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Replacement of fishmeal by yellow mealworm meal on the growth performance, feed utilisation and quality of large yellow croaker

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RESEARCH ARTICLE

Abstract

An 80-day feeding trial was conducted to evaluate the effects of yellow mealworm (*Tenebrio molitor*, TM) meal as substitute for dietary fishmeal on the growth performance, feed utilisation and flesh quality of large yellow croaker (initial body weight: 189.18±0.13 g). The control diet (TM0) was designed to contain 56% of fishmeal. Based on the TM0, graded levels of TM meal (15, 30, 45, 60, 75 and 100%, respectively) were used to replace fishmeal to formulate the other six experimental diets (TM15, TM30, TM45, TM60, TM75 and TM100), respectively. The results showed that the survival was not significantly affected by dietary TM meal levels (P>0.05). Compared with control group, the final body weight, weight gain rate and protein efficiency ratio decreased significantly when the replacement level over 30%, while feed conversion ratio increased significantly as replacement level over 45% (P<0.05). The total protein-bound amino acid content in muscle was significantly increased with the increase of dietary TM meal inclusion (P<0.05). With replacement level increasing, the percentage of eicosapentaenoic acid (EPA) and ∑n-3/∑n-6 poly-unsaturated fatty acids (PUFA) in muscle significantly decreased (P<0.05). Meanwhile, the skin redness (a*) and yellowness (b*) values in the ventral and bottom of ventral regions showed a decreasing and increasing trend, respectively (P<0.05). The TM100 group showed a higher myofibre diameter and lower myofibre density compared to the control group (P<0.05). Total replacement of fishmeal with TM meal significantly down-regulated and up-regulated the expression of *myf6* and *mstn*, respectively (P<0.05). The contents of inosine-5′-monophosphate and total free amino acids were significantly decreased with the increase of TM meal inclusion (P<0.05). In conclusion, TM meal can replace at least 30% of dietary fishmeal protein without negative effects on the growth, feed utilisation and flesh quality of large yellow croaker.

Keywords: large yellow croaker, *Tenebrio molitor*, fishmeal, growth, quality

1. Introduction

The shortage of fishmeal has been the key factor restricting the development of aquaculture industry in recent decades. In order to ensure the sustainable development of aquaculture, finding high quality protein sources to replace dietary fishmeal has always been a hot topic in aquaculture research. Many previous studies in some fish species have shown that some protein sources could partially replace dietary fishmeal without negative effect on growth and feed utilisation, such as soybean meal (gilthead sea bream) (Martínez-Llorens *et al.*, 2007), cottonseed meal (catfish) (Robinson and Li, 1994), meat and bone meal (Japanese flounder) (Kikuchi *et al.*, 1997) and poultry meal (cobia) (Watson *et al.*, 2014). However, these traditional protein sources still have some shortcomings. For example, the presence of anti-nutritional factors, poor palatability or unbalanced amino acid profile, make them difficult to replace high percentage of fishmeal in aquafeeds (Liu *et al.*, 2020; Sun *et al.*, 2015). Therefore, the research and developing for novel protein sources with better quality to replace fishmeal remain necessary.

Insects have attracted a lot of attention as novel protein sources used in food and feed fields, due to their excellent nutritional composition and the potential to meet sustainable and accessible principles (Nogales-Mérida *et al.*, 2018; Stamer, 2015; Van Huis, 2020). The yellow mealworm (*Tenebrio molitor*, TM) belongs to the coleoptera order, *Tenebrionidae* family. It can convert organic waste into available protein while cause less pollution and consume less resource (Van Huis, 2013; Van Huis and Dunkel, 2017). The TM larva is easy to breed and reproduce, and the products made by them contain high protein level (47-60%). As a result, the TM meal is widely used in poultry and fish feeds (Gasco *et al.*, 2018; Makkar *et al.*, 2014). Amounts of studies have reported the potential of TM meal as an alternative protein source for fishmeal in some carnivorous mariculture fish. For example, the TM meal could replace 25-50% dietary fishmeal without negative effect on growth performance of gilthead sea bream (Piccolo *et al.*, 2017), blackspot sea bream (Iaconisi *et al.*, 2017) and rainbow trout (Belforti *et al.*, 2016).

Large yellow croaker (*Larimichthys crocea*), one of the widely cultured carnivorous fish in China, is favoured by consumers due to its beautiful skin colour and delicious taste. Fishmeal is the main protein source in commercial feed of large yellow croaker. As a result, finding novel protein sources to replace fishmeal is also an urgent problem to be solved in large yellow croaker culture. Although many studies have shown that TM meal could successfully replace part of fishmeal in some carnivorous fish feed, the application of TM meal as substitute for dietary fishmeal for large yellow croaker has not been reported.

With the improvement of living standard, consumers pay more and more attention to the quality of aquaculture products. The quality is a complex concept, mainly including skin and meat colour, condition indices, flesh texture and flavour. It is affected by many factors, such as fish species, age, size, nutritional status, environmental factors and pre- or post-slaughter handling procedures (Bjørnevik *et al.*, 2017; Grigorakis, 2007). The nutritional status is one of the key factors affecting fish quality. At present, studies on fishmeal substitution mainly focus on growth performance and fish health. However, the fish quality affects the acceptance of consumers for farmed fish, especially for large yellow croaker. Thus, the effects of fishmeal replacement by TM meal on quality of large yellow croaker is also worth concerning.

The aim of the present study was to investigate the effects of TM meal as substitute for dietary fishmeal on the growth performance, feed utilisation and flesh quality of large yellow croaker.

2. Materials and methods

The present study was carried out strictly according to the recommendations in the Guide for the Use of Experimental Animals of Ocean University of China.

Experimental diets and design

Seven isonitrogenous (about 46% of crude protein) and isolipidic (about 9% of crude lipid) experimental diets were formulated (Table 1). To make floating extruded diets, the dietary starch contents were controlled. The micro-crystalline cellulose was used to adjust the experimental diets as isonitrogenous and isolipidic. The control diet used fish meal as main protein source (56% fish meal) and was named TM0. Based on the control diet, the fishmeal protein was replaced by 15, 30, 45, 60, 75 and 100% TM meal protein, respectively, and they were named as TM15, TM30, TM45, TM60, TM75 and TM100, respectively. The amino acid and fatty acid composition of the experimental diets are presented in Table 2 and Table 3.

The experimental diets were made in the Feed Research Institute of Chinese Academy of Agricultural Sciences (Beijing, China). All ingredients were crushed and ground to fine powder, then accurately weighed according to the feed formula and put into a mixer for mixing, and then pellets with a diameter of 6 mm was extruded by a twin-screw extruder (MY 56 × 2, Muyang, Yangzhou, China). After drying, the mixed fish oil, soybean oil and phospholipid oil were evenly sprayed onto the surface of the feed pellets by vacuum coater, and then all diets were packed into small bags and stored at -20 °C until use.

Feeding trial

The feeding trial was carried out in floating sea cages in Ningde, Fujian Province, China. Large yellow croakers were purchased from a commercial hatchery of Fufa (Ningde, Fujian Province, China). Before the formal experiment, the fish were temporarily fed in sea cages (4.0×8.0×4.0 m) for 2 weeks, during which they were fed with commercial diet to acclimate the feed and environment. At the beginning of the experiment, all fish were starved for 24 hours and weighed. Healthy fish of similar size (189.18±0.13 g) were selected and randomly distributed into 21 cages (2.0×2.0×2.0 m, 100 fish per cage). Each diet was assigned to three cages and the fishes were fed twice daily (5:00 and 18:00, respectively) until apparent satiation. Feeds feeding and fish mortality were recorded daily. During the 80-day feeding trial, the water temperature ranged from 19.8 to 28.5 °C, salinity 31.1 to 34.9, and the dissolved oxygen content >6 mg/l.

Table 1. Formulation and proximate composition of the experimental diets.

	TM0	TM15	TM30	TM45	TM60	TM75	TM100
Ingredients (%)							
TASA fish meal[1]	56.00	47.60	39.20	30.80	22.40	14.00	0.00
Tenebrio molitor meal	0.00	8.52	17.05	25.57	34.1	42.62	56.83
Tapioca starch	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Wheat flour	9.80	9.80	9.80	9.80	9.80	9.80	9.80
Wheat gluten	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fish oil	2.90	3.40	3.90	4.40	4.90	5.40	6.20
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lecithin	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calcium biphosphate	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Kelp powder	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Microcrystalline cellulose	14.20	13.58	12.95	12.33	11.70	11.08	10.07
Glycine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Betaine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Y_2O_3	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition (%, dry matter)							
Dry matter	95.75	95.79	95.90	96.29	95.57	95.54	95.52
Crude lipid	9.25	9.58	9.08	9.04	9.10	9.23	9.13
Crude protein	45.68	46.27	46.29	46.34	46.73	46.14	46.39
Ash	12.67	11.37	11.24	10.32	9.88	8.67	7.30
Gross energy (MJ/kg)	20.25	20.86	20.53	21.05	20.75	21.24	20.57

[1] TASA fish meal was supplied by Tecnológica de Alimentos S.A., Peru, belongs to TASA super steam dried fishmeal. See Table S1.

[2] Premix: vitamin premix (mg/kg diet): vitamin A, 20; vitamin D3, 10; vitamin E, 300; vitamin K3, 20; vitamin C, 600; inositol, 150; niacin acid, 80; calcium pantothenate, 40; vitamin B2, 15; vitamin B6, 15; vitamin B1, 10; folic acid, 10; vitamin B12, 8; biotin, 2; wheat middlings, 220. Mineral premix (mg/kg diet): $FeSO_4 \cdot H_2O$, 300; $MgSO_4 \cdot 7H_2O$, 1,200; $ZnSO_4 \cdot H_2O$, 200; NaCl, 100; $MnSO_4 \cdot H_2O$, 25; $CuSO_4 \cdot 5H_2O$, 30; $CoCl_2 \cdot 6H_2O$, 5; Na_2SeO_3, 5; KIO, 3. Others (mg/kg diet): Antioxidant, 50; Mould inhibitor, 200; Zeolite powder, 6,382.

Sample collection

At the end of the feeding trial, the fish were fasted for 24 hours and anesthetised with eugenol (1:10,000) (purity 99%, Shanghai Reagent, Shanghai, China). The fish were counted and weighted to calculate the survival (SR) and weight gain rate (WGR). Ten fish per cage were randomly sampled to measure body length and weight of body, viscera and liver for condition indices. Blood samples were collected from the caudal vein and allowed to clot for 4 h at 4 °C. The serum was separated by centrifugation (3,000 rpm/min, 10 min, 4 °C) and stored at -80 °C until use. The dorsal muscle and intestine samples were collected immediately frozen in liquid nitrogen and then stored at -80 °C until use. The dorsal muscle of another three fish in each cage were carefully sampled and fixed in 10% formaldehyde for histological analysis.

Another four fish from each cage were placed on ice and immediately delivered to the laboratory. Dorsal fillet above epaxial myotomes and below dorsal fin of one side was sampled for analysis of pH, drip loss, cooking loss, texture parameters and volatile compounds within 24 hours.

According to the method of Yi *et al.* (2014), six fish were randomly selected from each cage to measure the skin colour of the dorsal, ventral and caudal regions during 20:00 to 23:00 at night. A portable Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) was used. According to the recommendation of International Commission on Illumination (CIE, 1976), the skin colour parameters are represented by L*, a* and b* respectively for brightness, redness and yellowness.

Experimental diets and fish muscle composition analysis

The approximate composition of experimental diets and fish muscle were determined referring to AOAC standard method (AOAC, 1995). Moisture was determined by drying diets and muscle samples at 105 °C to constant

Table 2. Amino acid composition of the experimental diets (%, dry matter).[1]

	Diets						
	TM0	TM15	TM30	TM45	TM60	TM75	TM100
Arginine	2.58	2.64	2.56	2.63	2.59	2.63	2.61
Histidine	1.26	1.11	1.08	0.99	0.90	0.71	0.62
Isoleucine	1.81	2.00	1.88	1.95	1.92	1.94	1.92
Leucine	3.18	3.33	3.22	3.38	3.33	3.34	3.33
Lysine	3.03	3.08	2.98	3.06	2.96	2.92	2.86
Methionine	0.96	1.12	1.03	1.04	0.96	0.92	0.89
Phenylalanine	2.30	2.10	2.10	2.11	2.09	2.09	2.14
Threonine	1.80	1.84	1.71	1.80	1.70	1.74	1.70
Valine	2.18	2.43	2.36	2.52	2.56	2.66	2.72
EAA	19.10	19.65	18.92	19.48	19.01	18.95	18.79
Alanine	2.76	3.01	3.13	3.29	3.46	3.58	3.80
Aspartic acid	3.20	3.58	3.33	3.31	3.19	3.32	3.17
Cysteine	0.32	0.35	0.34	0.36	0.38	0.39	0.47
Glutamic acid	6.87	6.88	6.68	6.89	6.62	6.49	6.30
Glycine	2.23	2.18	2.06	2.08	1.96	1.86	1.74
Proline	1.90	1.89	2.07	2.35	2.40	2.35	2.45
Serine	1.67	2.04	2.20	2.54	2.78	3.01	3.39
Tyrosine	1.31	1.47	1.40	1.47	1.46	1.49	1.52
NEAA	20.26	21.40	21.21	22.29	22.25	22.49	22.84

[1] EAA = essential amino acids; NEAA = non-essential amino acids.

Table 3. Fatty acid composition of the experimental diets (% total fatty acids).[1]

	Diets						
	TM0	TM15	TM30	TM45	TM60	TM75	TM100
C14:0	5.87	5.88	5.78	5.01	4.45	4.28	3.30
C16:0	42.09	41.40	40.11	40.06	39.40	38.12	36.43
C18:0	12.31	12.30	11.96	13.33	14.53	13.85	15.05
C20:0	0.53	0.69	0.76	1.05	1.35	1.23	1.60
C16: n-7	2.74	2.45	2.48	2.36	2.05	1.94	1.38
C18: n-9	4.10	3.68	3.77	3.95	4.03	4.24	4.28
C18:2n-6	16.90	19.06	20.67	20.97	21.86	24.16	26.98
C20: n-9	0.90	0.86	0.86	0.95	1.04	0.93	1.08
C18:3n-3	2.28	2.43	2.89	2.82	2.88	3.45	3.78
C20:4n-6	0.29	0.26	0.26	0.22	0.19	0.17	0.11
C20:5n-3(EPA)	4.39	3.95	3.94	3.12	2.55	2.37	1.48
C22:6n-3(DHA)	4.65	4.10	3.98	3.23	2.68	2.37	1.52
Other FA[2]	2.94	2.97	2.80	2.93	2.99	2.89	3.02
∑SFA	62.95	62.45	60.44	61.60	61.95	59.56	58.66
∑MUFA	8.06	7.28	7.31	7.50	7.40	7.34	6.96
∑PUFA	28.99	30.28	32.25	30.90	30.65	33.10	34.39
∑n-3 PUFA	11.38	11.15	10.87	9.23	8.20	8.28	6.87
∑n-6 PUFA	17.61	19.74	21.38	21.67	22.45	24.82	27.52
∑n-3/∑n-6 PUFA	0.65	0.54	0.51	0.43	0.37	0.33	0.25

[1] DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; MUFA = mono-unsaturated fatty acids; n-3 = n-3 fatty acids; n-6 = n-6 fatty acids; PUFA = poly-unsaturated fatty acids; SFA = saturated fatty acids.

[2] Other fatty acid: C15:0, C17:0, C17:n-7, C18:3n-6, C21:0, C20:2n-6, C22:0, C20:3n-6, C22: n-9, C20:3n-3, C23:0, C22:2n-6, C24:0 and C24:n-9 were also detected but not reported in the table for low levels. They were utilised to calculate the fatty acid groups.

weight. Crude protein was determined using the Kjeldahl (2300-Kjeldahl apparatus, FOSS, Hillerød Denmark) method by measuring nitrogen (N×6.25). The Soxhlet method (Soxhlet extraction system B-811, FOSS) was used to measure crude lipid. Ash was determined by combustion. The sample was placed in muffle furnace and burned at 550 °C about 4 h to constant weight. Gross energy was determined using an Oxygen Bomb Calorimeter (Parr 6400, Parr Instrument Company, Moline, IL, USA).

Muscle samples used to determine protein-bound amino acids were freeze-dried and 30 mg of each sample was hydrolysed in 15 ml 6N HCl solution at 110 °C for 24 h. Amino acid profile were determined by automatic amino acid analyser (L-8900, Hitachi, Tokyo, Japan).

The analysis of free amino acids was carried out using the method of Wei *et al.* (2019b). One gram of muscle sample was homogenised with 3 ml 10% sulfosalicylic acid for 1 min. After centrifugation, the supernatant was extracted and filtered into the sample bottle for analysis using automatic amino acid analyser (L-8900, Hitachi).

For the analysis of fatty acids, the sample pretreatment method was referred to by Ma *et al.* (2019). Fatty acids were determined by gas chromatography-mass spectrometry (GCMS-QP2010, Shimadzu, Kyoto, Japan) fitted with an automatic sampler. Fatty acids were identified based on a mass spectrometry database retrieval (similarity >80%), and the results were expressed as the percentage of each fatty acid in total fatty acids.

Biochemical indexes assay in intestine

The fish intestine was divided into anterior intestine, mid-intestine and posterior intestine during sampling, and the midgut was taken for enzyme activity determination. The midgut of each group was weighed and homogenised into 0.01 M buffer solution. The activities of amylase (AMS), lipase (LPS), trypsin, chymotrypsin, creatine kinase (CK), γ-glutamyltransferase (γ-GT), Na^+K^+-ATPase and alkaline phosphatase (AKP) were measured by using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing,

China), and they were AMS (C016-1-1), LPS (A054-2-1), trypsin (A080-2-2), chymotrypsin (A080-3-1), CK (A032-1-1), γ-GT (C017-2-1), Na^+K^+-ATPase (A070-2-2) and AKP (A059-2-2), respectively. All procedures were carried out according to the instructions of the commercial kits.

Muscle pH, water holding capacity and texture analysis

Muscle pH value was determined using a digital display pH meter according to the method of Fuentes *et al.* (2010). The muscle (g) / distilled water (ml) = 1:9 was homogenised and measured with pH meter (PB-10, Sartorius, Göttingen, Germany).

Water holding capacity (WHC) was expressed by cooking loss and drip loss. The analysis of WHC was performed with previous studies with minor modification (Lv *et al.*, 2021; Sánchez-Alonso *et al.*, 2007). Fish fillet (5×3×1 cm) was cut from left side of the fish, weighted (W_1) and suspended in a plastic bag, where small holes had been made to drain the drip. Samples were placed at 2-4 °C for 48 h and weighed again (W_2) to determine the drip loss. Samples of the same size were weighed and placed in retort pouch then cooked in a water bath at 100 °C for 15 minutes. The fish fillets were taken out and the surface water were dried by paper towels, then reweighed to calculate the cooking loss according to the following equation:

$$\text{Drip loss (\%)} = 100 \times \frac{W_1 - W_2}{W_1}$$

$$\text{Cooking loss (\%)} = 100 \times \frac{\text{the weight before cooking} - \text{the weight after cooking}}{\text{the weight before cooking}}$$

Texture (hardness, cohesiveness, adhesiveness, springiness, chewiness and gumminess) analysis was performed by a texture analyser (TMS-TOUCH, Food Technology Corporation, West Sussex, VA, USA) with the method of Ginés *et al.* (2004). The muscle above the lateral line (1.5×1.5×1.0 cm) of fish was used for texture analysis. An 8 mm cylinder probe and double compression was applied to construct the texture profile analyses parameters. The test condition involved two consecutive cycles of compression with a constant speed of 30 mm/min with the deformation 60% of the original length, and the initial force was 0.1 N (Wei *et al.*, 2016). The shear force was determined by the combination single blade and meat shear cell.

Muscle hydroxyproline, collagen, water and salt soluble protein determination

The content of hydroxyproline was determined using the commercial kit (A030-2-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The collagen content was estimated by multiplying the hydroxyproline content by 8 (AOAC, 2000).

Water-soluble protein and salt-soluble protein were extracted with phosphate buffer (0.05 M, pH 7.0) alone or with 0.6 M KCl (Sigholt *et al.*, 1997), and the protein content was determined using the Coomassie brilliant blue method of TP commercial kit (A045-2-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Muscle nucleotides content and freshness assay

The nucleotide content was analysed by HPLC (LC-20AT, Shimadzu) with the method of Wei *et al.* (2019a), including inosine-5′-monophosphate (IMP), adenosine-5′-triphosphate (ATP), adenosine-5′-diphosphate (ADP), adenosine-5′-monophosphate (AMP), inosine (Ino) and hypoxanthine (Hx). The K and Ki values were used to evaluate muscle freshness, and the calculation equations are as follows:

$$K\ (\%) = 100 \times \frac{\text{Ino} + \text{Hx}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{Ino} + \text{Hx}}$$

$$Ki\ (\%) = 100 \times \frac{\text{Ino} + \text{Hx}}{\text{IMP} + \text{Ino} + \text{Hx}}.$$

Volatile compounds analysis

The content of volatile compounds in muscle was determined by gas chromatograph and ion mobility spectrometry (FlavourSpec®, G.A.S., Dortmund, Germany) (GC-IMS) equipped with an automatic sampling device. Briefly, muscle sample (3 g) was transferred into a 20 ml headspace bottle carefully and then incubated at 55 °C, while being stirred at 250 rpm for 15 min. After incubation, 500 ul headspace was injected using an 85 °C heated syringe into a FS-SE-54 capillary column in splitless mode. Pure nitrogen was used as the carrier gas with programmed flow: 2 ml/min for 2 min, ramp to 100 ml/min over 20 min, and maintained for 10 min until stopping. Analytes were driven to the ionisation chamber by a 3H ionisation source in positive ion mode. The 9.8 cm drift tube was operated at 45 °C with 150 ml/min nitrogen flow. Volatile compounds identification was mainly based on the comparison of retention index and drift time in GC-IMS library database.

Histology analysis

The sections were stained with picrosirius red using the slide stainer and observed under light microscope. Meanwhile, the micrograph of each group was taken and the muscle fibre density and diameter were calculated.

Gene expression in muscle

Total RNA was isolated from muscle using RNAiso Plus Kit (9109, Takara, Japan). The quality of RNA was detected by agarose gel electrophoresis at 1.2%, the concentration of RNA was assessed by Nano-Drop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The cDNA

was generated by using PrimeScript RT reagent Kit with gDNA Eraser (RR047A, Takara, Kusatsu, Japan) following the manufacturer's protocol. The mRNA levels of myogenic differentiation (*MyoD*), myogenin (*MyoG*), myogenic factor 5 (*myf5*), myogenic factor 6 (*myf6*), paired box 7 (*Pax-7*) and myostatin (*mstn*) were analysed by Real-time PCR system (Quant Studio 5, Applied Biosystems, Waltham, MA, USA). And β-actin was used as the internal reference to normalise the mRNA expression level of the control group. The results of gene expression were analysed according to $2^{-\Delta\Delta CT}$ method. The primers used for the real-time PCR analysis are shown in Table 4.

Calculations and statistical analysis

The survival, growth performance, feed utilisation and body condition indices of large yellow croaker were calculated as follows:

$$\text{Survival (SR, \%)} = 100 \times \frac{\text{Final fish number}}{\text{Initial fish number}}$$

$$\text{Weight gain rate (WGR, \%)} = 100 \times \frac{\text{final body weight} - \text{initial body weight}}{\text{initial body weight}}$$

$$\text{Protein efficiency ratio (PER, \%)} = 100 \times \frac{\text{Weight gain (g)}}{\text{Total protein fed (g, dry basis)}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed intake (g, dry basis)}}{\text{Weight gain (g)}}$$

$$\text{Feed intake (FI, \%/day)} = 100 \times \frac{\text{Total feed intake (g, dry basis)}}{\text{Number of feeding days} \times \frac{\text{final body weight + initial body weight}}{2}}$$

$$\text{Hepatosomatic index (HSI, \%)} = 100 \times \frac{\text{Hepatic weight}}{\text{Body weight}}$$

$$\text{Viscerosomatic index (VSI, \%)} = 100 \times \frac{\text{Viscera weight}}{\text{Body weight}}$$

$$\text{Condition factor (CF)} = 100 \times \frac{\text{Body weight}}{\text{Total length (cm}^3\text{)}}$$

All experimental data were analysed using the software of SPSS 25.0 (IBM Corp., New York, NY, USA). One-way analysis of variance (ANOVA) followed by Tukey's multiple range test was used to determine whether different levels of substitution had significant effects on the measured indicators. The significance level was set as $P<0.05$. All experimental results were presented as means ± SE (standard error of the mean).

3. Results

Growth performance and body condition indices

The data on growth performance and condition indices are shown in Table 5. There was no significant difference in survival (84.67-94.67%) among all the treatments ($P>0.05$). Compared with control group, the finial body weight, WGR and protein efficiency ratio significantly decreased when the level of replacement over 30% ($P<0.05$), while the FCR significantly increased as the proportion of substitution over 45% ($P<0.05$). The FI in TM75 and TM100 groups were significantly lower than those in the other groups ($P<0.05$). The TM100 group had significantly lower viscerosomatic index and hepatosomatic index than those in the other groups ($P<0.05$). The CF in TM60, TM75 and TM100 groups were significantly decreased compared with those in the other groups ($P<0.05$).

Muscle composition

The data on muscle composition are shown in Table 6. There was no significant difference in muscle moisture among the treatments ($P>0.05$). The contents of crude protein and ash in TM100 group were significantly higher than that in the control group ($P<0.05$), while crude lipid content showed an opposite trend. The contents of muscle hydroxyproline and collagen in the TM75 and TM100 groups were significantly lower than that in the control group ($P<0.05$). Water soluble protein content was not significantly affected by dietary composition ($P>0.05$). But a reduction of salt soluble protein content was found with the dietary inclusion of TM meal increasing ($P<0.05$).

Muscle protein-bound amino acid and fatty acid

According to Table 7, the essential amino acid (EAA) and non-essential amino acid (NEAA) contents significantly increased when the replacement level up to 45% ($P<0.05$).

Table 4. The primers used for the real-time PCR analysis.

Gene	Forward (5'-3')	Reverse (5'-3')	GenBank accession no.
MyoD	ACAGCAGCTCTTATTTCTCCGA	GTCATTCTTCAGACCGCCGTT	XM_010745476.3
MyoG	GGAGCTTTTCGAGACCAACCC	AGATTCCCACACAAGCCCAT	XM_010738811.3
myf5	CAACTGCTCTGACGGCAT	CGCACAGACTCTCATTCTTCG	XM_019276871.2
myf6	CCGAACCAGAGGCTACCCAA	TAACCGCTCGATGTAGCTGA	XM_010737255.3
Pax-7	ACCACCTTCACCGCTGAG	CTCGCCTGTTGCTAAACCAC	XM_010731346.3
mstn	GTCGCCCATCAACATGCTCT	GAGCATCCACAACGGTCCAC	XM_010733150.3
β-actin	GACCTGACAGACTACCTCATG	AGTTGAAGGTGGTCTCGTGGA	GU584189

Table 5. Effects of fishmeal replacement by TM meal on the growth performance and body condition indices of large yellow croaker.[1,2]

	Diets							
	TM0	TM15	TM30	TM45	TM60	TM75	TM100	*P*-value
Growth performance								
SR (%)	91.00±5.03	94.00±2.52	94.67±1.67	89.33±1.76	91.00±1.53	84.67±5.90	87.33±3.33	0.45
IBW(g)	189.27±0.27	189.47±0.13	189.93±0.35	188.73±0.29	188.93±0.07	188.73±0.35	189.2±0.50	0.15
FBW(g)	322.94±14.34^{a}	319.06±7.90^{a}	322.91±6.34^{a}	284.67±3.91^{b}	277.78±3.05^{b}	230.28±2.07^{c}	209.66±0.75^{c}	0.00
WGR (%)	70.62±7.55^{a}	68.40±4.29^{ab}	70.02±3.57^{a}	50.82±1.84^{bc}	47.03±1.66^{c}	22.02±1.27^{d}	10.82±0.61^{d}	0.00
FCR	1.48±0.03^{d}	1.52±0.11^{d}	1.46±0.06^{d}	1.94±0.02^{cd}	2.00±0.06^{c}	2.93±0.11^{b}	4.31±0.20^{a}	0.00
PER (%)	1.34±0.08^{a}	1.36±0.06^{a}	1.41±0.06^{a}	1.00±0.01^{b}	0.99±0.02^{b}	0.63±0.06^{c}	0.44±0.03^{c}	0.00
FI (%/d)	0.96±0.07^{a}	0.96±0.03^{a}	0.94±0.01^{a}	0.98±0.04^{a}	0.96±0.05^{a}	0.72±0.01^{b}	0.55±0.01^{b}	0.00
Body condition indices								
VSI (%)	4.97±0.15^{a}	5.13±0.14^{a}	5.10±0.14^{a}	5.10±0.14^{a}	5.12±0.19^{a}	5.03±0.14^{a}	4.51±0.14^{b}	0.04
HSI (%)	2.75±0.13^{a}	2.89±0.11^{a}	2.76±0.11^{a}	3.06±0.13^{a}	2.80±0.13^{a}	2.64±0.11^{a}	1.94±0.09^{b}	0.00
CF	1.83±0.02^{a}	1.81±0.02^{a}	1.80±0.01^{a}	1.76±0.02^{ab}	1.71±0.02^{b}	1.58±0.01^{c}	1.49±0.01^{d}	0.00

[1] CF = condition factor; FBW = finial body weight; FCR = feed conversion ratio; FI = feed intake; HSI = hepatosomatic index; IBW = initial body weight; PER = protein efficiency ratio; SR = survival rate; VSI = viscerosomatic index; WGR = weight gain rate.

[2] Values are mean ± SE. Mean values within the same row with different superscripts are significantly different (*P*<0.05).

Table 6. Effects of fishmeal replacement by TM meal on dorsal muscle composition of large yellow croaker (wet weight).[1]

	Diets							
	TM0	TM15	TM30	TM45	TM60	TM75	TM100	*P*-value
Moisture (%)	69.68±1.05	68.16±1.16	70.62±0.72	70.32±0.79	69.54±0.12	70.12±0.55	71.24±0.57	0.16
Crude protein (%)	16.32±0.06^{bc}	16.63±0.05^{abc}	15.88±0.24^{c}	16.31±0.23^{bc}	16.83±0.14^{ab}	16.78±0.27^{ab}	17.29±0.16^{a}	0.00
Crude lipid (%)	12.63±0.11^{a}	13.37±0.13^{a}	12.94±0.45^{a}	12.94±0.24^{a}	12.84±0.09^{a}	12.6±0.06^{ab}	11.64±0.22^{b}	0.00
Ash (%)	3.38±0.11^{b}	3.21±0.06^{b}	3.24±0.10^{b}	3.25±0.17^{b}	3.34±0.08^{b}	3.58±0.09^{ab}	3.88±0.06^{a}	0.00
Hydroxyproline (mg/g)	0.31±0.01^{a}	0.30±0.01^{a}	0.31±0.04^{a}	0.26±0.01^{ab}	0.25±0.01^{ab}	0.22±0.02^{b}	0.22±0.03^{b}	0.02
Collagen (mg/g)	2.45±0.03^{a}	2.40±0.05^{a}	2.52±0.28^{a}	2.07±0.01^{ab}	2.00±0.01^{ab}	1.79±0.19^{b}	1.79±0.25^{b}	0.02
Salt soluble protein (g/100 g)	6.85±0.02^{a}	6.45±0.16^{a}	5.58±0.23^{bc}	5.92±0.11^{b}	5.20±0.15^{cd}	5.41±0.10^{cd}	5.03±0.06^{d}	0.00
Water soluble protein (g/100 g)	4.36±0.18	4.18±0.22	3.98±0.07	3.91±0.07	3.91±0.07	3.97±0.06	3.92±0.06	0.11

[1] Values are mean ± SE. Mean values within the same row with different superscripts are significantly different (*P*<0.05).

However, the ratio of EAA/NEAA was not significantly affected by dietary TM meal levels (*P*>0.05).

According to Table 8, no significant difference was observed in docosahexaenoic acid (DHA) (C22:6n-3), saturated fatty acids, mono-unsaturated fatty acids and PUFA among all the treatments (*P*>0.05). The concentration of EPA (C20:5n-3) and Σn-3 PUFA were significantly decreased following the increase of TM meal inclusion (*P*<0.05). However, the percentage of Linoleic acid (C18:2n-6) and Σn-6 PUFA were significantly increased with increasing dietary TM meal levels (*P*<0.05). Therefore, this led to a significant reduction of the Σn-3/Σ n-6 PUFA ratio (*P*<0.05).

Enzyme activities in intestine

Results of intestinal enzyme activities are presented in Table 9. Digestive enzymes activity such as AMS, LPS, trypsin and chymotrypsin, were significantly decreased when the level of substitution over 45% (*P*<0.05). As for absorptive enzymes, the activities of CK and γ-GT significantly decreased as the dietary TM level increased above 30% (*P*<0.05), the AKP activity significantly decreased when the substitution level up to 60% (*P*<0.05). And $Na^{+}K^{+}$-ATPase activity was significantly lower in the TM75 and TM100 groups than that in the control group (*P*<0.05).

Table 7. Effects of fishmeal replacement by TM meal on protein-bound amino acid composition of large yellow croaker (%, dry matter).[1,2]

	Diets							
	TM0	TM15	TM30	TM45	TM60	TM75	TM100	*P*-value
EAA								
Arginine	3.32±0.17^{b}	3.29±0.01^{b}	3.28±0.02^{b}	3.27±0.09^{b}	3.57±0.01^{b}	3.48±0.07^{b}	4.04±0.10^{a}	0.00
Histidine	1.16±0.05^{b}	1.19±0.05^{ab}	1.29±0.03^{ab}	1.37±0.07^{ab}	1.41±0.05^{a}	1.28±0.05^{ab}	1.33±0.01^{ab}	0.03
Isoleucine	2.19±0.03^{c}	2.32±0.01^{bc}	2.58±0.04^{abc}	2.54±0.07^{abc}	2.63±0.13^{ab}	2.46±0.10^{abc}	2.78±0.12^{a}	0.00
Leucine	3.89±0.03^{d}	4.17±0.03^{cd}	4.15±0.05^{cd}	4.78±0.18^{ab}	4.65±0.01^{ab}	4.58±0.12^{bc}	5.10±0.11^{a}	0.00
Lysine	4.52±0.08^{b}	4.52±0.02^{b}	4.52±0.05^{b}	4.82±0.01^{b}	4.85±0.08^{b}	5.22±0.09^{a}	5.43±0.10^{a}	0.00
Methionine	1.50±0.04^{b}	1.61±0.04^{ab}	1.68±0.07^{ab}	1.83±0.06^{a}	1.63±0.05^{ab}	1.78±0.10^{a}	1.86±0.01^{a}	0.01
Phenylalanine	2.21±0.09^{b}	2.36±0.04^{ab}	2.50±0.03^{ab}	2.57±0.05^{ab}	2.70±0.14^{ab}	2.95±0.30^{a}	2.76±0.03^{ab}	0.02
Threonine	2.48±0.09^{b}	2.33±0.01^{b}	2.30±0.03^{b}	2.74±0.05^{a}	2.77±0.01^{a}	2.86±0.04^{a}	2.88±0.02^{a}	0.00
Valine	2.39±0.07^{b}	2.47±0.01^{b}	2.84±0.06^{a}	2.41±0.07^{b}	2.74±0.05^{ab}	2.58±0.02^{bc}	2.95±0.02^{a}	0.00
NEAA								
Alanine	2.82±0.03	2.95±0.04	2.97±0.01	2.92±0.08	2.92±0.04	3.20±0.27	3.21±0.03	0.18
Aspartic acid	4.88±0.05^{c}	4.98±0.05^{bc}	4.93±0.03^{c}	5.30±0.01^{abc}	5.12±0.01^{bc}	5.48±0.14^{ab}	5.75±0.25^{a}	0.00
Cysteine	0.44±0.01	0.46±0.01	0.49±0.03	0.46±0.03	0.46±0.02	0.48±0.03	0.45±0.01	0.79
Glutamic acid	7.47±0.06^{e}	7.63±0.04^{de}	7.64±0.08^{de}	8.13±0.21^{cd}	8.91±0.01^{b}	8.48±0.06^{bc}	9.63±0.16^{a}	0.00
Glycine	2.49±0.04^{c}	2.75±0.23^{bc}	3.07±0.04^{ab}	3.09±0.15^{ab}	3.03±0.02^{ab}	2.90±0.05^{abc}	3.29±0.01^{a}	0.00
Proline	1.64±0.03^{b}	1.69±0.05^{b}	1.85±0.07^{ab}	2.15±0.00^{ab}	2.18±0.02^{ab}	2.34±0.18^{a}	1.90±0.22^{ab}	0.00
Serine	2.20±0.05^{ab}	2.18±0.01^{ab}	2.09±0.02^{b}	2.33±0.06^{ab}	2.36±0.01^{b}	2.39±0.09^{b}	2.65±0.05^{a}	0.00
Tyrosine	1.75±0.05^{c}	1.81±0.06^{bc}	1.92±0.09^{abc}	2.11±0.10^{ab}	1.99±0.01^{abc}	2.09±0.07^{ab}	2.19±0.03^{a}	0.00
TAA	47.36±0.37^{d}	48.71±0.33^{d}	50.12±0.16^{cd}	52.82±1.12^{bc}	53.91±0.40^{b}	54.54±1.42^{ab}	58.21±0.73^{a}	0.00
EAA	23.67±0.28^{d}	24.27±0.12^{d}	25.15±0.07^{cd}	26.32±0.61^{bc}	26.95±0.34^{bc}	27.20±0.67^{ab}	29.13±0.41^{a}	0.00
NEAA	23.69±0.12^{d}	24.45±0.22^{d}	24.97±0.10^{cd}	26.50±0.52^{bc}	26.97±0.06^{b}	27.34±0.75^{ab}	29.08±0.33^{a}	0.00
EAA/NEAA	1.00±0.01	0.99±0.01	1.01±0.01	0.99±0.01	1.00±0.01	0.99±0.01	1.00±0.01	0.71

[1] EAA = essential amino acids; NEAA = non-essential amino acids; TAA: total amino acids.

[2] Values are mean ± SE. Mean values within the same row with different superscripts are significantly different (P<0.05).

Skin colour parameters

As shown in Table 10 and Figure 1, the skin colour of dorsal and caudal regions was not significantly affected by dietary treatments (P>0.05). With increasing dietary TM meal levels, the redness (a*) in the ventral regions and bottom of ventral of fish showed an obviously decreasing trend and the lowest value was found in the TM60 group, while the yellowness (b*) significantly increased and the maximum value appeared in TM60 group (P<0.05). The lightness (L*) did not show significant difference among all the treatments (P>0.05).

Muscle pH, water holding capacity and texture

The pH, WHC and texture parameters of muscle are presented in Table 11. No significant difference was observed for pH and cooking loss among all the groups (P>0.05). The drip loss significantly increased in TM75 and TM100 groups compared with that in the control group (P<0.05). The muscle hardness, springiness, chewiness and gumminess tend to decrease in TM75 and TM100 groups, however, there were no significant differences (P>0.05). The TM45 group had a higher muscle adhesiveness than that in TM0 group (P<0.05). Shear force in TM75 and TM100 groups were significantly lower than that in the control group (P<0.05).

Muscle histology

As shown in Figure 2, muscle cellularity was affected by the different levels of TM meal in the diets. The TM100 group showed a higher myofibre diameter and lower myofibre density compared to the control group (P<0.05). More intuitive results are shown with micrographs of muscle tissue sections.

Table 8. Effects of fishmeal replacement by TM meal on fatty acid composition of large yellow croaker (% total fatty acids).[1,2]

	Diets							
	TM0	TM15	TM30	TM45	TM60	TM75	TM100	*P*-value
Fatty acid								
C14:0	4.19±0.07	4.16±0.01	4.12±0.13	4.02±0.04	3.99±0.03	4.10±0.15	4.40±0.01	0.06
C16:0	52.48±0.20	52.87±0.27	51.34±0.52	51.62±0.50	51.38±0.21	51.62±0.85	51.93±0.23	0.22
C18:0	12.25±0.14	12.44±0.10	12.70±0.24	12.99±0.21	12.88±0.27	12.56±0.58	12.87±0.09	0.51
C20:0	0.43±0.04^{b}	0.59±0.06^{ab}	0.60±0.06^{ab}	0.58±0.01^{ab}	0.66±0.04^{ab}	0.62±0.06^{ab}	0.79±0.07^{a}	0.01
C16:n-7	4.73±0.00^{ab}	4.76±0.01^{a}	4.62±0.05^{abc}	4.54±0.02^{bcd}	4.42±0.06^{cd}	4.82±0.09^{a}	4.33±0.02^{d}	0.00
C18:n-9	4.32±0.18	4.10±0.10	4.48±0.17	4.11±0.02	3.98±0.06	4.54±0.17	4.36±0.04	0.05
C18:2n-6	10.28±0.02^{c}	10.47±0.18^{c}	11.15±0.25^{bc}	11.67±0.24^{ab}	11.65±0.10^{ab}	10.81±0.26^{bc}	12.39±0.32^{a}	0.00
C20:n-9	1.31±0.00^{ab}	1.04±0.14^{b}	1.06±0.15^{b}	1.22±0.12^{b}	1.26±0.15^{b}	1.37±0.20^{ab}	1.90±0.03^{a}	0.01
C18:3n-3	1.22±0.01^{bc}	1.26±0.02^{abc}	1.38±0.03^{a}	1.34±0.03^{ab}	1.35±0.03^{ab}	1.17±0.05^{c}	0.96±0.02^{d}	0.00
C20:4n-6	0.32±0.00	0.30±0.01	0.31±0.02	0.29±0.01	0.30±0.02	0.30±0.05	0.33±0.01	0.86
C20:5n-3(EPA)	2.21±0.04^{a}	2.00±0.02^{b}	1.98±0.03^{b}	1.79±0.01^{c}	1.72±0.02^{c}	1.69±0.09^{c}	1.51±0.01^{d}	0.00
C22:6n-3(DHA)	3.80±0.04	3.70±0.04	3.63±0.02	3.34±0.01	3.55±0.13	3.75±0.23	3.67±0.04	0.12
Other FA[3]	2.46±0.10	2.30±0.03	2.61±0.09	2.50±0.06	2.85±0.40	2.65±0.25	3.39±0.37	0.09
∑SFA	70.72±0.35	71.50±0.29	70.19±0.61	70.71±0.24	70.39±0.55	70.40±1.31	69.18±0.57	0.39
∑MUFA	10.92±0.25	10.33±0.05	10.85±0.25	10.4±0.16	10.46±0.38	11.27±0.49	11.42±0.48	0.19
∑PUFA	18.37±0.14	18.17±0.25	18.95±0.39	18.9±0.31	19.15±0.19	18.32±0.81	19.40±0.19	0.27
∑n-3 PUFA	7.36±0.09^{a}	7.03±0.08^{ab}	7.05±0.09^{ab}	6.54±0.05^{bc}	6.69±0.14^{abc}	6.66±0.37^{abc}	6.05±0.09^{c}	0.00
∑n-6 PUFA	11.01±0.05^{d}	11.14±0.18^{cd}	11.90±0.30^{bcd}	12.36±0.26^{abc}	12.47±0.04^{ab}	11.66±0.45^{bcd}	13.34±0.27^{a}	0.00
∑n-3/∑n-6 PUFA	0.67±0.01^{a}	0.63±0.01^{ab}	0.59±0.01^{bc}	0.53±0.01^{d}	0.53±0.01^{d}	0.57±0.01^{cd}	0.45±0.02^{e}	0.00

[1] DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; MUFA = mono-unsaturated fatty acids; n-3 = n-3 fatty acids; n-6 = n-6 fatty acids; PUFA = poly-unsaturated fatty acids; SFA = saturated fatty acids.

[2] Values are mean ± SE. Mean values within the same row with different superscripts are significantly different (*P*<0.05).

[3] Other fatty acid: C15:0, C17:0, C17: n-7, C18:3n-6, C21:0, C20:2n-6, C22:0, C20:3n-6, C22: n-9, C20:3n-3, C23:0, C22:2n-6, C24:0 and C24: n-9 were also detected but not reported in the table for low levels. They were utilised to calculate the fatty acid groups.

Table 9. Effects of fishmeal replacement by TM meal on digestive and absorptive enzymes activity in intestine of large yellow croaker.[1,2]

	Diets							
	TM0	TM15	TM30	TM45	TM60	TM75	TM100	*P*-value
Digestive enzymes								
AMS	0.22±0.01^{a}	0.21±0.01^{ab}	0.21±0.01^{abc}	0.20±0.00^{abc}	0.18±0.01^{bc}	0.18±0.01^{bc}	0.17±0.01^{c}	0.00
LPS	2.44±0.06^{a}	2.30±0.04^{a}	2.35±0.02^{a}	2.27±0.16^{a}	1.94±0.03^{b}	1.72±0.02^{bc}	1.57±0.03^{c}	0.00
Trypsin[3]	38.11±0.37^{a}	37.96±0.09^{a}	37.20±0.67^{a}	35.33±0.23^{a}	30.33±0.87^{b}	28.76±1.19^{bc}	26.00±0.92^{c}	0.00
Chymotrypsin[3]	1.07±0.05^{a}	1.02±0.06^{a}	1.02±0.06^{a}	0.90±0.01^{ab}	0.83±0.02^{b}	0.74±0.01^{b}	0.73±0.03^{b}	0.00
Absorptive enzymes								
CK	0.38±0.01^{a}	0.35±0.01^{ab}	0.35±0.01^{ab}	0.31±0.02^{b}	0.26±0.01^{c}	0.25±0.01^{c}	0.24±0.01^{c}	0.00
γ-GT	4.74±0.23^{a}	4.61±0.07^{ab}	4.29±0.04^{ab}	3.85±0.17^{bc}	3.46±0.15^{cd}	2.87±0.25^{d}	2.71±0.22^{d}	0.00
$Na^{+}K^{+}$-ATPase[3]	0.94±0.09^{a}	0.90±0.04^{a}	0.88±0.01^{ab}	0.82±0.02^{abc}	0.74±0.02^{abc}	0.67±0.05^{bc}	0.63±0.02^{c}	0.00
AKP	5.39±0.12^{a}	5.26±0.01^{a}	5.21±0.03^{a}	5.01±0.09^{a}	4.50±0.18^{b}	4.20±0.04^{b}	4.13±0.07^{b}	0.00

[1] AKP = alkaline phosphatase (king unit/g prot); AMS = amylase (U/mg prot); CK = creatine kinase (U/mg prot); γ-GT = γ-glutamyltransferase (U/L); LPS = lipase (U/g prot).

[2] Values are mean ± SE. Mean values within the same row with different superscripts are significantly different (*P*<0.05).

[3] in U/mg prot.

Table 10. Effects of fishmeal replacement by TM meal on skin colour parameters of large yellow croaker.[1,2]

	Diets							
	TM0	TM15	TM30	TM45	TM60	TM75	TM100	*P*-value
Dorsal region 1								
L*	46.68±1.60	50.80±1.81	47.23±1.54	49.78±1.61	47.34±1.59	46.96±2.10	49.88±2.22	0.49
a*	-0.02±0.62	0.07±0.47	-0.70±0.50	0.40±0.56	-1.02±0.45	-1.10±0.51	-1.49±0.74	0.13
b*	10.00±1.26	9.26±0.68	10.87±0.98	9.43±0.21	10.62±0.92	11.8±0.59	11.03±0.86	0.29
Dorsal region 2								
L*	63.66±2.05	63.78±1.82	61.10±2.75	64.86±3.13	66.99±3.26	69.4±2.89	70.21±2.05	0.23
a*	-0.31±0.50	-0.12±0.31	-0.79±0.66	0.10±0.40	-0.30±0.24	-0.84±0.29	-0.53±0.39	0.68
b*	14.94±0.66	14.87±0.85	14.48±0.94	13.75±0.67	14.23±1.12	13.55±0.83	14.84±0.93	0.85
Caudal region								
L*	84.51±1.19	84.65±1.10	85.53±0.76	85.59±1.27	85.30±1.99	88.50±0.56	86.32±0.35	0.16
a*	-3.35±0.68	-3.13±0.55	-3.74±0.98	-4.17±0.71	-3.93±1.16	-3.98±0.94	-4.18±0.67	0.95
b*	28.56±1.86	23.53±1.47	26.75±1.51	23.27±1.93	29.08±2.24	25.32±2.81	24.60±1.22	0.12
Ventral region 1								
L*	89.88±0.72	89.57±0.79	90.88±1.10	91.53±1.22	89.76±0.83	88.78±0.83	88.65±1.49	0.40
a*	-5.67±1.18^{ab}	-4.14±1.01^{a}	-3.46±0.61^{a}	-4.81±1.03^{ab}	-8.30±0.47^{b}	-7.09±0.87^{ab}	-7.07±0.39^{ab}	0.00
b*	32.75±4.09^{abc}	24.53±2.94^{bc}	21.86±1.59^{c}	24.91±4.38^{bc}	38.25±2.91^{a}	36.85±3.45^{ab}	34.6±2.08^{abc}	0.00
Ventral region 2								
L*	90.04±0.57	89.31±0.95	89.61±0.89	90.73±0.89	88.75±1.31	90.72±0.28	89.77±0.68	0.52
a*	-5.33±0.47^{a}	-4.74±0.39^{a}	-5.13±0.89^{a}	-4.95±0.55^{a}	-8.22±0.34^{b}	-5.95±0.86^{ab}	-6.11±0.53^{ab}	0.00
b*	27.64±1.14^{ab}	24.53±1.82^{ab}	26.11±1.71^{ab}	23.21±1.63^{b}	34.40±0.98^{a}	27.14±4.01^{ab}	29.29±3.11^{ab}	0.04
Bottom of ventral								
L*	78.54±0.63	78.50±0.78	78.62±0.98	80.33±1.08	81.33±0.62	80.41±0.90	80.41±0.58	0.60
a*	-0.05±1.21^{ab}	0.35±1.30^{a}	0.00±1.47^{a}	-2.77±0.86^{ab}	-6.57±0.84^{b}	-3.34±1.06^{ab}	-5.76±0.86^{b}	0.01
b*	47.11±5.28^{ab}	37.25±5.17^{b}	47.52±5.48^{ab}	50.52±3.51^{ab}	62.69±5.11^{a}	52.50±4.40^{ab}	57.64±3.39^{a}	0.01

[1] a* = redness; b* = yellowness; L* = lightness.

[2] Values are mean ± SE. Mean values within the same row with different superscripts are significantly different (P<0.05).

Muscle nucleotides content

There were no significant differences in the muscle content of ATP, ADP, AMP, Hx and Ino among all the treatments (P>0.05) (Table 12). Fish in the TM75 and TM100 groups had significantly lower IMP content than that in the control group (P<0.05). The K and Ki values in TM100 group were significantly higher than those in the other groups (P<0.05).

Muscle free amino acids

According to Table 13, nineteen kinds of free amino acids were detected in muscle. The content of total free amino acids was significantly decreased with the substitution level increasing (P<0.05). It is known that free amino acids contributed to the formation of five tastes (Kong *et al.*, 2017; Merlo *et al.*, 2021; Zhu *et al.*, 2021). The contents of sweet amino acids (Gly, Ala, Ser, Thr, Pro) and sour amino acids (Glu, Asp, His) significantly decreased when the proportion of substitution over 45% (P<0.05). The content of umami taste amino acids (Glu, Asp, Gly, Ala, Phe, Tyr) increased first and then decreased, and the lowest value appeared in the TM100 group, while the content of salty taste amino acids (Glu, Asp) showed an opposite trend with the increase of TM meal inclusion (P<0.05). The content of bitter taste amino acids (Met, Val, Ile, Leu, Arg) was not significantly affected by dietary treatments (P>0.05).

Identification of volatile compounds in muscle

As shown in Figure 3, all information given by the fingerprint analysis technique was used to qualitatively characterise. The relative contents of volatile components were measured by peak volume normalisation (Table 14), seventeen volatile compounds were detected by the GC-IMS Library. Among them, 7 aldehydes, 6 ketones, 1 ester, 1 alcohol, 1 acid and 1 phenol were identified. The control group showed higher trans-2-pentenal,

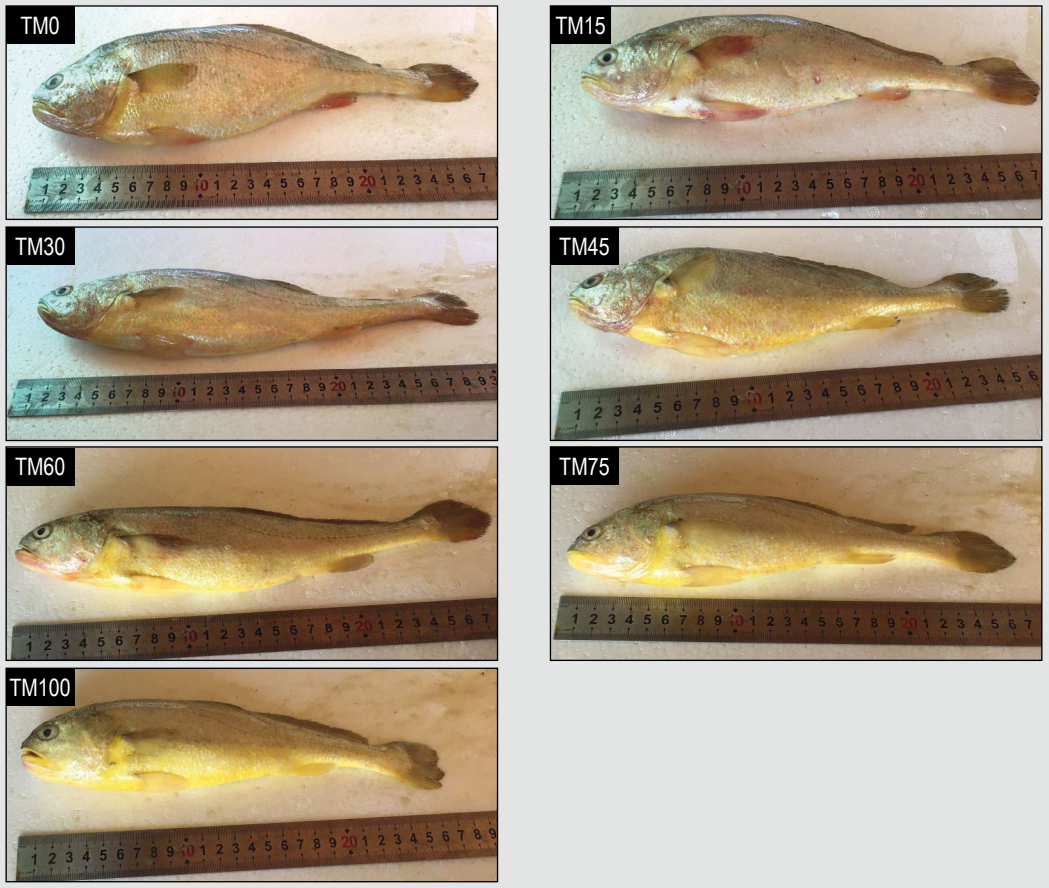

Figure 1. Large yellow croaker fed with different experimental diets after an 80-day feeding trial to show the skin colour.

Table 11. Muscle pH, water holding capacity and texture parameters of large yellow croaker.[1]

	Diets							
	TM0	**TM15**	**TM30**	**TM45**	**TM60**	**TM75**	**TM100**	***P*-value**
pH	7.02±0.03	7.08±0.05	7.02±0.04	7.07±0.03	7.05±0.03	7.07±0.02	7.04±0.08	0.93
Cooking loss (%)	7.29±0.81	8.69±0.62	9.14±1.03	9.49±0.87	9.36±0.54	10.08±0.89	10.74±0.93	0.16
Drip loss (%)	1.98±0.05^{b}	2.46±0.15^{ab}	2.69±0.09^{ab}	2.62±0.13^{ab}	2.75±0.12^{ab}	3.26±0.25^{a}	3.24±0.29^{a}	0.00
Texture parameters								
Hardness (N)	5.75±0.52	5.76±0.27	5.68±0.33	6.20±0.39	4.92±0.22	5.31±0.31	4.83±0.49	0.17
Springiness (mm)	1.43±0.08	1.32±0.09	1.41±0.09	1.45±0.07	1.32±0.05	1.29±0.05	1.11±0.11	0.07
Chewiness (mJ)	1.71±0.22	1.58±0.09	1.75±0.15	2.07±0.25	1.54±0.12	1.55±0.11	1.24±0.27	0.14
Cohesiveness	0.21±0.01	0.21±0.01	0.22±0.01	0.22±0.01	0.24±0.01	0.23±0.01	0.21±0.01	0.39
Adhesiveness (N*mm)	0.11±0.01^{b}	0.16±0.03^{ab}	0.15±0.01^{ab}	0.18±0.01^{a}	0.17±0.01^{ab}	0.17±0.01^{ab}	0.16±0.02^{ab}	0.03
Gumminess (N)	1.19±0.14	1.20±0.05	1.24±0.08	1.38±0.11	1.18±0.08	1.19±0.05	1.04±0.14	0.48
Shear force (N)	9.14±0.48^{a}	8.27±0.54^{ab}	8.77±0.20^{ab}	9.58±0.93^{a}	8.14±0.49^{ab}	7.36±0.23^{b}	7.21±0.32^{b}	0.02

[1] Values are mean ± SE. Mean values within the same row with different superscripts are significantly different (P<0.05).

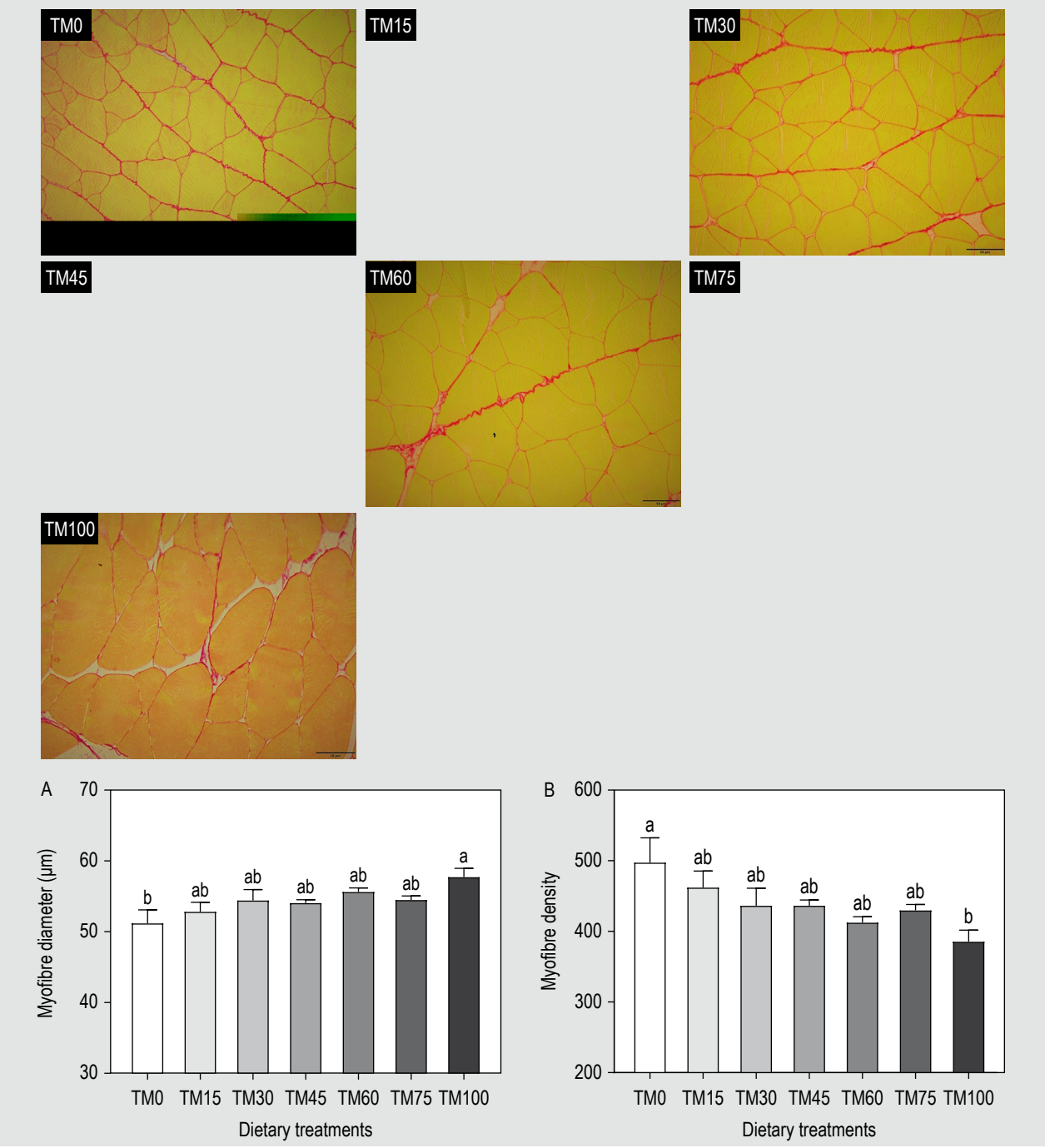

Figure 2. Muscle morphology of large yellow croaker stained with picrosirius red stain (bar=50 μm). Muscle cellularity (A): myofibre diameter (μm), (B): myofibre density. Data are shown as mean ± SE. Values with different letters means significant differences (*P*<0.05, Tukey's test).

2-hexanone, hexanal, benzaldehyde, 2-heptanone and pentanal contents compared to other groups. Meanwhile, higher levels of 2-methylbutanal, 3-methylbutanal, acetic acid, methylpropanal and 3-methylbutanol were detected mainly in TM75 and TM100 groups. The relative content of total volatile components increased significantly with the replacement level increasing (P<0.05).

Gene expression in muscle

The levels of gene expression in muscle are presented in Figure 4. The expression of *MyoD*, *MyoG*, *myf5* and *Pax-7* decreased generally by the increasing dietary TM meal levels, although no significant differences were detected between groups (P>0.05). The mRNA level of *myf6* first increased and then decreased, and the lowest value appeared

Table 12. Muscle nucleotides content (μmol/g, wet basis) and freshness (%) of large yellow croaker.[1,2]

	Diets							
	TM0	TM15	TM30	TM45	TM60	TM75	TM100	*P*-value
ATP	0.09±0.01	0.09±0.00	0.08±0.01	0.12±0.01	0.11±0.01	0.11±0.01	0.10±0.01	0.09
ADP	0.18±0.01	0.17±0.01	0.18±0.02	0.19±0.01	0.19±0.01	0.19±0.01	0.18±0.02	0.62
AMP	0.42±0.04	0.41±0.06	0.49±0.01	0.33±0.02	0.36±0.02	0.38±0.03	0.37±0.03	0.09
IMP	6.06±0.03^{a}	5.83±0.08^{ab}	5.99±0.14^{ab}	5.69±0.10^{ab}	5.76±0.02^{ab}	5.54±0.16^{bc}	5.12±0.13^{c}	0.00
Ino	0.15±0.01	0.13±0.01	0.15±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.62
Hx	0.23±0.01	0.22±0.01	0.23±0.01	0.23±0.01	0.23±0.01	0.22±0.01	0.23±0.01	0.81
K (%)	5.36±0.11^{b}	5.17±0.05^{b}	5.36±0.11^{b}	5.48±0.15^{b}	5.52±0.21^{b}	5.50±0.22^{b}	6.02±0.15^{a}	0.04
Ki (%)	5.93±0.12^{b}	5.72±0.10^{b}	5.99±0.11^{b}	6.07±0.16^{b}	6.11±0.22^{b}	6.13±0.25^{b}	6.74±0.18^{a}	0.03

[1] ADP = adenosine-5'-diphosphate; AMP = adenosine-5'-monophosphate; ATP = adenosine-5'-triphosphate; Hx = hypoxanthine; IMP = inosine-5'-monophosphate; Ino = inosine; K (%) = 100× [(Ino+Hx) / (ATP+ADP+AMP+IMP+Ino+Hx)]; Ki (%) = 100× [(Ino + Hx)/(IMP+Ino+Hx)].

[2] Values are mean ± SE. Mean values within the same row with different superscripts are significantly different (*P*<0.05).

Table 13. Effects of fishmeal replacement by TM meal on muscle free amino acids content of large yellow croaker (mg/100 g, wet basis).[1]

	Diets							
	TM0	TM15	TM30	TM45	TM60	TM75	TM100	*P*-value
Alanine	11.86±0.86^{ab}	12.27±0.41^{ab}	10.65±0.19^{bc}	9.69±0.77^{bc}	8.53±0.74^{c}	12.11±0.69^{ab}	14.25±0.66^{a}	0.00
Arginine	5.50±0.28^{a}	3.79±0.33^{ab}	3.89±0.27^{ab}	4.10±0.65^{ab}	2.87±0.58^{b}	2.46±0.12^{bc}	0.93±0.18^{c}	0.00
Aspartic acid	1.02±0.07^{bc}	1.31±0.13^{abc}	0.90±0.18^{c}	0.90±0.10^{c}	0.83±0.04^{c}	1.48±0.31^{ab}	1.54±0.09^{a}	0.02
Cysteine	6.46±0.74	6.32±0.19	5.92±0.33	6.55±0.11	6.07±0.26	6.58±0.40	6.65±0.32	0.78
Glutamic acid	7.14±0.35^{b}	7.20±0.40^{b}	5.54±0.44^{b}	6.97±0.51^{b}	6.13±0.24^{b}	7.54±0.38^{b}	10.15±0.64^{a}	0.00
Glycine	100.79±9.01^{abc}	131.45±10.05^{ab}	140.85±3.46^{a}	112.69±11.22^{ab}	122.70±11.22^{ab}	96.05±8.00^{bc}	58.16±6.26^{c}	0.00
Histidine	22.93±1.57^{a}	21.14±0.59^{ab}	21.90±1.29^{a}	19.95±0.21^{ab}	16.30±0.75^{bc}	13.02±1.43^{c}	6.32±1.24^{d}	0.00
Hydroxyproline	15.68±0.90^{a}	13.74±0.63^{ab}	16.56±0.76^{a}	10.15±1.43^{bc}	9.40±1.68^{bc}	5.58±0.57^{cd}	2.31±0.06^{d}	0.00
Isoleucine	2.14±0.12^{ab}	2.26±0.20^{a}	1.63±0.29^{ab}	1.69±0.05^{ab}	1.31±0.11^{b}	1.87±0.20^{ab}	2.25±0.22^{a}	0.02
Leucine	0.77±0.21	1.38±0.14	0.95±0.30	0.71±0.04	0.87±0.32	1.31±0.46	1.77±0.65	0.36
Lysine	30.22±1.42^{a}	25.87±0.48^{ab}	22.46±0.52^{b}	21.31±1.22^{bc}	15.93±1.55^{d}	16.28±1.31^{cd}	8.76±0.58^{e}	0.00
Methionine	2.31±0.89	2.51±0.45	2.87±0.34	3.38±0.06	2.95±0.57	2.07±0.63	2.61±0.35	0.67
Phenylalanine	1.61±0.39	1.99±0.32	1.19±0.09	1.05±0.09	1.08±0.04	2.32±1.38	1.26±0.17	0.61
Proline	98.49±8.57^{a}	60.58±4.33^{bc}	59.08±8.96^{bc}	76.80±2.49^{ab}	45.50±5.39^{cd}	60.21±5.28^{bc}	29.50±3.40^{d}	0.00
Serine	44.02±3.93	55.56±8.02	42.23±7.58	49.46±4.21	49.80±3.25	59.69±0.95	45.67±2.46	0.22
Taurine	89.00±4.25^{ab}	71.00±0.98^{ab}	76.60±4.29^{ab}	67.71±4.52^{b}	73.48±9.72^{ab}	75.04±2.68^{ab}	91.90±3.78^{a}	0.03
Threonine	49.67±5.02^{a}	19.24±5.46^{ab}	21.91±0.86^{b}	15.01±2.94^{ab}	11.17±2.77^{ab}	8.53±1.74^{ab}	3.82±0.28^{c}	0.00
Tyrosine	3.02±0.24	3.29±0.30	2.99±0.17	2.74±0.04	3.13±0.23	2.69±0.05	2.77±0.14	0.30
Valine	5.46±0.31	5.96±0.69	5.40±1.08	6.16±0.61	4.52±0.57	4.60±0.58	4.36±0.56	0.37
Total FAA	498.10±8.38^{a}	446.88±10.57^{b}	443.52±17.31^{b}	417.04±18.37^{bc}	382.55±14.68^{c}	379.44±3.99^{c}	294.96±12.49^{d}	0.00
EAA	120.61±3.38^{a}	84.15±5.62^{b}	82.20±0.69^{b}	73.36±5.23^{bc}	56.99±5.10^{cd}	52.47±4.93^{d}	32.07±1.14^{e}	0.00
NEAA	272.81±9.81^{a}	277.99±14.34^{a}	268.16±16.43^{a}	265.81±16.56^{a}	242.68±6.96^{a}	246.35±6.22^{a}	168.68±10.87^{b}	0.00
Umami taste[2]	125.44±10.25^{ab}	157.51±10.37^{a}	162.12±3.46^{a}	134.06±10.12^{a}	142.40±10.58^{a}	122.19±5.81^{ab}	88.11±5.23^{b}	0.00
Sweet taste[3]	304.83±11.12^{a}	279.10±11.48^{ab}	274.72±16.23^{ab}	263.66±19.91^{ab}	237.70±8.68^{b}	236.60±5.24^{b}	151.40±11.65^{c}	0.00
Salty taste[4]	8.17±0.33^{bc}	8.51±0.27^{bc}	6.44±0.56^{c}	7.88±0.42^{bc}	6.96±0.26^{bc}	9.01±0.40^{b}	11.69±0.73^{a}	0.00
Bitter taste[5]	16.18±1.48	15.90±0.46	14.74±0.91	16.04±1.31	12.51±1.87	12.31±1.58	11.91±1.41	0.13
Sour taste[6]	31.10±1.25^{a}	29.65±0.68^{a}	28.34±0.89^{a}	27.82±0.26^{ab}	23.25±0.99^{bc}	22.03±1.64^{cd}	18.01±0.51^{d}	0.00

[1] EAA = essential amino acids; NEAA = non-essential amino acids; Total FAA = total free amino acid content. [2] Umami taste: Glu + Asp + Gly + Ala +Phe +Tyr. [3] Sweet taste: Gly + Ala + Ser + Thr + Pro. [4] Salty taste: Glu + Asp. [5] Bitter taste: Met + Val + Ile + Leu + Arg. [6] Sour taste: Glu + Asp + His.

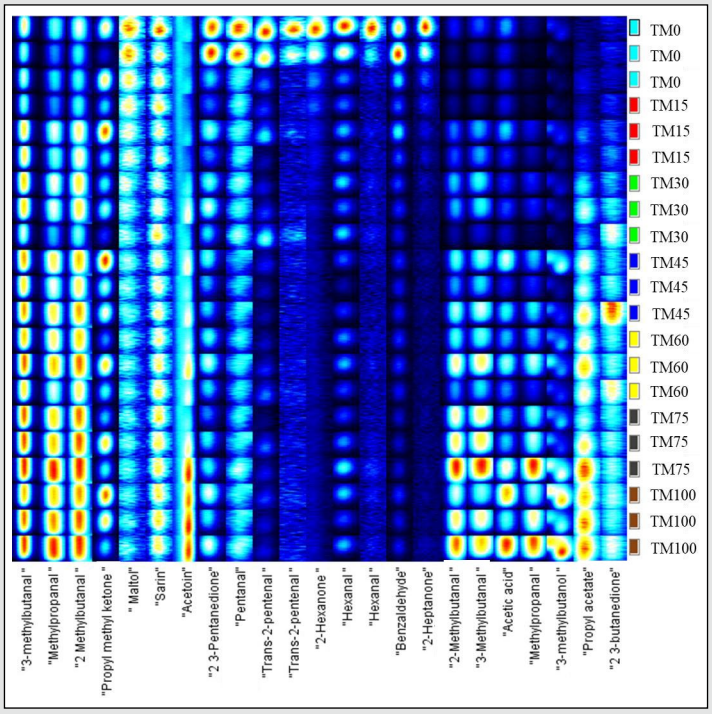

Figure 3. Fingerprints of all the samples generated by Gallery plot. Each row represents a sample (n=3), and each column represents a signal peak for a compound. Some compounds show two peaks, corresponding to monomers and dimers. The colours of the points qualitatively represent the concentration of the substance: light blue indicates low concentration, and red indicates greater concentration.

in the TM100 group ($P<0.05$). The transcription level of *mstn* in the TM75 and TM100 groups were significantly higher than those in the groups of TM15, TM30, TM45 ($P>0.05$).

4. Discussion

The TM meal as substitute for fishmeal has been studied in a variety of aquaculture animal species. However, there are no reports on the effects of dietary TM meal on growth performance of large yellow croaker. The present study found that replacement of fishmeal by TM meal had no significant effect on SR, and the TM meal could replace at least 30% of dietary fishmeal protein without significantly negative effect on the growth of large yellow croaker. Similarly, the African catfish showed equal growth performance when TM meal was used to replace 40% of dietary fishmeal compared with control group (Ng *et al.*, 2001). And previous study in European sea bass juveniles found that full-fat TM meal could be used 25% of inclusion in diet without affecting growth performance, while 50% content led to a worsening fish performance (Gasco *et al.*, 2016). In the present study, the FI decreased in the TM75 and TM100 groups, suggesting that high proportion substitution may affect palatability and thus reduce growth performance. The FCR significantly increased when the replacement level over 45%, indicating that feed utilisation was affected, which may be another reason for reduced growth performance.

The utilisation of nutrients depends on the activities of digestive and absorptive enzymes, which play key roles in breaking down and assimilating food. The exocrine pancreas of fish could synthesis and secrete many digestive enzymes into the intestinal lumen, such AMS, LPS, trypsin and chymotrypsin. Intestinal AKP is considered to be involved in absorption of nutrients, such as lipid, glucose and inorganic phosphate (Villanueva *et al.*, 1997), and γ-GT is involved in peptide transport (Griffith and Meister, 1980). The Na^+K^+-ATPase and CK play an important role in the energy metabolism of cells involved in the transport of phosphate, amino acids or glucose into the cells (Decking *et al.*, 2001; Geering, 1990). The present study showed that the activities of AMS, LPS, trypsin and chymotrypsin significantly decreased when the replacement of dietary fishmeal by TM meal over 45%. At the same time, the activities of AKP, γ-GT, Na^+K^+-ATPase and CK decreased following the inclusion of TM meal increasing. These data were consistent with the present results of growth performance, which indicated the high level of TM meal as substitution for fishmeal could reduce the activities of digestive and absorptive enzymes thus impair feed utilisation and growth performance of fishes. Some other research also found that high content of TM meal in

Table 14. Relative content of muscle volatile compounds of large yellow croaker by GC-IMS.[1]

	Diets							
	TM0	TM15	TM30	TM45	TM60	TM75	TM100	*P*-value
Aldehydes								
3-methylbutanal	1.00±0.06^{c}	1.29±0.01^{bc}	1.48±0.01^{b}	1.56±0.07^{b}	1.61±0.10^{b}	2.04±0.04^{a}	1.94±0.11^{a}	0.00
Methylpropanal	1.00±0.06^{e}	1.28±0.07^{de}	1.45±0.01^{cde}	1.86±0.14^{bcd}	1.94±0.24^{bc}	2.45±0.15^{ab}	2.61±0.14^{a}	0.00
2-methylbutanal	1.00±0.06^{d}	1.18±0.10^{cd}	1.36±0.12^{bcd}	1.75±0.10^{bc}	1.91±0.10^{ab}	2.45±0.20^{a}	2.35±0.09^{a}	0.00
Hexanal	1.00±0.03^{a}	0.48±0.04^{b}	0.55±0.04^{b}	0.35±0.04^{b}	0.47±0.05^{b}	0.51±0.08^{b}	0.50±0.05^{b}	0.00
Trans-2-pentenal	1.00±0.08^{a}	0.47±0.03^{b}	0.29±0.01^{bc}	0.27±0.02^{c}	0.30±0.04^{bc}	0.29±0.04^{bc}	0.29±0.03^{bc}	0.00
Pentanal	1.00±0.01^{a}	0.61±0.01^{b}	0.66±0.02^{b}	0.51±0.02^{c}	0.57±0.02^{bc}	0.59±0.03^{bc}	0.60±0.02^{bc}	0.00
Benzaldehyde	1.00±0.10^{a}	0.41±0.06^{b}	0.30±0.02^{b}	0.30±0.01^{b}	0.30±0.02^{b}	0.25±0.01^{b}	0.29±0.01^{b}	0.00
Ketones								
Propyl methyl ketone	1.00±0.01	1.00±0.05	0.68±0.04	0.82±0.03	0.87±0.14	0.86±0.14	0.81±0.07	0.17
Acetoin	1.00±0.02^{bc}	0.90±0.05^{c}	1.06±0.07^{bc}	1.19±0.05^{bc}	1.26±0.07^{abc}	1.34±0.15^{ab}	1.60±0.03^{a}	0.00
2,3-pentanedione	1.00±0.01^{a}	0.57±0.03^{b}	0.57±0.02^{b}	0.43±0.04^{b}	0.51±0.06^{b}	0.47±0.01^{b}	0.57±0.07^{b}	0.00
2-hexanone	1.00±0.18^{a}	0.47±0.04^{b}	0.38±0.02^{b}	0.35±0.02^{b}	0.33±0.01^{b}	0.37±0.01^{b}	0.34±0.01^{b}	0.00
2-heptanone	1.00±0.14^{a}	0.42±0.03^{b}	0.28±0.02^{b}	0.24±0.02^{b}	0.26±0.02^{b}	0.24±0.01^{b}	0.26±0.01^{b}	0.00
2,3-butanedione	1.00±0.02^{c}	1.31±0.03^{bc}	1.86±0.23^{abc}	2.09±0.21^{ab}	2.19±0.33^{a}	1.93±0.07^{ab}	1.78±0.14^{abc}	0.00
Alcohol								
3-methylbutanol	1.00±0.05^{c}	0.97±0.07^{c}	0.92±0.04^{c}	2.01±0.20^{b}	1.92±0.10^{b}	2.30±0.17^{b}	3.11±0.28^{a}	0.00
Acid								
Acetic acid	1.00±0.08^{d}	1.08±0.11^{cd}	1.14±0.05^{cd}	2.25±0.27^{bc}	1.98±0.25^{bcd}	2.35±0.39^{b}	4.23±0.33^{a}	0.00
Phenol								
Maltol	1.00±0.04^{a}	0.81±0.03^{b}	0.71±0.02^{bc}	0.70±0.02^{bc}	0.65±0.04^{c}	0.64±0.02^{c}	0.68±0.04^{bc}	0.00
Ester								
Propyl acetate	1.00±0.09^{d}	1.52±0.09^{cd}	2.24±0.16^{c}	3.52±0.38^{b}	4.10±0.15^{ab}	4.43±0.40^{ab}	4.92±0.17^{a}	0.00
Total	1.00±0.04^{cd}	0.97±0.04^{d}	1.03±0.02^{cd}	1.22±0.06^{cd}	1.29±0.09^{bc}	1.54±0.09^{ab}	1.62±0.06^{a}	0.00

[1] The relative contents of volatile components were measured by peak volume normalisation (the peak volume of the control group was regarded as 1). Values are mean ± SE. Mean values within the same row with different superscripts are significantly different (P<0.05).

diet could lead to worse feed utilisation. The reason could be the presence of chitin in TM meal, the main component of the insect exoskeleton, which could affect digestibility of the nutrients and feed utilisation (Dias *et al.*, 1998; Henry *et al.*, 2015; Kroeckel *et al.*, 2012; Longvah *et al.*, 2011).

Body condition index is often used to evaluate the health, growth and feeding of fish (Hartman and Margraf, 2006). It is also an important evaluation standard of fish quality. Meanwhile, body condition indices can reflect energy or nutrient reserves, as many studies have shown that they were significantly related to fish proximate composition (Brown and Murphy, 2004; Kaufman *et al.*, 2007; Pangle and Sutton, 2010). Lipid is the main energy storage material in fish (Tocher, 2003). In the present study, CF and hepatosomatic index were positively related to muscle lipid content, which is similar to the results from previous study in walleyes (Kaufman *et al.*, 2007). According to Kroeckel *et al.* (2012), the decrease of crude lipid content was mainly due to the reduction of feed intake with the increase of replacement level of fish meal with black soldier fly meal, which may also have affected physical indicators. In addition, the content of crude protein in TM100 group was significantly higher than that in the control group in the present study. Similar results were reported in rainbow trout (Belforti *et al.*, 2016), yellow catfish (Su *et al.*, 2017) and giant freshwater prawn (*Macrobrachium rosenbergii*) (Feng *et al.*, 2019). It is reported that the conversion of dietary protein to muscle is a heavy process that requires lipids as an energy source, resulting in a decrease in lipid content and an increase in protein content in muscle (Xu *et al.*, 2015). This is also a possible explanation and the exact metabolic mechanism needs to be further explored.

Amino acids are precursors of protein synthesis. In the present study, both protein-bound EAA and NEAA in muscle were significantly increased with higher TM meal, which was contrary to the trend of EAA and consistent with the trend of NEAA in feed. However, with the increase of replacement level, both free EAA and NEAA in muscle

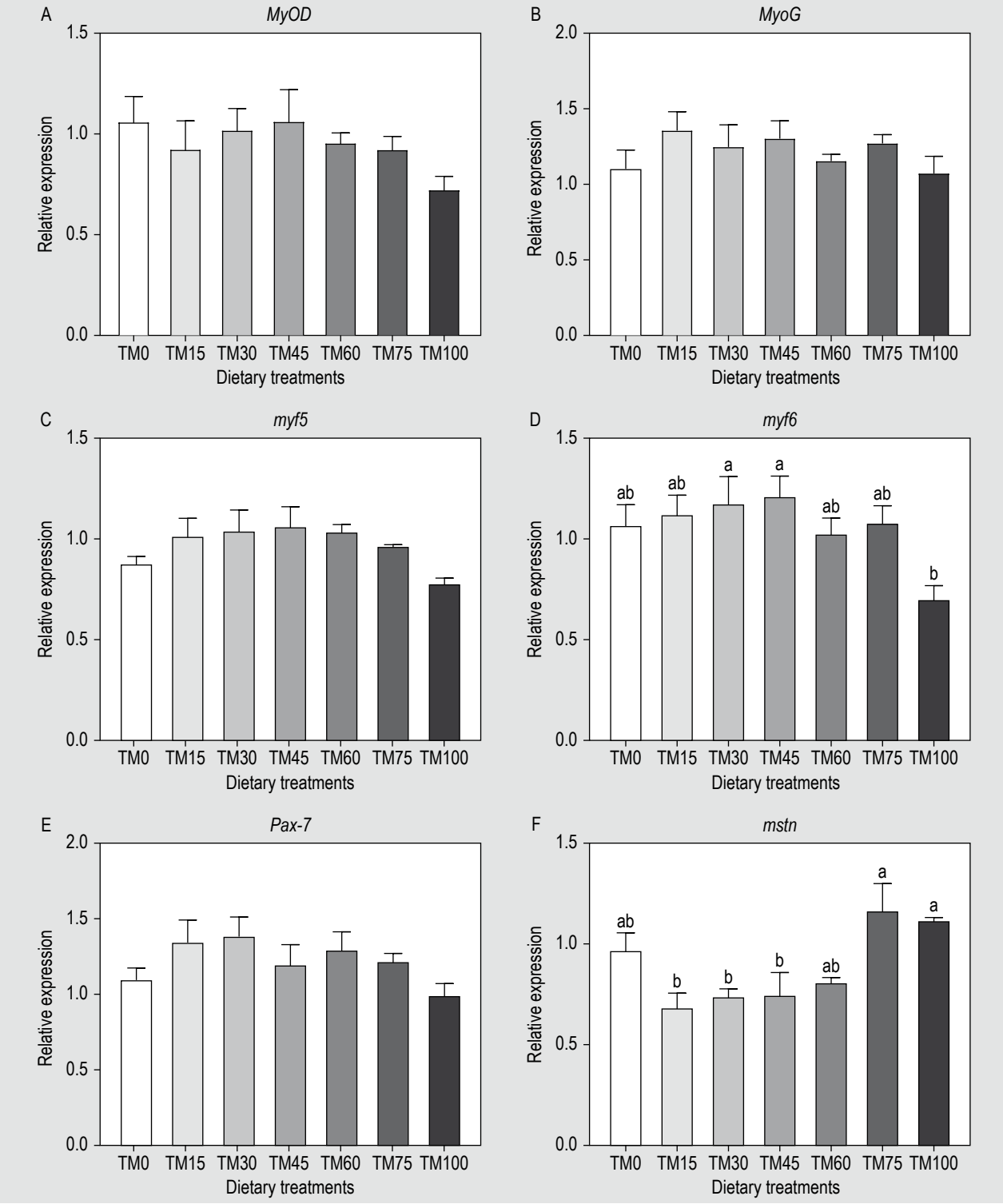

Figure 4. Expression of genes related to the muscle cell growth and differentiation: myogenic regulatory factors (*MyoD, MyoG, myf5, myf6*) (A-D), *Pax-7* (E) and *mstn* (F). Data are shown as mean ± SE. Values with different letters means significant differences (*P*<0.05, Tukey's test).

showed decreasing trend, which were completely contrary to the trend of protein-bound ones. The concentration of free amino acids (FAA) in tissues are mainly controlled by dietary amino acid absorption rate, amino acid oxidation and protein conversion (Carter *et al.*, 1995). According to Carter *et al.* (1995) and Houlihan *et al.* (1993), protein synthesis is the main factor in the removal of amino acid from FAA pools, which may explain the decrease trend

of FAA with the increase of protein-bound amino acids in muscle in the present study. There was no significant difference in crude protein content in muscle except for TM100, which further indicated that protein-bound and free amino acids could complement each other, so that the total amino acid content in muscle did not change significantly. In the research of Iaconisi *et al.* (2019), replacement of fish meal by TM meal also increased the contents of some essential and non-essential amino acids in muscle of gilthead sea bream and rainbow trout, but it did not provide a clear explanation, indicating that the relationship between amino acid utilisation and digestion and absorption needs further study.

Skin colour and appearance are important factors affecting the market acceptability of aquatic products (Haard, 1992a). Golden yellow skin colour is usually the first direct standard for consumers to evaluate the quality of large yellow croaker. The results in present study showed that the redness (a*) gradually decreased while the yellowness (b*) gradually increased in the ventral regions and the bottom of ventral of fish skin with the dietary TM meal level increasing. It is reported that mealworms contain a variety of carotenoids and riboflavin (Finke, 2002, 2015; Schabel *et al.*, 2010). This may explain that the dietary inclusion of TM meal positively affected skin colour of large yellow croaker, because several studies have shown that the addition of carotenoids to the diet can improve the skin colour of large yellow croaker, such as zeaxanthin, astaxanthin, xanthophylls (Yi *et al.*, 2014), lutein and canthaxanthin (Yi *et al.*, 2016). However, the present results were different from the other study in blackspot sea bream, in which a* value of skin significantly increased while L* value significantly decreased when TM meal replaced 0-50% fish meal (Iaconisi *et al.*, 2017). It was reported that the effectiveness of carotenoid source in terms of deposition and pigmentation is species specific and skin lightness (L*) seems to be influenced by environmental factors (Pavlidis *et al.*, 2006), which could partly explain the differences of pigmentation in different fish species.

Fish muscle is the main edible portion (Periago *et al.*, 2005), muscle quality is another factor affecting the market acceptability of fishes which could be affected by nutrient composition. In general, firm, elastic and juicy fish flesh is more acceptable to consumers. Fillet texture is one of the most important criterions to evaluate the muscle quality, of which the shear force is considered to reflect the flesh firmness of fish (Johnston *et al.*, 2006). In the present study, the fillet springiness and shear force tend to decrease with increasing dietary TM meal levels, which indicated that the muscle firmness tended to decrease. It has been reported that the muscle firmness may be positively correlated with collagen and hydroxyproline content (Johnston *et al.*, 2006; Ma *et al.*, 2019; Sato *et al.*, 1986; Wang *et al.*, 2015). In the present study, it was showed that muscle hydroxyproline and collagen content significantly decreased in TM75 and TM100 group. Water-holding capacity is also an important flesh quality parameter reflecting the juicy of fish flesh, which could be evaluated by cooking loss and drip loss. In the present study, it was found that drip loss significantly increased in TM75 and TM100 groups reflecting the poor WHC. Loje *et al.* (2007) showed that WHC was also positively correlated with collagen and hydroxyproline content. In the present study, the shear force, WHC, hydroxyproline and collagen contents showed the similar downward trend. In addition, pH is also an important factor affecting flesh quality (Haard, 1992b), the post-mortem pH decline causes the degradation of connective tissue and softer flesh, however, no dramatic changes were observed in the present study.

Fish muscle is mainly composed of white muscle and red muscle. Muscle growth mainly depends on the hypertrophy and hyperplasia of muscle fibres (Veggetti *et al.*, 1990). The number of muscle fibres is affected by various factors, such as species, strains, diet, and environmental temperature (Ayala *et al.*, 2001; Lopez-Albors *et al.*, 2003), of which diet is an important factor. Muscle cellularity (the number and size distribution of fibres) also affects flesh texture and thus changes the quality of fish (Hurling *et al.*, 1996; Johnston *et al.*, 2000). For example, several studies have demonstrated that average muscle fibre diameter is negatively correlated with hardness of flesh (Hatae *et al.*, 1990; Hurling *et al.*, 1996), while the positive correlation between muscle fibre density and textural parameters (such as hardness, springiness) was found by Johnston *et al.* (2000) and Periago *et al.* (2005). In the present study, the muscle cellularity was affected by dietary treatments. With increasing replacement levels, muscle fibre diameter gradually increased, while muscle fibre density showed a similar downward trend with texture parameters such as hardness and springiness, which again confirmed above conclusions of previous studies.

Muscle growth is controlled by multiple genes and complex signalling pathways, in which the growth and differentiation of muscle cells are mainly regulated by positive regulatory factors (such as MyoD family) and negative regulatory factors (such as myostatin). In the MyoD family (*MyoD*, *MyoG*, *myf5* and *myf6*), *MyoD* and *myf5* are called determinant factor and related to myoblasts proliferation, while *MyoG* and *myf6* are called differentiation factor and related to myoblasts differentiation and myofiber hypertrophy (Vélez *et al.*, 2017; Weintraub, 1993). Myostatin (*mstn*), a member of the TGF-β superfamily, negatively regulates muscle development by inhibiting the proliferation and differentiation of myogenic progenitor cells (Joulia *et al.*, 2003). *Pax7* also plays an important role in muscle development (Mao *et al.*, 2008). Many studies have shown that the expression of *MyoD* and *MyoG* are positively correlated with fillet texture (Østbye *et al.*, 2018; Richter *et al.*, 2021). In the present study, total replacement of fishmeal with TM meal down-regulated the expression of *myf6*

gene, and up-regulated *mstn* gene expression, indicating that the growth and differentiation of muscle cells may be regulated by feed composition, and *mstn* gene inhibits the proliferation and differentiation of muscle cells by negatively regulating the transcriptional activity of MyoD family. The results showed that the addition of TM meal could reduce muscle development by regulating genes expression, which resulted in the decrease of muscle fibre density and texture parameters such as firmness and shear force in the high proportion replacement group.

Flavour of fish mainly includes the taste and smell, which is also an important evaluation standard of fish quality for consumers. Volatile components, free amino acids, organic acids, nucleotides and peptides contribute to flavour formation (Konosu, 1979). Taste is made up of five basic qualities: sweetness, saltiness, bitterness, sourness and umami taste (Ninomiya, 1987). According to Fuke and Konosu (1991), free amino acids and nucleotides play important roles in producing the taste. Among them, glutamate and IMP are typical umami taste-active substance (Maruji *et al.*, 2010; Yamaguchi and Ninomiya, 2000). In fish muscle, ATP is degraded through the ATP - ADP - AMP - IMP - Ino - Hx metabolic pathway (Ocaño-Higuera *et al.*, 2011). In addition, K and Ki values were calculated from concentrations of ATP and its degradation products and used to evaluate the freshness of fish, which have a strong correlation with muscle changes in fish after death (Hamada-Sato *et al.*, 2005). The present study showed that the contents of IMP and total free amino acids decreased with the increasing dietary TM meal inclusion level, and K and Ki values in TM100 group significantly higher than those in the other groups. These results indicated that a higher proportion of substitution had a certain adverse effect on the taste of large yellow croaker. The change of IMP may be related to the decrease of its precursors such as AMP and the activity of related enzymes in the metabolic process. Since there is no significant difference in the content of Ino and Hx, it leads to the decrease of K and Ki values. But according to the report of Saito *et al.* (1959), fillets with K value below 20% are still very fresh products.

The formation of volatile compounds is mainly through thermal degradation, lipid oxidation and amino acid degradation, the volatile compounds contributing to aromatic odour in fresh fish mainly include volatile carbonyls (aldehydes and ketones) and alcohols, which are obtained from PUFA through lipoxygenase-mediated reactions (Alasalvar *et al.*, 2005). In the present study, aldehydes and ketones were mainly detected in fish fillet. The aromatic smell of fish muscle is derived mainly from n-3 PUFA (Serot *et al.*, 2001), such as 2, 3-Pentanedione, which can give the muscle a sweet, butter-like and fruity smell, decreased with the increase of replacement level. And 3-Methylbutanal and 3-Methylbutanol, the products of degradation and oxidative deamination of Leucine (Mu *et al.*, 2017), showed similar upward trend with Leucine content following the increase of TM meal inclusion. Previous study showed that the changes of fatty acid composition in fish fillet affect the total volatile compounds and thus affect the flavour of fish (Turchini *et al.*, 2013). Therefore, the difference of muscle volatile compounds among treatments may be due to changes in muscle fatty acid and amino acid composition. Meanwhile, the fatty acid profile of fish muscle reflects the dietary fatty acid composition, which affects the quality and nutritional composition of fish muscle (Sánchez-Muros *et al.*, 2014). In the present study, the inclusion of TM meal in diets affected the muscle fatty acid profile. The content of n-3 PUFA (such as EPA) significantly reduced, while the percentage of n-6 series PUFA, especially Linoleic acid (C18:2n-6) significantly increased with the increase of TM inclusion. Thus, a reduction of Σn-3/Σ n-6 PUFA ratio was detected following the increase of TM inclusion. Similar results were obtained in European sea bass (Gasco *et al.*, 2016; Mastoraki *et al.*, 2020), common catfish (Roncarati *et al.*, 2015) and rainbow trout (Belforti *et al.*, 2016). This is mainly because most terrestrial insects, including yellow mealworms, are notably deficient in EPA and DHA and rich in linoleic acid (Sánchez-Muros *et al.*, 2014). The dietary fatty acid composition of the present study was determined, and the content of EPA and DHA decreased with the level of TM meal increasing. However, EPA and DHA have important biological functions in fish, but the rate of synthesis is lower than the nutritional requirement, so their addition in the feed is necessary.

5. Conclusion

In the present study, *T. molitor* meal can replace at least 30% of fishmeal protein in the diet for large yellow croaker without negative effects on the growth, feed utilisation and fish quality. The inclusion of TM meal in the diet improved the skin colour of large yellow croaker. In addition, from the perspective of body composition, texture and flavour analysis, histology and gene expression, higher replacement levels (75 and 100%) can adversely affect the texture and flavour of fish fillets.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2021.0144.

Table S1. Composition of TASA fish meal.

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Conflict of interest

The authors declare no conflict of interest.

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