Animal Nutrition 10 (2022) 99–110

Contents lists available at ScienceDirect

Animal Nutrition

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

Original Research Article

A new single-cell protein from *Clostridium autoethanogenum* as a functional protein for largemouth bass (*Micropterus salmoides*)

Shifeng Ma [a, c], Xiaofang Liang [a], Pei Chen [a], Jie Wang [a], Xu Gu [a], Yuchang Qin [b], Christophe Blecker [c, *], Min Xue [a, *]

[a] National Aquafeed Safety Assessment Center, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing, 100081, China
[b] Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, 100193, China
[c] Food Science and Formulation, University of Liège, Gembloux Agro-Bio Tech, Avenue de la Faculté d' Agronomie, 2B, B-5030, Gembloux, Belgium

ARTICLE INFO

Article history:
Received 14 July 2021
Received in revised form
20 January 2022
Accepted 12 April 2022
Available online 21 April 2022

Keywords:
Clostridium autoethanogenum protein
Micropterus salmoides
Growth
Lipid metabolism
Hepatic and intestinal health
Gut-adherent microbiota

ABSTRACT

Clostridium autoethanogenum protein (CAP) is a new single-cell protein source originating from inactivated bacteria. An in vitro digestion experiment and an 8-wk growth experiment were conducted to evaluate the molecular weight distribution of the CAP hydrolysate, and the effects of dietary CAP levels on the growth performance, plasma parameters, hepatic and intestinal health, and the diversity of gut-adherent microbiota of largemouth bass (*Micropterus salmoides*). The fish (initial body weight of 47.99 ± 0.01 g) were fed diets where CAP gradually replaced 0% (CAP0), 12.5% (CAP12.5), 25% (CAP25), 37.5% (CAP37.5) and 50% (CAP50) of low-temperature steam dried anchovy fish meal (LTFM) in the diet. Results showed that the content of peptides below 1,000 Da in the CAP hydrolysate (0.56 mg/mL) was higher than that of the LTFM hydrolysate (0.48 mg/mL). Dietary CAP inclusion had no negative effect on growth performance, while whole-body lipid content significantly reduced in the CAP25 and CAP50 groups ($P < 0.05$). The plasma alanine aminotransferase activities and triglyceride concentrations in the CAP inclusion groups were significantly lower than those in the CAP0 group ($P < 0.05$). The plasma aspartate aminotransferase activity was significantly reduced in the CAP37.5 group ($P < 0.05$). The richness and diversity of the gut-adhesive microbiota and the proportion of *Clostridium sensu stricto 12* in the CAP50 group were significantly higher than those in the CAP0 group ($P < 0.05$). Dietary CAP inclusion inhibited inflammatory responses by down-regulating the mRNA levels of interleukin 1β (*IL1β*), *IL10* and transforming growth factor β1 ($P < 0.05$) in the liver. The mRNA levels of acetyl-CoA carboxylase 1 were significantly down-regulated in the CAP12.5, CAP25 and CAP37.5 groups ($P < 0.05$), while that of fatty acid synthase was significantly down-regulated in the CAP50 group ($P < 0.05$). These results demonstrate that dietary CAP inclusion could improve the hepatic and intestinal health of largemouth bass, and can be helpful to further develop CAP as a functional feed ingredient.

1. Introduction

Fish meal (FM) is valuable for its suitable amino acid composition, high digestibility and good palatability, and is thus the main protein source in aquafeed (Suárez et al., 2009). However, FM output is limited due to the deterioration of the marine ecosystem (Aghalino and Eyinla, 2009). It is necessary to find alternative protein sources for the sustainable development of aquaculture. The use of plant proteins is restricted because of the unbalanced amino acid composition, anti-nutritional factors, poor palatability, and occupation of arable land. The biosafety of animal proteins is often under scrutiny with risks such as salmonella, bovine

* Corresponding authors.
E-mail addresses: christophe.blecker@uliege.be (C. Blecker), xuemin@caas.cn (M. Xue).

Peer review under 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.2022.04.005

spongiform encephalopathy, and so on. Single-cell protein (SCP) is a kind of protein extracted from single-cell microorganisms such as algae, yeast, fungi, and bacteria. SCP has high nutritional value and production efficiency and does not occupy arable land. Several SCP, like microalgae and yeast, have been found to be functional food/feed ingredients for humans and animals (Vigani et al., 2015; Karim et al., 2020). The technology for converting lignocellulose in wastes or by-products from forestry and agriculture into protein sources through yeast fermentation has been developed (Øverland and Skrede, 2017). Yeasts have a high protein content, great amino acid composition, good palatability, and immunostimulant effects in feeding fish (Li and Gatlin, 2006; Ferreira et al., 2010; Øverland et al., 2013). However, the production of yeast protein from lignocellulosic biomass is high-cost, which limits its application in aquatic feed. Besides, several studies show that the bacterial biomass produced by natural gas fermentation with methanotroph (*Methylococcus capsulatus*) is a promising protein source (Feed-Kind®, Calysta, US) (Aas et al., 2006; Øverland et al., 2010). *Clostridium autoethanogenum* is an ethanol-producing bacteria utilizing CO as a carbon source (Abrini et al., 1994), and *C. autoethanogenum* protein (CAP, Richmore®) is a new single-cell protein source originating from the inactivated bacterial biomass produced in parallel with ethanol. It has a high protein content (>800 g/kg) and a well-balanced amino acid profile, which is similar to that of low-temperature steam dried anchovy fish meal (LTFM) (as shown in Table 1). Until now, CAP has been evaluated as a protein source in grass carp (*Ctenopharyngodon idllus*), black seabream (*Acanthopagrus schlegelii*) and juvenile Jian carp (*Cyprinus carpio* var. Jian) (Wei et al., 2018; Chen et al., 2020; Li et al., 2021a), respectively, all of which show that CAP is a promising protein material for aquafeeds.

Largemouth bass (*Micropterus salmoides*) is a high trophic level freshwater fish accustomed to eating chilled fish. With the acceptance of an artificial diet, the breeding scale of largemouth bass is in a stage of rapid growth. Several studies have tried to substitute the FM by some blended protein sources, like poultry by-product meal and soybean meal (Li et al., 2021c); shrimp hydrolysate, fermented soybean meal and corn gluten meal (Li et al., 2021b); or fermented soybean meal (He et al., 2020). These studies have reported that FM in the recipe could be reduced to 220 to 245 g/kg (He et al., 2020; Li et al., 2021c). However, because of the limited utilization of carbohydrates by this species, the commercial diets for largemouth bass generally contain 350 to 500 g/kg of high-quality FM.

Considering that the profile of CAP may provide a good protein source for carnivorous fish, this study determined the effects of replacing high-quality LTFM with CAP on the growth performance, plasma parameters, hepatic and intestinal histopathology, diversities of gut-adherent microbiota, lipid metabolism and inflammatory responses of largemouth bass.

2. Materials and methods

2.1. Animal ethics

In the present study, all fish were raised following the Laboratory Animal Welfare Guidelines of China (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of China, GB/T 35,892-2018).

2.2. Experimental formulas

The nutrient composition and amino acid content of LTFM and CAP are both shown in Table 1. The molecular weight distribution of the water-soluble part of LTFM and CAP hydrolysate are presented in Table 2. The calculated apparent digestibility coefficients of dry matter, protein and energy of the main ingredients (namely LTFM, CAP, cottonseed protein concentrate and soybean protein concentrate) are listed in Table 3. A formula containing 400 g/kg of LTFM was chosen as the control diet (CAP0). In the other 4 diets, 12.5%, 25%, 37.5% and 50% of the LTFM in the diet was replaced with CAP and were named as CAP12.5, CAP25, CAP37.5 and CAP50, respectively. The phosphorus content of CAP was 9.2 g/kg, while that of LTFM was 28.5 g/kg. As the CAP substitution levels increased, the addition of $Ca(H_2PO_4)_2$ was required to be balanced in the CAP diet. Due to the different nutrient compositions between LTFM and CAP, the formulas were balanced by adding microcrystalline cellulose. All the diets were isoenergetic and isonitrogenous. The formulation and

Table 1
Nutrient composition and amino acid content of the LTFM and CAP (g/kg, as is basis).

Item	LTFM	CAP
Nutrient composition		
Crude protein	720	800
Crude lipid	88.4	19.0
Ash	150	35.0
Acetic acid	–	20.0 [1]
Nucleobases		
Adenine	0.78	0.32
Cytosine	0.10	0.23
Guanine	5.66	7.88
Thymine	0.23	16.9
Uracil	0.14	1.01
Calculated total nucleotides	16.9	66.5
Indispensable amino acids		
Arginine	41.7	34.0
Histidine	14.5	16.8
Isoleucine	30.4	52.8
Leucine	52.6	62.2
Lysine	57.6	75.8
Methionine	21.0	22.9
Phenylalanine	28.2	30.0
Threonine	31.2	40.2
Tryptophan	7.00	6.20
Valine	35.5	54.4
Dispensable amino acids		
Alanine	44.0	46.3
Aspartic acid	67.2	95.4
Cystine	6.10	7.10
Glutamic acid	94.2	97.8
Glycine	41.7	38.7
Proline	26.1	24.0
Serine	30.0	32.1
Tyrosine	19.4	31.4
ΣAA [2]	648	768

LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein.
[1] The acetic acid content in CAP was provided by Beijing Shoulang Biotechnology Co. Ltd (China).
[2] ΣAA = sum of total amino acids.

Table 2
The molecular weight distribution of the water-soluble component of LTFM and CAP hydrolysate.

Molecular weight, Da	Relative quantity, %		Absolute content, mg/mL	
	LTFM	CAP	LTFM	CAP
>5,000	4.15	1.39	0.03	0.02
5,000 to 3,000	6.72	4.17	0.05	0.05
3,000 to 2,000	9.05	10.3	0.07	0.12
2,000 to 1,000	19.8	34.2	0.16	0.39
<1,000	60.3	50.0	0.48	0.56
Total	100	100	0.79	1.13

LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein.

Table 3
Apparent dry matter, protein and energy digestibility coefficients (%)[1] of the main ingredients for largemouth bass.

Ingredient	Dry matter	Protein	Energy
LTFM	79.0	86.2	86.9
CAP	82.1	87.2	83.9
Cottonseed protein concentrate	60.9	85.3	71.6
Soybean protein concentrate	71.3	89.8	81.4

LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein.
[1] The apparent digestibility coefficients of nutrients in CAP for largemouth bass are all quoted from Tan et al. (unpublished data). The apparent digestibility coefficients of nutrients in LTFM, cottonseed protein concentrate and soybean protein concentrate for largemouth bass are all quoted from Zou (2021).

composition of experimental diets are shown in Table 4. The dry ingredients were well mixed in a mixer (CH-100, The New Standard Powder Machinery Manufacturing Co., Ltd, Wuxi, China). The mixed material was ground through a hammer mill (JYNU30-15, Qingdao Jieyina Machinery Science & Technology Co., Ltd., Qingdao, China), and passed through a 0.180-mm sieve. A twin-screw extruder (EXT50A, Yanggong Machine, Beijing, China) was used to process 5 diets into extruded floating feed at the size of 3 mm diameter. The barrel of the extruder is divided into three temperature-controlled zones, which are heated by an electric heating system. The temperature of each zone along the material flow was 100, 120 and 150 °C respectively. The feeds were air-dried at room temperature (about 25 °C) and preserved at −20 °C during the growth trial. The amino acid compositions of the 5 formulas are listed in Table 5.

2.3. Experimental fish, rearing and sample collection

The fish were supplied by the Sanshui Platinum Aquatic Seedling Company (Foshan, China). After 24 h of starvation, the fish with an initial body weight of 47.99 ± 0.01 g were selected and distributed to 20 conical fiberglass tanks (256 L, water depth: 80 cm, volume: 0.25 m^3) at 25 fish/tank. There were four repetitions for each treatment. Fish were fed at 08:00 and 16:00 to meet the apparent satiation for 8 wk. The aquariums were installed in a recirculation system containing groundwater. The oxygen level in the tank was established using aeration and water flow rates through the tank. Aeration was performed 24 h per day. The water flow rate was set at 1.5 L/min. An oxygen meter (JPBJ-608, Shanghai INESA Scientific Instruments Co., Ltd, Shanghai, China) was used to measure the dissolved oxygen concentration and temperature of each tank daily. The ammonia nitrogen level and pH were both measured daily using test kits (Ammonia Nitrogen Detection Kit and pH Test Kit, Heliosense Biotechnologies, Inc, Xiamen, China). The water quality conditions were as follows: 23 ± 1 °C, pH = 7.5 to 8.5, ammonia nitrogen levels = 0.23 ± 0.06 mg/L, and dissolved oxygen (DO) > 7.0 mg/L. The ammonia nitrogen level and dissolved

Table 4
Ingredients and nutrient composition of experimental diets[1] (g/kg, as is basis).

Item	CAP0	CAP12.5	CAP25	CAP37.5	CAP50
Ingredients					
LTFM[2]	400	350	300	250	200
CAP[3]	0	45	90	135	180
Cottonseed protein concentrate[4]	148	148	148	148	148
Soybean protein concentrate[5]	148	148	148	148	148
Wheat gluten[6]	30	30	30	30	30
Spray-dried blood cell meal[7]	30	30	30	30	30
$Ca(H_2PO_4)_2$[8]	10	10	10	18	18
Lecithin oil[6]	20	20	20	20	20
Fish oil[9]	35	35	39	42	47
Soybean oil[6]	30	30	30	30	30
Vitamin and mineral premix[10]	14	14	14	14	14
Tapioca starch[11]	120	120	120	120	120
Kelp meal[12]	15	15	15	15	15
Microcrystalline cellulose[13]	0	5	6	0	0
Total	1,000	1,000	1,000	1,000	1,000
Nutrient compositions					
Crude protein	508.5	508.3	510.6	502.4	502.9
Crude lipid	119.0	113.4	109.6	109.3	106.6
Crude ash	97.9	91.5	85.7	87.1	80.4
Moisture	40.5	39.2	47.3	57.5	56.5
Gross energy, MJ/kg	20.5	20.6	20.5	20.2	20.2
Digestible phosphorus	11.1	10.4	9.73	10.9	10.2
Nucleotides	19.1	21.2	23.4	25.5	27.7

CAP = *Clostridium autoethanogenum* protein; LTFM = low-temperature steam dried anchovy fishmeal.
[1] CAP0 was the control diet. In the other 4 diets, 12.5%, 25%, 37.5% and 50% of the fish meal in the diet was replaced with CAP, named as CAP12.5, CAP25, CAP37.5 and CAP50, respectively.
[2] Supplied by Triple Nine Fish Protein Co., Ltd. (Denmark).
[3] Supplied by Beijing Shoulang Biotechnology Co., Ltd. (China).
[4] Supplied by Xinjiang Jinlan Plant Protein Co., Ltd. (China).
[5] Supplied by Yihai Kerry Investment Co., Ltd. (China).
[6] Supplied by Bohai Oil Co., Ltd. (China).
[7] Supplied by Beijing Hongshun Source Biotech Co., Ltd. (China).
[8] Supplied by Yunnan Phosphate Group Co., Ltd. (China).
[9] Supplied by JinhaiGrain and Oil Industry Co., Ltd. (China).
[10] Vitamin and mineral premix (mg/kg diet): vitamin A, 20; vitamin B_1, 12; vitamin B_2, 10; vitamin B_6, 15; vitamin B_{12} (1%), 8; niacinamide, 100; ascorbic acid (35%), 1,000; calcium pantothenate, 40; biotin (2%), 2; folic acid, 10; vitamin E (50%), 400; vitamin K_3, 20; vitamin D_3, 10; inositol, 200; choline chloride (50%), 4,000; corn gluten meal, 150; $CuSO_4 \cdot 5H_2O$, 10; $FeSO_4 \cdot H_2O$, 300; $ZnSO_4 \cdot H_2O$, 200; $MnSO_4 \cdot H_2O$, 100; KIO_3 (10%), 80; Na_2SeO_3 (10% Se), 67; $CoCl_2 \cdot 6H_2O$ (10% Co) 5; NaCl, 100; zeolite, 638.
[11] Supplied by Haid Group Co., Ltd. (China).
[12] Supplied by Qingdao Hisea Imp. & Exp. Co., Ltd. (China).
[13] Supplied by Huzhou City LinghuXinwang Chemical Co., Ltd. (China).

Table 5
Amino acid content of experimental diets[1] and dietary amino acid requirements of largemouth bass (g/kg, as-is basis).

Item	CAP0	CAP12.5	CAP25	CAP37.5	CAP50	Requirement[2]
Indispensable amino acids						
Arginine	35.8	35.4	34.4	34.3	33.6	20.0
Histidine	14.1	13.9	13.7	13.6	13.4	5.0
Isoleucine	20.1	20.6	21.6	22.8	23.4	9.0
Leucine	37.7	37.5	37.5	38.2	37.8	20.0
Lysine	34.5	34.8	35.4	36.5	36.7	21.0
Methionine	11.4	11.2	11.3	11.2	11.1	10.0[3]
Phenylalanine	24.0	24.1	23.8	23.9	23.6	17.0[4]
Threonine	20.1	20.2	20.4	20.8	20.9	11.0
Tryptophan	6.3	6.0	5.8	5.8	5.4	2.0
Valine	25.8	26.4	26.4	27.5	27.7	14.0
Dispensable amino acids						
Aspartic acid	48.9	49.2	50.3	51.6	51.9	–
Cystine	6.2	6.2	6.2	6.4	6.1	–
Glutamic acid	82.3	80.4	80.6	81.5	79.5	–
Glycine	26.3	25.9	25.4	25.5	25.4	–
Proline	23.9	23.5	23.9	23.2	22.6	–
Serine	22.5	22.6	22.6	22.6	22.7	–
ΣAA[5]	439.9	437.9	439.3	445.4	441.8	–

CAP = *Clostridium autoethanogenum* protein.
[1] CAP0 was the control diet. In the other 4 diets, 12.5%, 25%, 37.5% and 50% of the fish meal in the diet was replaced with CAP, named as CAP12.5, CAP25, CAP37.5 and CAP50, respectively.
[2] The dietary amino acids requirements of largemouth bass are quoted from Dairiki et al. (2007).
[3] Sum of methionine and cystine.
[4] Sum of phenylalanine and tyrosine.
[5] ΣAA = sum of total amino acids.

oxygen were both acceptable for largemouth bass (Subhadra et al., 2006). The light intensity was set to 400 lx with a photoperiod of 12D:12 L.

At the beginning and end of the feeding trial, 10 and 3 fish were randomly selected and stored frozen (−20 °C) respectively for the determination of whole fish composition. At the end of the feeding trial, the fish were weighed after 24 h of starvation to calculate final body weight (FBW) and specific growth rate (SGR). The feeding rate (FR), feed conversion rate (FCR), productive protein value (PPV) and productive lipid value (PLV) were all calculated by recording feed intake per tank. Twenty fish (5 fish per tank) were randomly selected from each group and were later narcotized with chlorobutanol (300 mg/mL). The body length, viscera weight, liver weight and visceral adipose weight were all recorded to calculate the morphological indexes including the condition factor (CF), viscera somatic index (VSI), hepatosomatic index (HSI) and visceral adipose index (VAI). Blood samples were collected from the caudal portion of the fish (2 fish per tank). Plasma was obtained by centrifuging the blood samples at 3,000 × *g* for 10 min at 4 °C. The liver near the bile duct (CAP0 and CAP50 groups) and distal intestine (CAP0 and CAP50 groups) were both sampled and fixed with 4% paraformaldehyde for histological determination. Liver samples were also snap-frozen in liquid nitrogen to extract RNA. At the end of the 8-wk feeding trial, four fish in CAP0, CAP12.5 and CAP50 groups were randomly selected at 6 h postprandially (the peak defecation time) and were anaesthetized with chlorobutanol (300 mg/mL). Both the chyme and cleaned gut samples were selected. Unfortunately, we failed to extract DNA from the chyme samples and only investigated the gut-adhesive bacteria. Except for the samples used for histological determination, all other samples were stored at −80 °C until testing.

2.4. Chemical analysis

All chemical analyses were conducted in duplicate according to AOAC (2006). The dry matter was measured at 105 °C. Crude protein was analyzed by the method of Kjeldahl (KjeltecTM 2300 Unit, Foss, Hillerød, Denmark), and the crude protein content was calculated by multiplying nitrogen by 6.25. Crude lipid was measured by acid hydrolysis (Soxtex System HT 1047 Hydrolyzing Unit, Foss, Hillerød, Denmark) followed by Soxhlet extraction (Soxtex System 1043, Foss, Hillerød, Denmark). Ash was obtained by burning the samples in a muffle furnace (CWF1100, Carbolite, Derbyshire, UK) at 550 °C for 16 h. The amino acids of the diets and protein materials were measured by using an amino acid analyzer (Hitachi 8900, Tokyo, Japan). The nucleobases in ingredients and feed were both measured following the method of Mydland et al. (2008) with some modifications. The samples were hydrolyzed with perchloric acid (20%) for 60 min at 100 °C. Then, the 5 kinds of nucleobases in the samples were analyzed by a Hitachi Chromaster high-performance liquid chromatography (HPLC) system (Hitachi Co. Ltd., Japan). The total nucleotide content of the samples is calculated, assuming that the molar fraction of each nucleotide is equal to that of its respective nucleobase (Romarheim et al., 2013).

2.5. In vitro digestion and molecular weight distribution of the protolysate

Both LTFM and CAP were subjected to an in vitro digestion. The methods were described by Chavan et al. (2001) with slight modifications. The in vitro digestion was carried out by a continuous pepsin-trypsin system. The protein samples (0.5 g) were dispersed in 20 mL of 0.05 mol/L HCl in 250 mL conical flasks, and adding 0.5 mL of 0.1 mol/L HCl dissolved in 5 mg of pepsin. The samples were gently shaken for 3 h at 37 °C and then neutralized with phosphate buffer (1.0 mol/L, pH 8). Appropriate trypsin (substrate-to-enzyme ratio is 100:1, wt/wt) was added to samples followed by a shake at 37 °C for 3 h. The protein content in the hydrolysate was determined by the Lowery method using a microplate reader (PowerWave XS2, BioTek, Winooski, VT, USA) assay at 650 nm (Lowry et al., 1951).

Following the methods of Zhuang et al. (2009), the molecular weight distribution of the protolysate was detected using a TSK gel G2000 SWXL 7.8 mm × 300 mm column (Tosoh, Tokyo, Japan) on the LC-15C HPLC system (Shimadzu, Japan).

2.6. Hematological parameters

Plasma concentrations of triglyceride (TG), total cholesterol (TC), glucose (GLU), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) and the activities of aspartate aminotransferase (AST) alanine aminotransferase (ALT) were all measured with a microplate reader (PowerWave XS2, BioTek, Winooski, VT, USA) and corresponding commercial kits from Jiancheng Bioengineering Institute, Nanjing, China (TG: A110-2-1, 96 T; TC: A111-2-1, 96 T; AST: C010-2-1, 96 T; ALT: C009-2-1, 96 T; HDL-C: A112-2-1, 96 T; LDL-C: A113-2-1, 96 T) and from Shanghai Rongsheng Biotech Inc., Shanghai, China (Glu: 361500, 96 T) following the protocols given by the suppliers (Yu et al., 2014).

2.7. RNA isolation, reverse transcription and real-time quantitative PCR (RT-PCR)

The core fragments of the genes were found in the RNA-seq database. A housekeeping gene namely elongation factor 1α (*EF1*α) (GenBank accession no. KT827794) was set as an endogenous reference. The gene-specific primers were used for mRNA quantification in RT-PCR (as shown in Table 6). RNA isolation, reverse transcription and RT-PCR were all performed according to the method of Yu et al. (2019).

2.8. Histopathological examination

All liver and distal intestine specimens were dehydrated and embedded in paraffin, and were cut into 6 μm slices. The samples were stained with hematoxylin and eosin (H&E for liver) and Alcian Blue (pH 2.5, for intestine) respectively, and were examined by a Leica DM2500 LED optical microscope (Solms, Germany).

2.9. Composition and diversity of gut-adhesive microbiota

Genomic DNA of the intestine was extracted with a PowerFecal DNA Isolation Kit (Mo Bio Laboratories, USA). The quality and purity of the genomic DNA were examined by electrophoresis using 1% agarose gels. The primers 338 F (ACTCCTACGGGAGGCAGCAG) and 806 R (GGACTACHVGGGTWTCTAAT) were used to amplify the V3–V4 hypervariable region of the bacterial 16 S rRNA gene. The purification, quantification and sequencing of the PCR products were all performed following the method of Wei et al. (2020).

FastQC (version 0.11.3) was used to control the quality of raw data. QIIME was used for dataset analysis. The sequences with 97% similarity were aggregated into operational taxonomic units (OTUs). The OTUs were then used to compute the richness and diversity indices. Based on the SILVA rRNA database, all sequences were categorized into taxonomic groups using the Ribosomal Database Project (RDP).

2.10. Statistics

SPSS statistics 19 (SPSS Inc., USA) was used to conduct data analysis. All data were analyzed by One-Way ANOVA. Homogeneity of variance was confirmed by Levene's test before ANOVA. Tukey's multiple range test was performed to analyze the differences among the means when significant differences were noticed ($P < 0.05$). Orthogonal polynomial contrasts were performed to indicate whether there were significant linear, quadratic and cubic effects responding to the increased CAP substitution levels. The graphics were drawn by OriginPro 9.0.0 (OriginLab Corporation, Northampton, USA).

3. Results

3.1. Molecular weight distribution of water-soluble part of the protolysate

The protein content in the CAP hydrolysate (1.13 mg/mL) was higher than that of LTFM hydrolysate (0.79 mg/mL) (Table 2). The chromatographic data showed that the content of peptides below 1,000 Da in the CAP hydrolysate (0.56 mg/mL) was higher than that of the LTFM hydrolysate (0.48 mg/mL).

3.2. Growth performance and morphometric parameters

The survival rate of all experimental groups was 100%. The growth performance indexes, morphological indexes and whole-body compositions are all listed in Table 7. When the LTFM in diets were gradually replaced by CAP to 50%, the growth performance including FBW, SGR, FR, FCR, PPV and PLV, and morphometric parameters including CF, HSI and VSI, and whole-body compositions including moisture, ash and crude protein all showed no significant difference ($P > 0.05$). The CAP substitution levels had significant linear effects on the VAI ($P = 0.004$) and whole-body crude lipid ($P = 0.001$). The CAP37.5 group had the lowest VAI value, and the CAP50 group had the lowest lipid content of the whole fish.

3.3. Hematological parameters

The results of hematological parameters are reported in Table 8. The CAP substitution levels had significant linear effects on plasma ALT activities ($P = 0.000$) and TG concentrations ($P = 0.001$). Both parameters in the CAP inclusion groups were significantly lower than those in the CAP0 group ($P < 0.05$). The replacement levels of CAP had significant linear ($P = 0.013$) and cubic ($P = 0.025$) effects on plasma AST activities. The plasma AST activity in the CAP37.5 group was the lowest. The CAP substitution levels had significant quadratic effects on the plasma GLU concentrations ($P = 0.001$). As the replacement levels of CAP

Table 6
Primer sequences for real-time PCR.

Gene	Primers	Sequence 5′-3′	Target size, bp	E-values, %	Temperature, °C
EF1α	Forward	TGCTGCTGGTGTTGGTGAGTT	147	102.8	60.4
	Reverse	TTCTGGCTGTAAGGGGGCTC			
ACC1	Forward	ATCCCTCTTTGCCACTGTTG	121	102.2	57.5
	Reverse	GAGGTGATGTTGCTCGCATA			
FASN	Forward	ATCCCTCTTTGCCACTGTTG	121	102.1	57.5
	Reverse	GAGGTGATGTTGCTCGCATA			
ATGL	Forward	CCATGATGCTCCCCTACACT	176	99.1	58
	Reverse	GGCAGATACACTTCGGGAAA			
HSL	Forward	ATCAGAGCTGGAGCACCCTA	122	99.3	60
	Reverse	GCAGAGGAGAGCAGAAAGGA			
PPARα	Forward	CCACCGCAATGGTCGATATG	144	104.3	59
	Reverse	TGCTGTTGATGGACTGGGAAA			
CPT1α	Forward	CATGGAAAGCCAGCCTTTAG	128	98.8	60
	Reverse	GAGCACCAGACACGCTAACA			
IL1β	Forward	CGTGACTGACAGCAAAAAGAGG	166	101.3	59.4
	Reverse	GATGCCCAGAGCCACAGTTC			
TNFα	Forward	CTTCGTCTACAGCCAGGCATCG	161	105.7	63
	Reverse	TTTGGCACACCGACCTCACC			
TGFβ1	Forward	GCTCAAAGAGAGCGAGGATG	118	95.6	59
	Reverse	TCCTCTACCATTCGCAATCC			
IL10	Forward	CGGCACAGAAATCCCAGAGC	119	113.6	62.1
	Reverse	CAGCAGGCTCACAAAATAAACATCT			

EF1α = elongation factor 1α; *ACC1* = acetyl-CoA carboxylase 1; *FASN* = fatty acid synthase; *ATGL* = adipose triglyceride lipase; *HSL* = hormone-sensitive lipase; *PPARα* = peroxisome proliferator activated receptor α; *CPT1α* = carnitine palmitoyltransferase 1α; *IL* = interleukin; *TNFα* = tumor necrosis factor α; *TGFβ1* = transforming growth factor β1.

Table 7
Effects of LTFM replacement by CAP on the growth performance, morphometric parameters and whole-body composition in largemouth bass[1].

Parameters		Groups[2]					Pooled SEM	Polynomial Contrasts		
		CAP0	CAP12.5	CAP25	CAP37.5	CAP50		Linear	Quadratic	Cubic
Growth performance	FBW[3], g	158	150	155	152	148	2.72	0.086	0.977	0.192
	SGR[4], %/d	2.20	2.11	2.17	2.13	2.09	0.03	0.088	0.975	0.195
	FR[5], % BW/d	1.72	1.70	1.74	1.70	1.69	0.02	0.368	0.355	0.769
	FCR[6]	0.87	0.89	0.89	0.88	0.89	0.01	0.058	0.235	0.054
	PPV[7], %	34.2	31.1	33.3	30.6	30.6	0.16	0.123	0.845	0.568
	PLV[8], %	99.3	91.5	93.5	89.7	88.5	4.31	0.123	0.656	0.611
Morphological index	CF[9], g/cm^3	1.86	1.81	1.79	1.81	1.77	0.02	0.016	0.399	0.123
	HSI[10], %	1.60	1.57	1.58	1.62	1.72	0.07	0.214	0.273	0.959
	VSI[11], %	8.50	7.98	8.27	7.90	8.16	0.16	0.190	0.186	0.736
	VAI[12], %	2.99^{a}	2.67^{abc}	2.78^{ab}	2.40^{c}	2.54^{bc}	0.10	0.004	0.307	0.794
Whole-body composition	Moisture, %	70.8	70.5	70.2	70.7	71.2	0.24	0.224	0.033	0.883
	Ash, %	3.30	3.44	3.26	3.27	3.33	0.07	0.711	0.889	0.160
	Crude protein, %	15.9	16.5	16.4	16.5	16.2	0.16	0.388	0.044	0.582
	Crude lipid, %	8.41^{a}	8.45^{a}	7.79^{b}	8.03^{ab}	7.68^{b}	0.13	0.001	0.813	0.784

LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein; SEM = standard error of treatment means.
$^{a\ to\ c}$ Within a row, means with different superscripts are significantly different (Turkey test; $P < 0.05$).
[1] Initial body weight (IBW) = 47.99 ± 0.01 (g), $n = 4$.
[2] CAP0 was the control diet. In the other 4 diets, 12.5%, 25%, 37.5% and 50% of the fish meal in the diet was replaced with CAP, named as CAP12.5, CAP25, CAP37.5 and CAP50, respectively.
[3] FBW (final body weight), $n = 4$.
[4] SGR (specific growth rate, %/d) = 100 × [ln (FBW/IBW)]/days, $n = 4$.
[5] FR (feeding rate, % BW/d) = 100 × feed intake (g, as is basis)/[(WF + WI)/2]/days, $n = 4$, where WF is the final total weight and WI is the initial total weight.
[6] FCR (feed conversion ratio) = feed intake/(WF − WI), $n = 4$.
[7] PPV (productive protein value, %) = 100 × (FBW × CFP−IBW × CIP)/(feed intake × feed protein content, %), where CFP (%) is final protein content in whole fish body and CIP (%) is initial protein content in whole fish body, $n = 4$.
[8] PLV (productive lipid value, %) = 100 × (FBW × CFL − IBW × CIL)/(feed intake × feed protein content, %), where CFL (%) is final lipid content in whole fish body and CIL (%) is initial lipid content in whole fish body, $n = 4$.
[9] CF (condition factor, g/cm^3) = 100 × (BW, g)/(body length, cm)^3, $n = 20$.
[10] HSI (hepatosomatic index, %) = 100 × (liver weight, g)/(BW, g), $n = 20$.
[11] VSI (viscera somatic index, %) = 100 × (viscera weight, g)/(BW, g), $n = 20$.
[12] VAI (visceral adipose index, %) = 100 × (visceral adipose weight, g)/(BW, g), $n = 20$.

Table 8
Effects of LTFM replacement by CAP on plasma biochemical parameters [1] of largemouth bass.

Parameters	Groups[2]					Pooled SEM	Polynomial contrasts		
	CAP0	CAP12.5	CAP25	CAP37.5	CAP50		Linear	Quadratic	Cubic
ALT, U/L	62.6^{a}	42.9^{b}	45.5^{b}	44.5^{b}	44.4^{b}	2.00	0.000	0.001	0.010
AST, U/L	24.1^{a}	24.2^{a}	20.5^{ab}	14.9^{b}	20.3^{ab}	1.96	0.013	0.249	0.025
GLU, mmol/L	5.82^{a}	3.81^{bc}	3.60^{bc}	3.00^{c}	5.31^{ab}	0.57	0.334	0.001	0.557
TG, mmol/L	5.61^{a}	3.47^{b}	3.41^{b}	2.61^{b}	3.26^{b}	0.42	0.001	0.010	0.667
TC, mmol/L	7.44^{b}	9.71^{a}	7.16^{b}	7.28^{b}	7.45^{b}	0.48	0.157	0.439	0.007
HDL-C, mmol/L	1.92^{bc}	2.94^{a}	2.06^{bc}	2.48^{ab}	1.43^{c}	0.22	0.049	0.002	0.565
LDL-C, mmol/L	3.07^{a}	2.53^{ab}	2.34^{ab}	1.65^{b}	2.35^{ab}	0.28	0.016	0.077	0.262
HDL-C to TC ratio	0.26^{a}	0.33^{a}	0.30^{ab}	0.38^{a}	0.19^{b}	0.04	0.561	0.013	0.180

LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GLU = glucose; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SEM = standard error of treatment means.
$^{a\ to\ c}$ Within a row, means with different superscripts are significantly different (Turkey test; $P < 0.05$).
[1] Values are means of 8 replicates.
[2] CAP0 was the control diet. In the other 4 diets, 12.5%, 25%, 37.5% and 50% of the fish meal in the diet was replaced with CAP, named as CAP12.5, CAP25, CAP37.5 and CAP50, respectively.

increased, the plasma GLU concentrations decreased first, and then increased when the replacement level reached 50%. The CAP substitution levels had significant cubic effects on the plasma TC concentrations ($P = 0.007$). The CAP12.5 group had the highest plasma TC concentration. Significant quadratic effects on plasma HDL-C concentrations ($P = 0.002$) and the HDL-C/TC ratios ($P = 0.013$) were observed responding to increased CAP substitution levels. Except for the CAP50 group, the CAP inclusion groups had higher plasma HDL-C concentration and the HDL-C-to-TC ratio compared with the control group. The CAP substitution levels had significant linear effects on the plasma LDL-C concentrations ($P = 0.016$). Compared with the CAP0 group, the CAP inclusion groups had lower plasma LDL-C concentration.

3.4. Intestinal histological examination and the composition and diversity of gut-adhesive microbiota

As shown in Fig. 1, numerous Alcian Blue-positive goblet cells were evenly distributed. Both the villi and columnar epithelial cells were normal in the CAP0 and CAP50 groups.

The alpha diversity indexes indicated that the richness and diversity of the gut-adhesive microbiota in the CAP50 group were significantly higher than the other two groups ($P < 0.05$) (Table 9). The composition and diversity of the gut-adhesive microbiota at phylum and genus levels are shown in Fig. 2A and B, respectively. At the phylum level, the intestinal bacteria were mainly Firmicutes and Fusobacteria, followed by Proteobacteria and Tenericutes

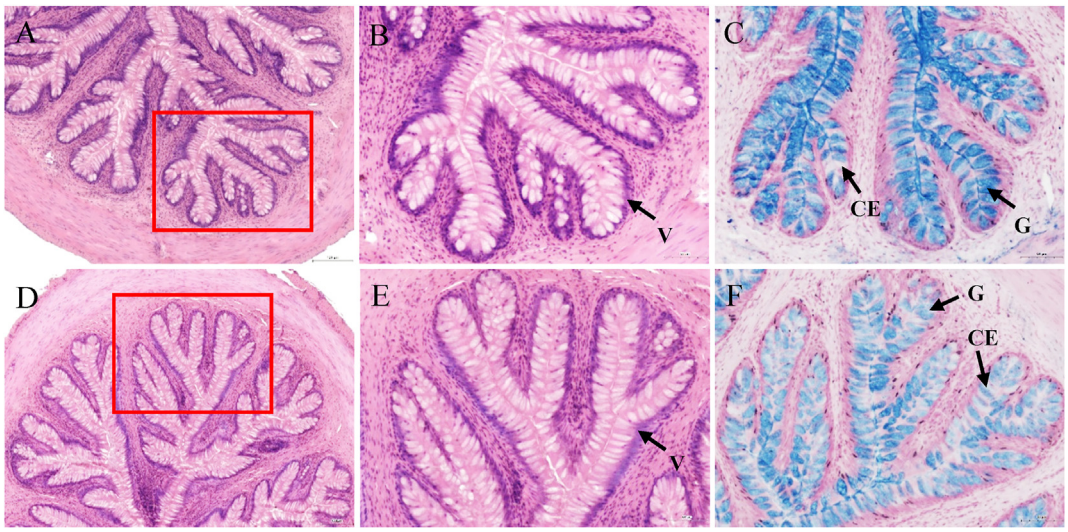

Fig. 1. Effects of LTFM replacement (0% and 50%) by CAP on the intestinal histopathology of largemouth bass. (A) CAP0, stained with hematoxylin and eosin (H&E) (scale bar = 100 μm); (B) CAP0, stained with H&E (scale bar = 50 μm); (C) CAP0, stained with Alcian Blue (AB) (scale bar = 50 μm); (D) CAP50, stained with H&E (scale bar = 100 μm); (E) CAP50, stained with H&E (scale bar = 50 μm); (F) CAP50, stained with AB (scale bar = 50 μm). V = villi; CE = columnar epithelial cells; G = goblet cells; LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein. CAP0 was the control diet. In the other 2 diets, 12.5% and 50% of the fish meal in the diet was replaced with CAP, named as CAP12.5 and CAP50, respectively.

Table 9
Alpha diversity index[1] of intestinal microbiota of largemouth bass fed diets with LTFM replaced by different CAP levels[2] for 8 wk.

Sample name	CAP0	CAP12.5	CAP50	Pooled SEM
Richness estimates				
Chao1	223[b]	277[b]	457[a]	22.5
Observed species	160[b]	204[b]	358[a]	26.0
Diversity estimates				
PD whole tree	37.8[b]	40.6[b]	124[a]	8.24
Shannon	1.18[b]	1.37[ab]	1.60[a]	0.10

LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein; SEM = standard error of treatment means.
[a, b] Within a row, means with different superscripts are significantly different (Turkey test; $P < 0.05$).
[1] Values are means of 4 replicates.
[2] CAP0 was the control diet. In the other 2 diets, 12.5% and 50% of the fish meal in the diet was replaced with CAP, named as CAP12.5 and CAP50, respectively.

(Fig. 2A). Compared with the CAP0 group, the Firmicutes were significantly enriched, whereas the proportion of the Fusobacteria was reduced in CAP12.5 and CAP50 groups (Fig. 2A, $P < 0.05$). At the genus level, *Clostridium sensu stricto 1*, *Cetobacterium* and *C. sensu stricto 12* were the three most abundant microorganisms in each group. Both *C. sensu stricto 1* and *C. sensu stricto 12* belong to Firmicutes, and *Cetobacterium* belongs to Fusobacteria. Although there was no significant difference, dietary CAP inclusion increased the proportion of *C. sensu stricto 1*. The proportion of *C. sensu stricto 12* in the CAP50 group was significantly higher than that in the CAP0 group, whereas the abundance of *Cetobacterium* in the CAP12.5 and CAP50 groups were lower than that in the CAP0 group (Fig. 2B, $P < 0.05$).

3.5. Hepatic histological examination, inflammatory cytokines and lipid metabolism

As presented in Fig. 3, the hepatic structure and hepatocytes were apparently normal in the CAP0 and CAP50 groups.

The relative mRNA levels of the genes related to inflammatory response and lipid metabolism in liver are presented in Fig. 4A and B, respectively. The CAP substitution levels had significant quadratic effects on the mRNA levels of interleukin 1β (*IL1β*) ($P = 0.000$), transforming growth factor β1 (*TGFβ1*) ($P = 0.007$) and *IL10* ($P = 0.000$). Dietary CAP inclusion down-regulated the mRNA levels of both *IL1β* and *IL10* ($P < 0.05$). Except for the CAP12.5 group, the mRNA levels of *TGFβ1* were significantly down-regulated in CAP inclusion groups ($P < 0.05$). The CAP substitution levels had no significant effect on the mRNA levels of tumor necrosis factor α (*TNFα*) ($P > 0.05$).

The replacement levels of CAP had significant quadratic effects on the mRNA levels of acetyl-CoA carboxylase 1 (*ACC1*) ($P = 0.000$) and peroxisome proliferator activated receptor α (*PPARα*) ($P = 0.000$). As the CAP replacement levels increased, the mRNA levels of *ACC1* and *PPARα* both decreased first, and then increased as the CAP replacement amount reached 50%. The CAP substitution levels had significant cubic effects on the mRNA levels of adipose triglyceride lipase (*ATGL*) ($P = 0.000$), carnitine palmitoyltransferase 1α (*CPT1α*) ($P = 0.000$) and hormone-sensitive lipase (*HSL*) ($P = 0.000$). The transcription of *ATGL* was inhibited in the CAP25 group, but was up-regulated in the CAP50 group ($P < 0.05$). The mRNA levels of *HSL* and *CPT1α* were both down-regulated in CAP25 and CAP37.5 groups, but were up-regulated in the CAP50 group ($P < 0.05$). The CAP substitution levels had significant linear effects on the mRNA levels of fatty acid synthase (*FASN*) ($P = 0.035$) with the lowest value observed in the CAP50 group ($P < 0.05$).

4. Discussion

There are several studies concerning the application of SCP as protein sources for different fish species. For example, a recent study showed that CAP could replace up to 58.20% FM in the diet of black sea bream, and the FM content could be reduced to 167 g/kg without any adverse effects observed on the growth performance (Chen et al., 2020). Li et al. (2021a) reported that a dietary inclusion of 200 g/kg CAP could improve the growth performance of juvenile Jian carp. Yeast from lignocellulosic biomass has been proven to be a potential alternative protein source for aquafeeds. Øverland et al. (2013) showed that the yeasts *Candida utilis* and *Kluyveromyces marxianus* were both able to replace 40% of the protein from high-quality FM in diets (remaining 347 g/kg FM) without adversely

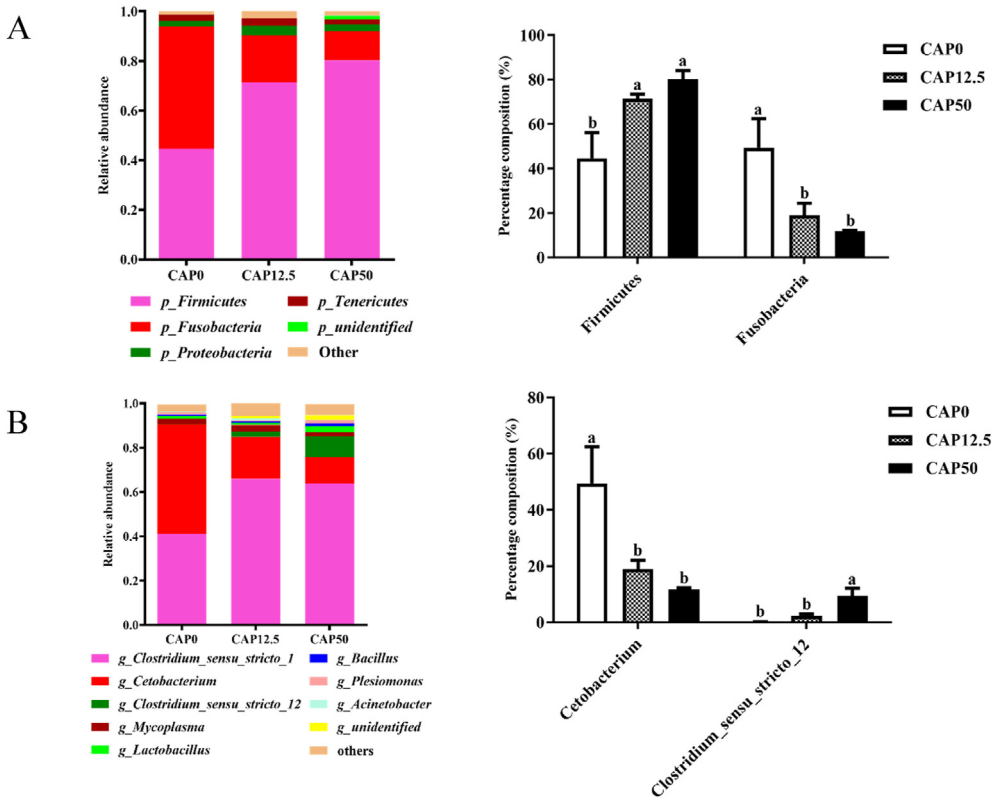

Fig. 2. Effects of LTFM replacement (0%, 12.5% and 50%) by CAP on the composition of gut-adherent microbiota in the distal intestine of largemouth bass. (A) Changes in gut-adherent microbiota at the phylum level among CAP0, CAP12.5 and CAP50 groups. (B) Changes in gut-adherent microbiota at the genus level among CAP0, CAP12.5 and CAP50 groups. [a,b]Bars with different letters are significantly different, Turkey test, $P < 0.05$, mean ± SEM (standard error of treatment means), $n = 4$. LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein. CAP0 was the control diet. In the other 2 diets, 12.5% and 50% of the fish meal in the diet was replaced with CAP, named as CAP12.5 and CAP50, respectively.

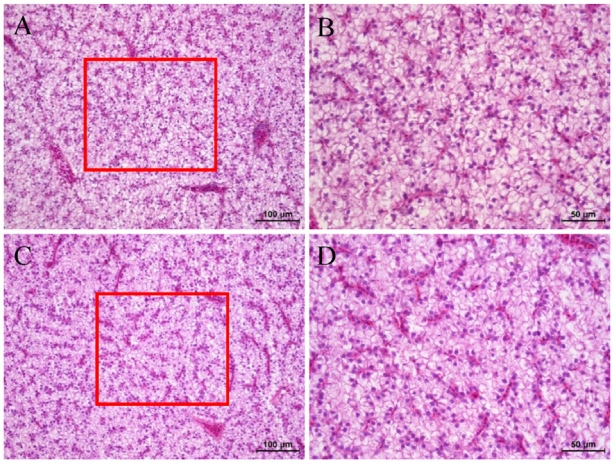

Fig. 3. Effects of LTFM replacement (0% and 50%) by CAP on the hepatic histopathology of largemouth bass. Hematoxylin and eosin (H&E) staining: (A) CAP0 (scale bar = 100 μm), (B) CAP0 (scale bar = 50 μm), (C) CAP50 (scale bar = 100 μm), (D) CAP50 (scale bar = 50 μm). LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein. CAP0 was the control diet. In the other 2 diets, 12.5% and 50% of the fish meal in the diet was replaced with CAP, named as CAP12.5 and CAP50, respectively.

affecting the growth performance of Atlantic salmon (*Salmo salar*). The nucleic acid content in yeast is 50 to 100 g/kg, which is much higher than that of most animal and plant protein sources (Øverland and Skrede, 2017). Rumsey et al. (1992) reported that a diet containing high levels of yeast nucleic acid extract (41.0 g/kg) did not affect the feed intake of rainbow trout (*Oncorhynchus mykiss*), but increased the growth rate and nitrogen retention. However, high dietary levels of brewer yeast (≥500 g/kg) could result in reduced palatability and growth in the same species (Rumsey et al., 1991). FeedKind (Calysta, US), extracted from the bacterial cultures of the methanotroph *M. capsulatus*, is another potential alternative protein source with high content of protein (700 g/kg) and nucleic acids (88.2 g/kg) (Romarheim et al., 2013). A diet containing 360 g/kg FeedKind had no adverse effects on the appetite of Atlantic salmon, but significantly increased the final body weight and specific growth rate (Aas et al., 2006). CAP contains 800 g/kg protein and 66.5 g/kg nucleotide and has a well-balanced amino acid profile similar to that of LTFM. The present study showed that CAP could replace 50% LTFM in the feed of largemouth bass without any negative effects on growth performance and feed utilization. Previous studies showed that the thick and rigid cell walls are the main factors that restricted the industrial production and utilization of yeast protein (Murray and Marchant, 1986; Kim et al., 1998; Yamada and Sgarbieri, 2005; Tukmechi and Bandboni, 2014). *C. autoethanogenum* is a gram-positive bacterium with much thinner cell wall than that of yeast. The apparent digestibility of CAP in largemouth bass was similar to that of LTFM, which confirmed that the cell wall of CAP did not limit its utilization. These results suggested that CAP is an excellent FM substitute for largemouth bass.

In addition to providing protein, different types of SCP contain many traces of functional substances. Yeast cell walls are rich in compounds with biological activity and immunostimulant effects,

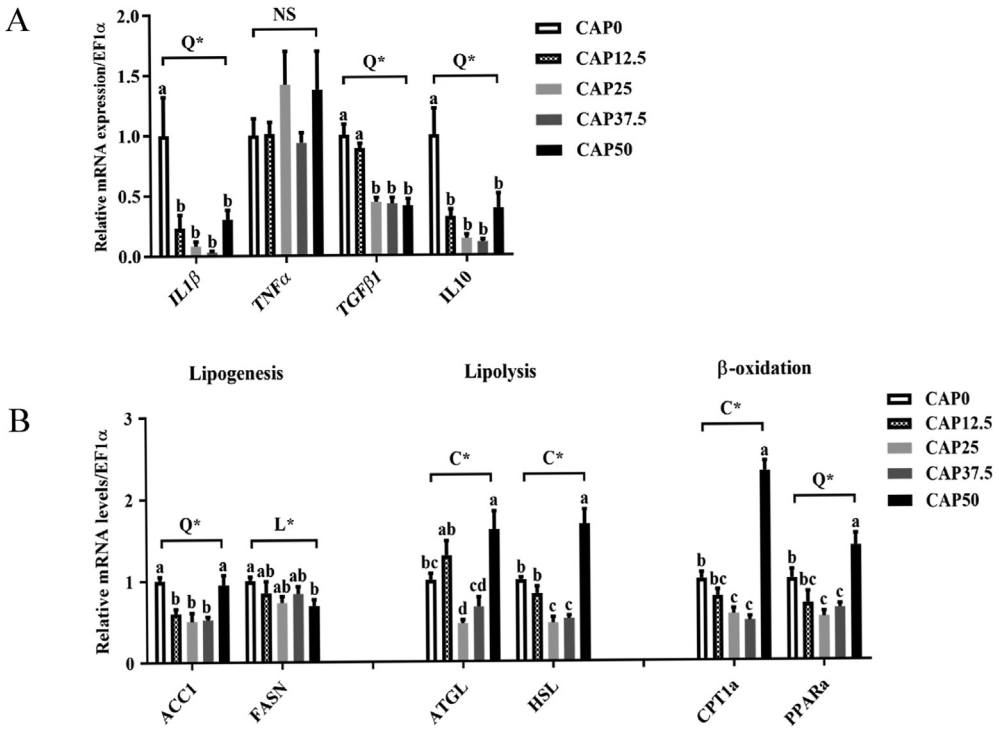

Fig. 4. Effects of LTFM replacement (0%, 12.5%, 25%, 37.5% and 50%) by CAP on the hepatic inflammatory responses and lipid metabolism of largemouth bass. (A) The mRNA levels of inflammatory cytokines (*IL1β* = interleukin 1β; *TNFα* = tumor necrosis factor α; *TGFβ1* = transforming growth factor β1; *IL10* = interleukin 10) in liver. (B) The mRNA levels of genes involved in lipogenesis (*ACC1* = acetyl-CoA carboxylase 1; *FASN* = fatty acid synthase), lipolysis (*ATGL* = adipose triglyceride lipase; *HSL* = hormone-sensitive lipase) and β-oxidation (*CPT1α* = carnitine palmitoyltransferase 1α; *PPARα* = peroxisome proliferator activated receptor α) in liver. [a to c] Bars with different letters are significantly different, Turkey test, $P < 0.05$, mean ± SEM (standard error of treatment means), $n = 8$. Significance of the linear (L), quadratic (Q) and cubic (C) orthogonal polynomial contrasts of the dependent variable across graded CAP inclusion level ($^{*}P < 0.05$). NS = no significance. LTFM = low-temperature steam dried anchovy fishmeal; CAP = *Clostridium autoethanogenum* protein. CAP0 was the control diet. In the other 4 diets, 12.5%, 25%, 37.5% and 50% of the fish meal in the diet was replaced with CAP, named as CAP12.5, CAP25, CAP37.5 and CAP50, respectively.

such as *β*-glucan and mannan oligosaccharides, which could stimulate the abundance of specific health-promoting bacteria like *Bacillus* in the intestine of largemouth bass (Yu et al., 2019). The nucleic acids in yeast may have a nitrogen-sparing effect in different fish species (Øverland and Skrede, 2017). Hassaan et al. (2018) found that diets supplemented with 15 g/kg of yeast extract could reduce the AST and ALT activities in Nile tilapia (*Oreochromis niloticus* L.), indicating that yeast nucleotides could improve the liver function of fish. FeedKind is rich in phospholipids, small peptides and nucleotides. Adeoye et al. (2021) observed that an inclusion of 93.0 g/kg of FeedKind in the diet to replace FM had no apparent effects on the serum AST and ALT activities of African catfish (*Clarias gariepinus*). But the nucleotides in the FeedKind could play a key role in the repair and regeneration of liver injury in the cirrhotic rat model (Pérez et al., 2004). Likewise, as a bacterial meal, both phospholipids and oligosaccharides are little in the CAP, and the water-soluble peptides are also much lower than that of FeedKind. However, in the present study, an improvement was observed in the health of fish in dietary CAP inclusion groups, as this could be partially related to the regulation of gut microbiota by the functional components (20 g/kg acetic acid and 66.5 g/kg nucleotide) in the CAP.

Feed ingredients may affect the composition and function of gut microbiota in fish (Visschers et al., 2013). Foreign bacteria in the gastrointestinal tract of fish are considered temporary, while others exist as members of the established microbiota connected with the intestinal mucosa. The beneficial adherent bacteria may protect fish from pathogen invasion, and mediate sorts of host immune functions (Rhee et al., 2004; Askarian et al., 2012). The present study evaluated the response of the gut-adherent microbiota to dietary CAP. The results indicated that dietary CAP inclusion increased the richness and diversity of bacterial communities. *C. sensu stricto* is an intestinal commensal species that plays an important role in maintaining the intestinal function of animals (including fish) (Wexler, 2007; Lopetuso et al., 2013). A relatively higher abundance of *C. sensu stricto 1* and *C. sensu stricto 12* was observed in CAP inclusion groups than that of the CAP0 group, suggesting that CAP could improve the intestinal mucous microbiota profiles, thereby improving the intestinal health. Dietary nucleotides have the potential ability to affect intestinal flora (Hossain et al., 2020). Singhal et al. (2008) reported that the supplement of nucleotides to the infant formula could increase the richness of Bifidobacterium. In the present study, the nucleotides in CAP may have probiotic effects on gut microbiology. However, Xu et al. (2015) demonstrated that dietary nucleotides (6 g/kg) inhibited the abundance of butyrate-producing bacteria in juvenile hybrid tilapia (*O. niloticus* ♀ × *Oreochromis aureus* ♂). Due to the limited understanding of supplemental nucleotides in fish diets, it is difficult to interpret the potential mechanisms underlying the beneficial effects of dietary nucleotides on the gut microbiota. The acetic acid contained in CAP may also cause changes in the intestinal microflora since several studies documented that dietary organic acid could impact the diversity and composition of intestinal flora in different fish species (Zare et al., 2021). However, the mechanism by which short-chain fatty acids modulate the microbiota in aquatic animals is still unclear. The digesta plays an important role in intestinal health. Jia et al. (2021) reported that the food digestion of juvenile hybrid grouper (*Epinephelus moara* ♀ × *E. lanceolatus* ♂) reached a peak at 6 h postprandially in intestine. After 6 h of enzymolysis, the content of oligopeptides was below 1,000 Da in the CAP hydrolysate

(0.56 mg/mL), which was higher than that in the LTFM hydrolysate (0.48 mg/mL), indicating that the CAP hydrolysate contains a higher proportion of peptides with 2 to 6 amino acid residues. Peptides containing 2 to 6 amino acids are more easily absorbed than proteins and free amino acids (Xie et al., 2008), and may be used as substrates for many intestinal bacteria (Hao et al., 2020). However, due to the high apparent digestibility of protein in both LTFM and CAP for largemouth bass, there should be no obvious difference on the protein digestibility among the test diets. Given the well-shaped intestinal histology, an improved gut-adhesive microbiota composition, and high content of low-molecular-weight peptides in the hydrolysate, CAP could improve intestinal health of largemouth bass.

Significantly improved liver functions were observed in the CAP inclusion groups in this study. Compared with the CAP0 group, plasma ALT activity was reduced in CAP inclusion groups, and plasma AST activity was reduced in the CAP37.5 group. In contrast to our study, Chen et al. (2020) found that dietary inclusion of 180 g/kg CAP had no significant effects on the liver functions of black sea bream. Maulu et al. (2021b) reported that including 150 g/kg CAP in the diet had no significant effects on the plasma ALT activity and the liver health of tilapia juveniles. These contradictory results may be a result of different fish species. Dietary nucleotides could affect the hematological parameters of both fish and shrimp. Hossain et al. (2020) reviewed the utilization of nucleotide in aquaculture, and found that optimal levels of dietary nucleotides would reduce plasma ALT and AST activities. However, the mechanism underlying the beneficial effects of dietary nucleotides on the liver health still remains to be studied.

Previous studies showed that largemouth bass has a poor starch utilization capacity, and high dietary levels of digestible carbohydrates (>10%) was therefore considered to be the primary factor inducing the metabolic liver disease (MLD) in this species (Ma et al., 2019; Zhang et al., 2020). In this study, the replacement of LTFM by CAP was achieved by using 120 g/kg tapioca starch, which could improve the intestinal and liver health of largemouth bass. Inflammation is a protective response that eliminates cell damage and initiates tissue repair. Inflammatory cytokines play an important role in inflammation (Karin and Clevers, 2016). Both *TNFα* and *IL1β* are pro-inflammatory cytokines, whereas *IL10* and *TGFβ1* are both anti-inflammatory cytokines (Low et al., 2003; Savan and Sakai, 2004; Ip et al., 2017). In this study, dietary CAP inclusions significantly down-regulated gene expressions of inflammatory cytokines (*IL1β*, *IL10* and *TGFβ1*), indicating that CAP could inhibit the inflammatory responses in largemouth bass. Bu et al. (2019) reported that a dietary inclusion of 10 g/kg yeast culture could reduce the production of peroxides, and indirectly down-regulate the expressions of pro-inflammatory cytokines for Ussuri catfish (*Pseudobagrus ussuriensis*). In contrast to the present study, Maulu et al. (2021a) found that dietary inclusions of 5% and 10% CAP up-regulated the expression of *TGFβ1* in tilapia juveniles. These conflicting results may be due to the different fish species. Excessive accumulation of lipids in hepatocytes promotes inflammation and fibrosis, causing a series of different degrees of histological damage (Liu et al., 2010). MLD induced by excess lipid accumulation has posed a great threat to the production of largemouth bass (Yu et al., 2019). Fatty acids (FAs) could supply and store energy. Both *FASN* and *ACC* are key enzymes for the synthesis of FA (Yu et al., 2019). In our study, the mRNA levels of *ACC1* were significantly down-regulated in the CAP12.5, CAP25 and CAP37.5 groups. The mRNA levels of *FASN* were also significantly down-regulated in the CAP50 group, indicating that CAP inclusion adversely affected lipogenesis in largemouth bass. These results were consistent with that of body lipid contents and plasma TG concentrations. Yu et al. (2019) obtained similar results on largemouth bass, showing that dietary autolyzed brewer's yeast inclusion significantly down-regulated the mRNA levels of both *ACC1* and *FASN*. ATGL is the rate-limiting enzyme in the first step of TG hydrolysis, whereas HSL is in charge of the subsequent degradation of diacylglycerol (Stringer et al., 2010). CPT1 is the main regulator of fatty acid oxidation (Kerner and Hoppel, 2000). PPARα regulates lipid catabolism in response to different energy requirements and nutritional status (Ji et al., 2011). In this study, the mRNA levels of *ATGL*, *HSL*, *CPT1α* and *PPARα* were all down-regulated in CAP25 or CAP37.5 groups, but were up-regulated in the CAP50 group, suggesting that the high CAP inclusion (50%) could enhance lipolysis, while the low level (25% and 37.5%) could inhibit lipolysis in largemouth bass. Somewhat similar results were also reported in tilapia juveniles fed with different levels of CAP (Maulu et al., 2021b). However, the low CAP inclusions (25% and 37.5%) also reduced lipid synthesis in largemouth bass in this study with a balanced state reached between lipogenesis and lipolysis. Overall, dietary CAP inclusions could prevent excessive accumulation of lipid by reducing the endogenous synthesis of FA, thereby inhibiting the inflammatory responses of largemouth bass. Gut bacteria play a role in maintaining the health of the gut–liver axis (Compare et al., 2012). *C. sensu stricto* may ferment undigested carbohydrates and amino acids into short-chain fatty acids (Lopetuso et al., 2013). Butyrate is the main source of energy for epithelial enterocytes, and can inhibit inflammation and improve lipid metabolism (Lopez-Siles et al., 2012; Ji et al., 2021). *C. sensu stricto 12* is a potential butyrate producer (Liu et al., 2020). Thus, the increased abundance of *C. sensu stricto 12* may explain the inhibited inflammatory responses, but this observation requires confirmation.

In conclusion, CAP can replace up to 50% of the high-quality LTFM in the diets of largemouth bass with no negative effects on growth performance and survival. CAP has a preferable amino acid profile with 66.5 g/kg of nucleotide and 20 g/kg acetic acid. It shows a high apparent digestibility with a large number of low-molecular-weight peptides in its hydrolysate. CAP inclusion improved the composition of gut-adhesive microbiota, suggesting that CAP could improve the intestinal health of largemouth bass. Dietary CAP inclusion also prevented excessive accumulation of lipid by reducing the endogenous synthesis of FA, and further suppressed the inflammatory responses. Additionally, the increased abundance of *C. sensu stricto* in the gut may play a role in improving liver health. Therefore, it is demonstrated that CAP is an excellent alternative protein source for largemouth bass.

Author contributions

Shifeng Ma: Conceptualization, Data curation, Methodology, Formal analysis, Writing – original draft. **Xiaofang Liang:** Visualization, Resources. **Pei Chen:** Methodology and histological experiment. **Jie Wang:** intestinal microbiota data analysis. **Xu Gu:** Data curation. **Yuchang Qin:** Validation. **Christophe Blecker:** Validation. **Min Xue:** Conceptualization, Data curation, Supervision, 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 study was supported by the National Key R&D Program of China (2019YFD0900200 and 2018YFD0900400), the National Natural Science Foundation of China (32172981 and 31902382), the

Agricultural Science and Technology Innovation Program of CAAS in China (CAAS-ASTIP-2017-FRI-08), and China Postdoctoral Science Foundation (2021M703544).

References

Aas TS, Grisdale-Helland B, Terjesen BF, Helland SJ. Improved growth and nutrient utilisation in Atlantic salmon (*Salmo salar*) fed diets containing a bacterial protein meal. Aquaculture 2006;259:365–76.

Abrini J, Naveau H, Nyns E-J. *Clostridium autoethanogenum*, sp. nov., an anaerobic bacterium that produces ethanol from carbon monoxide. Arch Microbiol 1994;161:345–51.

Adeoye AA, Akegbejo-Samsons Y, Fawole FJ, Olatunji PO, Muller N, Wan AHL, et al. From waste to feed: dietary utilisation of bacterial protein from fermentation of agricultural wastes in African catfish (*Clarias gariepinus*) production and health. Aquaculture 2021;531:735850.

Aghalino SO, Eyinla B. Oil exploitation and marine pollution: evidence from the Niger Delta, Nigeria. J Hum Ecol 2009;28:177–82.

Aoac. Association of official analytical chemists. Official methods of analysis 16th. Arlington, VA: AOAC; 2006. p. 66–961X.

Askarian F, Zhou Z, Olsen RE, Sperstad S, Ringø E. Culturable autochthonous gut bacteria in Atlantic salmon (*Salmo salar* L.) fed diets with or without chitin. Characterization by 16S rRNA gene sequencing, ability to produce enzymes and *in vitro* growth inhibition of four fish pathogens. Aquaculture 2012;326–329:1–8.

Bu X, Lian X, Wang Y, Luo C, Tao S, Liao Y, et al. Dietary yeast culture modulates immune response related to TLR2-MyD88-NF-kβ signaling pathway, antioxidant capability and disease resistance against *Aeromonas hydrophila* for Ussuri catfish (*Pseudobagrus ussuriensis*). Fish Shellfish Immunol 2019;84:711–8.

Chavan UD, McKenzie DB, Shahidi F. Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.). Food Chem 2001;74:177–87.

Chen Y, Sagada G, Xu B, Chao W, Zou F, Ng W, et al. Partial replacement of fishmeal with *Clostridium autoethanogenum* single-cell protein in the diet for juvenile black sea bream (*Acanthopagrus schlegelii*). Aquacult Res 2020;51:1000–11.

Compare D, Coccoli P, Rocco A, Nardone OM, De Maria S, Cartenì M, et al. Gut–liver axis: the impact of gut microbiota on non alcoholic fatty liver disease. Nutr Metabol Cardiovasc Dis 2012;22:471–6.

Dairiki JK, Dias CT, dos S, Cyrino JEP. Lysine requirements of largemouth bass, *Micropterus salmoides*: a comparison of methods of analysis of dose-response trials data. J Appl Aquacult 2007;19:1–27.

Ferreira I, Pinho O, Vieira E, Tavarela JG. Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. Trends Food Sci Technol 2010;21:77–84.

Hao Y, Guo R, Jia G, Zhang Y, Xia H, Li X. Effects of enzymatic hydrolysates from poultry by-products (EHPB) as an alternative source of fish meal on growth performance, hepatic proteome and gut microbiota of turbot (*Scophthalmus maximus*). Aquacult Nutr 2020;26:1994–2006.

Hassaan MS, Mahmoud SA, Jarmolowicz S, El-Haroun ER, Mohammady EY, Davies SJ. Effects of dietary baker's yeast extract on the growth, blood indices and histology of Nile tilapia (*Oreochromis niloticus* L.) fingerlings. Aquacult Nutr 2018;24:1709–17.

He M, Li X, Poolsawat L, Guo Z, Yao W, Zhang C, et al. Effects of fish meal replaced by fermented soybean meal on growth performance, intestinal histology and microbiota of largemouth bass (*Micropterus salmoides*). Aquacult Nutr 2020;26:1058–71.

Hossain MS, Koshio S, Kestemont P. Recent advances of nucleotide nutrition research in aquaculture: a review. Rev Aquacult 2020;12:1028–53.

Ip WKE, Hoshi N, Shouval DS, Snapper S, Medzhitov R. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. Science 2017;356:513–9.

Ji H, Li J, Liu P. Regulation of growth performance and lipid metabolism by dietary n-3 highly unsaturated fatty acids in juvenile grass carp, *Ctenopharyngodon idellus*. Comp Biochem Physiol B Biochem Mol Biol 2011;159:49–56.

Ji X, Chen M, Zhao M, Song Y, Lin Y, Yin H, et al. Effects of chitooligosaccharides on the rebalance of gut microorganisms and their metabolites in patients with nonalcoholic fatty liver disease. J Funct Foods 2021;77:104333.

Jia Y, Gao Y, Jing Q, Huang B, Zhai J, Guan C. Gastric evacuation and changes in postprandial blood biochemistry, digestive enzymes, and appetite-related genes in juvenile hybrid grouper (*Epinephelus moara* ♀ × *E. lanceolatus* ♂). Aquaculture 2021;530:735721.

Karim A, Gerliani N, Aïder M. *Kluyveromyces marxianus*: an emerging yeast cell factory for applications in food and biotechnology. Int J Food Microbiol 2020;333:108818.

Karin M, Clevers H. Reparative inflammation takes charge of tissue regeneration. Nature 2016;529:307–15.

Kerner J, Hoppel C. Fatty acid import into mitochondria. BBA-Mol Cell Biol L 2000;1486:1–17.

Kim JK, Tak K-T, Moon J-H. A continuous fermentation of *Kluyveromyces fragilis* for the production of a highly nutritious protein diet. Aquacult Eng 1998;18:41–9.

Li M, Liang H, Xie J, Chao W, Zou F, Ge X, et al. Diet supplemented with a novel *Clostridium autoethanogenum* protein have a positive effect on the growth performance, antioxidant status and immunity in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aqua Rep 2021a;19:100572.

Li P, Gatlin DM. Nucleotide nutrition in fish: current knowledge and future applications. Aquaculture 2006;251:141–52.

Li S, Dai M, Qiu H, Chen N. Effects of fishmeal replacement with composite mixture of shrimp hydrolysate and plant proteins on growth performance, feed utilization, and target of rapamycin pathway in largemouth bass. *Micropterus salmoides*. Aquaculture 2021b;533:736185.

Li X, Zheng S, Ma X, Cheng K, Wu G. Use of alternative protein sources for fishmeal replacement in the diet of largemouth bass (*Micropterus salmoides*). Part I: effects of poultry by-product meal and soybean meal on growth, feed utilization, and health. Amino Acids 2021c;53:33–47.

Liu B, Kleinsteuber S, Centler F, Harms H, Sträuber H. Competition between butyrate fermenters and chain-elongating bacteria limits the efficiency of medium-chain carboxylate production. Front Microbiol 2020;11:336.

Liu XJ, Luo Z, Xiong BX, Liu X, Zhao YH, Hu GF, et al. Effect of waterborne copper exposure on growth, hepatic enzymatic activities and histology in *Synechogobius hasta*. Ecotoxicol Environ Saf 2010;73:1286–91.

Lopetuso LR, Scaldaferri F, Petito V, Gasbarrini A. Commensal Clostridia: leading players in the maintenance of gut homeostasis. Gut Pathog 2013;5:23.

Lopez-Siles M, Khan TM, Duncan SH, Harmsen HJM, Garcia-Gil LJ, Flint HJ. Cultured representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii*can utilize pectin, uronic acids, and host-derived substrates for growth. Appl Environ Microbiol 2012;78:420–8.

Low C, Wadsworth S, Burrells C, Secombes C. Expression of immune genes in turbot (*Scophthalmus maximus*) fed a nucleotide-supplemented diet. Aquaculture 2003;221:23–40.

Lowry O, Rosebrough N, Farr AL, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.

Ma HJ, Mou MM, Pu DC, Lin SM, Chen YJ, Luo L. Effect of dietary starch level on growth, metabolism enzyme and oxidative status of juvenile largemouth bass, *Micropterus salmoides*. Aquaculture 2019;498:482–7.

Maulu S, Hualiang L, Ke J, Ren M, Ge X, Huang D, et al. Dietary *Clostridium autoethanogenum* protein modulates intestinal absorption, antioxidant status, and immune response in GIFT (*Oreochromis niloticus*) juveniles. Aquacult Res 2021a;52:5787–99.

Maulu S, Liang H, Ge X, Yu H, Huang D, Ke J, et al. Effect of dietary *Clostridium autoethanogenum* protein on growth, body composition, plasma parameters and hepatic genes expression related to growth and AMPK/TOR/PI3K signaling pathway of the genetically improved farmed tilapia (GIFT: *Oreochromis niloticus*) juveniles. Anim Feed Sci Technol 2021b;276:114914.

Murray AP, Marchant R. Nitrogen utilization in rainbow trout fingerlings (*Salmo gairdneri Richardson*) fed mixed microbial biomass. Aquaculture 1986;54:263–75.

Mydland LT, Frøyland JRK, Skrede A. Composition of individual nucleobases in diets containing different products from bacterial biomass grown on natural gas, and digestibility in mink (*Mustela vison*). J Anim Physiol Anim Nutr 2008;92:1–8.

Øverland M, Karlsson A, Mydland LT, Romarheim OH, Skrede A. Evaluation of *Candida utilis, Kluyveromyces marxianus* and *Saccharomyces cerevisiae* yeasts as protein sources in diets for Atlantic salmon (*Salmo salar*). Aquaculture 2013;402–403:1–7.

Øverland M, Skrede A. Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture. J Sci Food Agric 2017;97:733–42.

Øverland M, Tauson A-H, Shearer K, Skrede A. Evaluation of methane-utilising bacteria products as feed ingredients for monogastric animals. Arch Anim Nutr 2010;64:171–89.

Pérez MJ, Sánchez-Medina F, Torres M, Gil A, Suárez A. Dietary nucleotides enhance the liver redox state and protein synthesis in cirrhotic rats. J Nutr 2004;134:2504–8.

Rhee KJ, Sethupathi P, Driks A, Lanning DK, Knight KL. Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. J Immunol 2004;172:1118–24.

Romarheim OH, Landsverk T, Mydland LT, Skrede A, Øverland M. Cell wall fractions from *Methylococcus capsulatus* prevent soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*). Aquaculture 2013;402–403:13–8.

Rumsey GL, Kinsella JE, Shetty KJ, Hughes SG. Effect of high dietary concentrations of brewer's dried yeast on growth performance and liver uricase in rainbow trout (*Oncorhynchus mykiss*). Anim Feed Sci Technol 1991;33:177–83.

Rumsey GL, Winfree RA, Hughes SG. Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorhynchus mykiss*). Aquaculture 1992;108:97–110.

Savan R, Sakai M. Presence of multiple isoforms of TNF alpha in carp (*Cyprinus carpio* L.): genomic and expression analysis. Fish Shellfish Immunol 2004;17:87–94.

Singhal A, Macfarlane G, Macfarlane S, Lanigan J, Kennedy K, Elias-Jones A, et al. Dietary nucleotides and fecal microbiota in formula-fed infants: a randomized controlled trial. Am J Clin Nutr 2008;87:1785–92.

Stringer DM, Zahradka P, DeClercq VC, Ryz NR, Diakiw R, Burr LL, et al. Modulation of lipid droplet size and lipid droplet proteins by *trans*-10, *cis*-12 conjugated linoleic acid parallels improvements in hepatic steatosis in obese, insulin-resistant rats. BBA-Mol Cell Biol L 2010;1801:1375–85.

Suárez JA, Gaxiola G, Mendoza R, Cadavid S, Garcia G, Alanis G, et al. Substitution of fish meal with plant protein sources and energy budget for white shrimp *Litopenaeus vannamei* (Boone, 1931). Aquaculture 2009;289:118–23.

Subhadra B, Lochmann R, Rawles S, Chen R. Effect of dietary lipid source on the growth, tissue composition and hematological parameters of largemouth bass (*Micropterus salmoides*). Aquaculture 2006;255:210–22.

Tukmechi A, Bandboni M. Effects of *Saccharomyces cerevisiae* supplementation on immune response, hematological parameters, body composition and disease resistance in rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792). J Appl Ichthyol 2014;30:55–61.
Vigani M, Parisi C, Rodríguez-Cerezo E, Barbosa MJ, Sijtsma L, Ploeg M, et al. Food and feed products from micro-algae: market opportunities and challenges for the EU. Trends Food Sci Technol 2015;42:81–92.
Visschers RGJ, Luyer MD, Schaap FG, Olde Damink SWM, Soeters PB. The gut-liver axis. Curr Opin Clin Nutr Metab Care 2013;16:576–81.
Wei HC, Xing SJ, Chen P, Wu XF, Gu X, Luo L, et al. Plant protein diet-induced hypoimmunity by affecting the spiral valve intestinal microbiota and bile acid enterohepatic circulation in Amur sturgeon (*Acipenser schrenckii*). Fish Shellfish Immunol 2020;106:421–30.
Wei HC, Yu HH, Chen XM, Chao W, Zou FQ, Chen P, et al. Effects of soybean meal replaced by *Clostridium autoethanogenum* protein on growth performance, plasma biochemical indexes and hepatopancreas and intestinal histopathology of grass carp (*Ctenopharyngodon idellus*). Chinese J Anim Nutr 2018;30:4190–201.
Wexler HM. *Bacteroides*: the good, the bad, and the nitty-gritty. Clin Microbiol Rev 2007;20:593–621.
Xie Z, Huang J, Xu X, Jin Z. Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. Food Chem 2008;111:370–6.
Xu L, Ran C, He S, Zhang J, Hu J, Yang Yalin, et al. Effects of dietary yeast nucleotides on growth, non-specific immunity, intestine growth and intestinal microbiota of juvenile hybrid tilapia *Oreochromis niloticus*♀ × *Oreochromis aureus*. Anim Nutr 2015;1:244–51.
Yamada EA, Sgarbieri VC. Yeast (*Saccharomyces cerevisiae*) protein concentrate: preparation, chemical composition, and nutritional and functional properties. J Agric Food Chem 2005;53:3931–6.
Yu HH, Han F, Xue M, Wang J, Tacon P, Zheng YH, et al. Efficacy and tolerance of yeast cell wall as an immunostimulant in the diet of Japanese seabass (*Lateolabrax japonicus*). Aquaculture 2014;432:217–24.
Yu HH, Liang XF, Chen P, Wu XF, Zheng YH, Luo L, et al. Dietary supplementation of Grobiotic-A increases short-term inflammatory responses and improves long-term growth performance and liver health in largemouth bass (*Micropterus salmoides*). Aquaculture 2019;500:327–37.
Zare R, Kenari AA, Sadati MY. Influence of dietary acetic acid, protexin (probiotic), and their combination on growth performance, intestinal microbiota, digestive enzymes, immunological parameters, and fatty acids composition in Siberian sturgeon (*Acipenser baerii*, Brandt, 1869). Aquacult Int 2021;29:891–910.
Zhang Y, Xie S, Wei H, Zheng L, Liu Z, Fang H, et al. High dietary starch impaired growth performance, liver histology and hepatic glucose metabolism of juvenile largemouth bass, *Micropterus salmoides*. Aquacult Nutr 2020;26:1083–95.
Zhuang Y, Sun L, Zhao X, Wang J, Hou H, Li B. Antioxidant and melanogenesis-inhibitory activities of collagen peptide from jellyfish (*Rhopilema esculentum*). J Sci Food Agric 2009;89:1722–7.
Zou Y. Apparent Digestibility of key feed ingredients by largemouth bass (*Micropterus salmoides*). 2021.