

Article

Impact of *Atractylodes lancea* Ultrafine Powder on Crucian Carp (*Carassius auratus*) Growth, Body Composition and Hypoxia Tolerance

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Abstract

The goal of this study is to assess how dietary *Atractylodes lancea* ultrafine powder (AUP) affects crucian carp (*Carassius auratus*) growth, body composition, and hypoxia tolerance. A total of 630 crucian carp (13.21 ± 0.17 g) were fed diets containing 0.0%, 3.0%, 6.0%, 9.0%, 12.0%, 15.0%, and 18.0% AUP for 15 days. In crucian carp, dietary AUP was observed to improve protein content and decrease weight gain and fat content ($p < 0.05$). The ideal AUP addition level for fish was 13.04%, based on broken-line regression analysis of the protein concentration in fish bodies. The tissues and organs of crucian carp showed increased enzymatic antioxidant activities and non-enzymatic antioxidant levels, indicating that dietary AUP reduced the production of reactive oxygen species (ROS) and inhibited lipid oxidation ($p < 0.05$). This study shows that dietary AUP has a significant anti-obesity impact in fish, which is strongly linked to boosting the anabolism of protein and antioxidant defense as well as improving glucose metabolism to reduce glucose as the raw material for body fat synthesis. Additionally, under hypoxic conditions, the addition of AUP increased duration time (DT) and decreased crucian carp oxygen consumption rate ($p < 0.05$). The DT broken-line regression study verified that 6.05% is the ideal AUP level for fish. According to our research, dietary AUP strengthens fish's resistance to hypoxia by modifying the metabolism of proteins and carbohydrates for energy production and enhancing antioxidant defense in respiration-related tissues and organs. In conclusion, AUP may be a natural way to reduce fish obesity and hypoxic stress.

Keywords: *Atractylodes rhizome*; anti-obesity; anti-hypoxia; biochemical indicators; antioxidant



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Key Contribution: Dietary AUP enhances the hypoxia tolerance by adjusting the metabolism processes of carbohydrates and proteins for energy production in fish. By lowering weight increase and body fat content while raising body protein content in fish, AUP has a remarkable anti-obesity impact. Through both enzymatic and non-enzymatic antioxidant actions, AUP can lower the production of reactive oxygen species (ROS) and lipid oxidation in fish tissues and organs.

1. Introduction

The availability of dissolved oxygen (DO) in aquatic environments critically dominates fish growth [1]. DO can crucially deliver oxygen to tissues to support aerobic metabolism in the fish body and remarkably affects fish farming [2]. However, in shallow freshwater bodies the natural O₂ level can vary widely over the course of a day from low levels during the night to sometimes heavy oversaturation during the day [3]. Although aquaculture systems are commonly managed with aeration or water exchange, hypoxic episodes may still occur, particularly under high stocking density, elevated water temperature, and excessive accumulation of organic matter such as uneaten feed and metabolic wastes [4]. These conditions can increase O₂ consumption by fish and microorganisms while reducing O₂ availability in the water [5,6]. In addition, DO levels may fluctuate over time because of feeding activity, microbial decomposition, diurnal changes, or temporary limitations in aeration efficiency [4,7]. In captivity, fish usually face chronic and repetitive stressors, including hypoxia and the subsequent reoxygenation [5,6]. Hypoxic conditions in aquaculture can damage fish physiology and metabolism [7,8]. According to existing studies, hypoxia-reoxygenation retards fish growth performance and breaks their antioxidant defense mechanisms [1,9,10]. Consequently, it is important to further investigate strategies to alleviate the harmful effect induced by hypoxic stress.

Liu et al. reported that natural products, namely polysaccharides, alkaloids, saponins, flavonoids and active peptides, contribute well to anti-hypoxic treatment for high-altitude disease, which is a typical disease of hypoxia in plateau regions above 2500 m above sea level [11]. It has been reported that the antioxidant activity of herbs decides their anti-hypoxia efficacy [11]. Diets enriched with ascorbic acid, a common antioxidant, reduce the effects of hypoxia insult on juvenile tambaqui (*Colossoma macropomum*) [12]. *Atractylodes lancea*, which contains terpenoids, flavonoids, steroids, polysaccharides, etc., known for its many health-promoting qualities, has long been used for apparent antioxidative activities [13]. Therefore, it is reasonable to hypothesize that *Atractylodes lancea* could cut down hypoxic stress in fish. However, limited data are available regarding how *Atractylodes lancea* affects hypoxia tolerance in fish. Previous studies have shown that feeding *Atractylodes lancea* polysaccharides can improve the growth and productive performance in early-weaned piglets and laying hens, respectively [14]. Other reports indicated that *Atractylodes lancea* and its ethanol extract decreased the body weight and liver lipid content in obese mice [15,16]. Nevertheless, the impact of *Atractylodes lancea* on the growth performance and body composition in fish has not yet been reported.

The ultrafine powder of herbs presents better therapeutic efficacy than the regular powder of the herbs, known for their small diameter (less than 45 µm, up to 90% of the sample), nice surface properties, higher integrity in chemical composition, and improving dissolution and absorption in the gastrointestinal tract of animals [17]. Consequently, this study highlights the potential protective efficacy of *Atractylodes lancea* ultrafine powder (AUP) in mitigating hypoxia stress in crucian carp (*Carassius auratus*), thus assisting in applying it as a solution to improve hypoxia resilience in aquaculture systems, ultimately helping address the critical threats faced in fish farming.

2. Materials and Methods

2.1. Preparation of AUP

The roots of *Atractylodes lancea* were purchased from the Xuzhou Huaijingtang Trading Co., Ltd. (Xuzhou, China). First of all, the authenticity of *Atractylodes lancea* was determined using a pharmacopeia. Following the drying process in a blast drying oven (Jinghong DHG-9030A, Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China) at 50 °C, the roots were finely ground into powder using a Chinese medicine grinder (Ronghao

RHP-2000A, Changzhou Ronghao Machinery Manufacturing Co., Ltd., Changzhou, China) and passed through a 300-mesh sieve using the procedure described in the literature [18,19], ensuring that the particle size did not exceed 0.045 mm. The processes were kept at below 20 °C to prevent the active ingredients of *Atractylodes lancea* from deactivation. An amount of 630 g of resulting powder (AUP) was used for the next feed preparation. The dry AUP was stored in airtight containers, protected from light, and kept at −20 °C until further analysis.

Size distribution of AUP was measured by the hemocytometer method [6]. An amount of 10 mg of AUP was placed into a 10 mL centrifuge tube, added with 5 mL of ultrapure water, and shaken well. Then, the suspension of AUP was injected into the counting plate. The diameter of 200 particles was evaluated on the plate. The average size of particle was determined to be 0.0096 ± 0.0020 mm (Figure 1). Each sample was measured in triplicate.

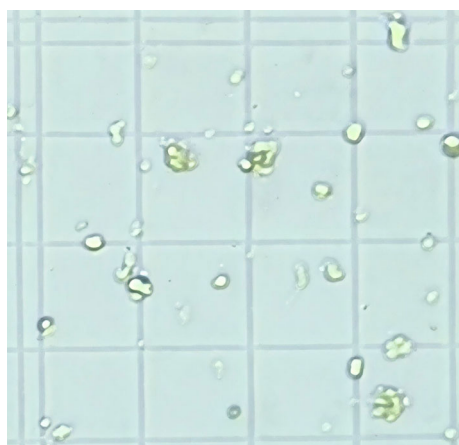


Figure 1. Size distribution of *Atractylodes lancea* ultrafine powder (AUP). The side length of each small square is 0.05 mm in hemocytometer.

2.2. Animal Experiment Diets

Firstly, the levels of moisture, crude protein, crude lipid, and ash in AUP were confirmed with the Association of Official Analytical Chemists (AOAC) methods [20,21]. Dry matter (moisture) was weighed according to weight loss drying at the temperature of 105 °C. Crude protein was calculated by the Kjeldahl method and multiplying the nitrogen by 6.25. The calculation of crude lipid was by Soxhlet extraction with petroleum ether. Ash was calculated from the weight loss after incineration of the samples at 550 °C in a muffle furnace (SX2-8-10, Shanghai SUN Experimental Instrument Co., Ltd., Shanghai, China). Dry matter, crude protein, crude lipid, and ash levels were illustrated in Table 1. The manufacturing procedure of basal and experimental feeds proceeded as followed in the previous literature [22].

Table 1. Composition of *Atractylodes lancea* ultrafine powder (AUP).

Ingredients	Content (%)
Dry matter	91.70 ± 0.41
Crude protein	7.04 ± 0.30
Crude lipid	4.01 ± 0.23
Ash	7.25 ± 0.33

Values are means $\pm$ SD of 3 replicates.

The basal diet composed 6.07% crude lipid and 34.07% crude protein, which was incorporated with 3.0%, 6.0%, 9.0%, 12.0%, 15.0%, and 18.0% AUP to formulate 6 types of experimental diets, respectively. In order to ensure that each experimental diet has

the same crude protein and crude fat content, wheat flour decreased by 12.96%, 25.93%, 38.89%, 51.86%, 64.82%, and 77.79%; corn oil decreased by 10.00%, 20.00%, 30.00%, 40.00%, 50.00%, and 60.00%; and rapeseed meal increased by 14.00%, 28.00%, 42.00%, 56.00%, 70.00%, and 84.00% for compensating for the AUP contribution, respectively. All diets were made into pellet feeds (1.5 mm) and dried at 50 °C. After cooling to room temperature, the feeds, sealed in plastic bags, were preserved at −20 °C for later use. The feeds underwent proximate analyses as per the AOAC methods. Table 2 lists the composition of the basal diet.

Table 2. Composition and proximate analysis on the basal diet.

Ingredients	%	Proximate Analysis	%
Fish meal	9.00	Dry matter	93.45
Meat bone meal	7.00	Crude protein	34.07
Silkworm pupa powder	6.00	Crude lipid	6.07
Soybean meal	26.00	Ash	6.11
Sunflower seed meal	7.00		
Rapeseed meal	5.00		
DDGS	6.00		
Wheat flour	28.00		
Lysine	0.50		
DL-methionine	0.70		
Threonine	0.70		
Ca(H ₂ PO ₄) ₂	0.40		
Fish oil	1.00		
Corn oil	0.70		
Vitamin mixture ¹	1.00		
Mineral mixture ²	1.00		

¹ Presented the per kg of vitamin mix below: folic acid (96%), 0.52 g; D-biotin (2%), 5.00 g; meso-inositol (99%), 52.33 g; D-calcium pantothenate (90%), 2.73 g; niacin (99%), 2.82 g; cyanocobalamin (1%), 0.10 g; ascorhyl acetate (93%), 7.16 g; riboflavine (80%), 0.63 g; pyridoxine HCl (81%), 0.92 g; menadione (23%), 0.43 g; thiamin nitrate (90%), 0.11 g; cholecalciferol (500,000 IUg^{−1}), 0.48 g; DL- α -tocopherol acetate (50%), 20.00 g; retinyl acetate (500,000 IUg^{−1}), 0.80 g. ² Presented the per kg of mineral mix below: CaCO₃, 897.98 g; KI (4% I), 2.90 g; ZnSO₄·7H₂O (23% Zn), 21.64 g; MnSO₄·H₂O (32% Mn), 4.09 g; Na₂SeO₃·5H₂O (1% Se), 2.50 g; CuSO₄·5H₂O (25% Cu), 1.20 g; FeSO₄·7H₂O (20% Fe), 69.70.

2.3. Feeding Trial

Fish farms in Neijiang, China, were the source of crucian carp. Their native habitat is carefully recreated in the laboratory; for example, the temperature is controlled at 22.0 ± 1.0 °C [23]. Experimenters changed the dechlorinated water each day and maintained a natural light cycle. Using tap water as the water source, in compliance with relevant water quality parameters. A cabinet air conditioner (Gree KFR-50LW, Gree Electric Appliances, Inc. of Zhuhai, Zhuhai, China) is used to regulate indoor temperature, thereby controlling water temperature. A mercury thermometer was employed for temperature monitoring, and a water quality analyzer (Octadern W-II, Wuxi Octadern Biotechnology Co., Ltd., Wuxi, China) served for measuring the water quality every 2 days. The fish were fed the baseline diet. A total of 630 crucian carps (average weight: 13.21 ± 0.17 g) were placed into 7 experimental groups. Each group had 3 tanks, and each tank had 30 fish. Among all these groups, the group fed with the basic diet was considered the control group, while the remaining 6 groups received diets supplemented with 3.0%, 6.0%, 9.0%, 12.0%, 15.0%, and 18.0% of AUP. Experimenters fed all fish groups 4 times each day and performed rigorous observation to ensure they were satiated with no overfeeding. Uneaten feed particles (feed residues) were counted, and their weight was calculated (weight of uneaten feed particles = number of uneaten particles × average weight of each particle) and used for correction of feed intake (FI).

After the 15 days of raising and fasting for 24 h, fish in each tank were sequentially counted and weighed. The initial number, final number, initial body weight (IBW), final body weight (FBW), and feed consumed were used to calculate the weight gain (WG), the specific growing rate (SGR) [21], FI, feed efficiency (FE) [24], and survival rate (SR) [25].

$$\text{WG (g fish}^{-1}\text{)} = \text{FBW (g fish}^{-1}\text{)} - \text{IBW (g fish}^{-1}\text{)}$$

$$\text{SGR (\% day}^{-1}\text{)} = 100 \times [\ln \text{FBW (g fish}^{-1}\text{)} - \ln \text{IBW (g fish}^{-1}\text{)}] / \text{experimental duration (day)}$$

$$\text{FI (g fish}^{-1}\text{)} = [\text{feed consumed (g)} - \text{feed residues (g)}] / \text{final number of fish}$$

$$\text{FE (\%)} = 100 \times \text{WG} / \text{FI}$$

$$\text{SR (\%)} = 100 \times (\text{final fish count} / \text{initial fish count})$$

At the commencement and conclusion of the feeding trial, body composition samples were collected as follows. Initial sampling: 30 fish were randomly selected from the source population. Final sampling: 5 fish were randomly sampled in tanks. The weight loss of fish after drying at 105 °C was taken into account to confirm the moisture content in the fish body. Subsequently, dried samples were homogenized and then analyzed via a FOSS DA1650 Near-Infrared Reflectance Spectrometer (Fosun Analytical Instruments (Suzhou) Co., Ltd., Suzhou, China) for the content of crude protein, crude lipid, and ash, as per the method of Cheli et al. (2012) and Reddersen et al. (2013) [26,27]. The calculation of body composition content was based on the following formula.

$$\text{Body composition (\%)} = \text{dried body composition (\%)} \times [1 - \text{moisture (\%)}]$$

2.4. Sampling

For the sample collection, 10 fish from each tank were anesthetized using a 50 mg L⁻¹ benzocaine solution for euthanasia, followed by being chilled on ice for the mitigation of tissue degradation [28]. Fish body weight and ruler measurement were executed for the calculation of condition factor (CF) [29]. Blood samples acquired from each group through caudal puncture were placed into heparinized syringes, followed by 3 min of centrifugation (1000 × g, 4 °C) within one hour. The levels of triglyceride (TG), glucose (GLU), total protein (TP), total amino acid (TAA), and plasma ammonia (PA) were measured in plasma that was collected from blood. The red blood cells (RBCs) were preserved at −80 °C for the confirmation of methemoglobin (MetHb), hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) levels. Subsequently, intestines were stripped from the hepatopancreas among the overall internal organs that were extracted from the abdominal cavity. The hepatosomatic and intestinal weights and intestinal length were measured for the calculation of the hepatosomatic index (HSI), intestosomatic index (ISI) [30], and intestine length index (ILI) [29].

$$\text{CF (\%)} = 100 \times [\text{body weight (g fish}^{-1}\text{)}] / [\text{body length (cm)}]^3$$

$$\text{HSI (\%)} = 100 \times [\text{hepatopancreas weight (g fish}^{-1}\text{)}] / [\text{body weight}]$$

$$\text{ISI (\%)} = 100 \times [\text{intestine weight (g fish}^{-1}\text{)}] / [\text{body weight}]$$

$$\text{ILI (\%)} = 100 \times [\text{intestine length (cm)}] / [\text{body length (cm)}]$$

All tissues then underwent liquid nitrogen freezing before being preserved at −80 °C. Intestinal tissues were for the assessment of anti-superoxide anion (ASA) and superoxide dismutase (SOD) activities, and glutathione (GSH) and MDA content. Hepatopancreatic tissues were for the assessment of glutamate-oxaloacetate transaminase (GOT), catalase (CAT), and glutathione peroxidase (GPx) activities and H₂O₂ content. Also, the gills were

quickly isolated and preserved at $-80\text{ }^{\circ}\text{C}$ for assays measuring GOT, glutamate-pyruvate transaminase (GPT), and GPx activities and GSH level. To conduct the assays for lactate dehydrogenase (LDH), GOT, and GPT activities as well as H_2O_2 levels, the muscle tissues were promptly harvested and stored at $-80\text{ }^{\circ}\text{C}$.

2.5. Hypoxia Trial

In our previous study [23], fishes (10 per tank) were weighed for body weight (BW, g) when the feeding experiment was finished. Hypoxia assays were employed using a saturated dissolved oxygen water volume (WV, L) of 29.23 times the BW of the fish. The oxygen consumption rate (OCR, $\text{mg g}^{-1} \text{h}^{-1}$) of the fish body was calculated based on the initial dissolved oxygen (IDO, mg L^{-1}) concentration and final dissolved oxygen (FDO, mg L^{-1}) concentration of the water and duration of hypoxia (DT, h: The period from the closure of the bottle mouth to the loss of balance in the fish was considered the DT) in an experimental bottle using the following formula.

$$\text{OCR} = [(\text{IDO} - \text{FDO}) \times \text{WV}] / (\text{DT} \times \text{BW})$$

2.6. Ethics Statement

The procedures described above were performed as per the ethical guidelines set by the Institutional Ethics Committee of the Chinese Institute of Chemical Biology. Experiments acquired the approval from the Institutional Animal Care and Use Committee of Neijiang Normal University, ensuring that the research adhered to relevant ethical considerations and guidelines for the humane treatment of animals (Approval Code: JM2023-11, Approval Date: 17 July 2023).

2.7. Biochemical Analysis

Tissue samples underwent homogenization in a solution encompassing 9 part (*w/v*)-refrigerated physiological saline. Following that, this homogenate was subjected to 20 min of centrifugation ($3200 \times g$, $4\text{ }^{\circ}\text{C}$), with the resulting supernatant stored for analyzing biochemical indicators. The measurement of GOT, GPT, and LDH activities followed the method in the literature [31]. The evaluations of SOD, CAT, GPx, and ASA activities and the GSH level were conducted using the known techniques [23,29]. The measurement of H_2O_2 and MetHb levels adhered to the method described in the literature [32]. MDA measurement followed the method of literature [33]. Measurement of total soluble proteins was conducted as per Bradford's methodology [34]. Hb content measurement was based on the following paper [35]. All biochemical indicators in plasma were ascertained using the commercial kits from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

2.8. Statistical Analysis

All experimental data were in the format of means $\pm$ standard deviation (SD). Prior to statistical testing, the Shapiro–Wilk test was used to verify the normality of data distribution, and the Levene test was applied to assess the homogeneity of variances for all variables. Once the assumptions for one-way analysis of variance (ANOVA) were satisfied, significant differences among the experimental groups were assessed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). If the ANOVA results demonstrated significant differences ($p < 0.05$), Duncan's multiple range test was conducted for post hoc comparisons, identifying the specific group pairs with significant differences. $p < 0.05$ indicated statistical significance.

3. Results

3.1. Effects of Dietary AUP on Growth Performance in Crucian Carp

As listed in Table 3, compared with participants not receiving the treatment, the supplement to AUP markedly reduced the WG, SGR, and FE (Table 3). A significant decline of WG, SGR, and FE was noted at 6.0%, 6.0%, and 3.0% of AUP supplementation, respectively ($p < 0.05$, Table 3), whereas fish fed the diet containing 18.0% AUP exhibited the lowest values.

Table 3. Effect of dietary supplementation with graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days on growth performance in crucian carp.

AUP (%)	IBW (g Fish ⁻¹)	FBW (g Fish ⁻¹)	FI (g Fish ⁻¹)	WG (g Fish ⁻¹)	SGR (% d ⁻¹)	FE (%)
0.0	13.32 ± 0.23 ^a	15.14 ± 0.39 ^d	5.03 ± 0.00 ^a	1.83 ± 0.20 ^d	1.07 ± 0.10 ^d	36.27 ± 3.92 ^d
3.0	13.36 ± 0.18 ^a	14.80 ± 0.11 ^{cd}	5.16 ± 0.26 ^a	1.45 ± 0.18 ^{cd}	0.86 ± 0.11 ^{cd}	28.01 ± 2.40 ^c
6.0	13.27 ± 0.21 ^a	14.57 ± 0.49 ^{bc}	5.09 ± 0.10 ^a	1.30 ± 0.34 ^{bc}	0.78 ± 0.19 ^{bc}	25.56 ± 6.43 ^{bc}
9.0	13.33 ± 0.04 ^a	14.61 ± 0.32 ^{bc}	5.03 ± 0.00 ^a	1.28 ± 0.28 ^{bc}	0.76 ± 0.16 ^{bc}	25.50 ± 5.57 ^{bc}
12.0	13.22 ± 0.20 ^a	14.19 ± 0.13 ^{ab}	5.09 ± 0.10 ^a	0.97 ± 0.16 ^{ab}	0.59 ± 0.10 ^{ab}	18.93 ± 2.80 ^{ab}
15.0	13.25 ± 0.15 ^a	14.28 ± 0.10 ^{ab}	5.15 ± 0.10 ^a	1.04 ± 0.10 ^{ab}	0.63 ± 0.06 ^{ab}	20.09 ± 1.57 ^{ab}
18.0	13.21 ± 0.17 ^a	14.02 ± 0.17 ^a	5.09 ± 0.10 ^a	0.81 ± 0.19 ^a	0.50 ± 0.12 ^a	15.92 ± 3.94 ^a

Values are means ± S.D. of 3 replicates, with 30 fish in each replicate. Values in the same column with the different superscripts are significantly different ($p < 0.05$). IBW, initial body weight; FBW, final body weight; FI, feed intake; WG, weight gain; SGR, specific growth rate; FE, feed efficiency.

3.2. Effects of Dietary AUP on Body and Organ Parameters of Crucian Carp

As presented in Table 4, CF, intestine weight, and ISI initially exhibited an increase followed by decreases with increasing dietary AUP levels in crucian carp. The parameters above showed the maximum value at 12.0%, 3.0%, and 12.0% AUP addition, respectively, but did not differ significantly compared with the control and subsequently declined as the increase in supplement of AUP (Table 4). The intestinal length and ILI at 3.0% of AUP addition were higher than those in the participants not receiving the treatment ($p < 0.05$); they peaked at 6.0% of AUP addition but subsequently. In contrast to the control, the hepatopancreas weight and HSI were significantly decreased at 9.0% of AUP addition ($p < 0.05$) and subsequently tended to stabilize (Table 4).

Table 4. Condition factor (CF), intestinal length, intestinal length index (ILI), intestine weight, intestosomatic index (ISI), hepatopancreas weight and hepatosomatic index (HSI) of crucian carp fed diets containing graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

AUP (%)	CF (%)	Intestinal Length (cm)	ILI (%)	Intestine Weight (g)	ISI (%)	Hepatopancreas Weight (g)	HSI (%)
0.0	4.56 ± 0.28 ^{ab}	19.70 ± 1.34 ^a	2.80 ± 0.11 ^a	0.405 ± 0.084 ^{ab}	2.541 ± 0.470 ^{ab}	0.53 ± 0.03 ^b	3.35 ± 0.14 ^b
3.0	4.69 ± 0.14 ^{ab}	21.61 ± 1.62 ^b	3.14 ± 0.11 ^{bc}	0.415 ± 0.060 ^b	2.530 ± 0.188 ^{ab}	0.56 ± 0.09 ^b	3.39 ± 0.31 ^b
6.0	4.79 ± 0.41 ^{ab}	22.96 ± 0.80 ^b	3.39 ± 0.23 ^d	0.413 ± 0.024 ^b	2.721 ± 0.157 ^b	0.51 ± 0.02 ^b	3.33 ± 0.11 ^b
9.0	4.75 ± 0.30 ^{ab}	22.44 ± 0.10 ^b	3.33 ± 0.02 ^{cd}	0.384 ± 0.014 ^{ab}	2.719 ± 0.002 ^b	0.42 ± 0.03 ^a	2.95 ± 0.30 ^a
12.0	4.97 ± 0.66 ^b	22.52 ± 1.72 ^b	3.36 ± 0.33 ^{cd}	0.404 ± 0.026 ^{ab}	2.776 ± 0.044 ^b	0.43 ± 0.02 ^a	2.98 ± 0.04 ^a
15.0	4.35 ± 0.02 ^a	22.00 ± 0.72 ^b	3.16 ± 0.08 ^{bcd}	0.384 ± 0.010 ^{ab}	2.599 ± 0.020 ^{ab}	0.43 ± 0.03 ^a	2.89 ± 0.16 ^a
18.0	4.32 ± 0.19 ^a	21.62 ± 0.40 ^b	3.08 ± 0.15 ^b	0.357 ± 0.008 ^a	2.397 ± 0.070 ^a	0.40 ± 0.04 ^a	2.70 ± 0.37 ^a

Values are means ± S.D. of 3 replicates, with 10 fish in each replicate. Values with the different superscripts in the same column are significantly different ($p < 0.05$).

3.3. Effects of Dietary AUP on Body Composition of Crucian Carp

As presented in Table 5, moisture levels showed no significance. Relative to the control group, the crude protein and true protein contents in crucian carp were significantly enhanced at 6.0% and 9.0% of AUP addition, respectively ($p < 0.05$), and reached the maximum value at 15.0% of AUP addition. According to broken-line regression analysis, the optimal dietary levels of AUP for crude protein content were determined to be 13.04% (Figure 2). The crude lipid content in crucian carp was significantly reduced at 6.0% of AUP

addition ($p < 0.05$), followed by a continuous decrease with rising AUP supplementation ($p < 0.05$, Table 5).

Table 5. The contents of moisture, crude protein, crude lipid, ash and true protein in crucian carp fed diets containing graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

AUP (%)	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Ash (%)	True Protein (%)
0.0	73.92 ± 0.39 ^a	13.92 ± 0.10 ^a	4.41 ± 0.15 ^d	7.72 ± 0.09 ^{ab}	8.36 ± 0.38 ^a
3.0	75.29 ± 2.64 ^a	14.47 ± 0.20 ^{ab}	4.31 ± 0.10 ^d	7.97 ± 0.14 ^{bc}	8.30 ± 0.44 ^a
6.0	73.64 ± 0.40 ^a	14.66 ± 0.03 ^b	4.02 ± 0.19 ^c	8.06 ± 0.27 ^c	9.02 ± 0.58 ^{ab}
9.0	74.51 ± 0.78 ^a	14.67 ± 0.04 ^b	3.52 ± 0.08 ^b	7.98 ± 0.30 ^{bc}	9.17 ± 0.40 ^b
12.0	74.40 ± 0.21 ^a	15.05 ± 0.18 ^{bc}	3.58 ± 0.08 ^b	7.95 ± 0.09 ^{bc}	9.21 ± 0.38 ^b
15.0	74.36 ± 0.55 ^a	15.28 ± 0.31 ^c	3.45 ± 0.06 ^b	7.80 ± 0.03 ^{bc}	9.42 ± 0.40 ^b
18.0	75.32 ± 0.66 ^a	14.89 ± 0.30 ^{bc}	3.07 ± 0.04 ^a	7.48 ± 0.07 ^a	9.29 ± 0.44 ^b

Values are means ± S.D. of 3 replicates, with 5 fish in each replicate. Values in the same column with the different superscripts are significantly different ($p < 0.05$).

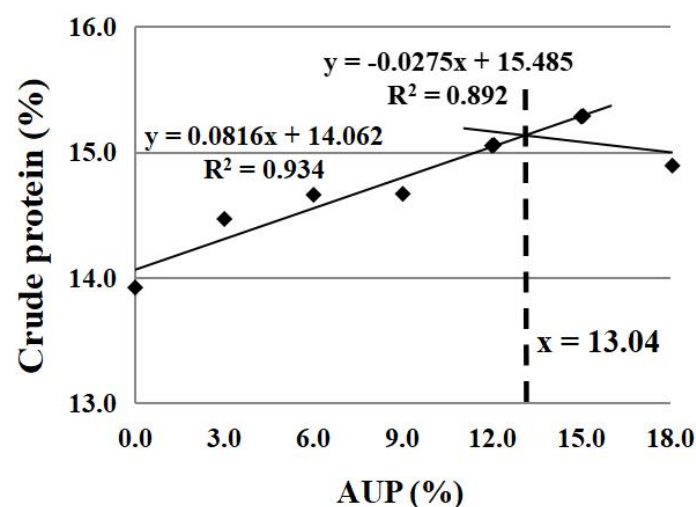


Figure 2. Broken-line regression analysis of crude protein content in crucian carp fed with different concentrations of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

3.4. Effects of Dietary AUP on Hypoxic Tolerance in Crucian Carp

Relative to the participants not receiving the treatment, 3.0% of the AUP treatment group exhibited remarkably prolonged hypoxic DT ($p < 0.05$) and obviously lowered OCR ($p < 0.05$), and the value reached its maximum and minimum at 6.0% of AUP addition, respectively (Table 6). According to regression analysis that adopted the broken-line model based on DT data, the optimum addition for hypoxia tolerance was 6.05% of AUP (Figure 3).

Table 6. Effect of dietary supplementation with graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days on the hypoxic tolerance indicators in crucian carp.

AUP (%)	BW (g Fish ⁻¹)	WV (L Bottle ⁻¹)	IDO (mg L ⁻¹)	DT (h)	FDO (mg L ⁻¹)	OCR (mg g ⁻¹ h ⁻¹)
0.0	14.32 ± 0.83 ^a	4.18 ± 0.04 ^a	8.17 ± 0.00 ^a	2.26 ± 0.28 ^a	0.10 ± 0.02 ^a	0.106 ± 0.017 ^b
3.0	14.27 ± 0.63 ^a	4.15 ± 0.16 ^a	8.17 ± 0.00 ^a	2.75 ± 0.29 ^b	0.11 ± 0.02 ^a	0.086 ± 0.011 ^a
6.0	14.12 ± 1.55 ^a	4.11 ± 0.28 ^a	8.17 ± 0.00 ^a	3.01 ± 0.13 ^b	0.11 ± 0.01 ^a	0.078 ± 0.001 ^a
9.0	14.42 ± 0.39 ^a	4.21 ± 0.12 ^a	8.17 ± 0.00 ^a	2.95 ± 0.32 ^b	0.10 ± 0.02 ^a	0.080 ± 0.006 ^a
12.0	14.42 ± 0.45 ^a	4.19 ± 0.11 ^a	8.17 ± 0.00 ^a	2.60 ± 0.20 ^{ab}	0.11 ± 0.02 ^a	0.090 ± 0.005 ^{ab}
15.0	13.93 ± 0.35 ^a	4.07 ± 0.22 ^a	8.17 ± 0.00 ^a	2.54 ± 0.16 ^{ab}	0.10 ± 0.01 ^a	0.093 ± 0.006 ^{ab}
18.0	13.57 ± 0.45 ^a	3.97 ± 0.23 ^a	8.17 ± 0.00 ^a	2.56 ± 0.23 ^{ab}	0.11 ± 0.01 ^a	0.093 ± 0.010 ^{ab}

Values are means ± S.D. of 3 replicates, with 10 fish in each replicate. Values in the same column with the different superscripts are significantly different ($p < 0.05$). BW, body weight; WV, water volume; IDO, initial dissolved oxygen; FDO, final dissolved oxygen; DT, durative time; OCR, oxygen consumption rate.

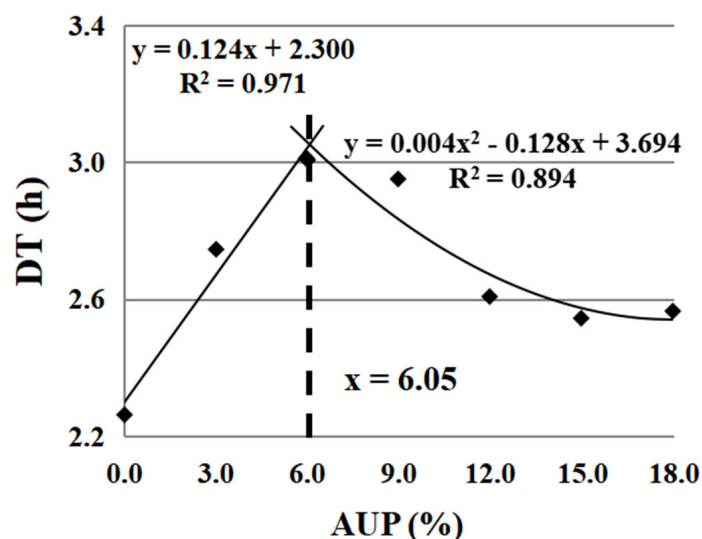


Figure 3. Broken-line regression analysis of durative time (DT) of crucian carp fed with different concentrations of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

3.5. Effects of Dietary AUP on Biochemical Parameters in Plasma of Crucian Carp

In the plasma of crucian carp treated with AUP, the levels of PA, TG, and GLU initially exhibited a decrease followed by increases with increasing dietary AUP levels in crucian carp. They achieved the lowest values at 9.0%, 12.0%, and 9.0% AUP, respectively, and then rose as the supplemental dose of AUP increased (Table 7). In the plasma of crucian carp treated with AUP, the levels of TP and TAA exhibited an increasing-to-decreasing trend. They peaked at 12.0% of AUP and then decreased as the supplemental level of AUP rose (Table 7).

Table 7. The content of plasma ammonia (PA), total amino acids (TAAs), total protein (TP), glucose (GLU) and triglyceride (TG) in plasma of crucian carp fed diets containing graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

AUP (%)	PA ($\mu\text{mol L}^{-1}$)	TP (mg mL^{-1})	TAA ($\mu\text{mol mL}^{-1}$)	TG (mmol L^{-1})	GLU (mmol L^{-1})
0.0	289.00 $\pm$ 18.88 ^{bc}	24.16 $\pm$ 4.49 ^{ab}	23.72 $\pm$ 1.53 ^a	1.66 $\pm$ 0.13 ^{ab}	10.74 $\pm$ 0.45 ^c
3.0	286.34 $\pm$ 24.41 ^{bc}	26.20 $\pm$ 3.54 ^{ab}	25.26 $\pm$ 1.98 ^{ab}	1.51 $\pm$ 0.12 ^a	12.15 $\pm$ 1.15 ^d
6.0	241.06 $\pm$ 22.01 ^a	25.56 $\pm$ 4.59 ^{ab}	26.79 $\pm$ 0.88 ^{ab}	1.52 $\pm$ 0.36 ^a	9.89 $\pm$ 0.29 ^{abc}
9.0	233.07 $\pm$ 6.10 ^a	26.71 $\pm$ 2.15 ^{ab}	27.93 $\pm$ 4.40 ^b	1.46 $\pm$ 0.13 ^a	8.87 $\pm$ 0.59 ^a
12.0	275.68 $\pm$ 6.92 ^{ab}	27.54 $\pm$ 1.19 ^b	28.32 $\pm$ 1.53 ^b	1.45 $\pm$ 0.03 ^a	9.21 $\pm$ 0.58 ^{ab}
15.0	290.33 $\pm$ 10.05 ^{bc}	20.46 $\pm$ 0.51 ^a	28.32 $\pm$ 0.88 ^b	1.97 $\pm$ 0.27 ^b	10.38 $\pm$ 0.28 ^{bc}
18.0	322.30 $\pm$ 48.83 ^c	21.10 $\pm$ 5.07 ^a	26.02 $\pm$ 2.16 ^{ab}	1.90 $\pm$ 0.20 ^b	9.66 $\pm$ 0.64 ^{abc}

Values are means $\pm$ S.D. of 3 replicates, with 5 fish in each replicate. Values in the same column with the different superscripts are significantly different ($p < 0.05$).

3.6. Effects of Dietary AUP on Biochemical Index in Intestines of Crucian Carp

The intestines of crucian carp fed 9.0%, 3.0%, and 9.0% of AUP showed a significant increase in ASA and SOD activities, as well as the GSH level, in comparison to the control group ($p < 0.05$), respectively. These values peaked at 9.0%, 9.0%, and 12.0% of AUP addition in feeds, respectively, and then decreased as the supplemental level of AUP increased (Table 8). Additionally, in the intestines of crucian carp treated with AUP, the MDA level initially exhibited a decrease followed by increases, meeting the minimum at 15.0% of AUP ($p < 0.05$) and then rising as the supplemental amount of AUP increased (Table 8).

Table 8. The activities of anti-superoxide anion (ASA) and superoxide dismutase (SOD) as well as the content of malondialdehyde (MDA) and reduced glutathione (GSH) in intestines of crucian carp fed diets containing graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

AUP (%)	ASA (U g ⁻¹ Protein)	MDA (nmol mg ⁻¹ Protein)	SOD (U mg ⁻¹ Protein)	GSH (mg g ⁻¹ Protein)
0.0	47.07 ± 0.74 ^a	11.78 ± 0.43 ^c	85.26 ± 1.25 ^a	8.79 ± 0.32 ^b
3.0	49.38 ± 0.68 ^a	11.69 ± 1.50 ^{bc}	92.92 ± 1.97 ^b	9.22 ± 0.62 ^b
6.0	48.52 ± 1.17 ^a	10.58 ± 0.38 ^{bc}	92.33 ± 7.00 ^b	9.47 ± 0.74 ^{bc}
9.0	56.49 ± 0.38 ^b	11.16 ± 1.22 ^{bc}	104.36 ± 4.86 ^c	10.27 ± 0.74 ^{cd}
12.0	56.23 ± 1.81 ^b	10.22 ± 0.32 ^{abc}	103.57 ± 4.12 ^c	10.71 ± 0.65 ^d
15.0	48.34 ± 1.77 ^a	8.71 ± 1.01 ^a	89.70 ± 1.34 ^{ab}	9.72 ± 0.67 ^{bc}
18.0	47.53 ± 3.88 ^a	10.01 ± 1.61 ^{ab}	90.91 ± 6.80 ^{ab}	7.72 ± 0.15 ^a

Values are means ± S.D. of 3 replicates, with 5 fish in each replicate. Values in the same column with the different superscripts are significantly different ($p < 0.05$).

3.7. Effects of Dietary AUP on Biochemical Index in Hepatopancreas of Crucian Carp

The GOT, CAT, and GPx activities in the hepatopancreas of crucian carp were significantly increased by dietary AUP supplementation at concentrations of 12.0%, 3.0%, and 3.0% in comparison to the control, respectively. These activities peaked at 12.0%, 6.0%, and 6.0% of AUP, respectively, and then decreased as the supplemental level of AUP increased (Table 9). In the hepatopancreas of crucian carp treated with AUP, the H₂O₂ level showed a decreasing-to-increasing trend, reaching its lowest values in 12.0% AUP ($p < 0.05$) and then rising as the supplemental level of AUP increased (Table 9).

Table 9. The activities of glutamate-oxaloacetate transaminase (GOT), catalase (CAT) and glutathione peroxidase (GPx), as well as level of hydrogen peroxide (H₂O₂), in hepatopancreas of crucian carp fed diets containing graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

AUP (%)	GOT (U g ⁻¹ Protein)	H ₂ O ₂ (mmol g ⁻¹ Protein)	CAT (U mg ⁻¹ Protein)	GPx (U mg ⁻¹ Protein)
0.0	34.05 ± 8.42 ^a	14.71 ± 1.74 ^b	12.35 ± 0.94 ^a	193.41 ± 7.17 ^a
3.0	40.56 ± 11.56 ^{ab}	14.90 ± 0.50 ^b	17.30 ± 2.29 ^b	228.25 ± 5.01 ^c
6.0	40.94 ± 8.23 ^{ab}	14.90 ± 1.59 ^b	26.66 ± 1.97 ^d	246.23 ± 8.49 ^d
9.0	39.49 ± 4.31 ^{ab}	14.55 ± 2.94 ^b	22.41 ± 1.91 ^c	224.04 ± 2.26 ^c
12.0	48.89 ± 2.35 ^b	10.12 ± 1.84 ^a	19.03 ± 1.36 ^b	216.25 ± 14.58 ^{bc}
15.0	45.21 ± 13.25 ^{ab}	12.18 ± 3.50 ^{ab}	19.12 ± 3.69 ^b	214.03 ± 13.86 ^{bc}
18.0	39.50 ± 3.66 ^{ab}	12.33 ± 1.83 ^{ab}	18.50 ± 1.48 ^b	206.71 ± 14.75 ^{ab}

Values are means ± S.D. of 3 replicates, with 5 fish in each replicate. Values in the same column with the different superscripts are significantly different ($p < 0.05$).

3.8. Effects of Dietary AUP on Biochemical Indicators in RBCs of Crucian Carp

The H₂O₂ and MDA levels exhibited a gradual decrease at rising concentrations of AUP addition in RBCs of crucian carp and were higher than those in the control group at 3.0% and 6.0% AUP additions ($p < 0.05$, Table 10). The MetHb content demonstrated a decreasing-to-increasing trend, reaching the lowest value at 12.0% of AUP addition ($p < 0.05$), subsequently ascended as the supplemental level of AUP increased (Table 10).

Table 10. The levels of methemoglobin (MetHb), hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) in red blood cells of crucian carp fed diets containing graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

AUP (%)	MetHb (%)	H ₂ O ₂ (mmol g ⁻¹ Protein)	MDA (nmol mg ⁻¹ Protein)
0.0	1.383 ± 0.006 ^b	59.50 ± 15.99 ^c	21.21 ± 2.68 ^d
3.0	1.359 ± 0.014 ^{ab}	36.67 ± 0.69 ^b	19.40 ± 3.26 ^d
6.0	1.378 ± 0.014 ^{ab}	15.80 ± 5.70 ^a	14.65 ± 1.70 ^c
9.0	1.372 ± 0.011 ^{ab}	10.70 ± 3.58 ^a	6.83 ± 1.48 ^b
12.0	1.339 ± 0.017 ^a	8.93 ± 3.16 ^a	3.32 ± 0.84 ^a
15.0	1.364 ± 0.041 ^{ab}	6.96 ± 0.63 ^a	1.59 ± 0.49 ^a
18.0	1.382 ± 0.029 ^{ab}	5.49 ± 0.94 ^a	1.42 ± 0.13 ^a

Values are means ± S.D. of 3 replicates, with 5 fish in each replicate. Values in the same column with the different superscripts are significantly different ($p < 0.05$).

3.9. Effects of Dietary AUP on Biochemical Parameters in Gills of Crucian Carp

In contrast to the control, dietary AUP supplementation significantly enhanced GSH level at 15.0% AUP addition ($p < 0.05$), which gradually increased with the AUP concentrations in diets in gills of crucian carp (Table 11). The GOT and GPT activities clearly rose at 3.0% and 9.0% AUP addition compared to the control group ($p < 0.05$), peaked at 6.0% and 9.0% AUP addition, respectively, and then declined as the AUP addition increased (Table 11). The activity of GPx peaked at 6.0% AUP addition and subsequently declined with higher AUP levels ($p < 0.05$, Table 11).

Table 11. The activities of glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT) and glutathione peroxidase (GPx) as well as the contents of reduced glutathione (GSH) in gills of crucian carp fed diets containing graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

AUP (%)	GOT (U g ⁻¹ Tissue)	GPT (U g ⁻¹ Tissue)	GSH (mg g ⁻¹ Tissue)	GPx (U mg ⁻¹ Tissue)
0.0	16.43 ± 1.44 ^a	9.02 ± 1.60 ^b	0.39 ± 0.02 ^a	47.22 ± 15.21 ^{ab}
3.0	24.06 ± 1.45 ^{bc}	10.36 ± 1.97 ^{bc}	0.35 ± 0.06 ^a	50.28 ± 9.00 ^{ab}
6.0	26.23 ± 0.66 ^c	11.47 ± 0.47 ^{bc}	0.48 ± 0.15 ^a	58.06 ± 10.46 ^b
9.0	22.19 ± 1.45 ^b	15.54 ± 1.20 ^d	0.55 ± 0.02 ^a	41.11 ± 10.00 ^{ab}
12.0	16.48 ± 1.16 ^a	13.87 ± 2.04 ^{cd}	0.72 ± 0.11 ^a	37.22 ± 7.73 ^a
15.0	16.57 ± 2.24 ^a	12.43 ± 3.52 ^{bcd}	2.83 ± 0.20 ^b	37.64 ± 3.47 ^a
18.0	15.91 ± 3.49 ^a	5.14 ± 0.08 ^a	3.23 ± 0.49 ^c	32.36 ± 9.15 ^a

Values are means ± S.D. of 3 replicates, with 5 fish in each replicate. Values in the same column with the different superscripts are significantly different ($p < 0.05$).

3.10. Effects of Dietary AUP on Biochemical Indicators in Muscle of Crucian Carp

Dietary AUP supplementation significantly raised the GOT activity in crucian carp muscle at 9.0% AUP addition ($p < 0.05$), peaked at 12.0% AUP addition, and then decreased as AUP addition increased (Table 12). At 3.0% and 9.0% AUP addition, respectively, dietary AUP supplementation markedly increased GPT and LDH activities in comparison to the control ($p < 0.05$). As AUP concentrations in feeds increased, these activities gradually increased in crucian carp muscle (Table 12). The H₂O₂ level in crucian carp muscle was considerably lowered by dietary AUP supplementation at 6.0% ($p < 0.05$) compared to the control, and it fell marginally as AUP concentrations increased in diets (Table 12).

Table 12. The activities of glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT) and lactate dehydrogenase (LDH), as well as the content of hydrogen peroxide, (H₂O₂) in muscle of crucian carp fed diets containing graded levels of *Atractylodes lancea* ultrafine powder (AUP) for 15 days.

AUP (%)	GOT (U g ⁻¹ Protein)	GPT (U g ⁻¹ Protein)	LDH (U g ⁻¹ Protein)	H ₂ O ₂ (mmol g ⁻¹ Protein)
0.0	17.97 ± 2.35 ^a	8.43 ± 2.32 ^a	254.39 ± 17.19 ^a	9.50 ± 1.85 ^b
3.0	19.46 ± 2.08 ^{ab}	14.91 ± 1.61 ^b	278.74 ± 18.34 ^{ab}	8.04 ± 0.74 ^b
6.0	20.32 ± 2.36 ^{abc}	14.81 ± 1.43 ^b	283.52 ± 14.15 ^{ab}	5.21 ± 0.80 ^a
9.0	25.64 ± 4.74 ^{bc}	16.56 ± 5.46 ^b	302.28 ± 10.30 ^{bc}	5.13 ± 1.48 ^a
12.0	26.96 ± 7.61 ^c	18.02 ± 1.67 ^b	301.38 ± 16.92 ^{bc}	4.32 ± 1.62 ^a
15.0	18.80 ± 2.16 ^{ab}	29.23 ± 4.89 ^c	316.40 ± 33.72 ^{bc}	4.37 ± 0.24 ^a
18.0	19.30 ± 1.54 ^{ab}	28.72 ± 3.17 ^c	329.24 ± 17.43 ^c	4.26 ± 0.61 ^a

Values are means ± S.D. of 3 replicates, with 5 fish in each replicate. Values in the same column with the different superscripts are significantly different ($p < 0.05$).

4. Discussion

4.1. Dietary AUP Reduced the Growth Performance in Fish

In fish, FE, WG, and SGR can reflect the growth performance [36,37]. The current results showed that crucian carp fed diets enriched with AUP decreased WG, SGR, and FE, which is consistent with previous findings that *Atractylodes lancea* therapy decreased mouse body weight [16]. According to our research, eating AUP reduces fish growth performance. The digestive and absorptive capacities of fish are favorably connected with

their growth [38]. The intestines and hepatopancreas are essential digestive organs in fish that are critical for the absorption and processing of nutrients [39]. According to our research, crucian carp's intestinal length and ILI were enhanced by dietary AUP. However, the intestine weight and ISI were not significantly improved in AUP-fed crucian carp. Moreover, a significant reduction in the hepatopancreatic weight and HSI was observed in AUP-fed crucian carp. The aforementioned findings imply that eating AUP may prevent fish from developing hepatopancreas. As of right now, no research has been done on the effects of AUP on fish digestive organs. Since digestive enzymes aid in the breakdown of nutrients and directly contribute to increased appetite and nutrient consumption, fish digestive capacity is primarily dependent on the effectiveness of digestive enzyme systems and the absorptive capacity of the intestinal epithelium [40]. Among these, lipase facilitates the hydrolysis of ester bonds in substrates that include phospholipids, cholesterol esters, and triglycerides [41]. One study found that the ethanol extract of *Atractylodes lancea* dramatically inhibited lipase in humans [15]. Taken together, dietary AUP reduces the growth performance by inhibiting the hepatopancreatic development and the lipase activity in fish.

4.2. The Anti-Obesity Effect of AUP in Fish

At present, the excessive accumulation of lipid in the fish body and liver has become one of the urgent problems to be solved in the aquaculture industry [42]. The anti-obesity effect manifests as the decreases in WG and body fat content in animals [16]. In this study, dietary AUP reduced the lipid content and increased the protein content in crucian carp. Broken-line regression analysis of protein content in the fish body indicated that the inclusion level of AUP at 13.04% in the diet showed the maximum level. It has been reported that *Atractylodes lancea* lowers the hepatic lipid content in mice [16]. The above results show that AUP has the anti-obesity effect on fish, conforming to the finding that the ethanol extract of *Atractylodes lancea* has the anti-obesity effect on obesity in mice [15].

Changes in protein content in fish bodies may be closely related to their metabolism. Protein is the primary energy source in fish [24]. Aminotransferases GOT and GPT are primarily responsible for controlling protein metabolism; their forward reactions direct amino acids into the TCA cycle for energy production by deamination [43,44], whereas their reverse reactions enable amino acid anabolism [45,46]. According to our research, crucian carp's hepatopancreatic GOT and muscle GOT and GPT activities were improved by dietary AUP supplementation. PA content is positively associated with the catabolic breakdown of protein because ammonia is the primary byproduct of protein catabolism in teleosts [47]. TP and TAA concentration in plasma are also positively correlated with the synthesis efficiency of protein [48]. According to our finding, dietary AUP reduced the PA content and enhanced the TP and TAA concentration in plasma of crucian carp. Existing studies have not studied how *Atractylodes lancea* affects the protein metabolism in fish. The results above indicate that AUP improves the anabolism of protein, which may be the reason for the increase in protein content in the fish's body.

The change in lipid content in a fish's body may be well related to the carbohydrate metabolism. Dietary carbohydrates are substrates essential for the synthesis of body fat and non-essential amino acids, which provide animals with energy to maintain their normal growth [49]. As a simple carbohydrate that can be directly digested and absorbed, GLU is one of the basic energy substances [50,51]. The GLU metabolism process can provide the energy for fish to maintain normal physiological functions, thereby reducing the fish body's dependence on protein and fat consumption [50,51]. The TG level in plasma is considered an important indicator related to lipid metabolism and can reflect the nutritional status of fish [52]. The experimental data showed that dietary AUP reduced the content of

TG and GLU in the plasma of crucian carp. The results in this study are consistent with the report that dietary *Atractylodes lancea* reduced the TG and GLU levels in the serum of high-fat diet (HFD)-fed mice [16]. The decrease in plasma TG content may be closely related to the reduced lipase activity [15]. As a vital enzyme in anaerobic glycolysis, LDH stimulates energy generation via GLU metabolism [53]. Our study confirmed the efficacy of dietary AUP supplementation in strengthening LDH activity in crucian carp muscle. Previous studies have not well elucidated how *Atractylodes lancea* affects the LDH activity in metabolizing organs of fish. In this study, dietary AUP improves the GLU metabolism for energy supply in fish. The results above confirm that dietary AUP can reduce the lipid content in a fish's body by reducing GLU as the raw material for synthesizing body fat.

4.3. Dietary AUP Intensified the Antioxidant Capacity in Fish

Organisms can continuously produce ROS during metabolic processes [54]. ROS, such as O_2^- , H_2O_2 and $\cdot OH$, oxidizes the cellular components of lipids and proteins, resulting in the lipid oxidation and inactivation of key enzymes in fish cells [53]. In our study, AUP addition considerably raised the ASA activity in intestines and reduced the H_2O_2 level in hepatopancreas and muscle of crucian carp. Dietary AUP restricted the formation of ROS in fish tissues and organs. This result conforms to the previous report that atractylodin, a major active ingredient of *Atractylodes lancea*, decreased the ROS level in the liver of HFD mice [55]. Lipid oxidation, manifested by higher MDA levels, is a crucial biomarker of ROS-induced damage [56]. In our study, dietary AUP reduced the MDA level in the intestines of crucian carp, consistent with the findings of other studies that dietary atractylodin considerably reduced the MDA level in the liver of HFD mice [55]. The results suggest that AUP addition decreases lipid oxidation by inhibiting ROS generation in fish.

The antioxidant systems in fish may dominate the effects of AUP on ROS and lipid oxidation. Fishes have evolved enzymatic antioxidants that counteract ROS in their organs and tissues [57]. SOD, a vital enzyme in antioxidant systems, transforms O_2^- into H_2O_2 and O_2 [56]. Subsequently, enzymes of GPx and CAT participate in the detoxification of H_2O_2 [58]. The administration of *Atractylodes lancea* polysaccharide produced a remarkably stronger SOD activity in the intestines of mice induced by cisplatin [59]. This study found that dietary AUP significantly increased the SOD activity in the intestines and CAT and GPx activities in the hepatopancreas of crucian carp. Fish neutralize intracellular ROS primarily by relying on non-enzymatic antioxidants of GSH [57]. The finding in the study that AUP addition elevated the GSH level in crucian carps' intestines conforms to a previous study demonstrating that dietary polysaccharides from *Atractylodes lancea* enhanced GSH levels in intestines of mice induced by cisplatin [59]. Previous studies seldom highlight the variation in the above parameters in fish digestive organs after AUP addition. These findings demonstrate that dietary AUP can scavenge ROS and prevent lipid oxidation by raising the levels of both enzymatic and non-enzymatic antioxidants in fish tissues and organs. This is one of the rare research projects that thoroughly investigates the effects of AUP on oxidative stress indicators in fish tissues and organs: it shows that dietary AUP may improve fish vitality and stress tolerance in aquaculture environments.

4.4. Dietary AUP Intensified the Hypoxia Tolerance in Fish

DO is a vital limiting factor in fish farming since sufficient oxygen levels essentially maintain the fishes' aerobic metabolism [2]. The oxygen concentration changes obviously in freshwater ecosystems seasonally and daily, severely restricting the growth and development of fish suffering hypoxia and subsequent reoxygenation events [3]. In this study, dietary AUP supplementation increased DT in hypoxic crucian carp, resulting in increased hypoxia tolerance. During this process, DO level in the water decreased from

8.17 mg L⁻¹ to 0.11 mg L⁻¹ in the experimental bottle. The ideal AUP addition levels for fish were found to be 6.05% using broken-line regression analysis of DT. Few studies have looked at how AUP affects fish in hypoxic environments. Fish usually have their metabolic rate adjusted considering the low-oxygen environments [60]. One important physiological adaptation mechanism that improves long-term survival performance in hypoxic conditions is metabolic rate depression [61]. OCR critically indicates the aerobic metabolic activity [62,63]. In this study, dietary AUP addition reduced OCR in crucian carp under hypoxic conditions, hinting at the efficacy of dietary AUP in intensifying fish hypoxia tolerance by attenuating aerobic metabolic activity. The fish gill is an osmoregulatory organ and a respiratory organ [64]. GOT and GPT primarily participate in fish protein metabolism [40,45]. Collectively, AUP supplementation increased GPT and GOT activities in the gills of crucian carp. According to our finding, dietary AUP benefits protein metabolism and energy supply in fish gills. Of note, in a hypoxic environment, animals produce energy primarily by virtue of anaerobic glycolysis [61,65]. On these accounts, AUP reduces protein catabolism and stimulates anaerobic glycolysis in fish, thereby intensifying the hypoxia tolerance. All these necessitate the elucidation of the promoting mechanisms of AUP against hypoxia resilience in fish in future research.

During breathing, RBCs are primarily responsible for the transportation of O₂ and the removal of CO₂ [66]. In RBCs, Hb is an O₂-carrying protein, which can bind to O₂ to form oxygenated Hb that takes charge of releasing O₂ to the animal tissues [23]. Oxygenated Hb can undergo spontaneous oxidation, producing O₂⁻ and MetHb incapable of binding or transporting O₂ in RBCs [67]. O₂⁻ dismutation promotes H₂O₂ to be formed, and the H₂O₂ reacts with heme Fe²⁺ for the generation of ·OH [66]. Hypoxia is accompanied by higher MDA levels in gills of *Mugil cephalus* [68]. The present investigation revealed the decreased levels of MetHb, H₂O₂ and MDA in RBCs of crucian carp fed with AUP. In harmony with the present results, the *Atractylodes lancea* polysaccharide decreased the H₂O₂ level in the intestines of mice induced by cisplatin [59]. These results demonstrate the reduced ROS production and the oxidation of cellular components. In aquatic creatures, antioxidants can play a critical role in preserving the health and functionality of tissues and organs [69]. Fish gills are susceptible to ROS damage because they are frequently subjected to environmental stress and exhibit large concentrations of hemoglobin and erythrocytes [64]. This study confirmed that AUP addition enhanced the GPx activity and GSH level in crucian carp gills, which conforms to a previous study demonstrating that dietary atractylodin elevated the GSH level in the liver of HFD mice [55]. Seldom have studies emphasized how AUP affects the aforementioned characteristics in fish gills and red blood cells. In our study, dietary AUP supplementation attenuates ROS generation and the oxidation of cellular components through strengthening the enzymatic antioxidant activity and non-enzymatic antioxidant level, indicating an improved antioxidant capacity in fish RBCs and gills. According to these theories, adding AUP to fish diets increases their ability to withstand hypoxia by boosting the antioxidant capacity of their respiration-related tissues and organs.

5. Conclusions

To sum up, dietary AUP reduces the growth performance of fish and has a remarkable anti-obesity effect. The broken-line regression analysis of protein content in the fish body confirms the optimal AUP addition levels for fish to be 13.04%. In our study, the anti-obesity effects of AUP on fish can be explained by the enhanced GLU metabolism for reducing GLU as the raw material for synthesizing body fat and improving the anabolism of protein and antioxidant defense. Additionally, dietary AUP increases antioxidant defense in fish respiration-related tissues and organs and modifies the metabolism of proteins and carbs for energy production, hence enhancing hypoxia tolerance. The ideal AUP addition level for

fish is 6.05%, according to the DT broken-line regression analysis. According to this study, AUP naturally reduces fish stress and obesity brought on by hypoxia, offering a possible dietary approach for enhancing fish obesity and resistance in low-oxygen environments.

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