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Animal Nutrition

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Short Communication

Partial replacement of soybean meal by yellow mealworm (*Tenebrio molitor*) meal influences the flesh quality of Nile tilapia (*Oreochromis niloticus*)

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ARTICLE INFO

Article history:

Received 10 May 2022

Received in revised form

23 September 2022

Accepted 27 September 2022

Available online 5 October 2022

Keywords:

Yellow mealworm meal

Novel feed protein source

Nile tilapia

Flesh quality

Texture property

ABSTRACT

This study investigated the effects of yellow mealworm meal (YM) replacing soybean meal (SBM) at different proportions (0%, 15%, 30% and 45%, referred as YM0, YM15, YM30 and YM45, respectively) on the flesh quality of Nile tilapia. A total of 360 fish (70.0 ± 0.12 g) were randomly divided into 4 groups (3 tanks per group). Fish were fed the experimental diet twice daily for 10 wk. The results showed that muscle protein content significantly decreased in YM30 and YM45, while the lipid content significantly decreased in YM45 ($P < 0.05$). The essential amino acids and flavor amino acids of the muscle were not affected by the YM substitution, while saturated fatty acid content decreased in YM30 and YM45 compared with YM0 ($P < 0.05$). Fillets in YM45 had higher hardness, gumminess, and a higher proportion of thin myofibers ($\leq 100 \mu\text{m}$, $P < 0.05$) than those in other groups. Further analysis revealed that apoptosis and atrophy related genes were up-regulated, while the muscle antioxidant capacity decreased significantly in YM45 ($P < 0.05$), which may be related to the high acid value in YM45 diet. Our findings indicated that YM could replace up to 30% SBM without substantially altering the flesh quality. When the replacement ratio increased to 45%, the flesh quality would change. Special attention should be paid to avoid feed rancidity which may affect the flesh quality of fish.

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1. Introduction

Aquaculture is one of the fastest-growing food-producing sectors. From 2000 to 2018, the contribution of global aquaculture production to global fish production increased from 25.7% to 46%, and it is expected to reach 50% by 2030 (FAO, 2020; Tran et al., 2022). Due to the high environmental cost of protein-rich

terrestrial plant cultivation, soybean meal (SBM), the most commonly used plant protein source for omnivorous fish, has become more expensive. The price of SBM has doubled in 2022 compared with that in 2019 in China (National Bureau of Statistics of the People's Republic of China, 2022, National Bureau of Statistics of the People's Republic of China, 2019). Exploring alternative protein sources is increasingly urgent (Hua et al., 2019).

Yellow mealworm (*Tenebrio molitor*) meal (YM) could be a promising protein source, because its crude protein content ranges from 47% to 60%, which is similar to or even higher than the protein content of SBM (Bernard, 2016; Makkar et al., 2014). In addition to having a favorable nutritional composition, YM also possesses environmental benefits (Makkar et al., 2014; Tran et al., 2022). Nowadays, the feasibility of YM as an alternative protein source for fishmeal has been explored in a variety of fishes (Henry et al., 2018; Sankian et al., 2018; Su et al., 2017). However, little attention has been paid to the effect of YM as a substitute for SBM.

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



With the increase in consumer health awareness, flesh quality has gained more and more attention. Sensory quality and nutritional value are two main aspects of flesh quality (Espe, 2008). Sensory quality is usually evaluated by texture parameters, including hardness, gumminess, cohesiveness, chewiness, and water-holding capacity (Ginés et al., 2004). Nutritional value includes the content of protein, lipid, unsaturated fatty acids, essential amino acids, etc (Mathew et al., 2019). It has been reported that dietary components are the main contributors to flesh quality (Wang et al., 2022). A study on rainbow trout (*Oncorhynchus mykiss*) revealed that substituting fishmeal with increasing levels of full-fat YM did not influence the fillet proximate composition, but decreased the ratios of polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) and n-3/n-6 (Iaconisi et al., 2018). Fillet firmness of *Litopenaeus vannamei* was unchanged, while the concentration of eicosapentaenoic acid and docosahexaenoic acid decreased with increasing levels of fishmeal substituted by YM (Panini et al., 2017). However, the effect of YM replacing SBM on fish flesh quality remains unknown.

Nile tilapia (*Oreochromis niloticus*) is the third-largest farmed fish all over the world with a total production of 4,525 thousand tonnes (Dawood et al., 2022). Nile tilapia has fast growth, physiological strength, high marketability, and excellent flesh quality (Khanjani and Sharifinia, 2021). Nowadays, SBM is the most commonly used plant protein source in tilapia feeds (El-Saidy and Saad, 2011). The feasibility of replacing SBM with YM has been verified in juvenile tilapia (Tubin et al., 2020), but its influence on fish flesh quality has not been investigated. Hence, in this study, YM was used to partially replace SBM to feed Nile tilapia for 10 wk, and the influence of the protein substitution on the fillet nutrient composition and texture properties was detected.

2. Materials and methods

2.1. Ethical statement

This experiment was approved by the Animal Experiment Ethics Committee of East China Normal University, and was conducted according to the requirements of the Care and Use of Laboratory Animals in China (20201002).

2.2. Experimental diets and feeding trial

Four isonitrogenous and isoenergetic experimental diets were formulated by replacing 0%, 15%, 30%, and 45% of SBM with YM referred to as YM0, YM15, YM30, and YM45, respectively. The formulation and proximate composition of the experimental diets were shown in [Supplementary Table S1](#). Feed processing operations were consistent with a previous study (Wu et al., 2022). The diets were stored in a dry and dark place at room temperature (25 ± 5 °C).

Nile tilapia (male) were purchased from Tianfa Fry Development Co. Ltd (Guangzhou, China). All fish were acclimated and reared to 70 g for the subsequent experiment. During the acclimation, fish were fed with a commercial feed containing 330 g/kg protein and 50 g/kg lipid (Tongwei, China). Three hundred and sixty healthy tilapia (70.0 ± 0.12 g) were randomly divided into 4 groups: YM0, YM15, YM30, and YM45 (3 tanks per group, 30 fish per tank). During the experiment, fish were fed twice daily (08:00 and 17:00). The feeding amount was 4% of body weight. After feeding, diet residue was collected and dried at 60 °C for the calculation of feed intake. The total weight of fish in each tank was weighed and recorded fortnightly and the feeding amount was adjusted accordingly. The experiment was carried out in an indoor-recirculating aquaculture system (Shanghai Haisheng, China). The

water temperature was maintained at 26 to 28 °C using an automatic temperature controller. The dissolved oxygen was maintained above 6.5 mg/L by continuous aeration. The ammonia nitrogen was <0.02 mg/L, and the photoperiod was set at a 12 h/12 h light/dark cycle using an automatic time controller. The experiment lasted for 10 wk.

2.3. Sample collection

Before sampling, all fish were fasted for 24 h and weighed to calculate the average body weight. Fish were anesthetized with MS-222 (120 mg/L, Sigma, USA). Nine fish from each treatment (3 individuals per tank) were used to measure body length, liver weight, condition factor (CF), hepatosomatic index (HSI), and carcass ratio. After removing scales and skin, the muscle above the right lateral line was dissected and sliced into 0.5-cm thick sections for texture analysis. The muscle above the left lateral line was collected for histological, biochemical, and molecular biological analysis. Another 6 fish (2 individuals per tank) were used for whole-body composition analysis. All samples were collected on ice and kept at -80 °C.

2.4. Whole-body, muscle, and feed biochemical composition analysis

The muscle moisture content was detected by drying the samples to a constant weight with a vacuum freeze dryer (Scientz-30ND, Scientz, China). The crude protein, crude lipid, and the moisture content of whole-body and feeding diet were determined according to our previous work (Wu et al., 2022). The content of hydroxyproline (A030-2-1) and glycogen (A043-1-1) in muscle was determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, China). The acid value of the diets was tested according to the GB/T 5009.229-2016 national standard.

2.5. Amino acid and fatty acid analysis

The amino acid composition was determined by using an L-8900 amino acid automatic analyzer (Hitachi, Japan), and the fatty acid methyl ester analysis was performed by GCMS-QP2010 SE (Shimadzu, Japan) according to the previous study (Wu et al., 2022). The relative proportions (% total fatty acids) of fatty acids were calculated by using the peak area ratio.

2.6. Texture analysis

A CT3-1500 texture analyzer (Brookfield, USA) was used to measure the texture parameters of 9 muscle samples from each treatment. The samples were compressed at a rate of 30 mm per minute using a 6 mm diameter metal probe. The target esteem of compression was 0.5 mm. The software TexturePro Adapt 2 (CNS Farnell Ltd., Great Britain) was used to calculate the hardness, gumminess, springiness, and cohesiveness based on force–time curves (Wu et al., 2021).

2.7. Histological analysis

Six dorsal white muscle samples of each treatment (2 individuals per tank) were used for histological analysis. Muscles were fixed in 4% paraformaldehyde and then embedded in paraffin. The 5- μ m thick muscle section was processed for hematoxylin-eosin analysis according to a previously reported method (Limbu et al., 2018) and was photographed using a 200 \times optical microscope (Nikon, Japan). The diameter of the muscle fiber was

represented by the longest axis length by using imaging software Nis-Elements version 4.60 (Nikon, Japan).

2.8. Quantitative real-time PCR

Total RNA extraction and cDNA synthesis were conducted according to the reference (Li et al., 2022) by using 6 muscle samples per group. Both elongation factor 1 α (*ef1 α*) and β -actin were used as the reference genes. The $2^{-\Delta\Delta Ct}$ method was used to estimate the relative expression level of genes. The primer sequences were listed in Supplementary Table S2.

2.9. Statistical analysis

The results were presented as mean \pm standard error of the mean (SEM). Normal distribution was tested by the Shapiro-Wilk test. Statistical analyses were conducted by using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. SPSS 23.0 (IBM, USA) was used to conduct statistical analysis. Bar graphs were produced by GraphPad Prism 7.0 software.

3. Results

3.1. Growth performance and whole-body proximate composition

As shown in Supplementary Table S3, with the increase in YM substitution level, there were no significant differences in weight gain (WG), specific growth rate (SGR), condition factor (CF), feed conversion ratio (FCR), protein efficiency ratio (PER) and hepatosomatic index (HSI) among the treatments ($P > 0.05$). The carcass ratio increased significantly in YM45 compared with YM0 ($P < 0.05$). Whole-body moisture, crude protein, and ash content were not affected by YM substitution ($P > 0.05$), while crude lipid content increased significantly in YM45 ($P < 0.05$).

3.2. Flesh nutritional value

As shown in Table 1, muscle moisture was similar among groups ($P > 0.05$). Muscle crude protein content in YM30 and

YM45 was significantly lower than that in YM0 ($P < 0.05$). Compared with YM0, muscle crude lipid content decreased significantly in YM45 ($P < 0.05$). The content of hydroxyproline and glycogen in muscle didn't differ significantly among treatments ($P > 0.05$). The content of essential amino acids (EAA), delicious amino acids (DAA), and flavor amino acids (FAA) in the muscle was not affected by the protein source replacement ($P > 0.05$). YM30 and YM45 showed significantly decreased SFA content compared to YM0 ($P < 0.05$). The amount of mono-unsaturated fatty acids (MUFA) increased in the muscle of YM30 and YM45, but only showed significance in YM30 ($P < 0.05$). The proportions of n-3 PUFA and n-6 PUFA, and the n-3/n-6 ratio were not affected by YM substitution ($P > 0.05$).

3.3. Texture properties

As shown in Fig. 1, muscle hardness and gumminess were significantly higher in YM45 than in YM0 ($P < 0.05$, Fig. 1a and b). The muscle springiness was significantly reduced in YM30 and YM45 ($P < 0.05$, Fig. 1c). The muscle cohesiveness and centrifugal loss were similar among groups ($P > 0.05$, Fig. 1d and e).

3.4. Myofiber histological characteristics and the expression level of myogenesis regulation and apoptosis-related genes

There were no significant differences in the average diameter of myofibers among treatments ($P > 0.05$), but the myofiber diameter distribution differed (Fig. 2e and f). In YM45, the proportion of myofiber with a diameter less than 100 μ m increased significantly ($P < 0.05$), while the proportion of myofiber with a diameter more than 100 μ m decreased significantly ($P < 0.05$, Fig. 2f).

To investigate the possible reason for the change in myofiber diameter, the expression levels of myogenesis (myogenic differentiation 2 [*myod2*] and myogenin [*myog*]), muscle atrophy (myostatin [*mstn*] and *atrogen-1*), and apoptosis (*caspase8*, *caspase9*, *caspase3*, and *caspase7*) related genes were assessed (Fig. 3). The expression level of *myod2* and *myog* were unchanged ($P > 0.05$), but the expression levels of *mstn* and *atrogen-1* in YM45 were significantly higher than those in YM0 ($P < 0.05$). Compared with YM0,

Table 1

Fillet proximate composition, amino acid, and fatty acid composition of Nile tilapia fed with the experimental diets for 10 wk¹.

Item	YM0	YM15	YM30	YM45
Proximate composition, % wet matter				
Moisture	77.46 \pm 0.24	77.45 \pm 0.23	77.05 \pm 0.28	78.12 \pm 0.52
Crude protein	21.08 \pm 0.31 ^a	20.63 \pm 0.16 ^{ab}	20.04 \pm 0.28 ^b	20.06 \pm 0.47 ^b
Crude lipid	2.32 \pm 0.10 ^a	2.04 \pm 0.10 ^{ab}	2.41 \pm 0.25 ^a	1.79 \pm 0.09 ^b
Hydroxyproline, μ g/g tissue	366.79 \pm 32.63	363.92 \pm 27.52	367.74 \pm 35.75	366.51 \pm 41.16
Glycogen, mg/g tissue	0.87 \pm 0.13	0.83 \pm 0.10	0.73 \pm 0.07	0.79 \pm 0.12
Amino acid composition, mg/g dry matter				
EAA ²	344.48 \pm 5.62	353.11 \pm 1.42	335.35 \pm 10.51	354.45 \pm 3.96
DAA ³	385.82 \pm 6.76	397.49 \pm 4.17	373.21 \pm 10.01	393.39 \pm 4.37
FAA ⁴	400.82 \pm 6.92	412.24 \pm 5.07	387.59 \pm 10.74	408.52 \pm 4.29
Fatty acid composition, % total fatty acids				
SFA	32.21 \pm 0.59 ^a	30.74 \pm 0.66 ^{ab}	30.16 \pm 0.63 ^b	30.40 \pm 0.29 ^b
MUFA	26.70 \pm 1.15 ^b	27.14 \pm 0.89 ^b	31.04 \pm 1.69 ^a	28.11 \pm 0.69 ^{ab}
PUFA	41.08 \pm 0.98	42.12 \pm 1.28	38.78 \pm 1.94	41.49 \pm 0.80
n-3PUFA	5.20 \pm 0.28	5.69 \pm 0.34	5.00 \pm 0.47	5.61 \pm 0.17
n-6PUFA	35.89 \pm 0.72	36.44 \pm 0.99	33.79 \pm 1.47	35.88 \pm 0.72
n-3/n-6	0.14 \pm 0.01	0.16 \pm 0.01	0.15 \pm 0.01	0.16 \pm 0.01

YM = yellow mealworm meal; EAA = essential amino acids; DAA = delicious amino acids; FAA = flavor amino acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Values were presented as mean \pm SEM (proximate composition and fatty acid composition $n = 6$, amino acid composition $n = 4$). Values in the same row with different superscripts were significantly different ($P < 0.05$).

¹ YM0, YM15, YM30, and YM45 represent using 0%, 15%, 30%, and 45% yellow mealworm meal to replace soybean meal, respectively.

² EAA includes threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine (Zhang et al., 2021).

³ DAA includes glutamic acid, alanine, aspartic acid, glycine, and arginine (Zhang et al., 2021).

⁴ FAA includes glutamic acid, alanine, aspartic acid, glycine, phenylalanine, and tyrosine (Zhang et al., 2021).

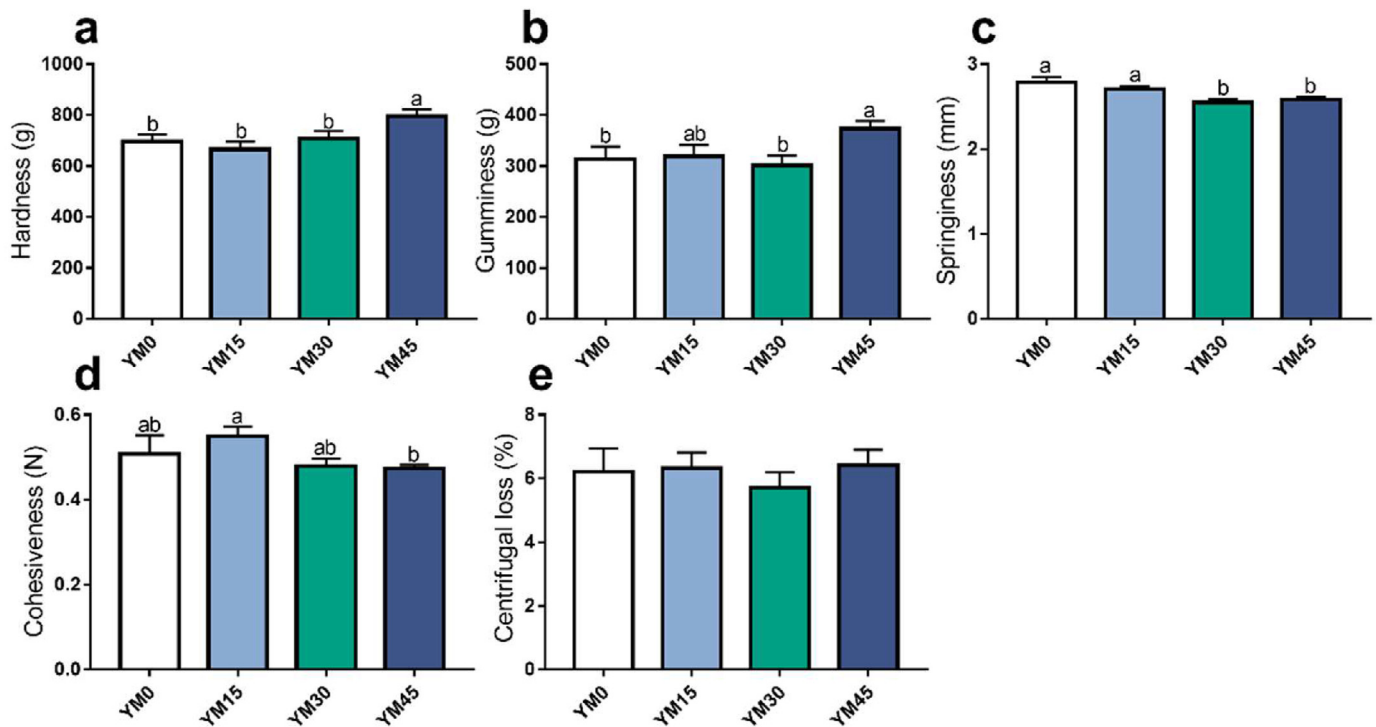


Fig. 1. Texture properties in the fillet of Nile tilapia fed with the experimental diets for 10 wk. YM = yellow mealworm meal. YM0, YM15, YM30, and YM45 represent using 0%, 15%, 30%, and 45% yellow mealworm meal to replace soybean meal, respectively. (a) Muscle hardness, (b) muscle gumminess, (c) muscle springiness, (d) muscle cohesiveness, and (e) muscle centrifugal loss. Values were presented as mean \pm SEM ($n = 9$). Bars with different letters indicated significant differences ($P < 0.05$).

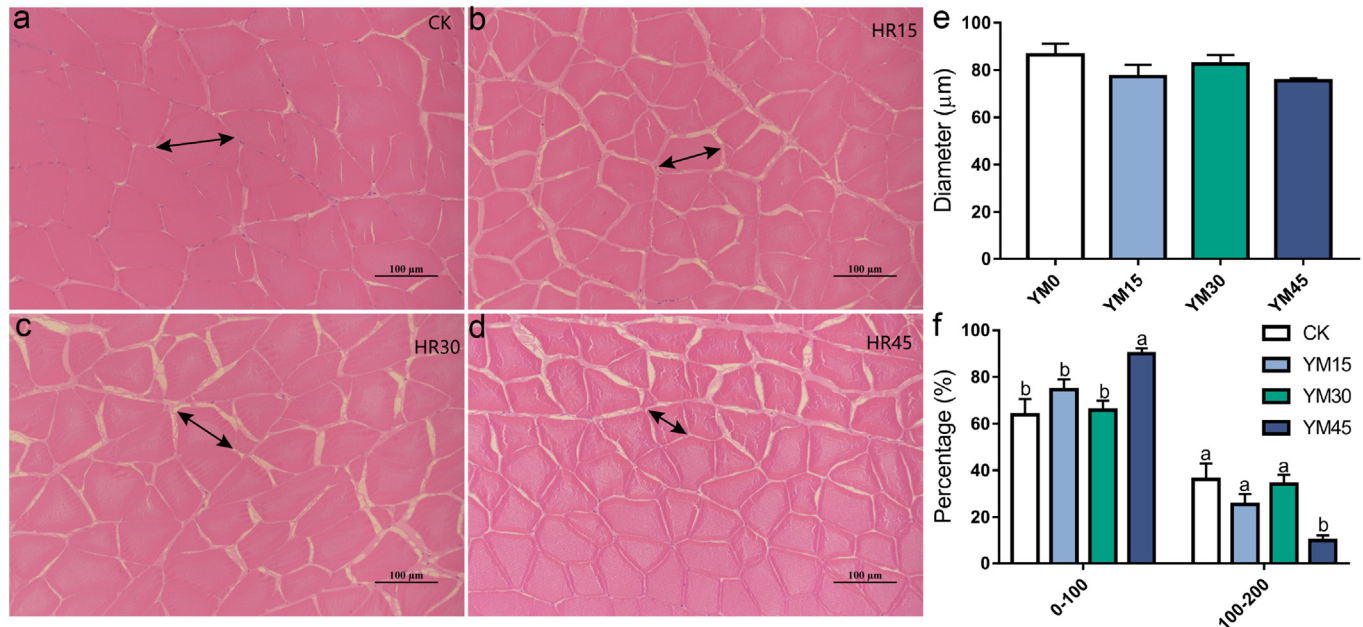


Fig. 2. Myofiber characteristics in the muscle of Nile tilapia fed with the experimental diets for 10 wk. YM = yellow mealworm meal. YM0, YM15, YM30, and YM45 represent using 0%, 15%, 30%, and 45% yellow mealworm meal to replace soybean meal, respectively (a–d) Muscle histological sections of YM0, YM15, YM30, and YM45 groups under $200\times$ magnification (scale bars indicate $100\ \mu\text{m}$, double-ended arrows illustrate long-axis distance). (e) The average diameter of myofiber (μm). (f) Myofiber diameter distribution (percentage of myofiber diameter within a certain range). Values were presented as mean \pm SEM ($n = 6$). Bars with different letters indicated significant differences ($P < 0.05$).

the transcript levels of *caspase8* and *caspase9* increased with a higher YM substitution level and showed significance in YM45 ($P < 0.05$). The apoptosis effector *caspase3* did not show a difference among treatments ($P > 0.05$), but *caspase7* was significantly up-regulated in YM45 ($P < 0.05$).

3.5. Anti-oxidative capacity of the muscle and the acid value of diets

In muscle of YM30 and YM45, superoxide dismutase (SOD) activity was significantly higher than that in YM0 ($P < 0.05$, Fig. 4a), while total antioxidation capacity (T-AOC) declined significantly as

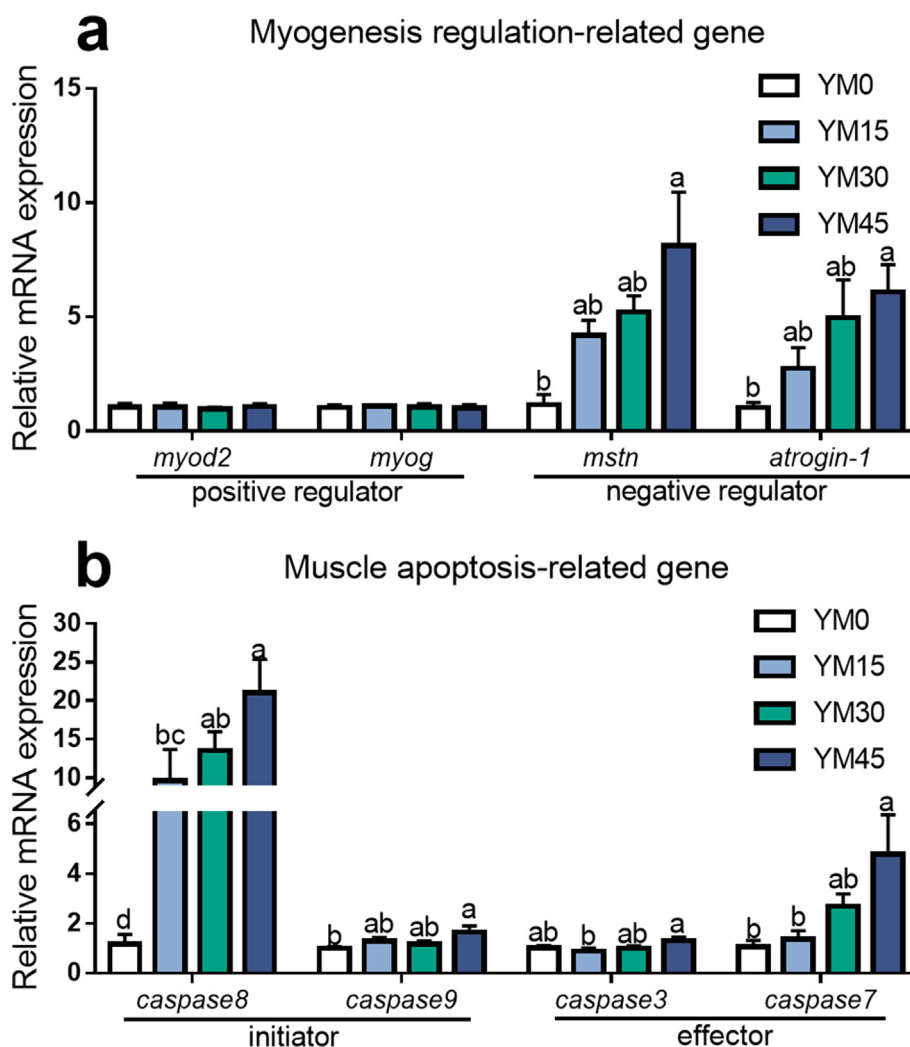


Fig. 3. The expression level of the genes related to myogenesis regulation and apoptosis in the fillet of Nile tilapia fed with the experimental diets for 10 wk. YM = yellow mealworm meal. YM0, YM15, YM30, and YM45 represent using 0%, 15%, 30%, and 45% yellow mealworm meal to replace soybean meal, respectively. (a) The relative expression level of myogenesis regulation-related genes. (b) The relative expression level of muscle apoptosis-related genes. *myod2* = myogenic differentiation 2; *myog* = myogenin; *mstn* = myostatin. Values were presented as mean \pm SEM ($n = 6$). Bars with different letters indicated significant differences ($P < 0.05$).

YM substitution levels increased with the lowest value observed in YM45 ($P < 0.05$, Fig. 4b). Besides, malondialdehyde (MDA) content increased significantly in YM substitution groups than in YM0 ($P < 0.05$, Fig. 4c).

At the beginning of the experiment, the acid value of diets was similar among groups (Fig. 5a, $P > 0.05$). While at the end of the experiment, the YM45 diet had a significantly higher acid value than the other groups (Fig. 5b, $P < 0.05$).

4. Discussion

It has been reported that including YM up to 10% in the diet did not influence the growth performance, carcass composition, or hematological indexes of Nile tilapia (Tubin et al., 2020). In the present study, the mealworm meal inclusion level in YM45 is 12.6% of the diet, which is similar to the result of Tubin et al. (2020). In the present study, we raised big fish with an initial weight of 70 ± 0.12 g for 10 wk to detect the flesh quality, thus the specific growth rate is relatively low compared with the juvenile tilapia (Soderberg, 1997).

Amino acid and fatty acid composition are important indicators of nutritional value. In the present study, muscle EAA was not affected by YM substitution. Similar results were found in

mandarin fish (*Siniperca scherzeri*) when they were fed with increasing levels of YM (Sankian et al., 2018). The proportions of DAA and FAA are important factors affecting flesh flavor (Zhang et al., 2021). In this study, the DAA and FAA in fillet muscle were not affected by YM substitution. It has been found that SFA and MUFA were not affected by YM substitution at 25% and 50% in blackspot seabream (*Pagellus bogaraveo*) fillet (Iaconisi et al., 2017), but the fillet of mandarin fish had higher SFA and MUFA when they were fed with diets containing 10% to 30% YM in place of fishmeal (Sankian et al., 2018). In the present study, the proportion of SFA decreased in the muscle of YM30 and YM45, while the MUFA increased in the muscle of YM30. The discrepancy may be caused by the different fish species or culturing environments (Xu et al., 2020).

Texture is a primary flesh sensory factor (Zhang et al., 2022). In the present study, we found that higher YM substitution (45%) changed flesh texture, as was manifested by the increased hardness, gumminess, and decreased springiness. It has been reported that high collagen content is associated with firm flesh texture (Nyu et al., 2007). However, the hydroxyproline content, which is used to reflect the collagen content, did not change among treatments. Reductions in myofiber diameter and muscle lipid content could

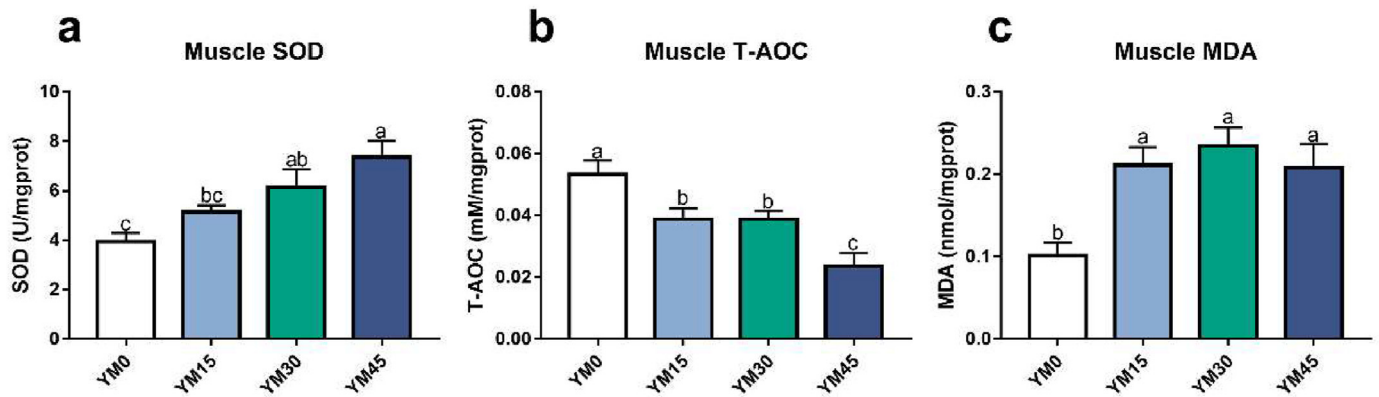


Fig. 4. The antioxidation parameters of fillet in Nile tilapia fed with the experimental diets for 10 wk. YM = yellow mealworm meal. YM0, YM15, YM30, and YM45 represent using 0%, 15%, 30%, and 45% yellow mealworm meal to replace soybean meal, respectively. (a) Superoxide dismutase (SOD) activity in fillet. (b) Total antioxidant capacity (T-AOC) of fillet. (c) Malondialdehyde (MDA) content in fillet. Values were presented as mean \pm SEM ($n = 6$). Bars with different letters indicated significant differences ($P < 0.05$).

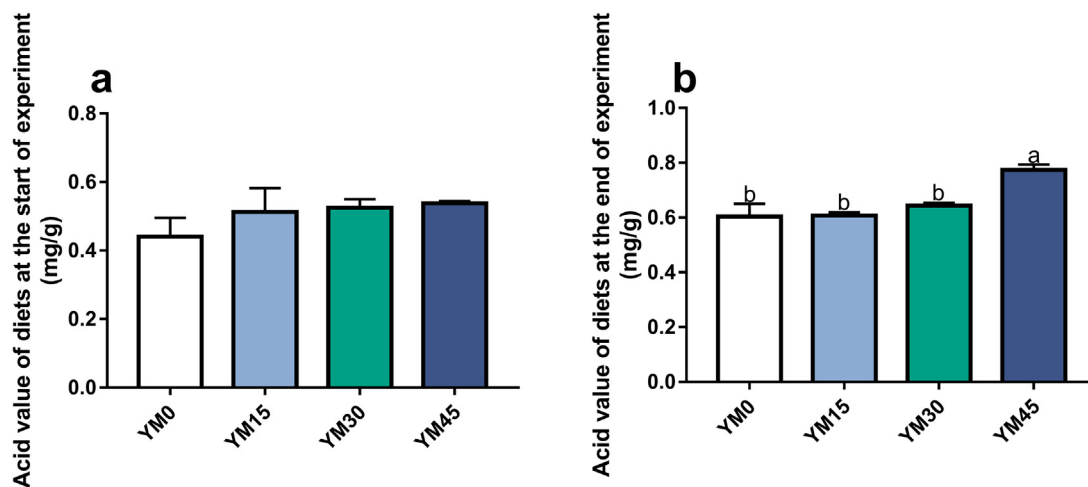


Fig. 5. The acid value of diets at the start and the end of the experiment. YM = yellow mealworm meal. YM0, YM15, YM30, and YM45 represent using 0%, 15%, 30%, and 45% yellow mealworm meal to replace soybean meal, respectively. (a) The acid value of the experimental diets at the start of the experiment. (b) The acid value of the experimental diets at the end of the experiment. Values were presented as mean \pm SEM ($n = 3$). Bars with different letters indicated significant differences ($P < 0.05$).

also increase muscle hardness (Zhang et al., 2021; Zhao et al., 2018). Consistently, we found that the proportion of small-diameter myofiber increased, while the crude muscle lipid decreased in YM45. However, it is noteworthy that the whole-body crude lipid increased in YM45. We detected the liver crude lipid content and mesenteric fat index, and found that both parameters increased significantly in YM45 (data not shown). We speculate that the protein source substitution might cause more lipid accumulation in visceral organs instead of muscle. In mammals, it has been reported that dietary amino acid imbalance and dietary protein malnutrition both induces fat deposition in the liver (Otani et al., 2020), suggesting that amino acid imbalance or decreased protein digestibility in YM substitution groups may disturb the lipid metabolism in fish.

The regulation of myofiber diameter is a complex process that is influenced by many factors, including myogenesis, muscle atrophy, and apoptosis. In a study of Atlantic salmon (*Salmo salar*), Østbye et al. (2018) indicated that muscle hardness had a positive correlation with myogenesis. Our results showed that the expression level of *myod* and *myog*, which are positive regulators of myogenesis, were unaffected, implying that myogenesis was not the main reason for myofiber diameter distribution change. Muscle atrophy could also influence the myofiber diameter (Liu et al.,

2020). The *mstn* and *atrogen-1* are two reliable atrophy indicators (Aravena-Canales et al., 2021; Rodriguez et al., 2014). The up-regulation of both genes indicated that muscle atrophy increased in YM45. During atrophy, protein degradation overwhelms protein synthesis, leading to the loss of muscle protein (Kitajima et al., 2020). Apoptosis activation was negatively correlated with myofiber diameter as well (Stratos et al., 2012). In this study, the expression of apoptosis initiator *caspase8* and *caspase9*, and apoptosis effector *caspase7*, were all up-regulated in YM45. These results suggested that muscle atrophy and apoptosis may account for the altered distribution of myofibers in YM45, resulting in the change in muscle texture.

Oxidized lipids could cause oxidative stress (Song et al., 2018), which can lead to atrophy and apoptosis (Andrianjafiniony et al., 2010; Powers et al., 2012). Fresh mealworms are perishable owing to microbial growth, lipid oxidation, the Maillard reaction, and enzymatic reactions (Borremans et al., 2020). Thus, the acid value of diets was measured. At the end of the experiment, the acid value of diets in YM45 increased significantly than that in other groups, suggesting that rancidity occurred in the feed of YM45. The significantly decreased T-AOC implied that the anti-oxidant capacity was disturbed in the muscle of YM45. We speculate that rancidity in YM45 caused muscle oxidative stress, apoptosis, and

atrophy, which altered myofiber diameter distribution, and finally influenced flesh texture.

5. Conclusion

In summary, our results indicated that YM could replace up to 30% SBM in Nile tilapia diets without substantially altering the flesh quality. When the replacement ratio was increased up to 45%, the hardness and gumminess increased, while springiness decreased in Nile tilapia muscle. More attention should be paid to the rancidity of feeding diet when using YM as a feed protein source.

Author contributions

Le Zhang: Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization. **Hong-Xia Wu:** Resources, Methodology, Writing – review & editing. **Wei-Jie Li:** Resources, Methodology, Data curation. **Fang Qiao:** Methodology, Supervision. **Wen-Bing Zhang:** Conceptualization, Project administration. **Zhen-Yu Du:** Conceptualization, Supervision. **Mei-Ling Zhang:** Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

We will give our thanks to Dr. Giuliana Parisi from Florence University for the valuable comments on the experimental design. This study was supported by the National Key Research and Development Program of China (2019YFD0900200) and the National Natural Science Foundation of China (31972798). Authors appreciate the help from the Chinese Academy of Agricultural Sciences Feed Research Institute for feed production.

Appendix supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aninu.2022.09.007>.

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