Effects of defatted yellow mealworm (*Tenebrio molitor*) on the feed qualities and the growth performance of largemouth bass (*Micropterus salmoides*)

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Abstract

The aims of the study were to investigate the effects of inclusion of defatted yellow mealworm (*Tenebrio molitor*, YM) replacing fishmeal on the pellet qualities of extruded feed and the growth performance of largemouth bass (*Micropterus salmoides*). Three formulations were used in the experimental diets, with replacing fishmeal at level (wt%): 0% (YM0), 50% (YM50) and 100% (YM100), respectively. Extrusion trials showed that the expansion rate and porosity of the YM50 feed pellets were higher than that of YM0 ($P<0.05$). However, the expansion rate and porosity of the YM100 was significantly lower than that of YM0 ($P<0.05$). Furthermore, the expansion rate, hydration time and hardness of the investigated diets had a strong correlation with the water absorption index and pasting properties of the raw recipe formulations. A total of 300 largemouth bass with about 80 g of initial weight were randomly distributed into 12 tanks and fed with one of the three isonitrogenous and isoenergetic diets for 8-week. The feeding trials revealed that the feed intake of fish in YM50 group was increased, but it did not compromise fish growth performance. However, the YM100 group had a negative impact on the growth performance of the largemouth bass. The apparent digestibility coefficient (ADC_d) and ADC_e in the YM50 group were significantly higher than that of other groups ($P<0.05$). In addition, the hydration time, hardness, expansion rate, microstructure parameters of the investigated diets had a strong correlation with the ADC of nutrients. The present study showed that YM can be used to replace up to 50% of fishmeal in the diet formulation without compromising the qualities of feed and the growth performance of the largemouth bass. But fully replacement negatively affected the qualities of feed and the growth performance of the fish.

Keywords: fishmeal, extruded floating diet, pellet quality of extruded feed, apparent digestibility coefficient

1. Introduction

Fishmeal is one of the most important ingredients for aquatic feeds, as it contains high protein content, rich in amino acid composition, fatty acids, vitamins and minerals. It also has a good palatability and high digestibility for aquatic animals (Hardy, 2010; Miles and Chapman, 2006; Tacon and Metian, 2008). In the aquaculture industry, demand for fishmeal is growing with rapid growth in the industry, but its limited availability is putting enormous pressure on a sustainable supply of fishmeal for the aquafeeds (Biswas *et al.*, 2020; Fawole *et al.*, 2020). At present, the continuous reliance on the limited fishmeal

in the aquaculture industry may not support the current rapid growth for aquaculture development. Therefore, some sustainable and high-quality ‘alternatives’ have been found to reduce environmental impacts and costs associated with aquaculture (Tacon and Metian, 2009). With an increase in global demand for food supply, the competition of some common staple food resources between human and animal production systems has been intensified, and non-grain protein sources for animal feeds have become a research hotspot.

As a new source of non-grain protein, insect protein is rich in amino acids, lipids, vitamins and minerals. It

contains extremely rich crude protein, which similar to the crude protein content of the fishmeal (up to 65%) (Barroso *et al.*, 2014; Rema *et al.*, 2019; Varotto Boccazzi *et al.*, 2017). Moreover, insect protein has relatively low production requirements and has been assessed to have a lower environmental impact than fishmeal (Smetana *et al.*, 2019). Insect farming can use agricultural co-products and waste-streams as nutritional substrates for insect growth. Thereby, insect farming will reduce environmental pollution and greenhouse gas emissions (Le Feon *et al.*, 2019; Van Huis and Oonincx, 2017).

As a fishmeal replacement, Stenberg *et al.* (2019) reported a positive effect on the transcription of antioxidant and stress-related genes in Atlantic salmon leukocytes by replacing fishmeal with black soldier fly in the diet of Atlantic salmon. Henry *et al.* (2018) demonstrated that replacing fishmeal with yellow mealworm (YM) can reduce the inflammatory response in serum in feeding European sea bass, while it enhanced the innate and adaptive immune response to pathogens. The substitution of fishmeal ingredients also has certain challenges in the extrusion processing of feed pellets. The feed pellets obtained from extrusion should have well-defined porosity and expansion rate leading to specific floatability and hydration time (Glencross *et al.*, 2010). Azzollini *et al.* (2018) showed that when the mealworm meal replaced 10% of fishmeal, it significantly improved the micro-structure and expansion rate of the feed pellets. When the replacement was 20%, the feed pellets showed a poor expansion rate, which was mainly due to the fat in the larvae. Irungu *et al.* (2018) showed that insect meal contains more fat and fibre than that of fishmeal, and an increase of fat in an extrusion process may affect the feed pellet expansion, and reduce its floatability.

Most studies are aimed at replacing fish meal with full-fat insect larvae, but with a large negative impact on pellet quality (Azzollini *et al.*, 2018; Weththasinghe *et al.*, 2021). Therefore, in order to overcome the processing challenges, defatted insect protein is more suitable for the production of extruded feed (Ottoni *et al.*, 2018; Rema *et al.*, 2019). Insect protein defatted process mainly includes chemical, physical, or mechanical (Ojha *et al.*, 2021). Different methods have different physiochemical sensorial characteristics and shelf life of YM (Son *et al.*, 2019). Nonetheless, the effect of replacing fishmeal with defatted YM on the physical qualities of extruded pellets and fish performance are still poorly investigated (Ido *et al.*, 2019; Renna *et al.*, 2017).

The aims of the study were to evaluate the effects of defatted YM replacing fishmeal on the physical qualities of extruded floating diet, pellet micro-structure, and growth performance of largemouth bass. It provides an experimental foundation for the application of defatted YM in the aquatic industry. Furthermore, it can also support the

development of aquatic industries that are environmentally friendly.

2. Materials and methods

Raw materials and extrusion process

The defatted YM protein used in this study was produced by Guangdong Zehecheng Biotechnology Co. (Guangdong, China). The microwave-dried YM were defatted by the CO₂ supercritical extraction method to prepare defatted YM protein (Sipponen *et al.*, 2018). The fishmeal was supplied by Tecnológica de Alimentos S.A., Ltd. (Lima, Peru). Fishmeal was produced from anchovy through the cooking-pressing-drying process. The chemical composition and physio-chemical property analysis results of fishmeal and YM are shown in Table 1.

The experimental diets consisted of three formulations with the YM contents at 0% (YM0), 50% (YM50) and 100% (YM100), respectively, all in wt%. The experimental diet formulations are presented in Table 2.

All diets were produced at a pilot plant of the Chinese Academy of Agricultural Sciences using a twin-screw extruder (TSE65, Yanggong, Beijing, China). In the production, the ingredients were mixed in a tank mixer (CH-100, Zhengtai, Zhejiang, China) for 10 min. After adding water (29%, wt) to the diets, the water and diets were allowed to continue mixing for 15 min. Before extrusion processing, the mixed materials need to be left for at least 30 min to allow an even distribution of water into the raw recipe formulations. The extruder has a twin-screw with diameter as 65 mm, and the length to diameter (L/D) ratio is 20:1. The die plate has two circular 3 mm holes with an open area of 14.23 mm². The cutting system was installed in the front of the die plate. The extruder was operated at a constant feed flow rate of 50 kg/h. The parameters of the extrusion process were given in Table 3.

Table 1. Chemical composition (g/kg, dry matter) and physio-chemical properties of fishmeal (FM) and yellow mealworm (YM).

	FM	YM
Moisture (g/kg)	78.0	70.0
Crude protein (g/kg DM)	671	668
Crude lipid (g/kg DM)	98.5	38.0
Ash (g/kg DM)	159	84.6
Chitin (g/kg DM)	ND ¹	146
Water solubility index (%)	3.33	18.1
Nitrogen solubility index (%)	13.6	19.4
¹ ND = not detected.		

Table 2. Formulation and compositions of experimental diets (g/kg).

Ingredients	YM0	YM50	YM100
Fishmeal ¹	485	242	0.00
Yellow mealworm ²	0.00	240	480
Cottonseed protein concentrated ³	100	100	100
Tapioca starch	50.0	50.0	50.0
Wheat flour ⁴	70.0	70.0	70.0
Wheat gluten meal ⁴	50.0	50.0	50.0
Spay-dried blood cell meal	40.0	40.0	40.0
α -cellulose	92.7	71.3	44.4
$\text{Ca}(\text{H}_2\text{PO}_4)_2$	6.00	13.0	25.0
Lecithin oil	20.0	20.0	20.0
Fish oil ⁵	28.1	35.7	43.2
Soybean oil	27.9	35.0	42.0
Kelp powder	15.0	15.0	15.0
Choline chloride	4.00	4.00	4.00
L-Lys.HCl	0.00	0.90	1.70
DL-Met	0.00	1.30	2.40
L-Thr	0.50	1.00	1.50
Vitamin and mineral premix ⁶	9.80	9.80	9.80
Y_2O_3	1.00	1.00	1.00
Total	1000	1000	1000
Nutrient compositions (g/kg, at dry matter basis)			
DM, dry matter (g/kg)	930	929	929
CP, crude protein (g/kg DM)	510	513	516
CL, crude lipid (g/kg DM)	97.1	94.4	118
Ash (g/kg DM)	116	100	88.0
GE, gross energy (MJ/kg)	20.7	21.0	21.0

¹ Fishmeal: anchovy, prime. Tecnológica de Alimentos S.A., Ltd. (Lima, Peru).

² Yellow mealworm: Guangdong Zehecheng Biotechnology Co. (Guangdong, China).

³ Cottonseed protein concentrated: Xinjiang Jinlan Plant Protein Co. Ltd. (Xinjiang, China).

⁴ Wheat flour and wheat gluten meal: Changping Nankou Flour Mill (Beijing, China).

⁵ Containing tertiary butylhydroquinone (200 mg/kg diet) as antioxidant.

⁶ Vitamin and mineral premix (mg/kg diets): Vitamin A 20; Vitamin B₁ 10; Vitamin B₂ 15; Vitamin B₆ 15; Vitamin B₁₂ (1%) 8; Niacinamide 100; Vitamin C phosphate (35%) 1000; Calcium pantothenate 40; Biotin (2%) 2; Folic acid 10; Vitamin E (50%) 400; Vitamin K₃ 20; Vitamin D₃ 10; Inositol 200; Corn protein powder 150; $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ 300; $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ 200; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 100; KI (10%) 80; Na_2SeO_3 (10% Se) 10; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (10% Co) 5; NaCl 100; $\text{MgSO}_4 \cdot 5\text{H}_2\text{O}$ 2,000; Zeolite 5,005.

Table 3. Extruder parameters during the production of experimental diets at three different yellow mealworm (YM) contents.

Processing parameters	YM0	YM50	YM100
Extrusion zone 1 (°C) ¹	100	100	100
Extrusion zone 2 (°C) ¹	100	100	100
Die temperature (°C)	140	140	140
Conditioning water content (wt%) ²	28.0	29.0	29.0
Screw speed (rpm)	166	166	166
Die diameter (mm)	3.00	3.00	3.00
Electric current (Am)	12.0	11.0	15.0
Throughput (kg/h)	47.7	46.9	53.9

¹ Heating the temperature in these parts of the extruder barrel.

² Water added in the mixer prior to extrusion. The water content was the actual measured value.

After holding the extrusion process for approximately 15 min to reach a steady-state, extrudate samples were collected. The extrusion process parameters (i.e. feed flow rate, barrel temperatures in different zones and screw speed) were recorded every 4 s by a computer system. About 10 kg extruded pellets were produced in each batch, three replicates were carried out (with an interval of 5 min between replicates). The pellet samples were forced air-dried at room temperature for 48 h. The samples were well sealed and stored at a temperature of 4 °C for analysis.

Fish experiment and rearing facilities

During the experiment period, all fishes were maintained in compliance with the Laboratory Animal Welfare Guidelines of China (Decree No. 2 of Ministry of Science and Technology, issued in 1988). A total of 300 largemouth bass were purchased from a single size-graded population (Tianjin Yuqing Aquatic Technology Company, Tianjin, China). All fish were acclimated under laboratory conditions and fed with the YM0 diet for 2 weeks, before the start of the trial. Fish (80±0.04 g) were selected after 24 h of starvation and divided into 12 tanks (256 l) with 25 fish per tank and 4 tanks as one trial group. The fish were fed apparent satiation twice a day at 08:00 and 17:00, respectively, for 56 days. The water conditions of the tanks were maintained at 26±2 °C, pH=7.4-7.6, dissolved oxygen >4.0 mg/l, ammonia nitrogen 0.1 mg/l. Each tank was aerated for 24 h/day with a photoperiod of 12 D: 12 L. During the feeding period, feed consumption was calculated by recording the weight of feed fed and the number of residual baits per day.

At the end of the feeding trial, the fish in each tank were weighed in batch after 24 h of starvation. The faeces were collected two weeks after the start of the feeding trial. Y₂O₃ was added as an exogenous marker in the experimental diets at 0.1% (wt%). The fish were collected at the peak faecal discharge period of 4-10 h after feeding, and the faeces were stored frozen at -20 °C, then dried at 70 °C and frozen for chemical analysis.

Physio-chemical properties analysis for raw materials

Water absorption index and water solubility index

The water absorption index (WAI) measures the amount of water absorbed by materials. The water solubility index (WSI) is related to the quantity of water-soluble molecules. The WAI and WSI were determined by Anderson (1982) according to the following method. The ingredients and raw recipe formulations were crushed and passed through a 70-mesh sieve. The crushed samples (2.5 g) were suspended in distilled water (30 ml) and placed in a 50 ml centrifuge tube. The suspension was stirred intermittently and centrifuged at 3,000×g for 10 min. The supernatant was poured into an

aluminium cup and dried at 135 °C for 2.5 h. The weight of the remaining gel in the centrifuge tube was measured, and the WAI and WSI were calculated as follows:

$$\text{WAI (\%)} = \frac{W_g - W_{ds}}{W_{ds}} \times 100 \quad (1)$$

$$\text{WSI (\%)} = \frac{W_{ss}}{W_{ds}} \times 100 \quad (2)$$

where W_g is the weight of colloid in the centrifuge tube after centrifugation (g), W_{ds} is the weight of sample (g), W_{ss} is the weight of dry matter in the Petri dish after drying (g).

Nitrogen solubility index

The nitrogen solubility index (NSI) was determined in three replicates according to the AACC approved method 46-23 with minor modifications (AACC, 1995). The samples (1 g) were weighed into 50 ml centrifuge tubes and added 30 ml of water, mechanically shaken at room temperature for 30 min and then centrifuged at 3,000×g for 10 min. The supernatant was collected and analysed for nitrogen by the standard Kjeldahl method in combination with the supernatant. NSI is the percentage of the measured nitrogen content divided by the protein content of the ingredients and raw recipe formulations.

Pasting properties (peak-viscous and final-viscous)

We used the method modified by Ragaee *et al.* (2006) to determine the pasting properties. The pasting properties of the raw recipe formulations were measured using a Rapid Visco-Analyzer (RVA, Perten, NSW, Australia). 5 g raw material sample and 25 ml (plus moisture adjustment) of distilled water were added to the sample canister and shaken for 5 s to disperse the sample. The Standard 1 temperature profile was used for the test procedure. Each treatment was tested in triplicate.

Oil absorption capacity

2.5 g of sample raw recipe formulations was weighed into a 50 ml centrifuge tube and mixed well with 30 ml of soybean oil. The mixture was centrifuged at 3,000×g for 10 min at 4 °C. The excess oil was removed after centrifugation and weighed (Samuelsen *et al.*, 2014). The oil absorption formula was calculated as follows.

$$\text{OAC(\%)} = \frac{W_g - W_{ds}}{W_{ds}} \times 100 \quad (3)$$

where W_g is the weight of colloid in the centrifuge tube after centrifugation (g); W_{ds} is the weight of dry matter sample (g).

Physical qualities of extruded floating diet analysis

Bulk density (BD)

The standard bulk density instrument (GHCS-1,000 series, Hangzhou Maizhe Instrument Co., Ltd., Zhejiang, China) with a capacity of 1 litre filled with feed pellets was weighted. The measurement was run in triplicate and reported as the average.

Water stability

The method modified by Baeverfjord *et al.* (2006) was used to determine the water solubility/stability. 10 g feed pellets samples (M_1 , accurate to 0.1 g) were placed in a 500 ml glass beaker in a steel-mesh cage and filled with water to a depth of 5.5 cm. The beaker was shaken in a water bath at 25 °C for 20 min and then dried at 135 °C for 2 h. The weight (M_2) of the pellets was measured after drying. The water stability (WS, %) was calculated as:

$$WS(\%) = \frac{M_1 \times (1-x) - M_2}{M_1 \times (1-x)} \times 100 \quad (4)$$

where M_1 is the weight of the feed pellets before the test; where M_2 is the weight of the feed pellet after drying; where x is the water content of the sample before measurement (as a weight percentage), measured by standard method.

Three replicates were taken for each group of samples and the average value was used as the result.

Oil absorption capacity and oil leakage

The oil absorption capacity (OAC) measures the amount of oil absorbed by feed pellets. The oil leakage (OL) reflects the ability of pellets to store oil. As described by Samuelsen and Oterhals (2016), a 100 g (A_1) pellet sample was weighed and placed in the measuring cup of a laboratory vacuum sprayer, which was subsequently emptied of air. Excess soybean oil was sprayed to the measuring cup and mixed followed by a slow release of air over a period of 3 min. The pellets were removed from the measuring cup. Excess oil was removed by lightly gently compressing the pellets with oil-absorbing wipes. The weight of the pellets was recorded (B_1).

Oil leakage was measured in samples from the oil absorption capacity analysis. The samples were placed on absorbent paper and stored at 40 °C for 24 h. Record the weight of the sample (B_2) and calculate the amount of oil leakage.

Three replicates were taken for each group of samples and the average value was used as the result. The Oil absorption capacity and oil leakage were calculated according to the following formula.

$$OAC(\%) = \frac{B_1 - A_1}{A_1} \times 100 \quad (5)$$

$$OL(\%) = \frac{B_2 - B_1}{A_1} \times 100 \quad (6)$$

Pellet hardness

Pellet hardness was measured using a Texture-Analyzer (TA-XT2, Model 1000 R, Stable Micro Systems, Blackdown Rural Industries, Surrey, UK), fitted with a 25 kg load cell and a principal component-operated remote control. The test probe (P10, 10 mm) was axially pressed on the pellet at a constant speed (0.8 mm/s) to achieve 60% compression. The major break of the pellet (the peak force) was measured and presented in Newton (N). Measurements were conducted for 20 pellets from each batch of the feed pellet samples and reported as the average (Samuelsen and Oterhals, 2016).

Expansion rate

Twenty pellets were randomly selected from each batch of samples. Their diameters were measured with a digital caliper. The average value of the ratio of the pellet diameter to the die hole diameter (3.0 mm) was taken as the expansion rate (Umar *et al.*, 2013).

Hydration time

According to a method of Wang *et al.* (2021), 150 pellets as one sample batch were placed in a 500 ml beaker containing 400 ml of distilled water at room temperature. Starting count time, two pellets were removed from the beaker every minute and cut to observe their cross-sections. When the pellet cross-section is completely soaked with water, and no hardcore, the time was recorded as the hydration time of the pellet. Three replicates from each batch of feed pellet samples were measured and reported as mean values.

Floatability

No less than 150 pellets were randomly selected and placed in a 500 ml beaker, containing 400 ml of distilled water at room temperature. After 30 minutes, the number of pellets floating on the surface of the water was recorded, with three replicates for each sample. Floatability (%) was calculated as: $N_1 / N \times 100$. Where N_1 is the number of floating pellets after 30 minutes; N is the total number of pellets in the water (Khater *et al.*, 2014).

Pellet durability index

The measurement was performed using a standard method ASAE S269.4 (Mina-Boac *et al.*, 2006). A 500 g pellet sample was placed in a pellet durability tester (ST136, Shengtai, Jinan, China). The equipment was run for 10 min. The sample was removed, sieved (i.e. greater than 5.60 mm), and the remaining pellet was weighed. The pellet durability

index (%) was calculated as: $Z_1 / Z_0 \times 100$. Where Z_1 is the weight of feed pellet after the test; Z_0 is the weight of the feed pellet before the test.

Micro-structure

The micro-structure characteristics of the feed pellets were analysed by scanning electron microscopy (SU3500, Hitachi, Tokyo, Japan). Feed pellets were cut with a blade and adhered to a holder using carbon adhesive tabs, and then gold sprayed with a vacuum ion jet (JEOL FineCoat JFC-1100, Tokyo, Japan) for 45 s. In the high vacuum mode, the gold-sprayed sample was observed with a Scanning electron microscopy at a voltage of 3 kV. The micro-structure parameters such as pore area and total porosity of the feed pellets were analysed using Image J software provided by the National Institutes of Health, Washington DC, USA (Ahmed *et al.*, 2010; Banjac *et al.*, 2021). Three replicates from each batch of feed pellet samples were measured.

Chemical analysis

The dry matter of diets and faeces were analysed by drying the samples at 105 °C to a constant weight. Crude protein was determined by Kjeldahl method using a Kjeldahl nitrogen tester (K1100F, Hanon, Shandong, China) (AOAC, 2006). The crude fat diets and faeces were acid hydrolysed with Soxhlet system HT 1047 hydrolysis unit followed by extraction of crude fat with Soxhlet system 1043 (Foss, Hillerød, Denmark). The ash of diets was analysed by combustion in a muffle furnace (CWF1100, Carbolite, Derbyshire, UK) at 550 °C for 3 h. The gross energy of the diets and faeces were determined using an IKAC2000 calorimeter (C2000, IKA, Staufen, Germany). The inert marker (Y_2O_3) of the diets and faeces was analysed using inductively coupled plasma atomic emission spectrometry at Guobiao (Beijing) Testing & Certification Co., Ltd. (Beijing, China).

Statistical analysis

The experimental data were expressed as the mean $\pm$ standard deviation (SD). All data were analysed by SPSS 22 software (IBM Corp., Armonk, NY, USA). Data were checked for normality and homogeneity of variances, followed by ANOVA or Kruskal-Wallis test when appropriate. The significant data were compared by Duncan's method for comparison of means, with differences considered significant at $P < 0.05$. Linear and quadratic polynomial comparisons were used to evaluate the relationship between different levels of YM replacement of fishmeal. The correlation between the expansion rate and water stability of extruded pellets with physio-chemical properties of the raw recipe formulations were analysed using the software OriginPro 2021 (OriginLab Corporation, Northampton, MA, USA).

3. Results

Physio-chemical properties of the raw recipe formulations

The physio-chemical properties of the raw recipe formulations are presented in Table 4. Table 4 shows that there was a significant difference in the water absorption index between the groups ($P < 0.05$), the raw recipe formulation of YM50 had the highest value, and the YM100 had the lowest values. The water solubility index in the raw recipe formulation of YM50 was significantly higher than YM0 ($P < 0.05$). The nitrogen solubility index in the diets of YM50 and YM100 were significantly higher than the YM0 group ($P < 0.05$). As the level of YM increased, the peak-viscous increased from 269 to 408 cp, and the final-viscous increased from 299 to 324 cp. There were no significant differences in the oil absorption capacity between the diets ($P < 0.05$).

Table 4. Physio-chemical properties of the raw recipe formulations at three different yellow mealworm (YM) contents (means $\pm$ SD, n=3).¹

	YM0	YM50	YM100
Water absorption index (%)	147 $\pm$ 0.8 ^b	152 $\pm$ 1.1 ^c	143 $\pm$ 0.2 ^a
Water solubility index (%)	5.85 $\pm$ 0.4 ^a	8.75 $\pm$ 0.5 ^b	7.08 $\pm$ 0.9 ^{ab}
Nitrogen solubility index (%)	14.4 $\pm$ 0.1 ^a	14.8 $\pm$ 0.1 ^b	14.8 $\pm$ 0.2 ^b
Oil absorption capacity (%)	87.6 $\pm$ 1.7	86.3 $\pm$ 2.2	87.4 $\pm$ 3.7
Peak-viscous (cp)	269 $\pm$ 4.0 ^a	339 $\pm$ 3.8 ^b	408 $\pm$ 6.2 ^c
Final-viscous (cp)	299 $\pm$ 5.4 ^a	304 $\pm$ 4.5 ^{ab}	324 $\pm$ 2.7 ^b

¹ Values (mean $\pm$ SD) in the same row with different superscript letters are significantly different ($P < 0.05$).

Physical qualities of extruded floating diet

The physical qualities of the extruded floating diet with an increased level of YM are presented in Table 5. The data in the Table 5 indicate that the bulk density, hardness, and hydration time of YM0 and YM50 diets were significantly lower than the diet YM100 ($P<0.05$). And the oil absorption capacity of YM0 and YM50 diets were significantly higher than the diet YM100 ($P<0.05$). There were significant differences in the expansion rate of the feed qualities between the groups ($P<0.05$), the diet YM50 had the highest value, and the diet YM100 had the lowest values. There was no significant difference in floatability between the groups ($P>0.05$), and the floatability was 100% in all diets.

Micro-structure of feed pellets

The micro-structure of feed pellet pore and quantitative micro-structural parameters of the feed pellets are presented in Figure 1A and Table 6, respectively. Figure 1A clearly shows the differences in micrographs of feed pore size and porosity with the addition of YM. In Figure 1A, the red areas represent the void pores, whereas the continuous solid matrix, mainly the pore walls is gray (non-pores). The yellow areas represent the shape edge of void pores. The feed pellet of diet YM50 was the largest and most aerated one, followed by YM0. The diet YM100 reduced the pore area and overall porosity of the feed pellets. Table 6 clearly shows the differences in microstructural parameters between the three diets. The pore area and total porosity of the diet YM50 were obviously higher than that of YM0 and YM100 ($P<0.05$). There was a significant difference in the pore counts for the three diets ($P<0.05$), the YM0 diet had the highest value, and the YM100 had the lowest value. Furthermore, an increase of the YM content in diets had a strong quadratic relationship with the pore area and porosity of the feed pellets ($P<0.01$). The pore counts are linearly decreased with increasing YM content in diets ($P<0.01$).

The pore size distribution of the feed pellets is presented in Figure 1B. It shows that the diet YM0, YM50, and YM100 have a pore area between 0.009–0.091 mm², 0.018–0.3288 mm², and 0.045–0.4725 mm², respectively. Similarly, the pore area of the diets increases with an increase of YM content in diet formulations.

Growth performance and apparent digestibility coefficients

The growth performance of the largemouth bass is given in Table 7, where 3 different diets with 3 levels of YM contents were applied. The final body weight (FBW) and specific growth rate (SGR) of the YM0 and YM 50 groups were significantly higher than that YM100 group ($P<0.05$). There was a significant difference in the feed conversion ratio (FCR) between the groups ($P<0.05$), the YM100 group had the highest value, and the YM0 group had the lowest value. According to ANOVA, there were no differences in FI between the three groups ($P>0.05$).

The apparent digestibility coefficients (ADC) of nutrients in the largemouth bass are given in Table 7. The ADC_p and ADC_l of the YM0 and YM50 groups were obviously higher than that of the YM0 group ($P<0.05$). The ADC_d and ADC_e of the YM50 group were obviously higher than that of the YM0 and YM100 groups ($P<0.05$).

According to the linear polynomial contrasts, the increase in the levels of YM in diets has a strong linear correlation with the FBW, SGR, and FCR of the largemouth bass ($P<0.05$). An increase of YM content in diets had a significant linear relationship with the ADC_d and ADC_e ($P<0.01$). Furthermore, a strong quadratic relationship was found between dietary YM levels and ADC_d ($P<0.01$). The Linear and quadratic relationships were observed between dietary YM levels and ADC_l ($P<0.01$).

Table 5. Physical qualities of extruded floating diet at three different yellow mealworm (YM) contents (means $\pm$ SD, n=3).¹

	YM0	YM50	YM100
Bulk density (g/l)	374 $\pm$ 1.3 ^a	366 $\pm$ 0.3 ^a	431 $\pm$ 0.3 ^b
Water stability (%)	4.10 $\pm$ 0.1 ^a	4.76 $\pm$ 0.1 ^b	4.54 $\pm$ 0.1 ^b
Oil absorption capacity (%)	64.1 $\pm$ 0.8 ^b	65.4 $\pm$ 1.0 ^b	48.2 $\pm$ 0.0 ^a
Oil leakage (%)	11.13 $\pm$ 0.0 ^b	10.92 $\pm$ 0.1 ^b	9.94 $\pm$ 0.1 ^a
Hardness (N)	86.4 $\pm$ 2.6 ^a	93.3 $\pm$ 4.6 ^a	129 $\pm$ 3.6 ^b
Expansion rate (%)	1.36 $\pm$ 0.0 ^b	1.39 $\pm$ 0.0 ^c	1.26 $\pm$ 0.0 ^a
Hydration time (min)	41.9 $\pm$ 0.7 ^a	42.2 $\pm$ 0.7 ^a	59.6 $\pm$ 0.1 ^b
Floatability (%)	100 $\pm$ 0.0	100 $\pm$ 0.0	100 $\pm$ 0.0
Pellet durability index (%)	98.6 $\pm$ 0.1 ^a	98.5 $\pm$ 0.0 ^a	98.7 $\pm$ 0.0 ^b

¹ Values (mean $\pm$ SD) in the same row with different superscript letters are significantly different ($P<0.05$).

Figure 1. Microstructure of feed pellets. (A) microscopy image feed pellets at three different yellow mealworm (YM) contents. YM substituted different levels of fishmeal 0% (YM0); 50% (YM50); 100% (YM100). **(B)** pore size distribution of the feed pellets (average from three replicates) with increased level of YM. The microstructural parameters such as pore area and total porosity of feed pellets were analysed by ImageJ software, and then the plots were represented by the software OriginPro 2021 software. The red areas represent the void pores. The pore walls is gray (non-pores). The yellow areas represent the shape edge of void pores.

Table 6. Scanning electron microscopy results for the microstructural parameters of feed pellets at three different yellow mealworm (YM) contents (means $\pm$ SD, n=3).^{1,2}

	YM0	YM50	YM100	P_{ANOVA}	P_{linear}	P_{quad}
Pore area (mm ²) ³	4.01 $\pm$ 0.1 ^a	5.69 $\pm$ 0.0 ^b	3.96 $\pm$ 0.1 ^a	0.000	0.696	0.000
Total porosity (%) ⁴	35.5 $\pm$ 0.9 ^a	38.1 $\pm$ 0.3 ^b	33.5 $\pm$ 0.7 ^a	0.009	0.081	0.005
Pore counts ⁵	749 $\pm$ 2.6 ^c	597 $\pm$ 9.1 ^b	339 $\pm$ 31.0 ^a	0.000	0.000	0.058

¹ Values (mean $\pm$ SD) in the same row with different superscript letters are significantly different ($P<0.05$).
² P_{ANOVA} = P -value of one-way ANOVA; P_{linear} and P_{quad} are the P -values of the linear and quadratic components of the polynomial comparison analysis of each parameter with increased level of YM.
³ Pore area (mm²): the area of pores in feed pellets.
⁴ Total porosity (%): the percentage of the pore area divided by the total area of the feed pellets.
⁵ Pore counts: the number of pores in the feed pellets.

Correlation analysis
(physio-chemical properties of the raw recipe formulations, physical qualities of extruded floating diet, micro-structure of feed pellets and nutritional data)

The correlation analysis between the physical qualities of extruded floating diet with physio-chemical properties of the raw recipe formulations are presented in Figure 2A and Table S1. Both clearly show that the WAI was significantly positively correlated with the expansion rate, pore area

and total porosity ($P<0.05$), and negatively correlated with hydration time and hardness ($P<0.05$). The WSI of the raw recipe formulations had a strong positive correlation with the water stability and pore area of the diets ($P<0.05$). The Pasting properties (PV and FV) of the raw recipe formulations had a strong positive correlation with the hydration time and hardness of the diets ($P<0.05$), and negatively correlated with expansion rate and total porosity ($P<0.05$).

Table 7. Growth performance and apparent digestibility coefficient (ADC) of nutrients (%) in largemouth bass, fed by experimental diets at three different yellow mealworm (YM) contents (means $\pm$ SD, n=4).^{1,2}

	YM0	YM50	YM100	P_{ANOVA}	P_{linear}	P_{quad}
Growth performance						
Final body weight (FBW) (g) ³	179 $\pm$ 3.6 ^b	169 $\pm$ 2.9 ^b	143 $\pm$ 4.7 ^a	0.00	0.000	0.131
Specific growth rate (SGR) (%/day) ⁴	1.44 $\pm$ 0.0 ^b	1.34 $\pm$ 0.0 ^b	1.08 $\pm$ 0.1 ^a	0.00	0.000	0.101
Feed intake (FI, %BW / d) (%) ⁵	1.52 $\pm$ 0.1	1.59 $\pm$ 0.1	1.51 $\pm$ 0.0	0.398	0.877	0.189
Feed conversion ratio (FCR) ⁶	1.10 $\pm$ 0.0 ^a	1.22 $\pm$ 0.0 ^b	1.44 $\pm$ 0.0 ^c	0.00	0.000	0.169
ADC of nutrients						
ADC _d (%) ⁷	71.4 $\pm$ 0.5 ^a	74.3 $\pm$ 0.4 ^b	72.1 $\pm$ 0.7 ^a	0.011	0.400	0.004
ADC _p (%) ⁸	88.1 $\pm$ 0.2 ^b	88.1 $\pm$ 0.1 ^b	86.1 $\pm$ 0.7 ^a	0.011	0.008	0.071
ADC _e (%) ⁹	77.4 $\pm$ 0.6 ^a	79.3 $\pm$ 0.2 ^b	76.6 $\pm$ 0.7 ^a	0.012	0.317	0.005
ADC _l (%) ¹⁰	95.0 $\pm$ 0.4 ^b	94.5 $\pm$ 0.3 ^b	89.0 $\pm$ 0.5 ^a	0.000	0.000	0.001

¹ Values (mean $\pm$ SD) in the same row with different superscript letters are significantly different ($P < 0.05$).

² P_{ANOVA} = P -value of one-way ANOVA; P_{linear} and P_{quad} are the P -values of the linear and quadratic components of the polynomial comparison analysis of each parameter with increased level of YM.

³ FBW: final body weight;

⁴ SGR (specific growth rate, %) = $100 \times [\ln (\text{FBW}/\text{initial body weight})] / \text{days}$.

⁵ FI (feed intake, % body weight/day) = $100 \times \text{total feed consumption (g)} / [(W_i + W_f + W_d) / 2] / \text{days}$.

⁶ FCR (feed conversion ratio) = feed intake / $(W_f + W_d - W_i)$. W_f is the final total weight; W_d is the total weight of dead fish; W_i is the initial total weight; n=4.

⁷ ADC of DM (ADC_d) (%) = $(1 - D_B / D_B') \times 100\%$, D_B is the content of marker in feed, D_B' is the content of marker in faeces.

⁸ ADC of CP (ADC_p) (%) = $[1 - (C_A' / C_A \times C_B / C_B')] \times 100\%$. C_A' is the protein content of the feed, C_A is the amount of protein in the faeces, C_B is the content of marker in feed, C_B' is the content of marker in faeces.

⁹ ADC of GE (ADC_e) [1 - $(G_A' / G_A \times G_B / G_B') \times 100\%$. E_A' is the energy content of the feed, E_A is the amount of energy in the faeces, E_B is the content of marker in feed, E_B' is the content of marker in faeces.

¹⁰ ADC of CL (ADC_l) (%) = $[1 - (L_A' / L_A \times L_B / L_B')] \times 100\%$. F_A' is the fat content of the feed, F_A is the amount of fat in the faeces, F_B is the content of marker in feed, F_B' is the content of marker in faeces.

Figure 2. Correlation analysis (A) the correlation analysis between the physical qualities of extruded floating diet with physico-chemical properties of the raw recipe formulations; (B) the correlation analysis between the physical qualities of extruded floating diet with apparent digestibility coefficient. ADC_d = ADC of DM; ADC_e = ADC of GE; ADC_l = ADC of CL; ADC_p = ADC of CP; ER = expansion rate; FV = final-viscous; H = hardness; HT = hydration time; NSI = nitrogen solubility index; OAC = oil absorption capacity; PA = pore area; PV = peak-viscous; TP = total porosity; WAI = water absorption index; WS = water stability; WSI = water solubility index. The white symbols * represent a significant correlation ($P < 0.05$). The white areas represent an uncorrelated relationship. The red areas represent the positive relationship, and the blue areas represent the negative relationship.

The correlation analysis between the physical qualities of extruded floating diet with apparent digestibility coefficient are presented in Figure 2B and Table S2. Both obviously show that the hydration time and hardness were significantly negatively correlated with the ADC_p and ADC_l ($P < 0.05$). The expansion rate of the diets had a strong positive correlation with the ADC_p and ADC_l ($P < 0.05$). The pore area of the diets had a strong positive correlation with the ADC_e and ADC_d ($P < 0.05$). The total porosity of the diets had a strong positive correlation with the ADC_p , ADC_e and ADC_l ($P < 0.05$).

4. Discussion

Physical qualities and micro-structure of feed pellets

From Table 5, it can be seen that the expansion rate and oil absorption capacity of diet YM50 were increased. However, the expansion rate of diet YM100 decreased. The correlation analysis results in Figure 2 show that the WAI has a strong positive correlation with the expansion rate and a strong negative correlation with the hydration time and hardness. However, the correlation between viscosity and qualities of feed pellets (expansion rate, hydration time and hardness) is exactly the opposite of the above. In this study, the raw recipe formulation of YM50 has a higher WAI than that of other groups, while the raw recipe formulation of YM100 shows higher pasting properties (Table 4). The WAI indicates the ability to retain the water of the diets, reflecting the water binding ability of the material (Lourenco *et al.*, 2016). As a universal plasticiser in foods, water can improve processability, cooking efficiency in the extrusion process, and final product performance of feed pellets (Hudson and Daubert, 2002; Oterhals and Samuelsen, 2015; Samuelsen *et al.*, 2013). Typically, a plasticiser may increase molecular mobility and reduce the glass and the melt transition temperature of biopolymers (Abiad *et al.*, 2009; Di Gioia and Guilbert, 1999; Igura *et al.*, 1997). Therefore, the raw recipe formulation of YM50 has a higher WAI, which means that it has increased hydration with water. This can also explain the high qualities of YM50 diet. However, the reason for the high WAI of YM50 will continue to be observed in the next phase of molecular structure research. In the aspect of raw material pasting properties, studies have shown that excessive softening of the melt limits the expansion of the material (Amaya-Llano *et al.*, 2007; Cha *et al.*, 2001; Chanvrier *et al.*, 2007). At the same time, the high viscosity of the melt increases specific mechanical energy and the roughness of the surface of the pellet (Draganovic *et al.*, 2014). Singh *et al.* (2014) shown that the decrease in pasting properties, thus favouring the formation and maintenance of melt pore bubbles, increases the floatation rate. From the trial results, the pasting properties increased with the increase of YM contents in the diets. The high pasting properties of the YM100 raw recipe formulation led to poor qualities of

pellet results. Under the same processing conditions with the same moisture content, the raw recipe formulation of the YM100 group has a higher viscosity than the other groups. This phenomenon can be balanced by increasing the moisture content (Draganovic *et al.*, 2011, 2014).

The micro-structure of the pellets can reflect the extrusion effect of the pellets. The extrusion feed pellets with a high expansion rate usually have high pore porosity. The number and area of pores on the pellets directly affect the floatability of the pellets. (Alcaraz *et al.*, 2021; De Cruz *et al.*, 2015). Table 6 indicates that the pore area and total porosity of the diet YM50 are significantly higher than those of the YM0 and YM100. Thus, the YM50 has the best pore micro-structure, which is characterised by high porosity and pore area. As can be seen from Figure 1 the diet YM0 has a higher number of pores and a smaller average pore size, while the diet YM100 has a lower number of pores and a larger average pore area. This is consistent with the data of bulk density and expansion rate. As with the physical qualities of feed, it may also be due to the high WAI and suitable pasting properties of YM50 which is conducive to the production of high-quality pore structures. However, the high pasting properties of YM100 make the extrudate a more rigid structure, thereby limiting the generation of pore structure (Lazou and Krokida, 2010). In the production of aquatic extruded floating feeds, oil in the formula is added mostly by spraying after vacuum. Therefore, the OAC and OL are important indicators of evaluation for floating extruded feed. The micro-structure such as the evenness of the outer surface and the permeability of the outer layer of the pellets play a fundamental role in the oil-infiltration pattern (Bouchon and Pyle, 2004). In this study, the oil absorption capacity of the diets YM0 and YM50 was higher than that of the YM100, and the oil leakage rate of diet YM100 was the lowest value. The diet YM50 has the best total porosity and expansion rate to provide the greatest oil absorption rate, which is similar to the results of Glencross *et al.* (2010). The YM100 group has less porosity and poor interconnection (thick pore walls) between the pores, resulting in a reduction in oil absorption, thereby reducing oil leakage (Alcaraz *et al.*, 2021). Once the oil is injected into the ball with a vacuum, the lower pore interconnectivity and less fragmented structure also hinder fat leakage (Draganovic *et al.*, 2013).

Growth performance and apparent digestibility coefficients

From Table 7, the growth performance of the YM50 group were not significantly different compared to the YM0 group. In addition, the YM100 group had a negative impact on the growth performance of the largemouth bass. The reasonable level of fishmeal replacement with YM depends on the different species and the growth stages of the experimental subjects according to their different

nutritional requirements (Kim *et al.*, 1991; Rahimnejad *et al.*, 2015). Gasco *et al.* (2016) found that the replacement of 25% of fishmeal with YM in the diets of European sea bass did not adversely affect its growth performance, but the growth performance was compromised when YM replacement levels reached 50%. While in rainbow trout diets, replacing 25-50% of fishmeal with YM could improve the growth performance of the rainbow trout (Belforti *et al.*, 2015). However, complete substitute of fishmeal with YM would reduce the WG of common catfish (Roncarati *et al.*, 2015). The present study results are in agreement with the results of Roncarati *et al.* (2015). The YM50 group does not compromise the fish growth performance. However, the YM100 group has a negative impact on the growth performance of the largemouth bass. The YM may be a potential alternative source of fishmeal in terms of its positive effect on the growth performance of fish. This may contribute to the composition of YM, whose protein content and limited amino acids similar to fishmeal (Henry *et al.*, 2015). Meanwhile, chitin in insect epidermis was present in a matrix containing proteins, lipids, and other compounds (Chapman, 1998; Kramer *et al.*, 1995; Tharanathan and Kittur, 2003). Previous studies reported that a reduction in the SGR of juvenile fish, where feeding diets containing black soldiers fly with chitin (1.6-7%) (Kroeckel *et al.*, 2012). Shiao and Yu (1999) also reported that diets contain 2-10% (wt) chitin resulted in a reduced weight gain in tilapia. In the study (Table 1), the chitin content in YM was 14% (wt, based on the dry matter). Such high chitin content in the YM100 group may be the main reason for the lower FCR in the growth performance of the largemouth bass, which is similar to the results of Roncarati *et al.* (2015).

In the study, Table 7 shows that the nutrient digestibility (crude protein, crude lipid and gross energy) of the YM100 group were lower than those of the YM0 and YM50 groups, which may be due to its excessive content of chitin in YM ingredient. Chitinases hydrolyse the polymer chitin to the level of disaccharides and trisaccharides, which in turn are broken down by chitinases to the sugar monomer β -N-acetyl-D-glucosamine (Danulat, 1987; Jeuniaux, 1963). Although, the reported chitinases are mainly present in the stomach and gut contents of fish that are chronic predators of crustaceans, degradation by chitinases appears to be limited in most fish (Kroeckel *et al.*, 2012; Magalhães *et al.*, 2017). Meanwhile, indigestible chitin may reduce the acquisition of digestive enzymes, thereby interfere with and reduce nutrients ADC (Henry *et al.*, 2015). Studies have shown that feeds containing chitin reduce nutrient digestibility in Atlantic salmon (Hansen *et al.*, 2010; Karlsen *et al.*, 2017). A study by Kroeckel *et al.* (2012) showed that juvenile turbot diets containing 33% defatted black soldier fly larval meal as a fishmeal substitute results in lower apparent digestibility of the fish. Some investigations also reported that chitin tends to reduce the water-holding capacity of digesta, increase faecal bulking and reduce

intestinal transit time, thereby hardening the action of digestive enzymes, while nutrients such as amino acids bound to chitin caused its digestive capacity to be weakened (Dias *et al.*, 1998; Finke, 2007; Henry *et al.*, 2015; Magalhães *et al.*, 2017; Piccolo *et al.*, 2017). In our study, the ADC_p , ADC_d , ADC_e , and ADC_l in the YM50 group were higher than those in the YM0 and YM100 groups. As shown in Figure 1, in the micro-structure of the YM50 diet, the pore area and porosity were the highest. The micro-structure of extruded pellet feed was also a key factor affecting digestibility. This is also consistent with the results of the correlation analysis between micro-structure of feed pellets and ADC of nutrients in Figure 2B. Chanvrier *et al.* (2007) hypothesised that the micro-structure might govern the accessibility of digestive enzymes to macromolecules, thereby controlling their digestion. The study of Azzollini *et al.* (2018) studied that due to high porosity and small wall thickness, the YM addition levels of 0% and 10% groups produced a high surface contact area and consequently higher diffusion of enzymes. In contrast, it also observed that the lower digestibility of the pellets obtained with the addition of 20% of YM might be due to higher compactness, which leads to a decrease in enzyme accessibility (Azzollini *et al.*, 2018). The lower porosity and thicker pore walls of the micro-structure of the YM100 group in this study might also be one of the important reasons affecting the digestibility, which is a match with the observation of Karkle *et al.* (2012). The correlation analysis of this study shows that hydration time and hardness was significantly negatively correlated with ADC_p and ADC_l . This may be because the feed with high hardness and hydration time makes the diffusion of digestive enzymes into its interior slower, hindering the gastric emptying, leaching loss, and non-synchronous absorption of pellet feed (Wei *et al.*, 2019; Zhang *et al.*, 2019). It has also been reported that the increase in pore cell wall and the decrease in porosity will increase the hardness and hydration time of the pellets (Ahmad *et al.*, 2019; Luo *et al.*, 2020). This is also consistent with the trend of microstructure and digestibility analysis. In addition, from Table 1, the WSI of YM was higher than that of fish meal, and the increase of WSI may increase the digestibility of nutrients. It may also be an important reason for the higher digestibility of the YM50 group, which is similar to the results of Irungu *et al.* (2018).

Based on the results of growth performance and digestibility, the digestibility of the YM50 group was even slightly higher than that of the YM0 group, and without significant difference in feed intake were observed. However, the growth performance of the YM50 group were still lower, which may be due to that insect contain antimicrobial peptides (AMPs). Wang *et al.* (2021) studied that high dose of AMPs may not improve growth performance, but rather act inhibit the growth of fish. It may be related to the energy over-consumption for immunostimulation (Klasing, 2007; Liu *et al.*, 2020; Ming *et al.*, 2020). Liu *et al.* (2020) reported

that 400 mg/kg AMPs synthesised by genetic engineering inhibited the growth performance of grass carp, which with a highest immunity response simultaneously. Whether the decrease in growth performance in this trial was related to the antimicrobial peptide needs further study.

5. Conclusion

The study showed that the defatted YM can replace up to 50% of dietary fishmeal in diet formulation without compromising the growth performance or nutrient digestibility of the largemouth bass. Meanwhile, it can improve the expansion rate of the extruded floating diet. At 100% YM replacement for fishmeal, the physical qualities of the extruded floating diet and the growth performance were reduced. It has been demonstrated from the trials that in defatted YM, the expansion rate, hydration time, and hardness of the investigated diets had a strong correlation with the WAI and pasting properties of the raw recipe formulations, and a strong correlation with apparent digestibility coefficient of the fish.

Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/JIFF2021.0125>

Table S1. The correlation analysis between the physical qualities of extruded floating diet with physio-chemical properties of the raw recipe formulations.

Table S2. The correlation analysis between the physical qualities of extruded floating diet with nutritional data.

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Conflict of interest

The authors declare no conflict of interest.

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