Animal Nutrition 19 (2024) 313–324

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Dietary astaxanthin alleviates black soldier fly oil-induced negative changes of fatty acids content and muscle quality on *Oncorhynchus mykiss* via mammalian target of rapamycin and AMP-activated protein kinase pathway

Yongkang Chen [a], Jian Zhong [b], Xuanqi Chen [a], Xiaomin Li [a], Haiqi Pu [a], Baoyang Chen [a], Yucai Guo [a], Anqi Chen [a], Wenjie Li [c], Peng Hu [d], Xinliang Zhu [d], Wei Zhao [a, *], Jin Niu [a, *]

[a] *State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory for Aquatic Economic Animals and Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China*
[b] *Zhanjiang Customs District, Zhanjiang 524000, China*
[c] *Qinghai Kunjie Environmental Protection Technology Co., Ltd., Xining 810000, China*
[d] *Key Laboratory of Subcritical Extraction of Anyang City, Henan Subcritical Biotechnology Co. Ltd., Anyang 455000, China*

ARTICLE INFO

Article history:
Received 21 March 2024
Received in revised form
2 July 2024
Accepted 18 July 2024
Available online 26 September 2024

Keywords:
Black soldier fly larvae oil
Astaxanthin
Flesh quality
DHA
mTOR pathway

ABSTRACT

This study evaluated the effect of black soldier fly (*Hermetia illucens*) larvae oil (BO) produced by a novel technique, subcritical butane extraction, on the flesh quality, lipid nutrients and muscle growth of rainbow trout (*Oncorhynchus mykiss*) fillet, and investigated the alleviating mechanisms of dietary astaxanthin (AST) supplementation. Two hundred and forty fish (215.16 ± 2.30 g) were distributed to three groups with four replicates. Fish were fed three experimental diets for 8 weeks: the control diet (CD diet), total fish oil of the CD diet was replaced with BO to formulate the BO100 diet, and then 1 g/kg AST was supplemented with the BO100 diet to formulate the AST diet. Results showed that the final body weight and the sarcomere length of fillet were significantly increased and the protein phosphorylation levels of mammalian target of rapamycin (mTOR) and p70 S6 kinase were enhanced in the BO100 group compared to the CD group ($P < 0.05$). However, there was a reduction in the hardness, springiness and chewiness of fillets, with a decrease in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in the fish of the BO100 group ($P < 0.05$). Supplementation of AST in the BO100 diet mitigated the impairment of springiness and chewiness and further promoted the sarcomere length of fillet ($P < 0.05$). Furthermore, dietary AST partially restored the EPA and DHA content of fillet by increasing the phosphorylation levels of serine/threonine kinase (AKT) and AMP-activated protein kinase α (AMPKα) ($P < 0.05$) and activating the gene expression of unsaturated fatty acid synthesis. To conclude, BO produced by subcritical butane extraction can be a readily available oil source for rainbow trout feed that can be used to promote muscle growth in rainbow trout. Further dietary AST supplementation can alleviate BO-induced lipid accumulation, restore DHA levels and improve the flesh quality of rainbow trout fillet.

1. Introduction

The advantages of insect farming in industrial culturing facilities are gradually realized: a lower environmental resource requirement, less greenhouse gas emissions and the transformation of low-value organic waste to high-value biomass (Van Huis and Oonincx, 2017; Van Huis and Gasco, 2023). As a potential source of protein, lipids and minerals, edible insects are controversial as human food, but there seems to be consensus on their use as feed

* Corresponding authors.
E-mail addresses: zhaow66@mail.sysu.edu.cn (W. Zhao), niuj3@mail.sysu.edu.cn (J. Niu).
Peer review under the responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2024.07.005

for farmed animals (Caparros Megido et al., 2016; Bessa et al., 2020). As a sustainable insect species, black soldier fly (*Hermetia illucens*) are suitable for producing biomass by feeding on organic waste such as kitchen waste and livestock manure (Amrul et al., 2022). The black soldier fly larvae (BSF) fed on kitchen waste typically contain greater than 30% crude protein and 30% crude lipid and are good sources of feed ingredients (Nyakeri et al., 2017; Shumo et al., 2019). To improve the utilization value, a novel technique, subcritical butane extraction, was developed in our previous study that the black soldier fly larvae oil (BO) could be extracted from BSF (Chen et al., 2023a). The optimal extraction process of BO could be conducted at 35 °C, 50 min/extraction and 5 extractions. However, BO extracted by this method needs further biosafety testing before it can be put into production.

To explore the availability of BO in animal feed, rainbow trout (*Oncorhynchus mykiss*) were selected for this study because they require high dietary fat (Meng et al., 2019). In addition, Chinese consumers' interest in rainbow trout is increasing rapidly, with China producing 37,345 tonnes of rainbow trout annually in 2022, a 4.27% increase from 2021 (35,814 tonnes) according to Bureau of Fisheries of MARA (2023). A previous study has shown that dietary full-fat BSF did not affect the color, water-holding capacity and nutritional value of rainbow trout fillets (Bruni et al., 2020). Besides, Fawole and colleagues reported dietary BO did not alter the nutrient retention of rainbow trout (Fawole et al., 2021). However, these studies also lacked details and sources regarding BO production methods. Additionally, changes in flesh quality, including texture analysis, and muscle growth of rainbow trout fillets have not been intensively studied. On the other hand, astaxanthin (AST) was not taken into account in these previous studies, which was considered an essential feed additive for salmonids (Lim et al., 2018). As a pigment carotenoid, AST is efficacious in coloring the flesh of salmonids, and thereby contributes to improving the product quality and price (Aquilina et al., 2014). What's more, AST is oil soluble and has a potential effect on lipid metabolism in rainbow trout (Kalinowski et al., 2023). However, it was unclear whether the efficacy of AST on fillet quality was affected by BO and the potential synergistic effect of AST and BO.

It should be emphasized that the utilization of insect ingredients in animal feed possibly leads to alterations in muscle texture and nutritional values (Gasco et al., 2019). The primary objective of this study was to investigate the effects of BO produced using subcritical butane extraction on the flesh quality and nutritional value of rainbow trout fillets. Additionally, the study examined the potential impact of further dietary supplementation with AST on these parameters. This study demonstrated how dietary BO affected fillet quality in rainbow trout and provided recent insights into AST mitigation of these adverse effects.

2. Materials and methods

2.1. Animal ethics statement

All animal studies were approved by the Experimental Animal Ethics Committee of Sun Yat-Sen University, in keeping with Chinese Ethical Guidelines for Experimental Animals. The study protocol, experimental procedures and fish in this study were reviewed and approved with an approval number of SYSU-IACUC-2023-B0475.

2.2. Experimental fish and diet

About 240 farmed triploid rainbow trout (*O. mykiss*) were provided by Qinghai Cold-Water Fishery Ecological Company (China). Before the experiment, the fish were acclimated and fed a commercial diet (Aller Aqua Qingdao Company, China) for 2 weeks in the cage of the Lasiwa Reservoir (China). The control diet (CD diet) was formulated to contain 41.70% crude protein and 17.86% crude lipid. Subsequently, total fish oil of the CD diet was replaced with BO to formulate the BO100 diet. Then, 1 g/kg 10% AST (BASF, Germany) was supplemented to the BO100 diet to formulate AST diet (Zhao et al., 2022). The BO was produced according to our previous study using subcritical butane extraction (Chen et al., 2023a). The three diets were produced using procedures previously reported (Meng et al., 2019). The experimental feed was extruded into 4.5-mm diameter pellets and stored at −20 °C before used. The formulation was showed in Table 1.

2.3. Experimental design

After acclimatization, 240 health and uniform-sized rainbow trout (average initial weight of 215.16 ± 2.30 g) were randomly distributed to three treatments with four replicates. Each 20 fish was cultured in a 2 m × 2 m × 3m cage and fed experimental diets for 8 weeks. The rainbow trout in each cage were fed twice a day (07:00 and 17:00) to apparent satiety. During the 8-week feeding trial, the water parameter was measured, with the dissolved oxygen ranging from 7.2 to 10.5 mg/L and water temperature ranging from 6.7 to 13.2 °C.

2.4. Determination of growth performance and feed utilization

After the feeding trial, fish were fasted for 24 h. Fish in each cage were counted and weighed to calculate the growth performance. The body weight and length of four individual fish from each cage were measured to determine the condition factor (CF). Afterwards, the livers of these four fish were separated and weighed to

Table 1
Composition and nutrient levels of diets (as-fed basis, %).

Item	Diet		
	CD	BO100	AST
Ingredients			
Fishmeal	42.00	42.00	42.00
Soybean meal	10.00	10.00	10.00
Soy protein isolate	12.00	12.00	12.00
Cassava starch	5.00	5.00	5.00
Wheat flour	13.00	13.00	12.90
Fish oil	12.00		
Black soldier fly larvae oil		12.00	12.00
Soy lecithin	3.00	3.00	3.00
$Ca(H_2PO_4)_2$	1.00	1.00	1.00
Vitamin premix[1]	0.50	0.50	0.50
Mineral premix[2]	0.50	0.50	0.50
Choline chloride	0.50	0.50	0.50
Vitamin C	0.50	0.50	0.50
Astaxanthin (10%)[3]			0.10
Total	100.00	100.00	100.00
Nutrient levels			
Crude protein	41.70	41.84	41.80
Crude lipid	17.86	17.55	17.38
Ash	10.23	10.21	10.15
Nitrogen free extract	23.22	22.39	22.08

[1] Vitamin premix (per kilogram diet): vitamin B_1 30 mg, vitamin B_2 60 mg, vitamin B_6 20 mg, nicotinic acid 200 mg, calcium pantothenate 100 mg, inositol 100 mg, biotin 2.5 mg, folic acid 10 mg, vitamin B_{12} 0.1 mg, vitamin K_3 40 mg, vitamin A 10,000 IU, vitamin D_3 2000 IU, vitamin E 160 IU.

[2] Mineral premix (per kilogram diet): $MgSO_4 \cdot H_2O$ 1090 mg, KH_2PO_4 932 mg, $NaH_2PO_4 \cdot 2H_2O$ 432 mg, $FeC_6H_5O_7 \cdot 5H_2O$ 181 mg, $ZnCl_2$ 80 mg, $CuSO_4 \cdot 5H_2O$ 63 mg, $AlCl_3 \cdot 6H_2O$ 51 mg, $MnSO_4 \cdot H_2O$ 31 mg, KI, 28 mg; $CoCl_2 \cdot 6H_2O$ 6 mg, $Na_2SeO_3 \cdot H_2O$ 0.8 mg.

[3] Astaxanthin was provided by BASF, Germany.

determine the hepatosomatic index (HI). These indicators were calculated as follows:

Specific growth rate (SGR, %/d) = 100 × [ln final body weight (FBW) − ln initial body weight (IBW)]/days;

Feed conversion ratio (FCR) = feed consumed/(FBW − IBW);

Feed intake (FI, g/per fish) = feed consumed/survival number;

HI (%) = 100 × liver weight/body weigh;

CF (g/cm^3) = 100 × FBW/(body length)^3.

2.5. Analysis of diets, whole body, liver and fillet composition

Another four fish were then collected for whole body composition analysis. The whole fish, liver and fillet were dried in an oven at 105 °C for 24 h till constant weight to determine the moisture content. The moisture was determined by the weight loss of dried samples according to GB 5009.3-2016. The crude protein content (N × 6.25) of diets, whole body, liver and fillet was determined by the Kjeldahl method (Tecator 1030 automatic analyzer, Tecator, Helsingborg, Sweden) according to GB/T 6432-2018. The crude lipid content of diets, whole body, liver and fillet was determined by the petroleum ether extraction method by a Soxtec System HT (Soxtec System HT6, Tecator, Hoganas, Sweden) according to GB 5009.6-2016. The ash of diets was determined by combusted in a combustion oven for 4 h at 550 °C till constant weight according to GB/T 6438-2007. Nitrogen free extract (NFE) of diets was calculated as: NFE = dry matter − ash − crude lipid − crude protein (Letelier-Gordo et al., 2015). The fatty acid composition of diets (Table 2) and fillet was determined by gas chromatography (GC–MS) method according to the GB 5009.168-2016. Briefly, the lipid was extracted, saponified and methylated to produce fatty acid methyl esters, which are then analyzed by GC–MS (GC7820A, Agilent, USA).

2.6. Measurement of flesh quality parameters

Dorsal fillets were carefully dissected for color determination, texture analysis and pH measurement. Firstly, the color of each fillet was measured using a colorimeter (CR-400, Minolta Camera Co., Ltd., Japan) and visually compared to the color card (SalmoFan, DSM, Netherlands). The result was presented as lightness (L*), redness (a*) and yellowness (b*). Texture analysis was performed at the fillet color measurement point using a texture analyzer (TMS-PRO, FTC, USA) following the method (Cao et al., 2023). The probe was loaded on the analyzer with the probe perpendicular to the surface of the fillet, and the parameter of texture analysis was set at test speed 60 mm/min and deformation 60% following a published report (Guan et al., 2023). Six main indices were used to characterize the results of the texture analysis including hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness (Cheng et al., 2021). After texture analysis, a pH meter (S220, Mettler, Switzerland) was used to measure the pH of fillet at the same point of color and texture analysis (Guan et al., 2023).

To determine the cooking loss of the fillet, fillet sample was blotted, weighed and placed in a filter bag. All the filter bags were cooking in a water bath at 75 °C for 25 min according to a published report (Mohsenpour et al., 2023). The cooked fillet sample was cooled, surface dried with a tissue and weighed. The cooking loss was calculated by the weight loss of fillet sample after cooking.

Table 2
Fatty acid composition of experimental diets (% of total fatty acid)[1].

Item	CD	BO100	AST
C12:0	0.17	16.91	16.20
C14:0	4.75	4.37	4.62
C14:1	0.10	0.25	0.32
C15:0	0.48	0.27	0.36
C16:0	21.47	18.72	20.27
C16:1	6.62	2.52	2.64
C17:0	0.79	0.39	0.52
C18:0	5.00	5.37	6.73
C18:1	20.21	19.82	19.47
C18:2n-6	13.03	18.01	15.88
C18:3n-6	0.28	0.07	0.07
C20:0	0.71	0.24	0.21
C18:3n-3	1.72	1.90	1.58
C20:1	1.79	1.00	1.04
C20:3n-6	0.19	0.03	0.03
C22:0	0.25	0.10	0.10
C20:4n-6	1.10	0.39	0.36
C22:1	0.27	0.17	0.15
C20:5n-3	8.36	3.96	3.88
C24:0	0.19	0.06	0.07
C24:1	1.12	0.57	0.59
C22:5n-6	0.37	0.05	0.06
C22:5n-3	1.29	0.31	0.31
C22:6n-3	9.40	3.92	3.88

[1] CD = control diet; BO100 = black soldier fly larvae oil replaces fish oil at 100% in control diet; AST = astaxanthin supplemented in BO100 diet.

2.7. Morphology analysis

Dorsal muscle of rainbow trout was sampled (0.5 mm × 0.5 mm × 0.5 mm) and fixed in 2.5% glutaraldehyde solution at 4 °C for 24 h. Then, it was fixed, dehydrated, embedded, sectioned and stained. The ultrathin sections were observed with a transmission electron microscopy (TEM, JEM-1400 Flash, Rigaku, Japan). The sarcomere length was measured by ImageJ software. Each section was randomly measured six times to determine the average length of sarcomere.

2.8. Real time -quantitative PCR (RT-qPCR)

Both liver and dorsal muscle were sampled and stored at −80 °C prior to RNA extraction. Total RNA was isolated using RNAiso Plus (#9109, TaKaRa, Japan). NanoDrop 2000 Spectrophotometer (Thermo Fisher, USA) was employed to verify the quantity of RNA. The RNA was then reverse transcribed to cDNA using a kit Evo M-MLV RT Kit with gDNA Clean for qPCR (#AG11711, Accurate Biotechnology Hunan Co., Ltd., China) following the protocol of manufacturer. RT-qPCR was conducted in LightCycler 480 (Roche Applied Science, Switzerland) with a SYBR Green Kit (#AG11701, Accurate Biotechnology Hunan Co., Ltd., China). The amplification program were performed as previously done in our laboratory (Chen et al., 2023b). The elongation factor-1 alpha (*ef-1α*) was used as the reference gene and the target gene were normalized and calculated by the $2^{-\Delta\Delta Ct}$ method. The primers for target genes are shown in Table 3.

2.9. Western blot analysis

The muscle sample of four fish in each group was lysed in RIPA lysis buffer (#FD009, Fude Biological Technology Co., Ltd., China), protease and phosphatase inhibitor (#FD1002, Fude Biological Technology Co., Ltd., China). After measuring the total protein concentration, samples were denatured with loading buffer at 100 °C for 5 min. Proteins were separated by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) (7.5% or 10% acrylamide gel containing 0.1% SDS) and transferred onto a polyvinylidene difluoride (PVDF) membrane. The membranes were blocked by 5% (w/v) skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) at room temperature for 2 h. The membranes were then incubated overnight at 4 °C in the appropriate concentration of primary antibody. Primary antibody mammalian target of

Table 3
Primers used for quantitative real-time PCR.

Gene	Sequences forward (5' to 3')	Sequences reverse (5' to 3')	Fragment length, bp	Accession no.
acc	TCATCAATGCCAAGGACCCC	CGTCAGAGTCCAGGTTTGCT	288	XM_036990266.1
fas	CCTCACCTCCAAAGACAGTCAG	CGACGGCGACACAGGAATAA	144	XM_021576226.2
elovl2	CTGGTGGAAACGCTACCTGA	ATCCAACGGGGAAACCACAA	104	KM244737
elovl5	CAATCTGGCCCTCACTCTATT	TGGTATCTGTTTCTCCCGCAC	119	AY605100
Δ5 fads	GCAGAGAGAACCGAGGATGG	GCAGTGCTTCTGGACCTCTT	136	JQ087459.1
Δ6 fads	CCATCGCAGATTAACCACCT	TTTGGATCCCCCATTAGACA	140	AF301910
cd36	CCTGCTCGTAGTCTTCGTCC	TTGCCGTAGTAAAAGAATGAAAAGC	104	AY606034.1
pparα	TCGAGTAACGGCTCTGAAGG	GCGTGAACTCCGTAGTGGTA	176	HM536190.1
pparβ	CTGGAGCTGGATGACAGTGA	GTCAGCCATCTTGTTGAGCA	59	AY356399.1
pparγ	CACGTTGGACTTGGCAGAGA	TGTGGTGGTGGGGACTGTT	122	HM536192.1
gpat	AAGTGCGACACACTACCTCG	AATGCAGGAGCTTGTTCCGA	146	XM_036946572.1
hsl	GGGCTGACCATCTGGCTAAT	ACATTGACATCACCCTCAGCA	200	HQ225622.1
myod	TGCCTGTCAAACATCGTGGA	TCCGTTCTCACTCGCTATGG	123	NM_001124720.1
myog	AGGTGAACGAGGCATTCGAG	GGTGCGGTATTGTAAGCCCT	179	NM_001124727.1
mstn	GGGCAGGCACATAGGTATCC	CAGCCATACGGACAGCACTT	100	NM_001124282.1
myf5	ACGCCATCCAGTACATCGAG	CAGTCAACCATGCTGTCGGA	133	NM_001124529.1
mrf4	AAAGTCTGCACCAACCGACA	GCGCTGCGTAAAATCTCCAC	150	XM_021563867.2
ghr	CAACTTCTGAACAAGCCCTGC	CACTCACTCCACTCTTTGGGA	184	NM_001124731.1
ef-1α	TCCTCTTGGTCGTTTCGCTG	ACCCGAGGGACATCCTGTG	59	AF498320

acc = acetyl-CoA carboxylase; *fas* = fatty acid synthase; *elovl2/5* = elongase of very-long fatty acid 2/5; *Δ5/6 fads* = Δ5/6 fatty acid desaturase; *cd36* = CD36/FAT fatty acid translocase; *myod* = myogenic determining factor; *myog* = myogenin; *mstn* = myostatin; *myf5* = myogenic factor 5; *mrf4* = myogenic factor 4; *ghr* = growth hormone receptor; *pparα/β/γ* = peroxisome proliferators-activated receptors-α/β/γ; *gpat* = glycerol-3-phosphate acyltransferase; *hsl* = hormone-sensitive lipase; *ef-1α* = elongation factor -1 α.

rapamycin (mTOR), p-mTOR, p70 S6 kinase (S6K) and p-S6K were provided by ABclonal Technology Co., Ltd., China. AMP-activated protein kinase α (AMPKα), p-AMPKα, serine/threonine kinase (AKT) and p-AKT were purchased from Cell Signaling Technology (Beverly, MA, USA). Membranes were washed three times in tris buffered saline with tween-20 (TBST) for 10 min each time and then incubated with horseradish peroxidase (HRP)-coupled secondary antibody for 1 h at room temperature. Protein bands were detected with an enhanced chemiluminescence (ECL) detection kit (#FD8020, Fude Biological Technology Co., Ltd., China), then visualized and recorded by Azure 300 (Azurebiosystems, CA, USA). The gray values were quantized using ImageJ 1.52a and then analyzed in SPSS 21.0 software (IBM, USA).

2.10. Proteomic analyses

Liver samples of four rainbow trout in each cage were collected and the samples of four replicates cages were homogenized and distributed to three sample pools. After being digested, desalted, the protein sample was transferred to peptide, which was then labeled and undergoing a high pH reverse phase separation by Ultimate 3000 system (Thermo Fisher scientific, MA, USA) with a reverse phase column (XBridge C18 column, 4.6 mm × 250 mm, 5 μm, Waters Corporation, MA, USA). The collected peptides were then analyzed by on-line nanospray LC-MS/MS on Orbitrap Fusion Lumos mass spectrometer to EASY-nLC 1200 system (Thermo Fisher Scientific, MA, USA). The data independent acquisition (DIA) results were analyzed by Spectronaut software. The proteins among groups with a fold change higher than 1.5 and false discovery rate value (FDR-value) lower than 0.05 were considered to be differentially expressed proteins (DEP). In addition, the functions and characteristics of DEP was analyzed and clustered by several database including Gene Ontology (GO, geneontology.org) and the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg) database.

2.11. Statistical analysis

The data were presented as mean ± standard error of the mean (SEM). Independent-sample *t*-test was conducted to evaluate the difference between the data. The statistical analysis was performed on SPSS 21.0 software (IBM, USA) and *P*-values < 0.05 were considered significant differences. When the *P*-value is greater than 0.05 and less than or equal to 0.10, it indicates a significant tendency.

3. Results

3.1. Growth performance and feed utilization of rainbow trout

The effect of dietary BO and AST supplementation on growth performance and feed utilization in rainbow trout is displayed in Table 4. Fish fed with the BO100 diet (BO100 group) exhibited a significantly higher FBW and a lower FCR compared to those fed with the control diet (CD group) ($P < 0.05$). Additionally, the BO100 group showed an increasing trend in weight gain (WG) and SGR ($0.05 < P < 0.10$). Survival, FI and CF did not differ significantly among the three groups ($P > 0.05$). However, the HI was significantly higher in the BO100 group compared to both the CD and AST groups ($P < 0.05$). The AST diet did not result in significant differences in WG, FBW, SGR, FCR, survival, FI and CF when compared to the BO100 group ($P > 0.05$). Nonetheless, an increasing trend in WG and FBW was observed in the AST group compared to the CD group ($0.05 < P < 0.10$). Furthermore, dietary AST significantly alleviated the HI of fish compared to the BO100 group ($P = 0.027$), although it remained significantly higher than the CD group ($P = 0.006$).

3.2. Whole body, liver and fillet composition

Regarding the major nutrient composition, no significant variations were observed in the crude protein levels of the whole body and liver among the different treatment groups ($P > 0.05$). However, the BO100 group exhibited a significantly lower crude protein content in the fillet compared to both the CD and AST groups ($P < 0.05$), as detailed in Table 5. In contrast, the crude lipid of fillet in BO100 group was significantly higher than the CD group ($P = 0.012$) and showed an increasing tendency compared to the AST group ($0.05 < P < 0.10$). Additionally, the crude lipid content of liver in BO100 and AST groups was significantly higher than the CD group ($P < 0.05$).

Table 4
Effects of dietary BO and AST on growth performance and feed utilization in *O. mykiss*[1].

Item	CD	BO100	AST	*P*-value of *t*-test[2]		
				CD vs BO100	BO100 vs AST	CD vs AST
WG, g	108.02 ± 5.478	139.19 ± 11.563	136.16 ± 13.213	0.051	0.869	0.097
FBW, g	323.18 ± 4.536	355.81 ± 11.413	352.33 ± 12.965	0.038	0.847	0.078
SGR, %/d	0.72 ± 0.032	0.88 ± 0.058	0.86 ± 0.066	0.056	0.869	0.104
FCR	1.50 ± 0.075	1.22 ± 0.057	1.35 ± 0.130	0.028	0.345	0.376
Survival, %	98.33 ± 1.178	100.00 ± 0.000	98.75 ± 1.250	0.207	0.356	0.816
FI, g/per fish	165.62 ± 16.261	166.67 ± 4.702	179.92 ± 11.083	0.919	0.129	0.251
HI, %	1.07 ± 0.048	1.37 ± 0.037	1.26 ± 0.016	0.006	0.027	0.012
CF, g/cm^3	1.71 ± 0.037	1.73 ± 0.040	1.69 ± 0.024	0.720	0.493	0.770

AST = astaxanthin; BO = black soldier fly larvae oil; WG = weight gain; FBW = final body weight; SGR = specific growth rate; FCR = feed conversion rate; FI = feed intake; HI = hepatosomatic index; CF = condition factor.
[1] CD = control diet; BO100 = black soldier fly larvae oil replaces fish oil at 100% in control diet; AST = astaxanthin supplemented in BO100 diet.
[2] *P*-value of *t*-test less than 0.05 indicated significant differences.

Table 5
Effects of dietary BO and AST on composition (**% of wet mass**) of whole body, fillet and liver in *O. mykiss*[1].

Item	CD	BO100	AST	*P*-value of t-test[2]		
				CD vs BO100	BO100 vs AST	CD vs AST
Whole body						
Moisture	69.79 ± 0.265	69.52 ± 0.705	70.74 ± 0.629	0.729	0.272	0.184
Crude protein	15.48 ± 0.205	15.87 ± 0.150	15.76 ± 0.138	0.174	0.618	0.269
Crude lipid	11.00 ± 0.256	11.23 ± 0.457	9.42 ± 0.871	0.688	0.116	0.261
Fillet						
Moisture	74.73 ± 0.252	74.50 ± 0.436	74.75 ± 0.171	0.668	0.616	0.948
Crude protein	21.49 ± 0.259	20.48 ± 0.231	21.38 ± 0.152	0.018	0.018	0.719
Crude lipid	2.66 ± 0.147	3.93 ± 0.417	3.10 ± 0.199	0.012	0.068	0.188
Liver						
Moisture	73.02 ± 0.583	72.28 ± 2.514	72.33 ± 0.277	0.104	0.156	0.328
Crude protein	21.99 ± 0.894	21.11 ± 1.867	21.52 ± 0.253	0.191	0.246	0.630
Crude lipid	3.88 ± 0.130	5.74 ± 0.307	5.19 ± 0.163	0.004	0.168	0.002

AST = astaxanthin; BO = black soldier fly larvae oil.
[1] CD = control diet; BO100 = black soldier fly larvae oil replaces fish oil at 100% in control diet; AST = astaxanthin supplemented in BO100 diet.
[2] *P*-value of *t*-test less than 0.05 indicated significant differences.

Furthermore, as illustrated in Table 6, the total n-3 polyunsaturated fatty acid (PUFA) content was significantly reduced in the fillets of the BO100 group compared to the CD group ($P < 0.001$), while the total n-6 PUFA content was increased ($P = 0.003$). Specifically, lauric acid (C12:0) and linoleic acid (C18:2n-6) accumulated in fillets of rainbow trout fed the BO100 diet, but the levels of arachidonic acid (C20:4n-6, ARA), eicosapentaenoic acid (C20:5n-3, EPA), and docosahexaenoic acid (C22:6n-3, DHA) were reduced ($P < 0.05$). As for the AST group, the lauric acid level of fillet was also increased as a marker of BO addition ($P < 0.001$). Compared to the BO100 group, dietary AST partially restored the total n-3 PUFA content of fillet and enhanced the DHA content ($P < 0.05$).

3.3. Flesh quality and fillet parameters

As shown in Table 7, there was no significant differences in the pH of the fillet among groups ($P > 0.05$). The hardness, springiness and chewiness of fillet in fish fed with the BO100 diet significantly decreased but the adhesiveness and cohesiveness increased compared to the fish fed the CD diet ($P < 0.05$). Surprisingly, dietary AST partly improved the springiness ($0.05 < P < 0.10$) and significantly promoted the chewiness of fillet compared to the BO100 group ($P = 0.009$). In addition, the cooking loss of fillet was decreased in the BO100 group compared to the CD group ($P = 0.024$) but restored in the AST group ($P = 0.032$).

The results of visual observation and TEM of fish fillets are shown in Fig. 1. The light and dark areas and Z-lines of myofibrils are exhibited apparently. Statistical results in Table 7 showed that the sarcomere length in the BO100 group was significantly longer than the CD group ($P = 0.002$), while that of the AST group was further longer than that in the BO100 group ($P < 0.001$). It was clear that the redness and yellowness of the flesh color in the AST group were deeper than the other two groups ($P < 0.05$). Also, the redness of fish fillets in the BO100 group was significantly lower than the CD group ($P = 0.008$).

3.4. Muscle growth

Both protein and mRNA expression were analyzed to illustrate the muscle growth of rainbow trout. As shown in Fig. 2, phosphorylation level of mTOR, S6K and AMPKα were significantly upregulated in the BO100 group compared to the CD group ($P < 0.05$). Furthermore, the phosphorylation level of AKT and AMPKα was significantly upregulated in the AST group compared to the BO100 group ($P < 0.05$). Additionally, phosphorylation level of S6K, AKT and AMPK was significantly upregulated in the AST group compared to the CD group ($P < 0.05$) as well as the mTOR ($P = 0.065$). In addition, the growth of muscle was studied on transcript level. Dietary BO100 remarkably upregulated the relative mRNA expression of myogenin (*myog*), myostatin (*mstn*) and myogenic factor 4 (*mrf4*), while downregulated the relative mRNA expression of growth hormone receptor (*ghr*) compared to the CD group ($P < 0.05$) (Fig. 3A). Additionally, dietary AST also upregulated the relative mRNA expression of myogenic factor 5 (*myf5*), while

Table 6
Fatty acid composition (% of total fatty acid) of fillet in *O. mykiss*[1].

Item	CD	BO100	AST	*P*-value of *t*-test[2]		
				CD vs BO100	BO100 vs AST	CD vs AST
C12:0	0.09 ± 0.002	6.02 ± 0.036	5.54 ± 0.044	<0.001	0.001	<0.001
C14:0	2.66 ± 0.097	2.74 ± 0.141	2.79 ± 0.013	0.655	0.757	0.259
C14:1	0.06 ± 0.001	0.12 ± 0.002	0.14 ± 0.001	<0.001	0.023	<0.001
C15:0	0.22 ± 0.016	0.18 ± 0.006	0.20 ± 0.001	0.074	0.024	0.290
C16:0	18.01 ± 1.913	17.71 ± 0.069	17.84 ± 0.098	0.883	0.352	0.932
C16:1	4.22 ± 0.568	3.39 ± 0.011	2.91 ± 0.006	0.217	<0.001	0.082
C17:0	0.45 ± 0.007	0.30 ± 0.002	0.33 ± 0.002	<0.001	0.002	<0.001
C18:0	4.65 ± 0.327	5.26 ± 0.028	5.45 ± 0.001	0.141	0.003	0.073
C18:1	21.21 ± 0.422	24.03 ± 0.155	22.57 ± 0.143	0.003	0.002	0.038
C18:2n-6	15.78 ± 0.563	20.20 ± 0.107	19.02 ± 0.141	0.002	0.003	0.005
C18:3n-6	0.29 ± 0.005	0.32 ± 0.002	0.28 ± 0.002	0.019	0.001	0.206
C20:0	0.27 ± 0.003	0.22 ± 0.001	0.20 ± 0.002	<0.001	0.002	<0.001
C18:3n-3	1.95 ± 0.028	1.87 ± 0.005	1.80 ± 0.006	0.050	0.001	0.007
C20:1	1.41 ± 0.070	1.29 ± 0.024	1.50 ± 0.021	0.200	0.004	0.301
C20:3n-6	0.56 ± 0.005	0.57 ± 0.004	0.61 ± 0.004	0.112	0.004	0.002
C22:0	0.18 ± 0.001	0.15 ± 0.005	0.17 ± 0.006	0.012	0.141	0.316
C20:4n-6	1.37 ± 0.058	0.86 ± 0.004	1.00 ± 0.060	0.001	0.078	0.012
C22:1	0.16 ± 0.003	0.12 ± 0.002	0.12 ± 0.001	<0.001	0.009	0.001
C20:5n-3	4.34 ± 0.062	2.11 ± 0.051	2.32 ± 0.028	<0.001	0.024	<0.001
C24:0	0.03 ± 0.000	0.03 ± 0.000	0.04 ± 0.000	<0.001	<0.001	0.307
C24:1	0.84 ± 0.003	0.51 ± 0.003	0.56 ± 0.001	<0.001	<0.001	<0.001
C22:5n-6	0.46 ± 0.007	0.22 ± 0.002	0.29 ± 0.002	<0.001	<0.001	<0.001
C22:5n-3	1.54 ± 0.022	0.67 ± 0.023	0.73 ± 0.002	<0.001	0.073	<0.001
C22:6n-3	15.98 ± 0.106	9.76 ± 0.052	12.41 ± 0.394	<0.001	0.003	0.001
ΣSFA	26.60 ± 1.603	32.65 ± 0.106	32.59 ± 0.108	0.020	0.683	0.020
ΣMUFA	27.91 ± 0.634	29.49 ± 0.172	27.81 ± 0.154	0.074	0.002	0.891
ΣPUFA n-3	23.83 ± 0.038	14.42 ± 0.104	17.27 ± 0.417	<0.001	0.003	<0.001
ΣPUFA n-6	18.48 ± 0.544	22.19 ± 0.109	21.22 ± 0.183	0.003	0.011	0.009
ΣPUFA n-3/ΣPUFA n-6	1.29 ± 0.039	0.65 ± 0.007	0.81 ± 0.026	<0.001	0.004	0.001

SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acids.
[1] CD = control diet; BO100 = black soldier fly larvae oil replaces fish oil at 100% in control diet; AST = astaxanthin supplemented in BO100 diet.
[2] *P*-value of *t*-test less than 0.05 indicated significant differences.

Table 7
Effects of dietary BO and AST on flesh quality and appearance in *O. mykiss*[1].

Item	CD	BO100	AST	*P*-value of *t*-test[2]		
				CD vs BO100	BO100 vs AST	CD vs AST
pH	5.99 ± 0.057	5.94 ± 0.044	6.05 ± 0.045	0.517	0.129	0.427
Hardness, N	3.11 ± 0.207	2.13 ± 0.153	2.60 ± 0.020	0.019	0.136	0.154
Adhesiveness, mJ	0.87 ± 0.043	1.38 ± 0.028	1.72 ± 0.241	0.001	0.229	0.026
Cohesiveness	0.22 ± 0.007	0.27 ± 0.006	0.28 ± 0.016	0.004	0.700	0.014
Springiness, mm	1.40 ± 0.048	0.70 ± 0.031	0.89 ± 0.059	<0.001	0.054	0.001
Gumminess, mJ	0.68 ± 0.056	0.63 ± 0.055	0.70 ± 0.034	0.578	0.387	0.779
Chewiness, mJ	1.08 ± 0.040	0.41 ± 0.035	0.78 ± 0.070	<0.001	0.009	0.019
Cooking loss, %	19.80 ± 0.441	18.51 ± 0.402	20.32 ± 0.608	0.024	0.032	0.475
Sarcomere length, μm	1.65 ± 0.013	1.73 ± 0.007	1.81 ± 0.006	0.002	<0.001	<0.001
L*	45.44 ± 0.471	45.35 ± 0.865	37.27 ± 1.137	0.928	0.001	0.001
a*	−1.28 ± 0.239	−2.28 ± 0.096	9.27 ± 0.115	0.008	<0.001	<0.001
b*	6.12 ± 0.909	4.85 ± 0.517	13.68 ± 0.254	0.270	<0.001	<0.001

AST = astaxanthin; BO = black soldier fly larvae oil; L* = lightness; a* = redness; b* = yellowness.
[1] CD = control diet; BO100 = black soldier fly larvae oil replaces fish oil at 100% in control diet; AST = astaxanthin supplemented in BO100 diet.
[2] *P*-value of *t*-test less than 0.05 indicated significant differences.

downregulated the relative mRNA expression of *myog* and *mstn* compared to the BO100 group ($P < 0.05$).

3.5. Lipid metabolism

As shown in Fig. 3B, the relative mRNA expression of fatty acid synthetase including acetyl-CoA carboxylase (*acc*) and fatty acid synthase (*fas*), elongase including elongase of very-long fatty acid 2 (*elovl2*) and 5 (*elovl5*), and desaturase such as Δ5 fatty acid desaturase (*Δ5 fads*) was upregulated in the BO100 group compared to the CD group ($P < 0.05$). Also, regulators of lipid metabolism were upregulated in the BO100 group compared to the CD group, such as CD36/FAT fatty acid translocase (*cd36*), peroxisome proliferators-activated receptors-α (*pparα*) and γ (*pparγ*) ($P < 0.05$) (Fig. 3C). In terms of the triglyceride metabolism, the relative mRNA expression of glycerol-3-phosphate acyltransferase (*gpat*) was upregulated, while the relative mRNA expression of hormone-sensitive lipase (*hsl*) was downregulated in the BO100 group compared to the control group ($P < 0.05$). In contrast, supplementing AST in the BO100 diet partly restored the relative mRNA expression of *acc*, *fas*, *elovl2*, *elovl5*, *cd36*, *gpat* and *hsl* in rainbow trout ($P < 0.05$).

Furthermore, the dynamic process of long chain unsaturated fatty acids (LC-PUFA) synthesis in rainbow trout was further explored using proteomics tools (Fig. 4). Principal component

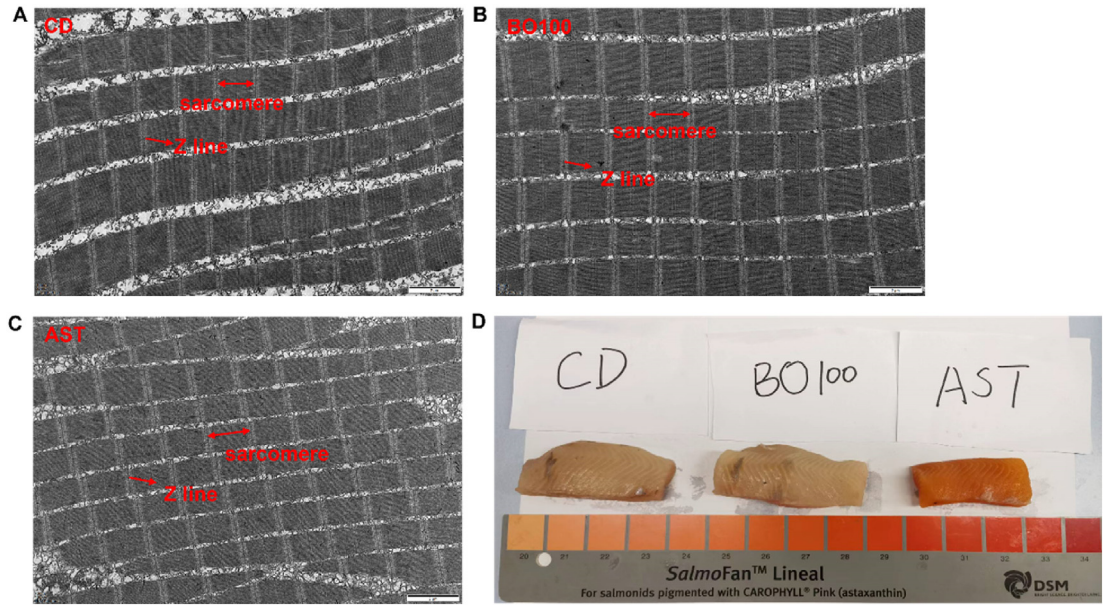

Fig 1. Effects of dietary black soldier fly larvae oil (BO) and astaxanthin (AST) supplementation on the myofiber morphology of rainbow trout. (A-C) Transmission electron microscope of longitudinal section. (D) Color appearance of fillet. CD = control diet; BO100 = BO replaces fish oil at 100% in control diet; AST = AST supplemented in BO100 diet.

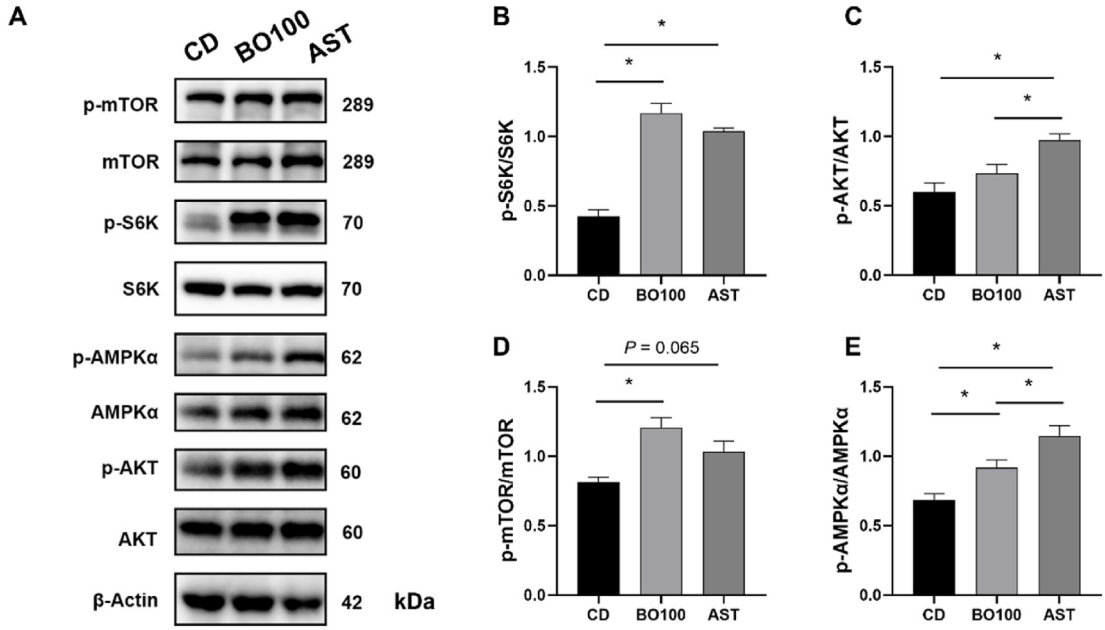

Fig 2. Effects of dietary black soldier fly larvae oil (BO) and astaxanthin (AST) supplementation on the protein expressions in the muscle sample of rainbow trout. (A) Western blot analysis of mammalian target of rapamycin (mTOR), p70 S6 kinase (S6K), AMP-activated protein kinase α (AMPKα), serine/threonine kinase (AKT) and their phosphorylation level, and β-actin. (B-E) Quantification of phosphorylation ratio of these proteins. Data are presented as mean ± SEM ($n = 4$). Asterisks (*) indicate significant differences between groups ($P < 0.05$). CD = control diet; BO100 = BO replaces fish oil at 100% in control diet; AST = AST supplemented in BO100 diet.

analysis (PCA) analysis clearly differentiated the three groups (Fig. 4A) and then DEP was defined by FDR value less than 0.05, fold change greater than 1.5 or less than −1.5 (Fig. 4B). Using the KEGG database for clustering, DEP were significantly enriched in lipid metabolism, endocrine system, amino acid metabolism, and carbohydrate metabolism in the comparison of the CD and BO100 groups (Fig. 4C). While in the contrast between BO100 and AST groups, DEP were enriched in transport and catabolism, circulatory system, translation and cell growth and death. As can be seen in Fig. 4D, fatty acid synthesis (ko00061), fatty acid elongation (ko00062), fatty acid degradation (ko00071), biosynthesis of unsaturated fatty acid (ko01040) and PPAR signaling pathways (ko03320) were significantly affected after complete replacement of fish oil by BO (CD-vs-BO100 and CD-vs-AST). Clustering heatmap showed that DEP of fatty acid transport (fatty acid-binding protein 1 [*fabp1*], *cd36*), synthesis (*acc* and *fas*), elongation (long chain acyl-coenzyme A synthetase 1 [*acsl1*], *elovl2* and *elovl6*) and desaturation (*Δ6 fads* and stearyl-CoA desaturase 1 [*scd1*]) were enriched in the BO100 group (Fig. 4E), while *elovl5* was enriched in the AST group.

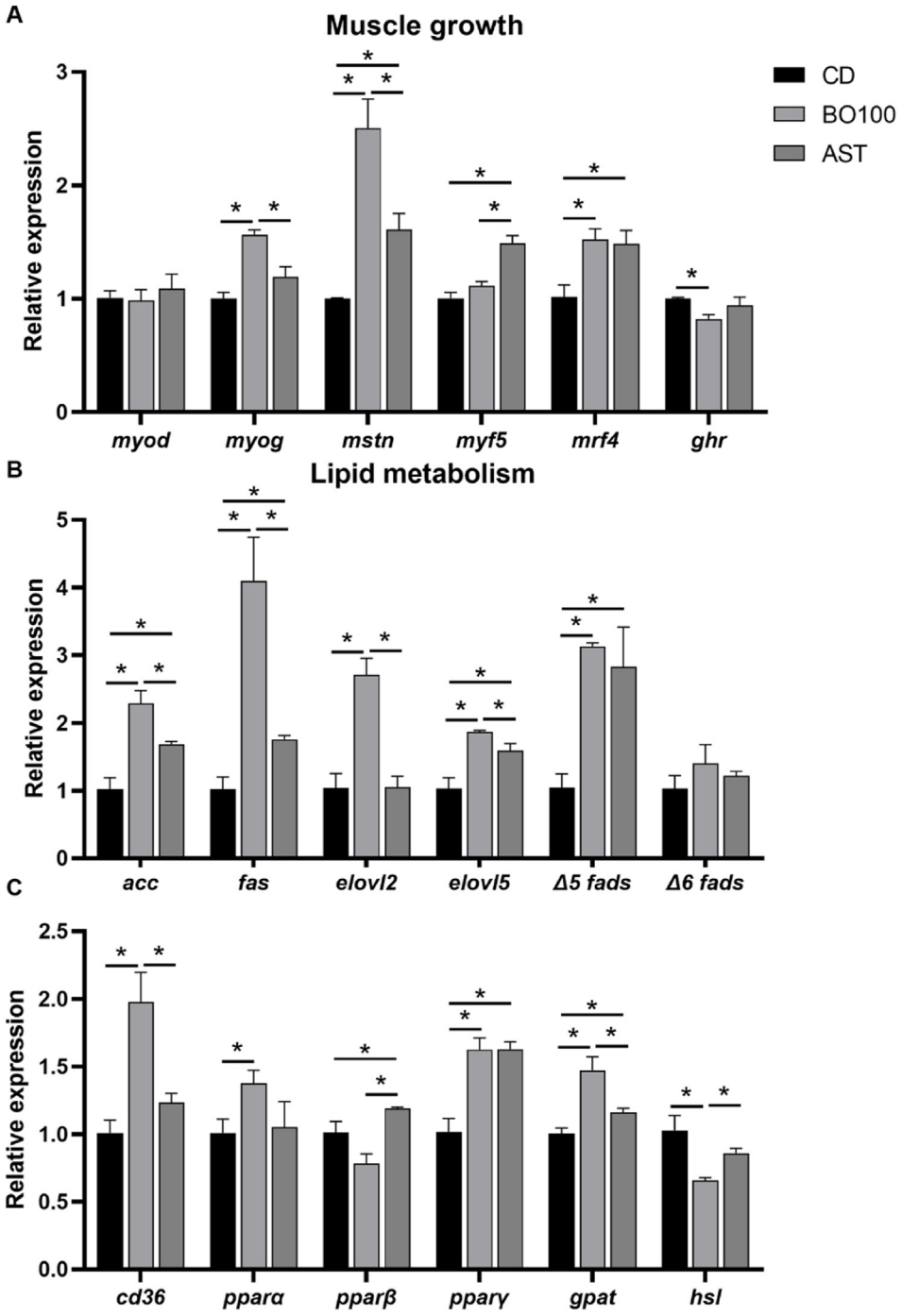

Fig 3. Relative mRNA expression of muscle growth and lipid metabolism in rainbow trout. (A) Genes related to muscle growth. (B and C) Genes related to lipid metabolism. Data are presented as mean ± SEM ($n = 4$). Asterisks (*) indicate significant differences between groups ($P < 0.05$). CD = control diet; BO100 = black soldier fly larvae oil replaces fish oil at 100% in control diet; AST = astaxanthin supplemented in BO100 diet. *acc* = acetyl-CoA carboxylase; *fas* = fatty acid synthase; *elovl2/5* = elongase of very-long fatty acid 2/5; *Δ5/6 fads* = Δ5/6 fatty acid desaturase; *cd36* = CD36/FAT fatty acid translocase; *myod* = myogenic determining factor; *myog* = myogenin; *mstn* = myostatin; *myf5* = myogenic factor 5; *mrf4* = myogenic factor 4; *ghr* = growth hormone receptor; *pparα/β/γ* = peroxisome proliferators-activated receptors-α/β/γ; *gpat* = glycerol-3-phosphate acyltransferase; *hsl* = hormone-sensitive lipase.

4. Discussion

The growth performance is the first consideration and most intuitive and primary indicator when introducing a novel oil source into the trout diet. Surprisingly, complete replacement of fish oil by BO significantly increased the FBW of rainbow trout in this experiment. This result showed a different trend in growth performance compared to the findings of Fawole et al. (2021), in which the growth of rainbow trout was not affected after dietary fish oil was totally replaced by BO. It is speculated that the difference may be due to the different extraction methods and the fatty acid composition of BO, which are closely related to the culture medium of the insects. Nevertheless, there are similarities between our findings and those of Fawole et al. (2021), such as elevated HI. These findings

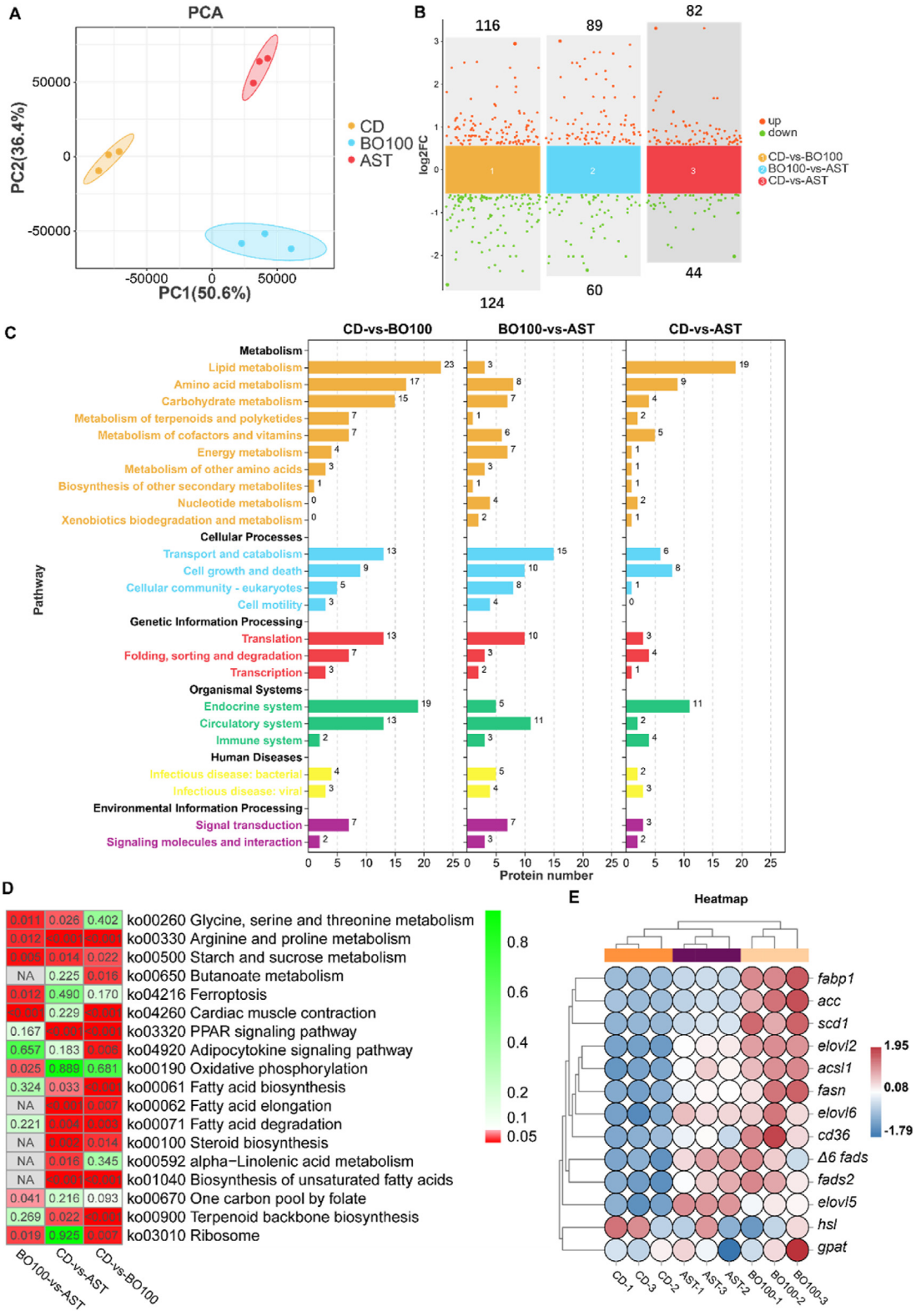

Fig 4. Proteomics analysis of rainbow trout fed with different diets. (A) Principal component analysis (PCA) analysis. (B) Distinguish of differentially expressed proteins (DEP) among groups. (C) DEP cluster according to Kyoto Encyclopedia of Genes and Genomes (KEGG) database. (D) Heatmap of influenced pathways in different comparisons (Values in the heatmap present the *P*-value of *t*-test between groups. *P*-values less than 0.05 indicate significant differences between groups. NA indicated that no DEP belonged to the specific pathway in the comparison). (E) Heatmap of DEP included in lipid metabolism. CD = control diet; BO100 = black soldier fly larvae oil replaces fish oil at 100% in control diet; AST = astaxanthin supplemented in BO100 diet. *fabp1* = fatty acid-binding protein 1; *acc* = acetyl-CoA carboxylase; *scd1* = stearyl-CoA desaturase 1; *elovl2/5/6* = elongase of very-long fatty acid 2/5/6; *Δ6 fads* = Δ6 fatty acid desaturase; *acsl1* = long chain acyl-coenzyme A synthetase 1; *fas* = fatty acid synthase; *cd36* = CD36/FAT fatty acid translocase; *fads 2* = fatty acid desaturases 2; *hsl* = hormone-sensitive lipase; *gpat* = glycerol-3-phosphate acyltransferase.

indicated that BO produced by subcritical butane extraction is safe for rainbow trout. However, the elevated HI and crude lipid content of the liver in the BO100 group suggested potential liver stress, which may be due to an excessive saturated fatty acid intake leading to the synthesis and storage of triglyceride (Rosqvist et al., 2014). The liver size of rainbow trout was reduced after AST supplementation, suggesting that dietary AST may alleviate lipid deposition and liver burden induced by dietary BO. This perspective is also confirmed by a recent study, in which rainbow trout fed with dietary AST showed a reduced liver size compared to the control group (Kalinowski et al., 2023).

In terms of the flesh quality, dietary BO enhanced the crude lipid content of fillet. In addition, the fatty acid content of the fillets followed the same trend as that of the feed, suggesting that dietary BO affected the lipid metabolism of rainbow trout. Similar results were also reported in some teleost such as barramundi (*Lates calcarifer*), striped catfish (*Pangasianodon hypophthalmus*) and Jian carp (*Cyprinus carpio* var. Jian) (Li et al., 2016; Hender et al., 2021; Sudha et al., 2022). Since BO typically consists of a high percentage of lauric acid and extremely low levels of EPA and DHA, similar results would be observed when fish were fed the BO diets. Notably, dietary AST partially restores the DHA content of fillet, suggesting that AST may function to regulate unsaturated fatty acid synthesis in rainbow trout. On the other hand, the observed decrease in the protein content of the fillet in the BO100 group may raise concerns; however, dietary AST appears to have mitigated this nagetive effect. In addition to the nutritional composition, variations in feed types can simultaneously affect the sensory properties and flesh quality of aquatic products (Luo et al., 2021), which directly affects consumer preference, consumption trend and commercial value. Fish fillet color is one of the main reasons influencing the willingness to consume because the visual effect is always direct and strong. Supplementation of AST in BO100 diets significantly improved the redness and yellowness of flesh colors, indicating that total replacement of fish oil by BO does not affect the coloring function of AST.

Flesh quality and chewing sensation of fish fillets are associated with the textural characteristics. The results showed that the hardness, springiness and chewiness of the fillets in the BO100 group decreased, but the adhesiveness increased. This suggested that the fillets of rainbow trout became softer when fed with dietary BO, which may be less attractive to consumers (Espe, 2008). Interestingly, dietary AST partially restored the springiness and chewiness of the fillets. Since textural changes in fish fillets are closely related to muscle structure and growth, muscle microstructure, protein expressions and growth-related genes were investigated to understand the mechanism by which this occurred (Lefaucheur, 2010). Results of TEM illustrated that dietary BO100 significantly increased the sarcomere length, which was further enhanced by AST supplementation. The growth of myofiber is largely dependent on hypertrophy and hyperplasia of myofiber, which is associated with myogenic regulatory factors (MRF) in skeletal muscle (White et al., 2018). Myogenic determining factor (myod) and myf5 are the primary MRF members that contributed to the transformation of proliferating somatic cells to myogenic cells, while the secondary mrf, myog and mrf4, further promoted differentiation and maturation of myoblast into myofibers (Sabourin and Rudnicki, 2000). In the present study, dietary BO facilitated the gene expression of secondary *mrf*, *myog* and *mrf4*, which promoted the generation of myofibers and thus explained why the sarcomere length was longer in the BO100 group. Meanwhile, the elevated expression of *mstn* may represent negative feedback on increased myofiber generation. A previous study also indicated that relative mRNA expression of muscle *myod*, *mrf5* and *myog* significantly upregulated in large yellow croaker (*Larimichthys crocea*) fed with 750 mg/kg glycerol monolaurate (Wang et al., 2022a). Different from the BO100 group, although muscle sarcomere length was further increased in rainbow trout fillets fed the AST diet, the relative mRNA expression of *myog* and *mstn* was downregulated, whereas *myf5* relative mRNA expression was upregulated, suggesting that dietary AST may target different mechanisms of muscle growth. As an antioxidant, AST has been shown to attenuate muscle atrophy in rats by inhibiting oxidative stress and protein hydrolysis in the findings of Shibaguchi et al. (2016). Furthermore, another study claimed that combined intake of AST, β-carotene, and resveratrol promoted protein synthesis during muscle hypertrophic (Kawamura et al., 2020). Therefore, it is speculated that AST may have influenced the textural characteristic of fish fillets by modulating oxidative stress and protein synthesis. This hypothesis is also supported by the fillet crude protein results, in which the crude protein significantly increased in the fillet of the AST group compared to the BO100 group. More importantly, the protein expression results supported this hypothesis. Dietary BO100 significantly increased the phosphorylation levels of mTOR and S6K, which may be activated through the AMPK pathway. On the other hand, AST supplementation may have activated the mTOR pathway through the activation of the AMPK pathway as well as the phosphoinositide 3-kinase-AKT (PI3K-AKT) pathway. In summary, the replacement of total fish oil by BO in the feed may provide protein for myofiber differentiation and maturation through activation of the AMPKα-mTOR-S6K pathway, thereby affecting the textural characteristics of rainbow trout fillets. Furthermore, supplementation of the BO100 diet with AST improved the appearance of fillets and may further activate the PI3K-AKT pathway for protein synthesis to promote the growth of sarcomere.

The enrichment of unsaturated fatty acid nutrients in fish fillets, especially n-3 LC-PUFA, which is highly beneficial to human health (Ruxton et al., 2004), is one of the reasons for consumers choose them. In the present study, n-3 LC-PUFA (including EPA and DHA) content was reduced and the n-3 PUFA/n-6 PUFA ratio was also lower in the BO100 group compared to the CD group. This impairment was partially restored in the AST group. Dietary AST had been prove to enhance DHA content in eggs by improving PUFA stability through antioxidant effects (Wang et al., 2022b). Others also reported an enhancement effect of EPA and DHA content in Chinese mitten crab (*Eriocheir sinensis*) feed supplement with 1.2 g/kg 10% AST (Zhang et al., 2024). To further explore how BO affected the synthesis of PUFA in rainbow trout, studies on the transcript level were conducted. The peroxisome proliferators-activated receptors (ppar) signaling pathway was speculated to play an important function in BO-affected lipid metabolism since the relative mRNA expression of *pparα* and *pparγ* was upregulated in the BO100 group compared to the CD group. Similar activations of pparα and pparγ were also observed in rice field eel (*Monopterus albus*) fed BSF meal (Hu et al., 2020). This process may be mediated by the uptake and transport of fatty acids by cd36, which was activated in the BO100 group (Su and Abumrad, 2009; Pepino et al., 2014). The collective up-regulation of these genes suggests that dietary BO in this context immensely promotes lipid metabolism in rainbow trout, including fatty acid synthesis, elongation and desaturation processes. In this case, AST supplementation restored mRNA expression of multiple genes to a lower level, including *acc*, *fas*, *cd36*, *gpat* and *hsl*. This suggested that dietary AST may promote fatty acid catabolism and inhibit fatty acid synthesis de novo, thereby attenuating lipid deposition and enhancing the liver health of rainbow trout, which was consistent with trends in the crude lipid content of whole fish and fillet (Zhao et al., 2023). The DEP clustering heatmap further supported the results of the transcripts that the fatty acid synthesis process in rainbow trout was enhanced by the use of BO, including de novo synthesis, elongation, and desaturation. On the one hand, the addition of AST may have

attenuated the de novo synthesis of fatty acids and the storage of triglyceride, which could have alleviated the hepatic burden of rainbow trout and reduced the crude lipid content of the fillets. On the other hand, AST supplementation improved DHA content in fillets of rainbow trout fed with the BO100 diet, which may be mediated through the promotion of mRNA expression and protein levels of fatty acid desaturase.

5. Conclusion

In this study, we have explored the underlying mechanisms of BO produced by subcritical butane extraction as a substitute for fish oil in the diet of rainbow trout. Our findings indicated that a complete substitution of fish oil by BO could potentially compromise the flesh quality (lower hardness, springiness and chewiness), change the nutritional components (lower protein and higher fat) and lead to a reduction of LC-PUFA (DHA and EPA) contents within the trout fillets. A promising strategy to mitigate these negative effects is the inclusion of dietary AST. Supplementation of AST in the BO diet could alleviate the changes in springiness and chewiness, partially restore DHA levels and improve the visual appeal of the trout fillets. Significantly, the inclusion of dietary AST enhanced the crude protein content and reduced the crude lipid content in the fillets. This effect is likely mediated by the upregulation of AKT and AMPK protein phosphorylation, which in turn modulates downstream lipid metabolism. Consequently, using AST as an additive in the feed of BO-fed rainbow trout may be instrumental in enhancing the overall quality of the fillets.

Credit Author Statement

Yongkang Chen: Conceptualization, Investigation, Writing – Original draft, Writing – Review & Editing. **Jian Zhong:** Resources. **Xuanqi Chen:** Investigation. **Xiaomin Li:** Investigation. **Haiqi Pu:** Data curation. **Baoyang Chen:** Data curation. **Yucai Guo:** Data curation. **Anqi Chen:** Methodology. **Wenjie Li:** Supervision. **Peng Hu:** Resources. **Xinliang Zhu:** Resources. **Wei Zhao:** Writing – Review & Editing. **Jin Niu:** Funding acquisition, 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

This research was supported by the Project of National Natural Science Foundation of China (32172982), and National Natural Science Foundation of China (32303014), and Guangdong Basic and Applied Basic Research Foundation (2024A1515010444), and Project of Science and Technology of Guangdong Province (2023A1515012627). Many thanks to Xiaoying Li and Yingying Wang from Qinghai University for their help with sampling and fillet texture determination.

References

Amrul NF, Kabir Ahmad I, Ahmad Basri NE, Suja F, Abdul Jalil NA, Azman NA. A review of organic waste treatment using black soldier fly (*Hermetia illucens*). Sustainability 2022;14(8):4565.

Aquilina G, Bampidis V, Bastos MDL, Costa LG, Flachowsky G, Gralak MA, Hogstrand C, Leng L, Secundino López-Puente Giovanna Martelli BM, Ramos F, Renshaw D, Rychen G, Saarela M, Sejrsen K, Beelen PV, Wallace RJ, Westendorf J. Scientific Opinion on the safety and efficacy of astaxanthin (CAROPHYLL® Pink 10% CWS) for salmonids and ornamental fish. EFSA J 2014;12(6):3725.

Bessa LW, Pieterse E, Marais J, Hoffman LC. Why for feed and not for human consumption? The black soldier fly larvae. Compr Rev Food Sci Food Saf 2020;19(5):2747–63.

Bruni L, Randazzo B, Cardinaletti G, Zarantoniello M, Mina F, Secci G, Tulli F, Olivotto I, Parisi G. Dietary inclusion of full-fat Hermetia illucens prepupae meal in practical diets for rainbow trout (*Oncorhynchus mykiss*): lipid metabolism and fillet quality investigations. Aquaculture 2020;529:735678.

Cao S, Guan L, Li C, Sun G, Tian H, Sun R, Tu J, Meng Y, Ma R. Effects of dietary protein and lipid levels on growth, metabolism, antioxidative capacity, and fillet quality of adult triploid rainbow trout farmed in net cage. Aquacult Nutr 2023;2023:4733343.

Caparros Megido R, Gierts C, Blecker C, Brostaux Y, Haubruge É, Alabi T, Francis F. Consumer acceptance of insect-based alternative meat products in Western countries. Food Qual Prefer 2016;52:237–43.

Chen Y, Wang Z, Liu J, Guo Y, Chen A, Chen B, Hu P, Zhu X, Li W, Zhao W. Optimization of extraction factor and nutritional characterization of black soldier fly larvae oil via subcritical butane extraction. LWT 2023a;186:115221.

Chen Y, Zhuang Z, Liu J, Wang Z, Guo Y, Chen A, Chen B, Zhao W, Niu J. Effects of *Hermetia illucens* larvae meal on the Pacific white shrimp (*Litopenaeus vannamei*) revealed by innate immunity and 16S rRNA gene sequencing analysis. Comp Biochem Physiol Genom Proteonomics 2023b;46:101080.

Cheng X, Li M, Leng X, Wen H, Wu F, Yu L, Jiang M, Lu X, Gao W, Zhang W. Creatine improves the flesh quality of Pacific white shrimp (*Litopenaeus vannamei*) reared in freshwater. Food Chem 2021;354:129498.

Espe M. Understanding factors affecting flesh quality in farmed fish. In Improving farmed fish quality and safety. Elsevier; 2008. p. 241–64.

Fawole FJ, Labh SN, Hossain MS, Overturf K, Small BC, Welker TL, Hardy RW, Kumar V. Insect (black soldier fly larvae) oil as a potential substitute for fish or soy oil in the fish meal-based diet of juvenile rainbow trout (*Oncorhynchus mykiss*). Animal Nutrition 2021;7(4):1360–70.

Gasco L, Biasato I, Dabbou S, Schiavone A, Gai F. Animals fed insect-based diets: state-of-the-art on digestibility, performance and product quality. Animals 2019;9(4):170.

Guan L, Zhuo L, Tian H, Li C, Li J, Meng Y, Ma R. Canola oil substitution doesn't affect growth but alters fillet quality of triploid rainbow trout. Aquaculture 2023;569:739385.

Hender A, Siddik MA, Howieson J, Fotedar R. Black soldier fly, *Hermetia illucens* as an alternative to fishmeal protein and fish oil: impact on growth, immune response, mucosal barrier status, and flesh quality of juvenile barramundi, *Lates calcarifer* (Bloch, 1790). Biology 2021;10(6):505.

Hu Y, Huang Y, Tang T, Zhong L, Chu W, Dai Z, Chen K, Hu Y. Effect of partial black soldier fly (*Hermetia illucens* L.) larvae meal replacement of fish meal in practical diets on the growth, digestive enzyme and related gene expression for rice field eel (*Monopterus albus*). Aquaculture Reports 2020;17:100345.

Kalinowski CT, Betancor MB, Torrecillas S, Sprague M, Larroquet L, Véron V, Panserat S, Izquierdo MS, Kaushik SJ, Fontagné-Dicharry S. More than an antioxidant: role of dietary astaxanthin on lipid and glucose metabolism in the liver of rainbow trout (*Oncorhynchus mykiss*). Antioxidants 2023;12(1):136.

Kawamura A, Aoi W, Abe R, Kobayashi Y, Wada S, Kuwahata M, Higashi A. Combined intake of astaxanthin, β-carotene, and resveratrol elevates protein synthesis during muscle hypertrophy in mice. Nutrition 2020;69:110561.

Lefaucheur L. A second look into fibre typing—relation to meat quality. Meat Sci 2010;84(2):257–70.

Letelier-Gordo CO, Dalsgaard J, Suhr KI, Ekmann KS, Pedersen PB. Reducing the dietary protein:energy (P:E) ratio changes solubilization and fermentation of rainbow trout (*Oncorhynchus mykiss*) faeces. Aquacult Eng 2015;66:22–9.

Li S, Ji H, Zhang B, Tian J, Zhou J, Yu H. Influence of black soldier fly (*Hermetia illucens*) larvae oil on growth performance, body composition, tissue fatty acid composition and lipid deposition in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquaculture 2016;465:43–52.

Lim KC, Yusoff FM, Shariff M, Kamarudin MS. Astaxanthin as feed supplement in aquatic animals. Rev Aquacult 2018;10(3):738–73.

Luo J, Monroig Ó, Zhou Q, Tocher DR, Yuan Y, Zhu T, Lu J, Song D, Jiao L, Jin M. Environmental salinity and dietary lipid nutrition strategy: effects on flesh quality of the marine euryhaline crab *Scylla paramamosain*. Food Chem 2021;361:130160.

Meng Y, Qian K, Ma R, Liu X, Han B, Wu J, Zhang L, Zhan T, Hu X, Tian H, Li C. Effects of dietary lipid levels on sub-adult triploid rainbow trout (*Oncorhynchus mykiss*): 1. Growth performance, digestive ability, health status and expression of growth-related genes. Aquaculture 2019;513:734394.

Mohsenpour M, Nourani M, Enteshary R. Effect of thawing under an alternating magnetic field on rainbow trout (*Oncorhynchus mykiss*) fillet characteristics. Food Chem 2023;402:134255.

Nyakeri E, Ogola H, Ayieko M, Amimo F. Valorisation of organic waste material: growth performance of wild black soldier fly larvae (*Hermetia illucens*) reared on different organic wastes. Journal of Insects as Food and Feed 2017;3(3):193–202.

Pepino MY, Kuda O, Samovski D, Abumrad NA. Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. Annu Rev Nutr 2014;34:281–303.

Rosqvist F, Iggman D, Kullberg J, Cedernaes J, Johansson H-E, Larsson A, Johansson L, Ahlström H, Arner P, Dahlman I. Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. Diabetes 2014;63(7):2356–68.

Ruxton C, Reed SC, Simpson M, Millington K. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J Hum Nutr Diet 2004;17(5):449–59.
Sabourin LA, Rudnicki MA. The molecular regulation of myogenesis. Clin Genet 2000;57(1):16–25.
Shibaguchi T, Yamaguchi Y, Miyaji N, Yoshihara T, Naito H, Goto K, Ohmori D, Yoshioka T, Sugiura T. Astaxanthin intake attenuates muscle atrophy caused by immobilization in rats. Physiological reports 2016;4(15):e12885.
Shumo M, Osuga IM, Khamis FM, Tanga CM, Fiaboe KK, Subramanian S, Ekesi S, van Huis A, Borgemeister C. The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. Sci Rep 2019;9(1):10110.
Su X, Abumrad NA. Cellular fatty acid uptake: a pathway under construction. Trends Endocrinol Metabol 2009;20(2):72–7.
Sudha C, Ahilan B, Felix N, Uma A, Chidambaram P, Prabu E. Replacement of fish oil with black soldier fly larvae oil and vegetable oils: effects of growth, whole-body fatty acid profile, digestive enzyme activity, haemato-biochemical responses and muscle growth-related gene expression of juvenile striped catfish, *Pangasianodon hypophthalmus*. Aquacult Res 2022;53(8):3097–111.
Van Huis A, Gasco L. Insects as feed for livestock production. Science 2023;379(6628):138–9.
Van Huis A, Oonincx DG. The environmental sustainability of insects as food and feed. A review. Agronomy for Sustainable Development 2017;37:1–14.
Wang J, Jiang H, Alhamoud Y, Chen Y, Zhuang J, Liu T, Cai L, Shen W, Wu X, Zheng W, Feng F. Integrated metabolomic and gene expression analyses to study the effects of glycerol monolaurate on flesh quality in large yellow croaker (*Larimichthys crocea*). Food Chem 2022a;367:130749.
Wang H, He W, Mahukpégo Dansou D, Zhang H, Dwi Nugroho R, Tang C, Guo X, Yu Y, Zhao Q, Qin Y, Zhang J. Astaxanthin improved the storage stability of docosahexaenoic acid-enriched eggs by inhibiting oxidation of non-esterified poly-unsaturated fatty acids. Food Chem 2022b;381:132256.
White DP, Baumgarner BL, Watanabe WO, Alam MS, Kinsey ST. The effects of dietary β-guanidinopropionic acid on growth and muscle fiber development in juvenile red porgy, *Pagrus pagrus*. Comp Biochem Physiol B Biochem Mol Biol 2018;216:48–58.
Zhang L, Zhang R, Jiang X, Wu X, Wang X. Dietary supplementation with synthetic astaxanthin and DHA interactively regulates physiological metabolism to improve the color and odor quality of ovaries in adult female *Eriocheir sinensis*. Food Chem 2024;430:137020.
Zhao W, Guo Y-C, Huai M-Y, Li L, Man C, Pelletier W, Wei H-L, Yao R, Niu J. Comparison of the retention rates of synthetic and natural astaxanthin in feeds and their effects on pigmentation, growth, and health in rainbow trout (*Oncorhynchus mykiss*). Antioxidants 2022;11(12):2473.
Zhao W, Yao R, Wei H-L, Guo Y-C, Chen A-Q, Chen B-Y, Jin N. Astaxanthin, bile acid and chlorogenic acid attenuated the negative effects of high-fat diet on the growth, lipid deposition, and liver health of *Oncorhynchus mykiss*. Aquaculture 2023;567:739255.