directly by noting the fruiting bodies on each substrate bag. Striped thickness was taken using a caliper. Diameter of pileus was determined with a meter rule. Measuring from one edge of the pileus across the stripe to the other edge. While the fresh weight of fruit bodies was measured with a digital weighing balance.

Dry matter loss of mushrooms was calculated according to Rubina and Aivars (2021).

- Dry matter loss = Initial Weight – Final Weight

  Initial Weight (Rubina and Aivars, 2021).

% Biological Efficiency = Total fresh weight of harvested mushroom × 100%

Dry weight of substrate (Yang *et al.*, 2013)

Statistical Analysis

The data obtained was subjected to statistical analysis using Analysis of variance (ANOVA). Separation of means with significant difference was done using Duncan Multiple Range Tests (DMRT) at 5% probability level.

Results

The mean ammonia levels in the different treatments evaluated were; T1 (with pure water) 0.00mg/l, T2 (Fish effluents without mushroom inclusion) 0.231mg/l, T3 (1.0% mushroom inclusion) 0.269mg/l, T4 (1.5% mushroom inclusion) 0.286mg/l, T5 (2.0% mushroom inclusion) 0.427mg/l and T6 (2.5% mushroom inclusion) 0.429mg/l. The mean water temperature and pH during the study were within acceptable range**.**

Proximate Composition of Experimental Diets

Table 1 shows that moisture content in all the treatments diets were lower than the control diet (T1), crude fibre content in the experimental diets increased as the inclusion levels of mushroom in the diet increased. Nitrogen free extract (NFE) was about same range in all experimental diet except in diet T4 with NFE of 36%. T1 had the highest ethyl extract compared to other experimental diets, with crude protein increaing across the row with Treatment T1 having the lowest value. Ash content reduced gradually across each treatment (T1 - T5),

Table 1: Proximate Composition of Experimental Diet

(%)	**TREATMENTS**				
	T1	T2	T3	T4	T5
Moisture content	14.23	13.28	11.32	11.37	11.11
Crude Fibre	1.90	2.94	4.76	4.97	5.14
Nitrogen free extract	30.17	31.14	31.32	36.00	31.84
Ethyl extract	13.34	11.79	12.80	10.47	12.26
Crude protein	24.75	28.53	29.47	25.40	28.00
Ash content	15.61	12.32	10.33	11.79	11.65
Total	100	100	100	100	100

***Pleurotus ostreatus* Growth Performance**

Height of fruiting bodies (mm) results show that the mushroom fed effluent without any supplementation of feed (T2) had the least mean height of fruiting body as shown in Table 2. There was no significant difference in the mean height of T1, T5 and T6, but T4 had the highest fruiting body height as shown in Table 1. The mean stripe thickness of the fruiting bodies reveals that T2, T6 and T1 (5.34mm, 8.83mm and 9.64mm) had the least stripe thickness that were statistical different from T3, T5 and T4 (10.79mm, 11.09mm and 11.50mm) respectively. The mean diameter of pileus shows that T2 (72.22 ± 33.83mm) and T4 (74.93 ± 19.27mm) diameters were not significantly different. But were significantly different from all the other treatments (T1 55.90 ± 8.16mm, T3 58.71 ± 17.41mm, T5 53.13 ± 23.59mm and 55.96 ± 18.92mm) respectively.

The results show that the mean fresh weight was irregularly varied between treatments, and T4 had the highest mean fresh weight closely followed by treatment T2 (78.67g) and T1 (74.67g), while all the other treatments were significantly different as shown in Table 1. And the percentage dry matter loss in all the treatments were closely related between treatments. Biological Efficiency (%) results show that T4 had the highest percentage biological efficiency followed by T2 and T1, while the other treatments were marginally different.

Table 2: Growth Characteristics of *Pleurotus ostreatus*, Raised with Fish Effluents Fed Experimental Diets Supplemented with Mushroom.
NOTE: Means with different superscripts across rows are statistically different ($p<0.05$).

	TREATMENT							
PARAMETERS	T1	T2	T3	T4	T5	T6	F-value	P
HFB (mm)	57.27±14.55^{b}	53.91±12.18^{a}	61.57±23.50^{c}	70.91±10.20^{d}	58.75±13.56^{bc}	56.85±13.11^{b}	1.782	0.133
ST (mm)	9.64 ± 4.12^{b}	5.34 ± 7.11^{a}	10.79 ± 4.43^{bc}	11.50 ± 3.51^{c}	11.09 ± 8.30^{c}	8.83±3.45^{b}	1.700	0.151
DP (mm)	55.90 ± 8.16^{b}	72.22±33.83^{d}	58.71±17.41^{c}	74.93±19.27^{d}	53.13±23.59^{a}	55.96±18.92^{b}	1.919	0.107
FW (g)	74.67	78.67	60.00	83.33	56.67	60.00		
DML (%)	88.00	91.00	91.00	91.00	91.00	92.00		
BE (%)	7.50	7.90	6.00	8.30	5.70	6.00		
MNFB	5.00	6.00	8.00	11.00	5.00	2.00		

HFB: Height of fruiting body, ST: Stripe thickness, DP: Diameter of pileus, FW: Fresh weight, DML: Dry matter loss and BE: Biological Efficiency.
T1: 0%H2O control, T2: 0% control, T3: 1.0%, T4: 1.5%, T5: 2.0%, T6: 2.5% Mushroom inclusion levels respectively.

Clarias gariepinus Growth Parameters

The result of the growth performance and nutrient utilization of *Clarias gariepinus* juvenile fed basal diets supplemented with mushroom at different inclusion levels are as shown in Table 3;

Specific growth rate increased gradually across treatment T1 - T5 and was highest in T3 (37.87 ± 5.32) at the 3rd week, and as the study continued, growth rate increased gradually across treatments and was not significantly different ($p>0.05$) across treatments T2 - T5 but T1 (70.88 ± 6.09) was significantly different ($p<0.05$) from that of other treatments.

Absolute (T2 - T5) and relative growth rates(T1-T5), were not significantly different ($p>0.05$) in treatments but there was significant difference ($p<0.05$) between treatment T1 and other treatments. T1 (11.42 ± 2.29 g/day) had the lowest absolute growth rate. While the relative growth rate showed significant difference ($p<0.05$) between treatments T2 (969.2 ± 123.05%) with the highest relative growth rate and T1 (751.96 ± 156.03%) the lowest.

Mean weight gain as shown in Table 3, increased gradually across each treatment and highest weight gain was obtained in T3 although it was not significantly different ($p>0.05$) in treatments T2 -T5 but there was significant difference ($p<0.05$) between treatment T1 and other treatments. With T1 (102.80 ± 20.61g) having the lowest weight gain. Feed conversion ratio at the 3rd week of study was higher than the subsequent weeks of study and was not significantly different ($p>0.05$) across treatments but

there was significant difference ($p<0.05$) at treatments T3 and T5 while there was no significant difference ($p>0.05$) between the other treatments, with T2 (0.53 ± 0.07) having the lowest value.

Table 3: Growth Parameters of *Clarias gariepinus* Fed Different Levels of Supplementation with Oyster mushroom

Parameters	Week	Treatments					F	Sig.
		T1	T2	T3	T4	T5		
Specific growth rate (%)	3	30.16±8.22^{a}	34.06±4.59^{ab}	37.87±5.32^{bc}	30.39±6.59^{a}	35.32±7.05^{ab}	5.183	0.001
	6	52.63±5.66^{a}	58.61±3.90^{ab}	61.30±3.77^{bc}	58.11±3.43^{b}	59.33±4.16^{b}	11.560	0.000
	9	70.88±6.09^{a}	78.77±3.94^{bc}	76.80±4.75^{bc}	77.40±3.73^{bc}	76.55±4.14^{bc}	8.627	0.000
Absolute growth rate (g/day)		11.42±2.29^{a}	14.84±1.68^{ab}	14.16±2.04^{b}	14.39±1.86^{b}	13.93±1.79^{b}	9.571	0.000
Relative growth rate (%)		751.96±156.03^{a}	969.21±123.05^{bc}	911.27±149.99^{abc}	925.56±113.65^{abc}	901.04±121.57^{abc}	7.569	0.000
Weight gain (g)	3	21.15±8.77^{a}	24.90±5.26^{ab}	30.15±6.49^{bc}	21.42±7.10^{a}	26.81±7.46^{b}	5.680	0.000
	6	53.42±10.08^{a}	66.73±8.46^{ab}	74.63±9.20^{c}	66.32±7.93^{b}	68.98±8.16^{b}	15.630	0.000
	9	102.80±20.61^{a}	133.58±15.14^{bc}	127.45±18.34^{bc}	129.52±16.75^{bc}	125.36±16.09^{bc}	9.560	0.000

Cont'd

Feed conversion ratio	3	1.42±0.59[a]	1.12±0.36[a]	1.47±0.32[a]	2.44±0.73[a b]	1.80±0.55[a]	17.998	0.000
	6	0.49±0.10[a]	0.50±0.08[a b]	0.56±0.06[a]	0.49±0.06[a]	0.56±0.07[a b]	4.776	0.001
	9	0.77±0.15[a]	0.53±0.07[a]	0.65±0.09[a]	0.66±0.08[a]	0.66±0.09[a]	14.712	0.000
protein efficiency ratio	3	3.28±1.36[a]	2.50±0.53[a]	3.22±0.69[a]	1.82±0.60[a]	2.15±0.60[a]	12.523	0.000
	6	8.28±1.56[a]	6.41±0.81[a b]	6.94±0.85[a b]	8.44±1.01[a]	6.47±0.76[a b]	17.897	0.000
	9	5.31±1.06[a]	6.70±0.76[a b]	5.34±0.77[a]	6.22±0.80[a b]	5.53±0.71[a]	10.946	0.000
Feed intake (g)	3	25.40±0.00	34.92±0.00	31.75±0.00	47.62±0.00	44.44±0.00	4.26	0.000
	6	25.40±0.00	36.51±0.00	36.51±0.00	31.75±0.00	38.10±0.00	1.88	0.000
	9	76.19±0.00	69.84±0.00	80.95±0.00	84.13±0.00	80.95±0.00	8.66	0.000
Survival rate (%)	3	100.00	95.24	100.00	98.41	100.00	13.34	0.00
	6	100.00	95.24	96.83	88.89	100.00	10.78	0.00
	9	88.89	80.95	77.78	73.02	95.24	7.16	0.00

NOTE: Mean values with same superscript across rows are not significantly different ($p>0.05$)

Protein efficiency ratio during the period of study (Table. 3), was highest at week 6 and also not significantly different ($p>0.05$) in treatments T1 and T4 and lowest at week 3, where it was not significantly different ($p>0.05$) across all treatments (T1-T5). and at week 9 was not significantly different ($p>0.05$) in treatments T2 and T4 and between treatments T1, T3 and T5, but there was significant difference ($p<0.05$) across all treatments with T2 (6.70 ± 0.76) having the highest Protein efficiency ratio.

Feed intake at week 3 of this study was significantly different across each treatments and increased gradually across treatments T1-T5. Feed intake at week 6 of this study was significantly different ($p<0.05$) and increased across treatments T1 - T5 except at T4 which was less than T3. Feed intake at week 9 of this study was significantly different ($p<0.05$) with treatments T3 (80.95g) and T5 (80.95g)

having the highest feed intake and T2 (69.84g) having the lowest. Survival rate during the period of study reduced gradually from week 3 to week 9. Survival rate at the 9th week of this study reduced gradually from T1 to T4 with T5 (95.24%) having the highest survival rate.

Discussion

Proximate Composition of Diet

Moisture content in all the treatments were lower than the control diet (T1), which suggests the fast growth of mould in experimental diets T1 and T2 with the least inclusion level of mushroom. This therefore suggests that high moisture contents aid the growth of mould. This was previously reported by Kabir *et al*. (2015) in their study. They reported that moisture content of fish feeds affects the quality of feed, and that high moisture in feeds sometimes encourages bacterial growth.

Crude fibre content in the experimental diets increased as the inclusion levels of mushroom in the diet increased. Mushrooms are high in fibre, hence aids digestion and better feed utilization. This suggests an increase in feed utilization as the mushroom inclusion level increased due to fibre present in mushroom. This agrees with the report of Ngoc *et al.* (2013) on their study on the impact of fibre intake in pigs as increase in fibre increased digestibility of feed by the pigs.

Nitrogen free extract (NFE) was about same range in all experimental diet except in T4, NFE of feed encourages increase in fat of test animal like the Sea bass fed the highest level of NFE (35.2%) in diet as reported by Lanari *et al*. (1999). This suggests why the fish fed diet T4 had the second highest weight gain at the end of the study period.

Treatment T1 got rancid and had off-flavour faster (at about 3wks) than other treatments which stayed longer with treatment T5 being the last to get rancid during this study. This may have been due to T1(Control diet) having the highest Ethyl extract level compared to the other experimental diets. This observation agrees with Magala *et al*. (2022) who reported that metabolic interaction between lipids and oxygen may result in the generation of short-chain molecules from long-chain fatty acids by a process referred to as rancidity. With the possibilities of unfavourable storage conditions, these chemical processes can produce highly reactive molecules resulting in rancid fats and oils, which can cause disagreeable aromas and flavour. Chemical processes deplete feed of nutrients and in some situations, rancidity and vitamin degradation occur very quickly. Due to the high degree of unsaturation, the fatty acids are particularly susceptible to oxidative rancidity. Feed manufacturers attempt to prevent oxidation in lipid sources such as fish oil by stabilizing them with antioxidants. Mushrooms which were added to the experimental diets must have acted as antioxidants that helped to stabilize the fish oil. This also suggests the reason why the treatment T1 had lower feed intake and acceptability of feed.

Crude protein increased across the row with Treatment T1 having the lowest value which can be attributed to low nutritional value. *Clarias gariepinus* are carnivorous in nature and high protein content plays a significant role in the nutrition of carnivores in terms of balanced amino acid, fatty acid content and high digestion as reported by Huang *et al.* (2023) while working on juvenile Cobia (*Rachycentron canadum*). Treatment T1 had the lowest crude protein value, this may have resulted in the low growth rate of fish fed with T1 experimental diet as growth across all other experimental diets increased during the study. Ash content reduced gradually across each treatment (T1 - T5) and the mean feed intake increased with reducing ash content, this suggests that the ash content of the diet has effect on the utilization of the diet. This is in agreement with Shearer *et al.* (1992) who studied juvenile *Atlantic salmon* and reported that feed efficiency was reduced as the level of dietary ash increased.

The fish effluents analyzed showed that there were significant levels of ammonia (0.02%), that supported the overall growth of *Pleurotus ostreatus* in the various treatments. And this is in agreement with Hoa and Wang (2015) report, who observed increased mycelium growth in *Pleurotus ostreatus* with increase in the concentration of ammonium chloride from 0.01% to 0.09%.

Fruiting body heights from the different treatments though were not statistically significant ($p<0.05$ but the observed values varied between treatment. Higher mean values of stipe length obtained in T3 and T4, may probably have been due to the mushroom inclusion levels in the feed which resulted in the moderate ammonia level in the effluent used in the substrate preparation and watering. This is in agreement with Chukwurah *et al*. (2013) report where they reported that different mean values of Stipe length were observed in mushrooms, grown in different types of agricultural waste given different substrate treatment. The highest number of fruiting bodies was observed in T4, and this may have resulted from the moderate inclusion level of mushroom in the experimental diet, whose influence on the effluents used resulted in not too low or high nitrate levels. This is in agreement with the report of Patel and Trivedi (2015), who postulated that mushroom will fruit at an increased rate with increasing available nutrients. But Laursen (2018) stated that increasing the amount of nutrients available in a substrate could result in increasing mycelial production only to a certain extent, after which, further increase may result in contamination by fungi and decrease in mycelial growth. The decreased mean number of fruiting bodies observed in T5 (2.0% inclusion) and T6 (2.5% inclusion) might therefore be due to the high mushroom inclusion level in the experimental diet that may have affected *P. ostreatus* fruitification in this study. T4 had the median level of ammonia concentration and the highest stipe thickness value during the study. Since high mean values were also observed in T3 and T5 respectively, this might have probably been due therefore to better utilization of the available CO_2, in the fruiting chambers. This agrees with the report of Belletini *et al.* (2019) who stated that stipe thickness was dependent on available carbon dioxide concentration. The values of mean stipe thickness recorded during this study were within the range of 5mm to approximately 12mm. According to Habib *et al.* (2005) and Sarker *et al*. (2007), respectively reported that the stipe thickness of wild *Pleurotus ostreatus* was within the range of 4.5mm to 7.0mm and 5 to 8.0mm. Increased thickness might have been as a result of the addition of $CaCO_3$ in the substrate, that acted as a stabilizer capable of neutralizing pH in growing medium (pH=7). At the right concentration, it can increase stipe density, this assertion is in agreement with Royse and Sánchez (2003) findings, as they recorded an increase of 24.9% in stipe thickness of *Lentinula edodes* supplemented with $CaCO_3$ compared to substrate which were not supplemented. The different mushroom inclusions levels in the basal diets may have affected the structure and properties of the substrate, leading to variations in pileus diameter. According to Tesfay *et al*. (2020), Pileus diameter of oyster mushroom depends on the structure, compactness and physical properties of the type of substrates used. Synytsya *et al*. (2008) also stated that fruiting bodies with larger pileus (cap) and shorter stipes (stalk) were better than that with smaller pileus and longer stipes. In the present study, T4 provided better quality of mushroom with larger pileus diameter (74.93±19.27) as compared to the other treatments.

Biological Efficiency: This study aligns with Islam *et al.* (2009) observation that sawdust is consistently the best medium for mycelia growth and fruitification, as the substrate used during this study supported the growth of *P. ostreatus;* a mixture of sawdust, rice bran and chalk. The positive influence of fish culture effluents on the substrate efficiency coupled with the physico-chemical parameters which were at optimal levels during the study may have been responsible for the high percentage biological efficiency especially in T4. Generally, substrate quality has been reported to affect the yield and

biological efficiency of *Pleurotus ostreatus.* Onyeka *et al.* (2017) observed that different substrates gave varying mushroom yield because of the biological and chemical difference of the substrates. Although the substrate used across all treatment here was similar, the inclusion levels of mushroom in the experimental diets were different and this might have resulted in the variation observed in the biological efficiency across the treatments.

Dry matter loss across all treatments was high. Thus, the use of fish culture effluents for mycelia growth in this study might have been responsible for the high dry matter loss. As according to Van Loon *et al*. (2000), the use of a water saturated substrate in mushrooms culture has the ability to increase moisture content. But their observation as it relates to the existent of a linear decrease of yield of mushroom with decreasing dry matter loss levels which holds true for treatments T2 and T4 that had the highest mean fresh weight at the end of the study, does not hold same but contrast the results obtained in treatments T1, T3, T5 and T6. As T6 had the highest dry matter loss but with one of the lowest mean fresh weights at harvest.

Growth Performance

Mushroom supplementation in diets of *Clarias gariepinus* post juveniles resulted in better body weight gain, protein efficiency ratio, feed conversion ratio, feed intake, survival rate, specific, absolute and relative growth rate. This is in agreement with the study of Ashley-Dejo *et al.* (2022), they earlier reported that 50% substitution level of oyster mushroom meal in *Oreochromis niloticus* diet produced better result than without substitution.

Treatment T2 with 1% inclusion level of oyster mushroom had the lowest and best mean feed conversion ratio which might have resulted in its having the highest protein efficiency ratio and other growth parameters. This result agrees with Hardy *et al*. (2021), report that the lower the FCR of a fish the higher the quality and utilization of the feed, and since Oyster mushroom possess antioxidants and prebiotic properties, that enhence digestion, increase immune system efficiency, resulting therefore in healthy fish production with good digestion capabilities that enables it grow better (Wan-Mohtar *et al*., 2020). Treatment T5 with 2.5% supplementation of oyster mushroom had the highest mean survival rate of 98.41%, Bawadekji *et al*. (2017) had earlier reported that the oyster mushroom has antimicrobial activities and antibiotics properties which may have in this study helped to increase the immunological indices of *Clarias gariepinus*. Hence, the observed high survival rate of fish in treatment T5 due to the better immunity of the fish in this treatment. Treatment T4 with 2% supplementation of oyster mushroom had the highest mean feed intake of 84.13g, this shows higher acceptability, palatability digestibility, and flavour in this treatment which are basically feed quality indicators (Joshua and Adogbeji, 2017).

Conclusion

Pleurotus ostreatus and fish are both highly nutritious food items, and integrating them in a fish-cum-mushroom production system can from this study create a sustainable and profitable agricultural venture. Providing both household nutritional security and high employment opportunities that can create small Cottage industry started with limited resources.

This study confirms that fish culture effluents especially when basal feed has been supplemented can be utilized for mushroom cultivation as it supports the growth performance of and biological efficiency of *Pleurotus ostreatus*. Therefore, this study suggests that 1.5% mushroom inclusion level in fish feed formulation, gave the optimum level required to achieve best growth performance of *Pleurotus*

ostreatus. Mushroom though can solely be cultured, but integrating its production by the use of fish effluent rich in nutrient can support its growth. The use of fish culture effluents for mushroom cultivation is therefore a promising approach that can help address some of the environmental challenges facing the aquacultural sub-sector in Nigeria. Using mushroom and its by-products like the spent substrate and mushroom stalks, to enrich the nutrient content of fish feed will open up channels of producing high quality protein foods with minimal resources. It can also create a diversified income stream for aquaculturists, thereby reducing their dependence on fish and reduce the risk of culture failure. This type of integration can also create new job opportunities for women and youth, such as in mushroom harvesting and processing, that can lead to additional economic benefits for the populace.

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