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Effects of Different Mushroom By-Product Types and Levels on Growth Performance and Survival Rate in Dietary of Nile Tilapia (*Oreochromis niloticus*)

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The utilization of 2 different types of mushroom by-product, *Pleurotus sajor-caju* (Fr.) Sing (MBPP) and *Schizophyllum commune* (MBPS), in Nile tilapia diets was investigated. Seven isonitrogenous (crude protein 32%) and isolipidic (crude lipid 7%) practical diets were formulated by replacing fishmeal protein with mushroom by-product protein (MBP) at 3 different levels (20, 40 and 60%) of each MBP type comparing to control diet (Diet 1). Each diet group was randomly allocated to triplicate groups of fish in aquaria (80 Liters) and each aquarium was stocked 10 fish with initial weight 7.5 g/fish. Fish were fed by hand twice daily with apparent satiation for 6 weeks. Two-way ANOVA was used for evaluation of main effects of variables, i.e. MBP type and inclusion level, as well as interaction effects between MBP type and inclusion levels on survival rate, growth performance, protein efficiency ratio (PER) and fish production cost from feed. The results showed that MBP type affected growth performance, PER and fish production cost in that fish fed diet containing MBPP showed the higher result than those fed MBPS diets ($p<0.05$). However, the inclusion level of MBP was not significantly difference. Those fish fed the diet containing MBPP replacement fishmeal protein at 20-60% and fish fed diet containing MBPS replacement fishmeal protein at 20% was not significantly difference to control diet fed group ($p>0.05$).

Keywords: Mushroom by-product, protein replacement fishmeal, Nile tilapia diet

Introduction

Fish meal is considered as the major protein source and the best ingredient for fish feed because of the compatible with the protein requirement (Alam *et al*., 1996). However, it is the most expensive protein source since the increasing demand and unstable supply. Finding the alternative protein sources as a protein replacement fish meal protein in fish

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feed has been studied. Plant and animal derived protein have been investigated (El-Seyed, 1998, 1999; Bhosale *et al*., 2010; Gonzales *et al*., 2007; Lim *et al*., 2007; Nguyen *et al*., 2009). In addition, by-product from agricultural waste was also investigated. Mushroom by product is one of the alternative sources for replacement fishmeal protein in fish with the advisable effect on growth performance compared to fishmeal protein (Phromkunthong *et al.,* 2014). Undesirable mushroom, mushroom stalk, contained a rich source of protein, polysaccharide and antioxidant resulting in application either replacement fish meal protein (Phromkunthong et al., 2014) or stress reduction by PH fluctuation (Ahmed *et al.*, 2017). In addition, mushroom beta glucan mixture has been investigated in grouper diet and proven to be safer, environmental friendly, pollutant free, improve disease resistance, enhance immunity and decrease mortality (Chang *et al*., 2013). Chou *et al.*, (2013) mention that stalk cut off is 25 to 33% of total production. Mushrooms are well known for their quality protein such as glutathione, single cell protein and rich amount of essential amino acids and are used as dietary supplement (Mukhopdhay and Guha, 2015).

Thailand is a particularly good place to grow tropical mushrooms because of the warm climate favorable for mushroom growing**.** *Pleurotus sajor-caju* and *Schizophyllum commune* known as “Hed Nangpha” and “Hed Krang”, respectively are the popular edible mushrooms and is commercially cultivated in Thailand especially in the southern Thailand. Since the high amount of undesirable mushroom part regarding the processing before package to the market, the aim of this study was therefore to evaluate the possible interactions between Mushroom by-product types and replacement fishmeal protein level on survival rate and growth performance including the feed cost for production Nile tilapia.

Materials and methods

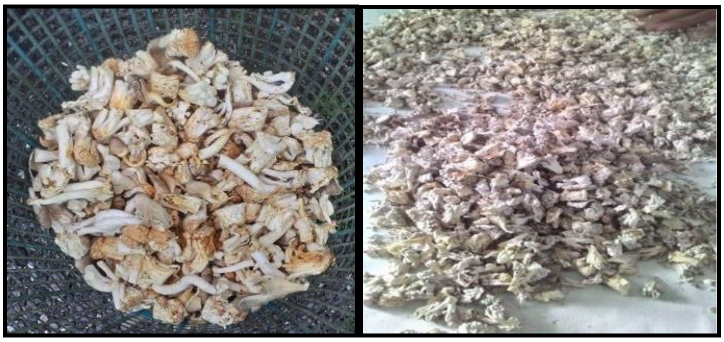
Experimental diet

Fresh mushroom by-product (MBP) consisting of stalk and cap deterioration of two different types; *Pleurotussajor-caju* (Fr.) Sing (MBPP) and *Schizophyllum commune* (MBPS) were obtained locally from a commercial mushroom farm at Ronphiboon District, Nakhon Si Thammarat province (Figure 1). Both of MBPP and MBPS were washed and dry with hot air oven at 70^{o}C overnight. Then ground thoroughly as MBP powder and stored at 4^{o}C. Moisture, protein, fat and ash contents were determined according to the method of AOAC (1990): dry matter by drying in an oven at 105^{o}C, ash by combustion at 550^{o}C in a muffle furnace for 3 h, protein content using Kjeldahl method (Gerhardt, Germany) and fat using dichloromethane (Soxtec System HT6). MBP powders were then incorporated into the diets at different ratios of fishmeal protein to MBP

Table 1. The formulation and approximate composition analysis of the diets (g/ 100 g)

Ingredient	Control diet		MBPP			MBPS	
Diet (Fishmeal protein substitution)	**1 (0%)**	**2 (20%)**	**3 (40%)**	**4 (60%)**	**5 20%)**	**6 (40%)**	**7 (60%)**
Fishmeal	10.00	8.00	6.00	4.00	8.00	6.00	4.00
Soybean meal	50.00	50.00	50.00	50.00	50.00	50.00	50.00
MBPP	0.00	10.78	21.56	32.33	0.00	0.00	0.00
MBPS	0.00	0.00	0.00	0.00	6.55	13.15	19.75
Rice bran	10.00	10.00	10.00	7.99	10.00	10.00	10.00
Cassava meal	12.00	12.00	7.14	0.00	12.00	12.00	10.70
Rice husk	12.99	4.07	0.00	0.00	8.26	3.48	0.00
Soybean oil	3.24	3.38	3.53	3.91	3.42	3.6	3.78
Vitamin/Mineral premix[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride[1]	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Salt	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Monocalcium-phosphate[1]	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Proximate composition of diet (g/100 g feed)							
Protein	32.79±0.16	33.71±0.17	31.86±0.29	31.97±0.23	32.13±0.05	33.97±0.02	33.56±0.23
Lipid							
Ash	9.69±0.04	8.38±0.26	7.45±0.09	7.72±0.31	8.58±0.27	8.13±0.14	7.28±0.27
Moisture	9.24±0.40	8.12±0.13	9.90±0.36	8.73±0.61	9.18±0.03	6.38±0.36	8.29±0.13

[1] Donated from Thai Union Feed Mill Co., Ltd.



(A) (B)

Figure 1. Characteristic of mushroom by-product types; A) *Pleurotussajor-caju* (Fr.) Sing (MBPP), B) *Schizophyllum commune* (MBPS)

protein: 80:20, 60:40, and 40:60. The diets were formulated to be isonitrogenous (32% protein) and isolipid (7% lipid). The formulation and approximate composition analysis of the feeds are shown in Table 1.

Feeding trial

Seven hundred healthy juvenile Nile tilapia were obtained from the private farmer at Phatthalung province, Thailand. Fish were stocked under the 2,000 Liter fiber glass tank with 1,000 Liter of water. Fish were fed 2 times daily with commercial diet for 2 weeks. Prior the feeding trial, similar size of fish were counted to 15 fish per aquarium and acclimatized to the aquarium for one week. Fish were randomly counted and weighted in to the aquarium at the density by ten fish per aquarium with the initial fish weight 7.5 g/fish. They were then randomized into 21 aquariums by triplicate tanks of each diet. The fish were fed respective diets to satiation two times daily at 9:00 am and 5.00 pm for 6 weeks. During the experimental period, the rearing aquaria were cleaned, the dead fish were counted and water exchanged by 50% daily.

Data collection and Statistical analysis

Fish were weighted and counted the remaining fish every two week to monitor the fish growth and survival rate. Consumed of each diet was recorded daily. At the end of feeding experimental period, remaining fish were counted and weighed in the replicate groups. Survival rate, weight gain, specific growth rate, feed conversion ratioand protein efficiency ratiowere calculated by the following equations:

$$\text{Survival rate} = 100 \times \frac{(\text{Initial No.- Final No.})}{\text{Initial No}}$$

$$\text{Weight gain} = \text{final weight (g/fish)- initial weight (g/fish)}$$

Specific growth rate (%/day; SGR) =

$$\frac{(\ln(\text{final body weight}) - \ln(\text{initial body weight}))}{\text{Numbers of feeding period (day)}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Feed consumed (g/fish)}}{\text{Weight gain (g/fish)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g/fish)}}{\text{Protein consumed (g/fish)}}$$

Two way ANOVA was used for evaluation of main effects of MBP types (MBPP and MBPS) and substitution level of fishmeal protein by MBP (20, 40 and 60%). The interaction effects between MBP types and substitution level on survival rate and growth performance were evaluated. Differences between means were determined and compared by Duncan multiple ranged test. The differences were regarded as significantly different at <0.05.

Table 2. Proximate composition of MBP and ingredient incorporated in diet (g/100 g).

Ingredients	**Protein**	**Lipid**	**Moisture**	**Ash**
MBPP	12.08 ± 0.11	1.27±0.26	9.43±0.10	6.24±0.05
MBPS	19.79±0.09	1.39±0.10	7.57±0.05	5.64±0.03
Fishmeal	65.10±0.23	13.45±0.55	5.25±0.02	18.15±0.11
Soybean meal	49.16±0.51	2.30±0.04	10.76±0.04	6.72±13.44
Rice bran	7.17±0.17	12.00±0.25	11.49±0.32	6.56±0.18
Cassava meal	3.45±0.10	0.50±0.00	11.61±0.05	3.96±0.07

Data shown as Mean±SD

Results

Proximate composition of MBP and ingredient were shown in Table 2. Protein content of MBPS was higher than MBPP by 19.79±0.09% and 12.08± 0.11%, respectively. Two-way ANOVA showed that there was no interaction effect between MBP type and substitution level on growth performance and survival rate. However, MBP type affected weight gain, FCR, specific growth rate, PER and survival rate significantly (p<0.05) in that fish fed MBPP diet showed the higher growth performance than those fed MBPS diet at all substitution levels (p<0.05). The substitution level was not affected growth performance (Table 3). Comparing to the control diet fed group, growth performance of those fish fed the diet containing MBPP replacement fishmeal protein at 20-60% and fish fed diet containing MBPS replacement fishmeal protein at 20% was not significantly difference to fish fed control diet (p>0.05) (Table 3). Survival rate was not significantly different among treatment (p>0.05).

Cost of the diet with the different substitution level was reduced with the higher level of MBP protein comparing to control diet. Feed cost for fish production was also affected by the MBP type in that the cost of fish fed MBPP was lower price than the cost of fish obtained MBPS (Table 4).

Table 3. Weight gain, FCR, specific growth rate, PER and survival rate of Nile tilapia after 6 weeksfeeding periodand two way ANOVA of MBP types andsubstitution levels of MBP.

Treatment	Weight gain (g/fish)	SGR (%/day)	PER	Survival rate (%)
1.Control (0% MBP)	9.12±1.32[a]	2.26±0.22[a]	1.60±0.07[a]	80±10[a]
MBPP				
2.MBPP 20%	8.71±0.65[a]	2.21±0.13[a]	1.55±0.17[a]	80±14[a]
3.MBPP 40%	9.06±1.11[a]	2.26±0.20[a]	1.59±0.08[a]	85±7[a]
4.MBPP 60%	8.62±0.36[a]	2.18±0.06[a]	1.43±0.03[a]	75±14[a]
MBPS				
5.MBPS 20%	8.46±1.51[a]	2.13±0.26[a]	1.51±0.23[a]	90±14[a]
6.MBPS 40%	4.89±0.44[b]	1.44±0.09[b]	1.10±0.03[b]	65±7[a]
7.MBPS 60%	5.51±0.99[b]	1.58±0.21[b]	1.14±0.13[b]	65±7[a]
ANOVA (*p*)				
MBP type (T)	0.003	0.002	0.013	0.406
Substitution level (L)	0.088	0.070	0.098	0.317
T * L	0.059	0.057	0.143	0.317
MBP type				
MBPP	8.80±0.63[a]	2.22±0.12[a]	1.52±0.11[a]	78±12[a]
MBPS	6.28±1.90[b]	1.72±0.36[b]	1.25±0.23[b]	73±15[a]

Data shown as Mean±SD with different superscripts for the same row are significantly different ($p<0.05$).

Table 4. Cost of diet, FCR and feed cost for fish production (Baht/ kg fish) of those fed different type and inclusion level of MBP for 6 weeks[1].

Treatment	Cost of diet[2] (Baht/ kg feed)	FCR	Feed cost for fish production (Baht/ kg fish)[3]
1.Control (0% MBP)	18.65	1.91±0.08[a]	35.57±1.58[a]
MBPP			
2.MBPP 20%	17.82	1.92±0.21[a]	34.26±3.72[a]
3.MBPP 40%	16.51	1.98±0.09[a]	32.68±1.55[a]
4.MBPP 60%	14.89	2.19±0.04[a]	32.68±0.66[a]
MBPS			
5.MBPS 20%	17.84	2.09±0.33[a]	37.33±5.82[a]
6.MBPS 40%	17.03	2.69±0.06[b]	45.76±1.10[b]
7.MBPS 60%	16.09	2.62±0.31[b]	42.21±4.96[b]
ANOVA (*p*)			
MBP type (T)	-	0.011	0.005
Substitution level (L)	-	0.070	0.346
T * L	-	0.268	0.246
MBP type			
MBPP	-	2.03±0.17[a]	33.21±2.00[a]
MBPS	-	2.47±0.36[b]	41.77±5.12[b]

[1]Data shown as Mean±SD with different superscripts for the same row are significantly different ($p<0.05$); [2]Feed cost calculated from the price of each ingredient (Baht/kg): fishmeal 45, soybean meal 20, rice bran 10, soybean oil 50, cassava meal 10, vitamin/mineral premix 50, NaCl 10, mono-calcium phosphate 23, rice husk 5 and choline chloride 31.5; [3]Feed cost for fish production (Baht/ kg fish) was calculated by Feed cost * FCR .

Discussion

Protein content of MBPS was higher than MBPP may be due to the MPBS consisting of the higher amount of cap mushroom deterioration than MBPP. Phromkhunthong *et al.*, (2014) revealed that the protein amount of whole mushroom was higher than the stalk of mushroom. Substitution fishmeal protein with different types of MBP and different inclusion level in the diet of Nile tilapia was investigated. The results revealed that there were no interaction effect between MBP type and substitution level on growth and survival rate of fish and the substitution level was not affected the growth performance. Only the MBP type was affected on growth. Fish fed MBPP showed the greater result than fish fed MBPS. This is because of the smell of MBPS was disagreeable smell (Won *et al.*, 2012) resulted in the lower acceptance of those fish fed MBPS than those fed MBPP. In addition, the spitting out of feed was also observed in the high level of MBPS. However, the incorporation of MBPS in the diet at the low level, 20% or 6.55 % by weight , gave the best result of growth while at 40 – 60% replacement led to reduction in both growth and survival rate. MBPP protein can be substituted in the diet at 20-60% of fishmeal protein or 10.78-33.33 % by weight of diet with no significantly different to control diet fed group. Similar phenomena were also reported previously by Phromkunthong *et al.*, (2014) and Muin *et al.*, (2015). Phromkunthong *et al.*, (2014) recommended that the replacement of protein from MBPP for fish meal protein should not higher than 30%. In addition, the best benefit on production cost was 28%. Muin *et al.*, (2015) found that an agriculture waste, mushroom stalk from *Pleurotus sajor caju* can replace fishmeal protein at 33% or 10% by weight of diet with the good growth in Nile tilapia fingerlings.

This investigated found that MBP protein can be substituted fishmeal protein for Nile tilapia production this might because of the amino acid content of MBP was suit for digestibility and metabolism (Chirinang and Intarapichet, 2009). Their quality protein from mushroom such as glutathione, single cell protein and rich amount of essential amino acid are previously described (Mukhopdhay and Guha, 2015). In addition, Bano *et al.*, (1963) concluded that the supplementation of this mushroom in diet would help to overcome lysine deficiency. However, at the higher level of MBP may negative effect on fish growth since the higher content of chitin from cell wall of mushroom (Vetter, 2007) which is difficult to digest by digestive enzyme of fish.

Conclusion

In this study, protein from mushroom by-product can be substituted fish meal protein in the Nile tilapia diet. Type of MBP affected on the growth performance of fish. By-product from *Pleurotussajor-caju* (Fr.) Sing (MBPP) showed the better result of growth performance and production cost from feed to produce fish 1 kg than by-product from *Schizophyllum commune* (MBPS). However, at the low level of MBPS can be substitution in Nile tilapia diet (20%) with no significant difference to control diet fed group. The result indicated that the use of MBPP protein at the level of 20-60 % replacement fishmeal protein or 10.78-32.33% by weight of diet is a potential protein source in Nile tilapia diet.

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