

Article

Effects of Fermented Mulberry Leaf Powder on Growth Performance, Meat Quality, Antioxidant Capacity, and Fat Metabolism in Grass Carp (*Ctenopharyngodon idellus*)

Hongquan Wang ¹, Shuang Wu ¹, Xiwen Zhang ², Wei Tang ², Xiangyue Zou ^{3,*} and Yurong Zhao ^{2,*}

¹ College of Fisheries, Hunan Agricultural University, Changsha 410128, China; zwx577258@163.com (H.W.)

² College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China

³ Institute of Cotton and Sericulture, Hunan Academy of Agricultural Sciences, Changsha 410128, China

* Correspondence: zwx18985450689@stu.hunau.edu.cn (X.Z.); 17508508445@stu.hunau.edu.cn (Y.Z.); Tel.: +86-0731-84612685 (Y.Z.); Fax: 0731-84619706 (Y.Z.)

Abstract

The use of plant-based fermentation products as substitutes for costly feed ingredients like fishmeal and soybean meal has been at the forefront of aquaculture research. This study investigated the effects of dietary fermented mulberry leaf powder (FMLP) on grass carp (0.86 ± 0.07 kg). Fish were fed a basal diet supplemented with 0%, 5%, 10%, or 15% FMLP for 70 days to assess impacts on growth, muscle quality, antioxidant status, and lipid metabolism. Results indicated that FMLP did not significantly affect growth performance ($p > 0.05$). However, it reduced the hepatosomatic index, viscerosomatic index, condition factor, and intraperitoneal fat ratio ($p < 0.05$). While muscle basic nutrition was unchanged, FMLP improved post-mortem muscle water-holding capacity, drip loss, cooking yield, and texture ($p < 0.05$). FMLP lowered malondialdehyde and elevated glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities in grass carp ($p < 0.05$). Concurrently, the gene expression of *GSH-Px*, *Nrf2*, and *SOD* was upregulated in the liver ($p < 0.05$). Regarding lipid metabolism, FMLP increased serum high-density lipoprotein cholesterol and decreased hepatic total cholesterol and triacylglycerol ($p < 0.05$). It promoted gene expression related to fatty acid oxidation (*PPAR α* , *CPT-1a*, *CPT-1b*) while inhibiting the genes involved in lipogenesis (*SREBP*, *FAS*). Quadratic regression analysis determined that an inclusion level of approximately 7.55% to 11.40% (with the data suggesting an optimal range near 8–10%) improves muscle quality, enhances antioxidant defenses, and promotes healthier lipid metabolism in grass carp.

Keywords: fermented mulberry leaves; muscle quality; antioxidation; fat metabolism; grass carp



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Key Contribution: Adding 8–10% fermented mulberry powder does not impair the growth performance of grass carp, while also improves meat quality and regulates lipid metabolism.

1. Introduction

Grass carp (*Ctenopharyngodon idellus*) is valued for its tender flesh, delicious taste, and rich nutritional profile, making it an important source of high-quality aquatic protein. Large-scale grass carp farming depends heavily on the use of high-quality protein ingredients such as fish meal and soybean meal [1]. However, fish meal and soybean meal are often

in short supply on the market, leading to a significant increase in grass carp farming costs. Therefore, the development and utilization of high-quality protein feed ingredient alternatives to fish meal and soybean meal have become a research focus for reducing grass carp production costs [2].

Mulberry leaves are rich in protein, amino acids, fatty acids, vitamins, minerals, and various bioactive substances (such as quercetin, chlorogenic acid, etc.), exhibiting multiple pharmacological activities including antioxidant and immunomodulatory effects [3,4]. However, raw mulberry leaves contain antinutritional factors such as fiber and tannins, which limit their direct application in fish feed. Microbial fermentation can effectively improve the feeding value of mulberry leaves. Research has shown that after fermentation, the content of antinutritional factors such as fiber and tannins in mulberry leaves significantly decreases, while the levels of nutrients including crude protein, crude fat, amino acids, and minerals relatively increase [2]. Meanwhile, the fermentation process also enhances the polysaccharide content and hydroxyl radical scavenging capacity of mulberry leaves, strengthening their functional properties [3,4]. Preliminary research by our team has revealed that co-fermentation using yeast and lactic acid bacteria can effectively reduce the tannin content in mulberry leaves. Moreover, supplementation with fermented mulberry leaf powder (FMLP) at a 5% level significantly promotes growth performance, enhances antioxidant capacity, and increases intestinal enzyme activities in crucian carp [5].

To further explore the application value of FMLP in grass carp, this study, based on the nutritional requirements of grass carp, reduced the fish meal inclusion level and incorporated fermented mulberry leaf powder to formulate isolipidic and isonitrogenous basal diets. Building on this foundation and incorporating the team's previous research findings, the study further investigated the effects of supplementing the diet with 5%, 10%, and 15% FMLP on the growth, muscle quality, antioxidant capacity, and lipid metabolism of large-sized grass carp, as well as the optimal inclusion level. The results showed that the addition of FMLP, without negatively affecting growth performance, significantly reduced the hepatosomatic index and viscerosomatic index, improved muscle quality, enhanced antioxidant capacity, promoted lipid metabolism, and inhibited excessive lipid deposition in grass carp. These findings provide a reference for the application of fermented mulberry leaves in aquaculture, the development of high-quality unconventional feed resources, and the formulation of environmentally friendly feed for grass carp.

2. Materials and Methods

2.1. Experimental Design

2.1.1. FMLP Fermentation Method and Feed Preparation

Based on the research findings of Zhu et al. [5] on the screening of tannin-degrading bacteria and fermentation techniques for mulberry leaves, the fermented mulberry leaves used in this experiment were prepared as follows: solid-state fermentation with a solid-to-liquid ratio of 1:1.1; compound fermentation using lactic acid bacteria and yeast; an inoculum size of 9.8%, and a fermentation duration of 44.6 h. The feed was formulated according to the nutritional standard for formulated feed of grass carp (GB/T 36205-2018) [6] (Table 1). The feed ingredients were ground and passed through a 60-mesh sieve, uniformly mixed using the gradual expansion method, and then processed into 3.0 mm diameter pellets using an SLX-80 pellet feed machine. The pellets were air-dried naturally and stored in a freezer at -20°C until use. The conventional nutritional composition of FMLP is shown in Table S1 [5]. All feed ingredients used in the experiment were purchased from COFCO Group Co., Ltd. The fish meal was red fish meal, Grade 2 (GB/T 19164-2021) [7]; the soybean meal (GB/T 19541-2017) [8], cottonseed meal (GB/T 21264-2007) [9], rapeseed meal (GB/T 23736-2009) [10], wheat bran (NY/T 119-2021) [11], rice bran (NY/T

122-1989) [12], corn meal (GB/T 17890-2008) [13], and wheat flour (GB/T 1355-2021) [14] were all Grade 2 feed ingredients, while the soybean oil (GB/T 1535-2017) [15] was Grade 3.

Table 1. Feed composition and nutrient level (%).

Materials	CON	5% FMLP	10% FMLP	15% FMLP
Fish meal	1.50	1.50	1.50	1.50
Soybean meal (43%)	17.00	17.00	17.00	17.00
Cottonseed meal	12.00	12.00	12.00	12.00
Wheat flour	18.00	18.00	18.00	18.00
Corn meal	5.00	4.00	3.00	1.00
Soybean oil	0.40	0.60	0.80	1.10
Rapeseed meal	16.40	16.50	16.20	16.10
Wheat bran	13.00	11.00	10.00	9.00
Rice bran	10.00	8.00	6.00	4.00
FMLP	0.00	5.00	10.00	15.00
Ca(H ₂ PO ₄) ₂	1.50	1.60	1.70	1.80
Bentonite	1.70	1.30	0.30	0.00
Premix ¹	3.50	3.50	3.50	3.50
Nutritional level ²				
Crude protein	26.14	26.14	26.14	26.15
Crude fat	3.80	3.75	3.72	3.76
Crude fibre	6.30	6.48	6.69	6.90
Total phosphorus	1.17	1.16	1.16	1.16
Lysine	1.31	1.33	1.34	1.35
Sulfur amino acid	1.02	1.02	1.01	1.00

¹ The premix provides the following per kg of complete feed: VA, 2000 IU; VD3, 400 IU; VE 100 IU, VK3 10 mg; VB1, 5 mg; VB2, 10 mg; calcium pantothenate, 40 mg; nicotinic acid 100 mg, VB6 10 mg; biotin, 1 mg; folic acid, 5 mg; VB12, 0.02 mg; VC, 300 mg; inositol, 200 mg; antioxidant, 1250 mg; betaine, 1000 mg; NaCl, 5000 mg; Cu (as copper sulfate), 6 mg; Fe (as ferrous sulfate), 160 mg; Mn (as manganese sulfate), 33 mg; Zn (as zinc sulfate), 120 mg; I (as potassium iodide), 0.3 mg; Se (as sodium selenite), 0.2 mg; Co (as cobalt sulfate), 5 mg. ² Except for lysine and sulfur amino acids, all other nutrients are measured values. Abbreviation: CON = control group; FMLP = fermented mulberry leaf powder.

2.1.2. Experimental Design for Grass Carp Feeding

A total of 360 grass carp of similar body size (0.86 ± 0.07 kg) were randomly assigned to four treatment groups, each with three replicates of 30 fish per replicate. The fish were fed experimental diets containing 0%, 5%, 10%, or 15% FMLP for a 70-day feeding trial. Fish were fed to satiation twice daily (07:00 and 17:00), and the feed amount was adjusted every three days based on the growth performance of the grass carp. During the rearing period, the water temperature ranged from 26 °C to 30 °C, pH from 7 to 9, dissolved oxygen from 3 to 5 mg/L, ammonia nitrogen was below 0.5 mg/L, and nitrite nitrogen was below 0.005 mg/L. Although dissolved oxygen ranged from 3 to 5 mg/L, these levels are within the acceptable range for adult grass carp under the experimental conditions [16].

2.2. Sample Collection

At the end of the feeding trial, all grass carp were fasted for 12 h, and then collected, counted by replicate and weighed; the data were used to calculate the weight gain ratio, specific growth ratio, and feed conversion ratio. Two fish with body weights close to the average were randomly selected from each replicate, anesthetized with clove oil, and blood samples were collected from the caudal vein using a 1 mL medical syringe. Blood was centrifuged at 3500 rpm for 10 min, and the serum was separated and stored at −20 °C for subsequent analysis of serum biochemical parameters. The fish were then dissected on an ice tray. Liver samples were collected, immediately frozen in liquid nitrogen, and stored at −80 °C for gene expression analysis by real-time quantitative PCR. Dorsal muscle samples were collected for analysis of muscle texture characteristics, routine nutrient

composition, and muscle quality. Additionally, three fish with body weights close to the average were selected from each replicate, anesthetized with clove oil, weighed individually, and measured for body length and body height. They were then dissected on an ice tray to separate the viscera, hepatopancreas, and visceral fat, and each was weighed separately to calculate indices.

2.3. Growth Performance

After the experiment, the evaluation of grass carp growth performance and morphological indices primarily included initial body weight (IBW), final body weight (FBW), weight gain ratio (WGR), specific growth ratio (SGR), feed conversion ratio (FCR), condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), and intraperitoneal fat index (IPFI). The calculation formulas were adapted from previous studies [1,16], as shown below:

$$\text{WGR (\%)} = (\text{final body weight} - \text{initial body weight}) / \text{initial body weight} \times 100$$

$$\text{FCR} = \text{total amount of the feed consumed} / (\text{final body weight} - \text{initial body weight})$$

$$\text{SGR (\%/d)} = (\text{final body weight} - \text{initial body weight}) / \text{day} \times 100$$

$$\text{CF (g/cm}^3\text{)} = \text{final body weight} / (\text{body length})^3 \times 100$$

$$\text{HSI (\%)} = \text{Liver weight} / \text{body weight} \times 100$$

$$\text{VSI (\%)} = (\text{viscera weight} / \text{body weight}) \times 100$$

$$\text{IPFI (\%)} = (\text{Abdominal lipid weight} / \text{bodyweight}) \times 100$$

2.4. Determination of Body Composition and Meat Quality

The determination of grass carp body composition was performed according to the method of Xu et al. [17] with the measured indicators mainly including moisture, crude protein, and crude ash. The determination of grass carp muscle quality was performed according to the methods of Gao et al. [18] and Li et al. [19], with the measured indicators mainly including pH values at 45 min and 24 h, water-holding capacity, shear force, drip loss, cooking loss, and texture properties (hardness, springiness, gumminess, cohesiveness, chewiness).

2.5. Determination of Hydrolyzed Amino Acid Content in Muscle

The determination method of Zhao et al. [20] was used, employing an automated amino acid analyzer (L-8080, Hitachi, Tokyo, Japan). The operating procedure was briefly as follows: accurately weigh 0.1 g of freeze-dried muscle sample, then pipette 1 mL of hydrochloric acid (6 mol/L) into a hydrolysis tube sealed with an alcohol blowtorch (110 °C, 24 h) for hydrolysis. After washing with deionized water, dilute the solution to a volume of 50 mL. Pipette 1 mL of the filtrate, pass it through a 0.22 µm filter membrane, and collect the filtrate in a brown sample vial. Subsequently, load the sample into the instrument to determine the amino acid content.

2.6. Physiological and Biochemical Parameter Testing

Grass carp muscle levels of malondialdehyde (MDA), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and total antioxidant capacity (T-AOC) were quantified using a microplate reader (Infinite M PLEX; Tecan, Victoria, Austria). Both serum and hepatic cholesterol (TC), triacylglycerol (TG), MDA, T-SOD, GSH-Px, and T-AOC levels were measured with the same microplate reader (Infinite M PLEX; Tecan, Victoria, Austria).

using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocols.

2.7. Detection of Liver Antioxidant and Lipid Metabolism-Related Indicators

The expression of genes related to antioxidant defenses and lipid metabolism in the liver was detected by RT-qPCR. Total RNA was extracted from the tissues using the RNA extraction kit (SteadyPure, Ecoray Biology, Changsha, China). The reverse transcription kit (Evo M-MLVA, Ecoray Biology, Changsha, China) was utilized to obtain cDNA, and the quantitative kit (AG11701, Ecoray Biology, Changsha, China) was used to detect the relative expression of genes. Primers were synthesized in Beijing Qingke Biotechnology Co., Ltd., Beijing, China, and their sequences are listed in Table 2. The reaction procedure was based on the study by Zhang et al. [21]. The gene expression levels were analyzed using the $2^{-\Delta\Delta C_t}$ method after normalization to the internal reference genes β -actin and 18S.

Table 2. Sequences of primers.

Gene	Primer	Sequences (5'→3')	Accession Number
<i>PPARγ</i>	<i>PPARγ-F</i>	CTGAAGACCAACCATCCAGAC	EU847421.1
	<i>PPARγ-R</i>	GTGTAAGCACCAGTCCAACCTCC	
<i>PPARα</i>	<i>PPARα-F</i>	CAAATGCAAAGCACGGACCA	FJ623265.1
	<i>PPARα-R</i>	GTGCACTGGCAGCAATGAAA	
<i>ATGL</i>	<i>ATGL-F</i>	GTGTCACCTCACACGGGTCAT	HQ845211.2
	<i>ATGL-R</i>	TGGTGTCTTTCAGCTCCGAC	
<i>HSL</i>	<i>HSL-F</i>	CGAAACTTTGACAGGCACGG	HQ446238.1
	<i>HSL-R</i>	CGACACTCGCGCATTTTCAT	
<i>CPT-1a</i>	<i>CPT-1a-F</i>	ATGGAGAGTGGACGGGCTAT	KP262350.1
	<i>CPT-1a-R</i>	GGCCAGACCTGTAAACGTGA	
<i>SREBP</i>	<i>SREBP-F</i>	AAGCAGAACAGAGGAGTGCG	KJ162572.1
	<i>SREBP-R</i>	GAAGGAAGGGGGCAGATAGC	
<i>CPT-1b</i>	<i>CPT-1b-F</i>	CCCCTCAACAGCAGCTCAAT	KT601069.1
	<i>CPT-1b-R</i>	ACCGTCATCAGCAACAGGC	
<i>FAS</i>	<i>FAS-F</i>	TGGGGTCGTTTCAGCCATTAC	MK111644.1
	<i>FAS-R</i>	ACTTTGCCGATGTGTTTGCC	
<i>ACC</i>	<i>ACC-F</i>	AGGTGGACCAGAGGTTCCAT	XM_051892882.1
	<i>ACC-R</i>	GCATCTTATGGTTGGCGCAG	
<i>Nrf2</i>	<i>Nrf2-F</i>	AAGCCTGGATGGAGTTGCTC	KX243419.1
	<i>Nrf2-R</i>	CTGTGCTTCTCCGGAAGGTT	
<i>Keap</i>	<i>Keap-F</i>	TCTTCGAAAACCCCTGCCTC	KF811013.1
	<i>Keap-R</i>	ATCCCATGTCAGCCAGCTTC	
<i>SOD</i>	<i>SOD-F</i>	GGACCAACCGATAGTGAAAG	XM_051910923.1
	<i>SOD-R</i>	GAACAAAGCAGAAATCAGAGG	
<i>GSH-Px</i>	<i>GSH-Px-F</i>	GGGCTGGTTATTCTGGGC	EU828796.2
	<i>GSH-Px-R</i>	AGGCGATGTCATTCCTGTTC	
β -actin	β -actin-F	GCTATGTGGCTCTTGACTTCG	DQ211096.1
	β -actin-R	GGGCACCTGAACCTCTCATT	
18S	18S-F	ATTTCGACACGGAGAGG	XR_007928648.1
	18S-R	CATGGGTTTAGGATACGCTC	

2.8. Regression Analysis

SPSS 26.0 (Version 26.0, IBM, USA) was used to conduct quadratic regression analysis with the additive amount of FMLP as the independent variable and the significant difference index of grass carp as the dependent variable, and the quadratic function, R^2 and ANOVA values were obtained. The ANOVA value less than 0.05 indicated that there was a significant quadratic relationship between the dose and the difference index of grass carp. Then,

according to the quadratic function, Microsoft Excel 2021 (Version 2021, Microsoft Corp, USA) was used to calculate the optimal dose value of FMLP [21].

2.9. Statistical Analysis

Data were collated in Microsoft Excel 2021 (Microsoft Corp, USA). Outliers were identified using Grubbs' test ($\alpha = 0.05$) and removed via SPSS 26.0 (IBM, USA), followed by Shapiro–Wilk normality tests and Levene's variance homogeneity tests. To avoid pseudoreplication, the experimental unit for statistical analysis was defined according to the nature of each response variable. For growth performance, survival, feed conversion, and morphometric indices (e.g., HSI, VSI, IPFI, CF), data from individual fish within the same tank were first averaged to obtain a single value per tank. These tank means were then used as the experimental unit ($n = 3$ per treatment) for one-way ANOVA. For meat quality, biochemical parameters (serum, liver, muscle), and gene expression, measurements were performed on individual fish ($n = 6$ per treatment). One-way ANOVA and Duncan's multiple-range test were applied, $p < 0.05$ indicates a significant difference. Linear and quadratic analyses were conducted using the SPSS regression curve estimation model, and $p < 0.05$ was considered statistically significant. Results were expressed as mean and standard error of the mean. Graphs were generated using OriginPro 2022 (Version 2022, OriginLab, USA).

3. Results

3.1. FMLP Improves the Growth Performance of Grass Carp

As shown in Table 3, the inclusion of FMLP in the diet had no significant effects on FBW, WGR, FCR, or SGR in grass carp ($p > 0.05$). However, it reduced HSI, VSI, IPFI, and CF, with these changes exhibiting both linear and quadratic trends ($p < 0.05$). With increasing levels of FMLP supplementation, the CF, VSI, and HSI of grass carp decreased ($p < 0.05$). Furthermore, the addition of FMLP reduced the IPFI of grass carp, with the lowest value observed in the group receiving 10% FMLP ($p < 0.05$).

Table 3. Effects of FMLP on the growth performance in grass carp.

Items	CON	FMLP Supplementation Dosage			SEM	<i>p</i> -Value		
		5%	10%	15%		Treatment	Linear	Quadratic
IBW, kg	0.85	0.87	0.87	0.85	0.069	0.981	0.947	0.909
FBW, kg	1.82	1.82	1.88	1.83	0.089	0.890	0.661	0.839
SGR, %/d	1.09	1.11	1.18	1.11	0.051	0.398	0.409	0.356
WGR, %	114.40	117.92	127.97	117.65	8.176	0.428	0.473	0.405
FCR, %	3.11	2.97	2.69	2.99	0.200	0.263	0.359	0.247
HSI, %	2.10 ^a	1.95 ^{ab}	1.64 ^{bc}	1.56 ^c	0.189	0.023	0.002	0.010
VSI, %	7.89 ^a	7.96 ^{ab}	6.95 ^{bc}	6.59 ^c	0.197	<0.001	<0.001	<0.001
IPFI, %	1.66 ^a	1.49 ^b	1.18 ^c	1.41 ^{bc}	0.103	0.001	0.008	0.001
CF	2.04 ^{ab}	2.11 ^a	1.96 ^b	1.95 ^b	0.049	0.008	0.016	0.037

Results were expressed as mean and standard error of mean. Different lowercase letters indicate significant differences. The statistical analysis sample size for IBW, FBW, WGR, SGR, and FCR is three, and for HSI, VSI, IPFI, and CF, it is six. Abbreviation: IBW = initial body weight; FBW = final body weight; WGR = weight gain ratio; SGR = specific growth ratio; FCR = feed conversion ratio; HSI = hepatosomatic index; VSI = viscerosomatic index; IPFI = intraperitoneal fat index; CF = condition factor; CON = control group; FMLP = fermented mulberry leaf powder.

3.2. FMLP Improves the Body Composition and Meat Quality of Grass Carp Muscle

The inclusion of FMLP increased the water-holding capacity, cooking yield, and hardness of grass carp muscle, while reducing drip loss, chewiness, cohesiveness, and springiness, exhibiting linear or quadratic trends ($p < 0.05$; Table 4). However, it had no

significant effects on muscle moisture, crude protein, crude ash, shear force, adhesiveness, gumminess, or pH values at 45 min and 24 h ($p > 0.05$; Table 4). Compared with the control group, drip loss was reduced in the 5% and 10% FMLP groups; the cooking yield and water-holding capacity were increased in the 5% FMLP group, while chewiness, cohesiveness, and springiness were decreased in the 10% FMLP group ($p < 0.05$; Table 4). Additionally, supplementation with 15% FMLP increased muscle hardness and decreased springiness in grass carp ($p < 0.05$; Table 4).

Table 4. Effects of FMLP on the body composition and muscle meat quality in grass carp.

Items	CON	FMLP Supplementation Dosage			SEM	<i>p</i> -Value		
		5%	10%	15%		Treatment	Linear	Quadratic
Moisture, %	79.55	79.36	79.61	79.56	0.305	0.866	0.776	0.919
Crude protein, %	19.98	20.11	19.79	19.76	0.339	0.687	0.344	0.608
Crude ash, %	0.34	0.35	0.25	0.34	0.059	0.283	0.692	0.584
pH _{45 min}	6.30	6.43	6.49	6.32	0.094	0.164	0.631	0.085
pH _{24 h}	6.20	6.23	6.26	6.23	0.044	0.572	0.354	0.380
Water holding capacity, %	64.15 ^b	67.82 ^a	65.04 ^b	63.35 ^b	1.269	0.008	0.265	0.013
Cooking yield, %	71.94 ^b	74.54 ^a	72.87 ^b	72.55 ^b	0.783	0.015	0.951	0.048
Drop loss, %	1.72 ^a	1.48 ^b	1.41 ^b	1.68 ^a	0.086	0.002	0.576	0.001
Shear force, N	7.57	7.26	7.80	7.49	0.678	0.881	0.885	0.990
Hardness, N	24.01 ^b	23.30 ^b	23.76 ^b	33.22 ^a	2.489	0.002	0.005	0.001
Chewiness, MJ	4.66 ^{ab}	5.70 ^a	2.79 ^c	3.64 ^{bc}	0.777	0.008	0.049	0.149
Adhesiveness, N·mm	0.11	0.10	0.11	0.09	0.007	0.007	0.222	0.058
Cohesiveness	0.17	0.16	0.13	0.15	0.016	0.102	0.080	0.095
Springiness, mm	1.08 ^a	1.08 ^a	0.83 ^b	0.92 ^b	0.056	<0.001	0.002	0.007
Gumminess, N	4.91	4.31	3.76	4.67	1.010	0.771	0.830	0.641

Results were expressed as mean and standard error of mean ($n = 6$). Different lowercase letters indicate significant differences. Abbreviation: CON = control group; FMLP = fermented mulberry leaf powder.

3.3. FMLP Had No Significant Effect on the Hydrolyzed Amino Acid in Grass Carp Muscle

The addition of different doses of FMLP had no significant effects on the hydrolyzed amino acid content in grass carp muscle ($p > 0.05$; Table 5). There were no significant differences among the groups in amino acid content and composition, including total amino acid content, total essential amino acid content, and total umami amino acid content. Furthermore, neither linear nor quadratic trends were significant ($p > 0.05$; Table 5).

Table 5. Effect of FMLP on the content and composition of hydrolyzed amino acids in grass carp muscle (g/100 g dry weight).

Items	CON	FMLP Supplementation Dosage			SEM	<i>p</i> -Value		
		5%	10%	15%		Treatment	Linear	Quadratic
EAA								
Lysine	1.93	1.90	1.87	1.92	0.427	0.620	0.865	0.493
Methionine	0.61	0.55	0.56	0.61	0.393	0.320	0.857	0.165
Arginine	1.16	1.14	1.15	1.17	0.192	0.798	0.640	0.608
Threonine	0.86	0.84	0.84	0.86	0.192	0.737	0.752	0.524
Leucine	1.61	1.57	1.55	1.52	0.027	0.350	0.815	0.216
Isoleucine	0.90	0.87	1.55	1.62	0.045	0.301	0.732	0.171
Histidine	0.70	0.69	0.68	0.69	0.022	0.936	0.661	0.807
Phenylalanine	0.89	0.88	0.85	0.88	0.023	0.301	0.380	0.262
Valine	0.96	0.91	0.91	0.97	0.031	0.247	0.952	0.120

Table 5. Cont.

Items	CON	FMLP Supplementation Dosage			SEM	<i>p</i> -Value		
		5%	10%	15%		Treatment	Linear	Quadratic
NEAA								
Alanine	1.15	1.08	1.07	1.11	0.400	0.207	0.450	0.100
Cysteine	0.20	0.14	0.16	0.19	0.034	0.390	0.991	0.221
Glutamic acid	3.34	3.27	3.28	3.34	0.079	0.726	0.968	0.516
Glycine	0.88	0.86	0.85	0.86	0.021	0.625	0.247	0.409
Proline	0.61	0.58	0.58	0.57	0.019	0.272	0.083	0.166
Serine	0.79	0.77	0.77	0.78	0.019	0.672	0.878	0.456
Tyrosine	0.72	0.70	0.69	0.73	0.034	0.426	0.864	0.264
Aspartic acid	2.01	1.98	1.98	2.02	0.428	0.772	0.786	0.578
EAA	7.75	7.53	7.45	7.78	0.209	0.326	0.961	0.186
NEAA	11.56	11.22	11.22	11.49	0.253	0.472	0.792	0.277
TAA	19.30	18.75	18.67	19.27	0.458	0.390	0.899	0.216
FAA	9.00	8.77	8.73	8.96	0.209	0.502	0.788	0.302

Results were expressed as mean and standard error of mean ($n = 6$). Abbreviation: CON = control group; FMLP = fermented mulberry leaf powder; EAA = essential amino acids; FAA = flavor amino acid, include Ala, Asp, Glu, Gly and Ser; NEAA = non-essential amino acids; TAA = total amino acid, include EAA and NEAA.

3.4. FMLP Improved the Lipid Metabolism and Antioxidant Capacity of Grass Carp

As shown in Table 6, the addition of FMLP increased the activities of T-SOD in the serum, liver, and muscle of grass carp, elevated the levels of T-AOC in the liver and muscle, reduced the content of MDA in the serum and liver, enhanced the activity of GSH-Px in the serum and liver, and decreased the levels of TC and TG in the liver. It also increased the concentration of HDL-C in the serum ($p < 0.05$). Except for liver TG, all other indicators with significant differences exhibited linear or quadratic trends ($p < 0.05$). However, the addition of FMLP had no significant effects on the concentrations of LDL-C, TC, TG, and T-AOC in the serum, GSH-Px activity in the liver, or MDA content in the muscle of grass carp ($p > 0.05$).

In the serum, compared to the control group, the FMLP groups showed increased concentrations of HDL-C, T-SOD, and GSH-Px, along with a reduction in MDA concentration ($p < 0.05$). Among these, the 5% FMLP group exhibited the highest T-SOD and GSH-Px activities and the lowest MDA concentration ($p < 0.05$). Additionally, the 10% FMLP group had the highest HDL-C concentration ($p < 0.05$).

In the liver of grass carp, compared to the control group, the FMLP groups showed increased levels of T-AOC and T-SOD, along with reduced contents of TC, TG, and MDA ($p < 0.05$). Among these, the 10% FMLP group had the lowest TG and MDA contents ($p < 0.05$). Furthermore, the 15% FMLP group exhibited the highest T-AOC and T-SOD activity and the lowest TC content ($p < 0.05$), though these values were not significantly different from those of the 10% FMLP group ($p > 0.05$).

In the muscle of grass carp, compared to the control group, the FMLP groups showed increased levels of T-AOC, T-SOD, and GSH-Px ($p < 0.05$). Among these, the 10% FMLP group had the highest T-SOD activity ($p < 0.05$). Additionally, the 15% FMLP group exhibited the lowest T-AOC and GSH-Px contents ($p < 0.05$), though its GSH-Px activity was not significantly different from that of the 10% FMLP group ($p > 0.05$).

Table 6. Effects of FMLP on lipid metabolism and antioxidant in grass carp.

Items	CON	FMLP Supplementation Dosage			SEM	<i>p</i> -Value		
		5%	10%	15%		Treatment	Linear	Quadratic
Serum								
TC, mmol/L	6.04	5.95	6.40	5.88	0.294	0.264	0.842	0.552
TG, mmol/L	1.18	1.11	1.16	1.13	0.081	0.840	0.709	0.858
HDL-C, mmol/L	2.71 ^b	3.01 ^a	3.12 ^a	2.57 ^b	0.124	0.001	0.575	<0.001
LDL-C, mmol/L	2.26	2.05	2.42	2.37	0.138	0.079	0.247	0.445
MDA, nmol/mL	8.93 ^a	6.14 ^{bc}	6.70 ^b	5.24 ^c	0.516	<0.001	<0.001	<0.001
T-AOC, U/mL	7.87	8.17	8.23	7.16	0.629	0.407	0.379	0.246
T-SOD, U/mL	351.16 ^b	442.63 ^a	406.40 ^{ab}	342.87 ^b	32.956	0.017	0.574	0.008
GSH-Px, U/mL	496.91 ^b	537.78 ^a	538.44 ^a	543.90 ^a	12.337	0.004	0.003	0.002
Liver								
TC, umol/gprot	165.04 ^a	146.49 ^{ab}	137.11 ^{bc}	120.36 ^c	10.882	0.005	<0.001	0.001
TG, umol/gprot	317.44 ^a	329.26 ^a	221.63 ^b	299.31 ^a	34.364	0.023	0.213	0.240
MDA, nmol/mgprot	1.11 ^a	0.81 ^b	0.70 ^b	0.71 ^b	0.065	<0.001	<0.001	<0.001
T-AOC, U/mgprot	0.22 ^b	0.30 ^a	0.33 ^a	0.30 ^a	0.024	0.001	0.004	<0.001
T-SOD, U/mgprot	243.09 ^c	260.76 ^b	272.78 ^{ab}	286.43 ^a	11.047	0.006	<0.001	0.002
GSH-Px, U/mgprot	2521.05	2534.02	2580.68	2558.84	203.555	0.991	0.797	0.961
Muscle								
MDA, mmol/gprot	3.00	2.85	2.61	2.54	0.218	0.158	0.072	0.076
T-AOC, U/mgprot	0.11 ^c	0.21 ^b	0.20 ^b	0.25 ^a	0.017	<0.001	<0.001	<0.001
T-SOD, U/mgprot	85.27 ^c	161.74 ^a	134.82 ^b	127.01 ^b	11.215	<0.001	0.108	<0.001
GSH-Px, U/mgprot	352.05 ^c	465.71 ^b	425.89 ^b	524.27 ^a	24.340	<0.001	<0.001	<0.001

Results were expressed as mean and standard error of mean ($n = 6$). Different lowercase letters indicate significant differences. Abbreviation: CON = control group; FMLP = fermented mulberry leaf powder; MDA = malondialdehyde; T-SOD = total superoxide dismutase; GSH-Px = glutathione peroxidase; T-AOC = total antioxidant capacity; TC = cholesterol; HDL-C = high density lipoprotein cholesterol; TG = triacylglycerol; LDL-C = low density lipoprotein cholesterol.

3.5. FMLP Regulates Hepatic Antioxidant Genes and Fat Metabolism Genes

The FMLP upregulated the relative expression levels of *Nrf2*, *SOD*, *GSH-Px*, *ATGL*, *CPT-1a*, *CPT-1b*, *HSL*, *PPAR α* , and *SREBP* genes in the liver of grass carp, while downregulating the relative expression levels of *ACC*, *FAS*, and *PPAR γ* genes, all of which exhibited either linear or quadratic trends ($p < 0.05$; Figure 1). However, FMLP had no significant effect on the relative expression level of the *Keap* gene in the liver of grass carp ($p > 0.05$; Figure 1). Compared with other groups, the 15% FMLP group showed the highest relative expression levels of *Nrf2*, *SOD*, and *GSH-Px* genes ($p > 0.05$; Figure 1A). Compared with the control group, the relative expression levels of *Nrf2*, *SOD*, and *GSH-Px* genes were upregulated in the 10% FMLP group, and the relative expression level of the *Nrf2* gene was upregulated in the 5% FMLP group ($p < 0.05$; Figure 1A). Compared with other groups, the relative expression levels of the lipid synthesis-related genes *ACC* and *FAS* were the lowest in the 5% and 10% FMLP groups, while the relative expression levels of the lipolysis-related genes *CPT-1a*, *CPT-1b*, and *HSL* were the highest in the 10% FMLP group, and the relative expression level of the *ATGL* gene was the highest in the 15% FMLP group ($p < 0.05$; Figure 1B). Furthermore, the relative expression levels of the transcriptional regulatory factors *PPAR α* (upregulated) and *SREBP* genes (downregulated) peaked in the 10% FMLP group, whereas that of *PPAR γ* was the lowest in the 5% FMLP group ($p < 0.05$; Figure 1B).

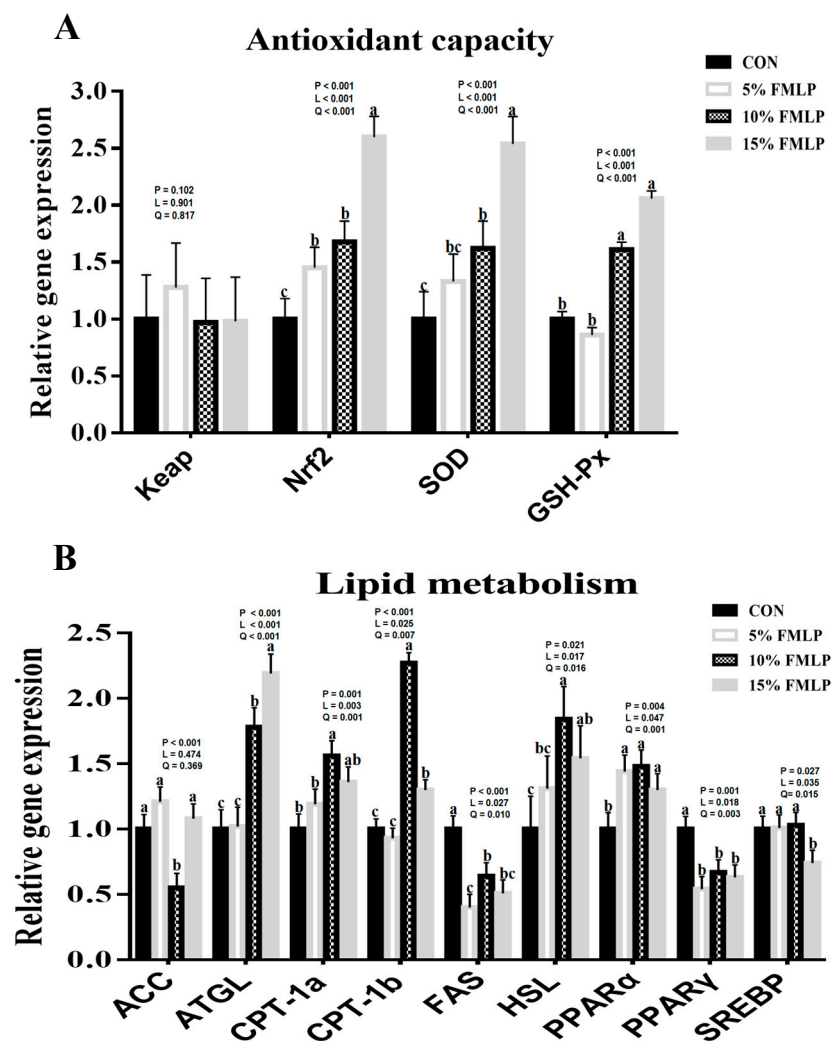


Figure 1. Effects of FMLP on hepatic antioxidant capacity and lipid metabolism in grass carp. Results are expressed as mean and standard error of mean ($n = 6$). Different lowercase letters indicate significant differences. (A). Gene expression levels related to antioxidant capacity in grass carp liver; (B). Gene expression levels related to lipid metabolism in grass carp liver. Abbreviation: CON = control group; FMLP = fermented mulberry leaf powder. P, L, and Q in the figure denote p -value for treatment effect, linear trend, and quadratic trend, respectively.

3.6. Regression Analysis and Optimal Dose Screening

As shown in Table 7, regression analysis indicated that the optimal dosages of FMLP for quadratic changes in meat quality-related indicators in grass carp muscle, namely, water holding capacity, cooking yield, and drip loss, were 6.53%, 7.55%, and 7.97%, respectively ($p < 0.05$). For antioxidant capacity-related indicators in grass carp, the optimal dosages for maximizing T-SOD and *Nrf2* in liver were 8.67% and 18.65%, respectively; for minimizing liver MDA and maximizing liver T-AOC, the optimal dosages were 11.59% and 10.46%, respectively; and for maximizing serum T-SOD and GSH-Px, the optimal dosages were 7.04% and 11.40%, respectively ($p < 0.05$). Regarding lipid metabolism-related indicators in grass carp, the optimal dosage for maximizing serum HDL-C was 7.14%, while the optimal dosages for maximizing the relative expression levels of hepatic *HSL*, *CPT-1a*, *CPT-1b*, and *PPARα* were 11.04%, 11.21%, 9.98%, and 9.03%, respectively ($p < 0.05$). The optimal dosages for minimizing the relative expression level of hepatic *FAS* and *PPARγ* were 10.06% and 9.76%, respectively ($p < 0.05$). In conclusion, the inclusion of FMLP in grass carp diets at a dosage range of 7.55% to 11.40% can improve muscle quality, antioxidant capacity, and lipid metabolism in grass carp.

Table 7. Investigation of the optimal supplementation level of FMLP.

Items	Regression Equation	R ²	p-Value	Optimal Supplemental Level (%)
Muscle quality				
Water holding capacity	$y = -0.0536x^2 + 0.7004x + 64.527$	0.750	0.013	6.53
Cooking yield	$y = -0.0292x^2 + 0.4412x + 72.221$	0.575	0.048	7.55
Drop loss	$y = 0.0051x^2 - 0.0803x + 1.7285$	0.979	0.001	7.97
Antioxidant capacity				
Muscle-T-SOD	$y = -0.8428x^2 + 14.608x + 91.395$	0.751	<0.001	8.67
Liver-Nrf2	$y = 0.0017x^2 + 0.0634x + 1.0601$	0.686	<0.001	18.65
Liver-MDA	$y = 0.0032x^2 - 0.0742x + 1.1098$	0.720	<0.001	11.59
Liver-T-AOC	$y = -0.001x^2 + 0.0208x + 0.2221$	0.531	<0.001	10.40
Serum-T-SOD	$y = -1.5871x^2 + 22.361x + 356$	0.368	0.008	7.04
Serum-GSH-Px	$y = -0.3592x^2 + 8.1892x + 499.14$	0.446	0.002	11.40
Lipid metabolism				
Serum-HDL-C	$y = -0.0086x^2 + 0.1228x + 2.6833$	0.729	<0.001	7.14
Liver-CPT-1a	$y = -0.0039x^2 + 0.0874x + 0.9622$	0.467	0.001	11.21
Liver-CPT-1b	$y = -0.009x^2 + 0.1798x + 0.814$	0.376	0.007	9.98
Liver-FAS	$y = 0.004x^2 - 0.0806x + 0.8728$	0.355	0.010	10.06
Liver-HSL	$y = -0.0061x^2 + 0.1347x + 0.9468$	0.324	0.016	11.04
Liver-PPAR α	$y = -0.0062x^2 + 0.112x + 1.0093$	0.471	0.001	9.03
Liver-PPAR γ	$y = 0.0042x^2 - 0.082x + 0.9624$	0.431	0.003	9.76

4. Discussion

This study aimed to investigate the effects of FMLP on growth performance, muscle quality, antioxidant capacity, and lipid metabolism in grass carp (0.86 ± 0.07 kg). The experimental results showed that although dietary supplementation with FMLP did not significantly promote the growth of grass carp, it effectively reduced the HSI, VSI, and IPFI, improved muscle water-holding capacity and texture characteristics, and enhanced antioxidant capacity while optimizing lipid metabolism. In this study, no significant differences were observed in WGR, SR, or FCR between the FMLP-supplemented groups and the control group, indicating that FMLP can be used as a safe protein ingredient in grass carp feed without compromising growth performance. This finding is consistent with the study by Zhu et al. [5] on crucian carp, which reported that dietary supplementation with 5–10% fermented mulberry leaf improved immunity without negatively affecting growth. It is worth noting that the initial body weight of grass carp in this study was relatively large (0.86 kg), and their growth rate was inherently lower than that of juveniles, which may have partially masked the growth-promoting effects of FMLP. However, when FMLP supplementation exceeded 10%, growth performance, serum antioxidant capacity, and intestinal digestive enzyme activities of crucian carp decreased, and the homeostasis of the intestinal microbiota was disrupted [5]. In this study, grass carp exhibited good tolerance to 15% FMLP, which may be attributed to the fact that herbivorous fish have greater adaptability to plant-based feed ingredients than omnivorous fish.

Notably, muscle quality is a key factor influencing the commercial value of grass carp. In this study, although FMLP supplementation did not alter the conventional nutritional composition or amino acid profile of the muscle, it significantly improved muscle water-holding capacity, cooking loss, drip loss, and texture characteristics. The 5% FMLP group exhibited the highest water-holding capacity and cooking yield, the 10% FMLP group showed the lowest drip loss and the best springiness, while the 15% FMLP group demonstrated significantly enhanced hardness. These findings suggest that the effects of FMLP on muscle quality are dose-dependent, with varying optimal thresholds for different quality

indicators. The improvement in muscle water-holding capacity may be closely associated with the antioxidant effects of FMLP [22]. Lipid peroxidation can disrupt cell membrane integrity, leading to increased drip loss [23]. In this study, FMLP significantly reduced serum and liver MDA contents, indicating effective inhibition of lipid peroxidation, thereby preserving the structural integrity of muscle cell membranes. Furthermore, bioactive compounds in mulberry leaves, such as flavonoids, may influence texture characteristics by affecting the cross-linking state of muscle proteins [24]. It is noteworthy that the amino acid composition of the muscle remained unchanged, suggesting that the mechanisms by which FMLP improves muscle quality primarily involve regulation of lipid oxidation status and functional properties of proteins, rather than alterations in the amino acid profile [24,25].

Oxidative stress is a critical limiting factor affecting fish health and production performance in intensive aquaculture [26]. In this study, FMLP supplementation significantly increased T-SOD and GSH-Px activities in grass carp, reduced MDA content, and up-regulated the hepatic gene expression of *Nrf2*, *SOD*, and *GSH-Px*. These results indicate that FMLP enhances the transcriptional activity of the antioxidant enzyme system, likely through the Nrf2 signaling pathway, thereby improving overall antioxidant defense capacity. Similarly, Zhu et al. [5] and Abbas et al. [22] also confirmed that FMLP supplementation enhanced antioxidant capacity by upregulating the expression of antioxidant genes such as *Nrf2*, *SOD*, and *GSH-Px*. These studies collectively suggest that the regulation of antioxidant function in fish through the Nrf2 signaling pathway by fermented plant-based feed additives may represent a conserved mechanism. Nrf2 is a key transcription factor regulating the expression of antioxidant enzymes, and its activation initiates the coordinated expression of a series of antioxidant genes [27]. In this study, the upregulation of Nrf2 gene expression by FMLP was accompanied by corresponding increases in the expression of its downstream target genes *SOD* and *GSH-Px*, along with decreased MDA content, providing strong correlational evidence for the involvement of this signaling pathway. Dose–response analysis revealed that hepatic T-SOD activity increased linearly with rising FMLP supplementation levels, reaching the highest level in the 15% group. In contrast, serum T-SOD and GSH-Px activities peaked in the 5% and 10–15% groups, respectively, while muscle T-AOC was highest in the 15% group. This tissue specificity may be related to the metabolic characteristics of each tissue and the distribution of bioactive components from FMLP. Regression analysis indicated that the optimal FMLP supplementation level for enhancing antioxidant function ranged from 7.04% to 11.59%, suggesting that in practical production, the appropriate inclusion level can be selected based on the target tissue or indicator of interest.

Furthermore, FMLP significantly reduced the HSI, VSI, CF, and IPFI in grass carp, exhibiting both linear and quadratic trends. This finding has important production implications, as the reduction in visceral mass suggests an increase in the edible portion of the grass carp, thereby improving the economic efficiency of aquaculture. In the present study, the intraperitoneal fat ratio reached its lowest value (1.18%) in the 10% FMLP group, representing a 28.9% reduction compared to the control group, demonstrating the significant efficacy of FMLP in regulating body fat deposition in grass carp. It is noteworthy that lipid metabolism disorders are a common trigger for nutritional diseases in aquaculture [28]. In this study, FMLP supplementation reduced hepatic TC and TG contents, increased serum HDL-C concentration, upregulated the expression of fatty acid oxidation-related genes (*PPAR α* , *CPT-1a*, *CPT-1b*), and downregulated the expression of lipogenesis-related genes (*SREBP*, *FAS*). This indicates that FMLP bidirectionally regulates lipid metabolism in grass carp by promoting lipid catabolism and oxidation while inhibiting lipid synthesis, thereby reducing hepatic and intraperitoneal fat deposition [29]. Mechanistically, the upregulation of *PPAR α* a master regulator of fatty acid oxidation directly enhances the transcription of

CPT-1a and *CPT-1b*, which are rate-limiting enzymes for mitochondrial β -oxidation, thereby accelerating fatty acid utilization. Concomitantly, the downregulation of *SREBP* (a key transcription factor for lipogenic genes) reduces the expression of *FAS* and *ACC*, suppressing de novo fatty acid synthesis. This coordinated shift from lipogenesis to lipolysis leads to a net reduction in lipid stores, as reflected by decreased hepatic TG and intraperitoneal fat. The bioactive flavonoids and polyphenols in mulberry leaves may act as natural agonists of *PPAR α* and inhibitors of *SREBP*, as reported in mammalian studies [29–32].

It is noteworthy that, although FMLP generally enhanced serum T-SOD and HDL-C, both parameters in the 15% supplementation group returned to levels comparable to the control group. A plausible explanation for this observation is that excessive intake of polyphenol-rich FMLP may trigger a negative feedback regulation of the Nrf2/Keap1 pathway [33,34]. While moderate doses regulate the expression of genes related to the Nrf2 and upregulate downstream antioxidant enzymes, prolonged or high-dose exposure can induce compensatory suppression of Nrf2 transcriptional activity, possibly through the accumulation of reactive oxygen species at a level that overwhelms the cellular redox buffering capacity, as reported in other studies on flavonoid supplementation in aquatic animals [34]. Additionally, the decline in serum HDL-C at 15% might reflect an altered cholesterol reverse transport or hepatic lipoprotein metabolism, potentially due to high-level mulberry leaf components interfering with bile acid enterohepatic circulation or intestinal cholesterol absorption efficiency [33,35].

Regression analysis indicated that the optimal FMLP supplementation level for improving lipid metabolism-related parameters ranged from 7.14% to 11.21%. Specifically, the optimal dosage for promoting fatty acid oxidation (*CPT-1a*, *CPT-1b*, *PPAR α*) and lipolysis (*HSL*) was 9.03–11.21%, while the optimal dosage for inhibiting lipogenesis (*FAS*, *PPAR γ*) was 9.76–10.06%. This suggests that the regulation of lipid metabolism by FMLP is bidirectional and coordinated, with the net effect being a shift toward lipid catabolism and oxidation. The study by Wang et al. [36] on glycerol monolaurate also demonstrated that functional additives can optimize lipid metabolism by regulating the activities of key genes such as *ACC*, *FAS*, *ATGL*, *HSL*, and *CPT-1*, a regulatory pattern highly consistent with the findings of the present study. Based on the comprehensive regression analysis, the appropriate dietary FMLP supplementation range for improving muscle quality, antioxidant capacity, and lipid metabolism in grass carp in this study was 7.55–11.40%. This range encompasses the intersection of the optimal dosages for each indicator: 7.55–7.97% for optimizing muscle quality, 7.04–11.59% for optimizing antioxidant function, and 7.14–11.21% for optimizing lipid metabolism. It is noteworthy that if considering only growth performance and muscle nutritional composition, FMLP supplementation up to 15% produced no negative effects, providing flexibility in adjusting the inclusion level in production according to specific requirements.

However, it is important to acknowledge that several regression models in Table 7 had relatively low R^2 values (e.g., serum T-SOD: $R^2 = 0.368$; liver *CPT-1b*: $R^2 = 0.376$; liver *HSL*: $R^2 = 0.324$; serum GSH-Px: $R^2 = 0.446$). Such low coefficients of determination indicate that the quadratic model explains only a modest proportion of the variance in these specific parameters, and the calculated “optimal” inclusion levels derived from these equations should be interpreted with caution. Several factors may contribute to this limited explanatory power. First, biological responses to dietary additives are often inherently variable due to individual differences in metabolism, nutrient absorption, and physiological status, which cannot be fully captured by a simple quadratic function [37]. Second, the sample size per treatment may not be sufficient to precisely estimate the curvature of dose–response relationships, especially when the true response is shallow or plateaued [38]. Third, the effects of FMLP on some indicators might follow a more complex pattern than

a quadratic model can adequately describe. Therefore, while the significant quadratic p -values (all < 0.05) suggest a genuine non-linear trend, the exact optimal points from models with low R^2 should be regarded as approximate references rather than definitive recommendations [39]. In practice, our regression analysis suggests that the most beneficial effects are achieved within a range of approximately 7.55% to 11.40%, with the majority of key indicators peaking near 8–10% inclusion. This range should be considered a practical guideline rather than a fixed prescription, given the limitations of the dose levels tested.

In summary, this study reveals that FMLP possesses multiple functions, including improving flesh quality, enhancing antioxidant capacity, and regulating lipid metabolism, supporting its use as a functional feed additive. The grass carp used in this study were of a size close to market size, allowing the results to directly inform production practices. The study by De-Leon et al. [40] on replacing soybean meal with fermented rice bran also emphasized the significant value of fermentation technology in developing local protein sources. However, this study has certain limitations. The specific active compounds in FMLP responsible for the observed effects have not been fully identified; subsequent research could employ metabolomic analysis to characterize these key functional substances. Furthermore, while this study focused on the Nrf2 and PPAR signaling pathways, whether FMLP regulates metabolism through other pathways (e.g., AMPK, mTOR) requires further investigation. Notably, the role of the intestinal microbiota in metabolizing the active components of mulberry leaves and mediating their functions warrants further study. Additionally, as this was a 70-day feeding trial, the long-term effects of FMLP and its suitability under different aquaculture systems remain to be validated.

5. Conclusions

All in all, dietary supplementation with FMLP at approximately 7.55% to 11.40% (with data suggesting an optimal range near 8–10%) effectively improves muscle quality, antioxidant capacity, and lipid metabolism in grass carp (0.86 ± 0.07 kg) without compromising growth performance. FMLP reduces hepatosomatic and viscerosomatic indices while enhancing muscle water-holding capacity and texture. Mechanistically, FMLP may regulate the expression of genes related to the Nrf2 antioxidant pathway and modulate lipid metabolism by upregulating fatty acid oxidation genes (*PPAR α* , *CPT-1a*, *CPT-1b*) and downregulating lipogenesis genes (*SREBP*, *FAS*). Notably, FMLP supplementation up to 15% shows no adverse effects on growth or muscle nutrient composition in grass carp.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes11080459/s1>, Table S1: Conventional nutritional value of fermented mulberry leaf powder.

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