Figure S1. Heatmap of differentially expressed genes (DEGs) in the zebrafish liver after 14 days of dietary supplementation with the mealworm bioactive substance. Samples (n = 4) are represented in columns, while DEGs are displayed in rows. Sample groupings are labeled as control (C) and 100 μg/g treatment (100), with individual sample numbers indicated.

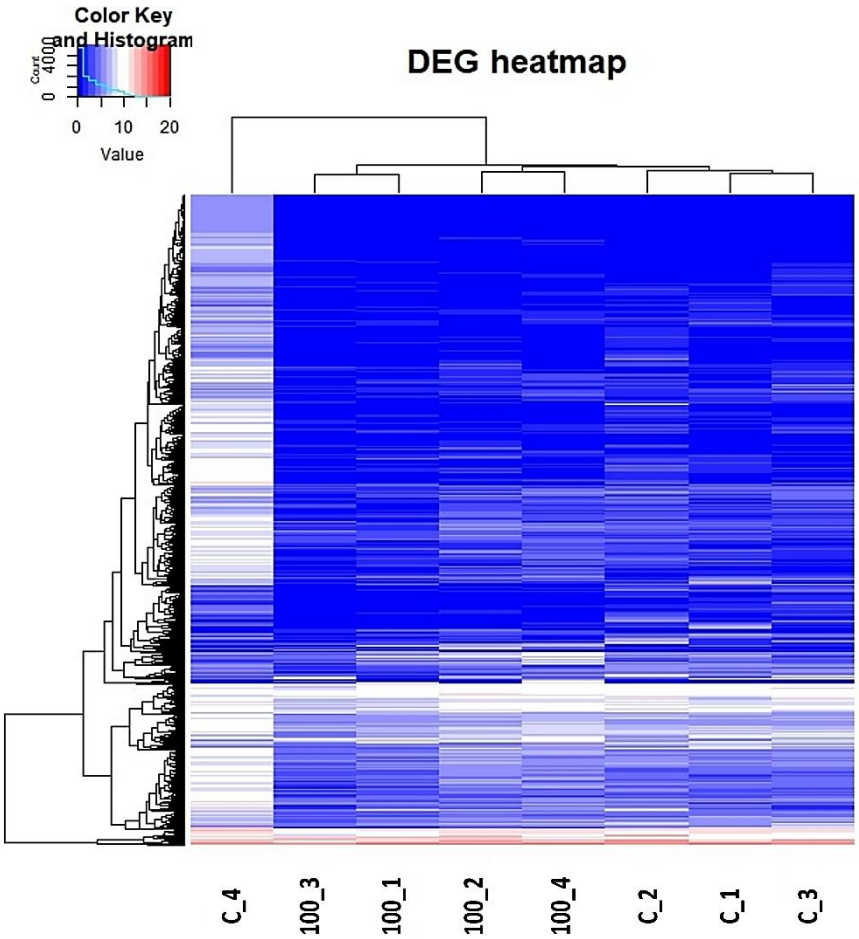


Figure S2. Heatmap of differentially expressed genes (DEGs) in the zebrafish intestine after 14 days of dietary supplementation with the mealworm bioactive substance. Samples (n = 4) are represented in columns, while DEGs are displayed in rows. Sample groupings are labeled as control (C) and 100 μg/g treatment (100), with individual sample numbers indicated.

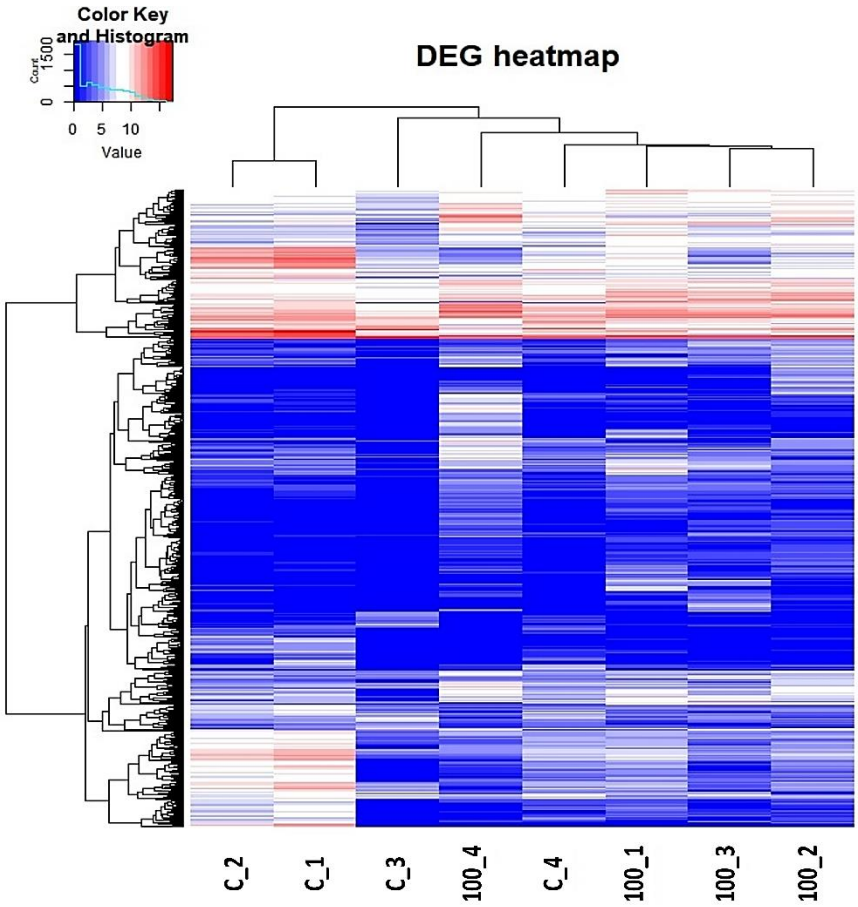
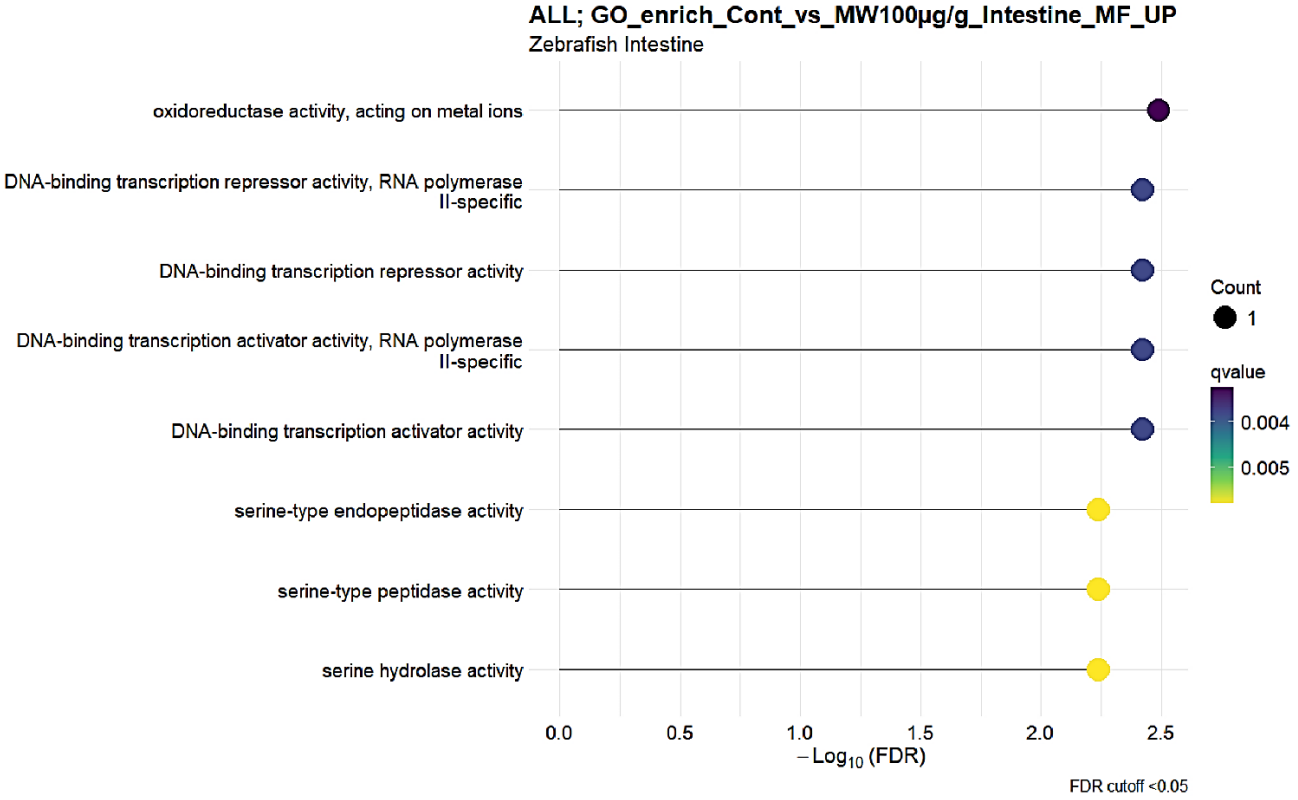
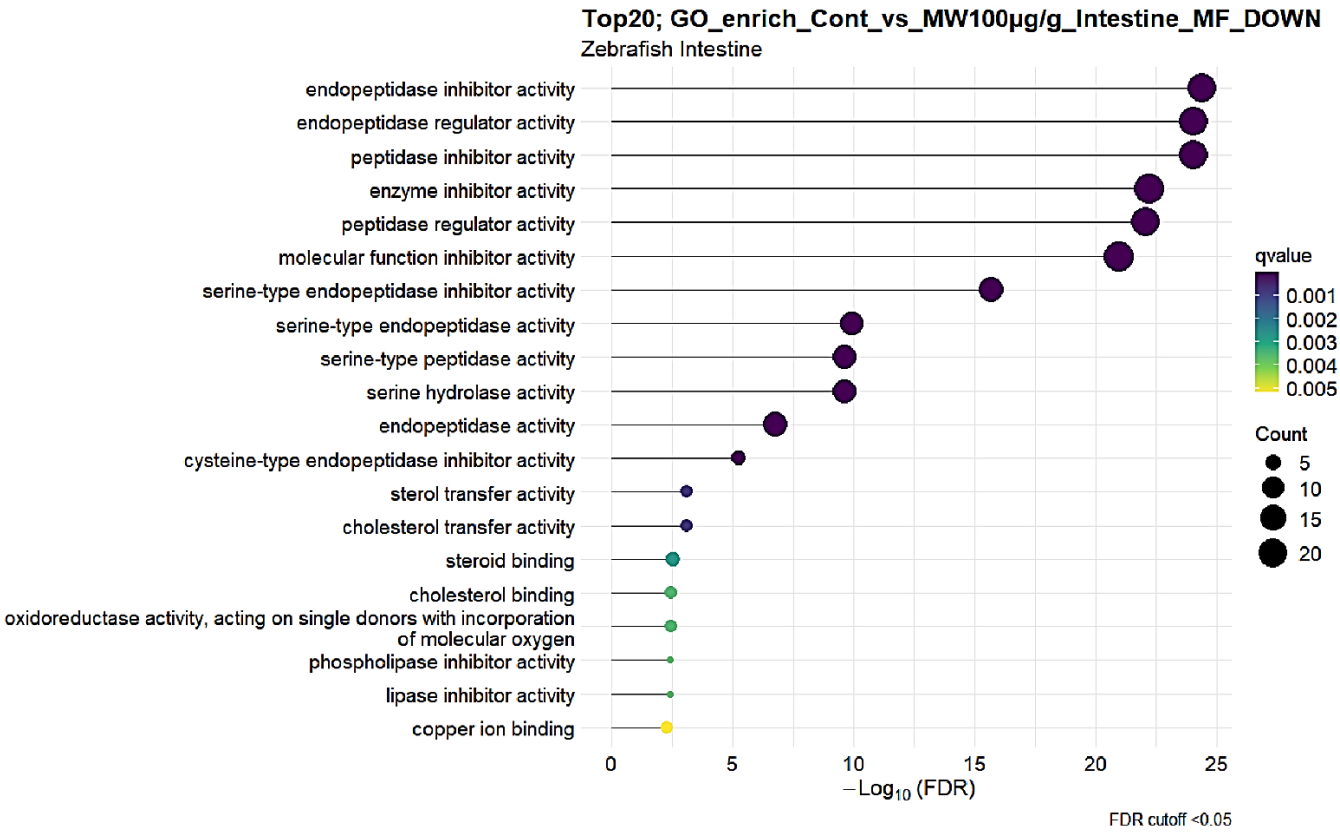


Figure S3. Gene Ontology (GO) analysis of molecular function (MF) terms in the zebrafish intestine based on differentially expressed genes (DEGs) identified in the comparison between the mealworm bioactive substance group and the control group. (a) Up-regulated DEGs; (b) Down-regulated DEGs

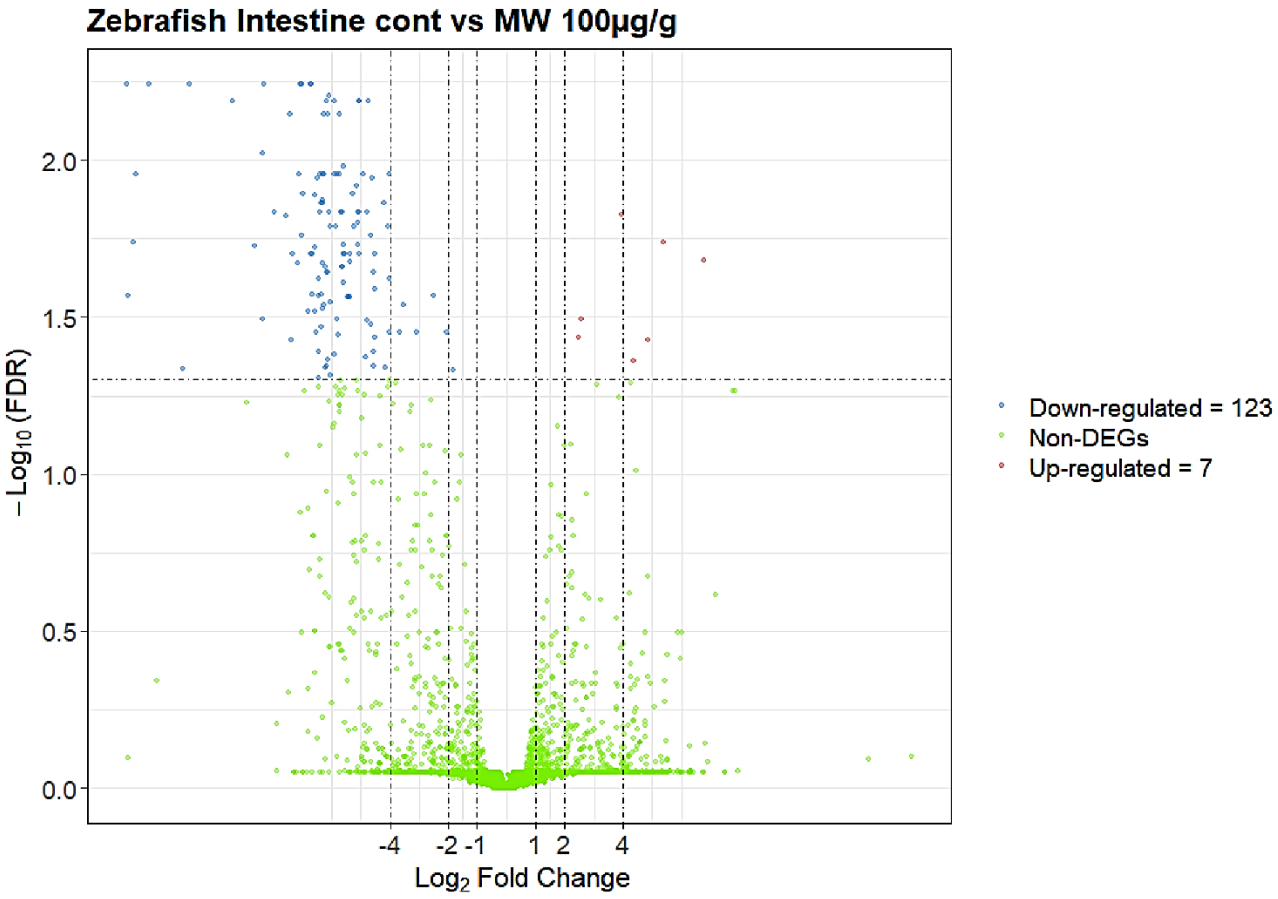


(a)

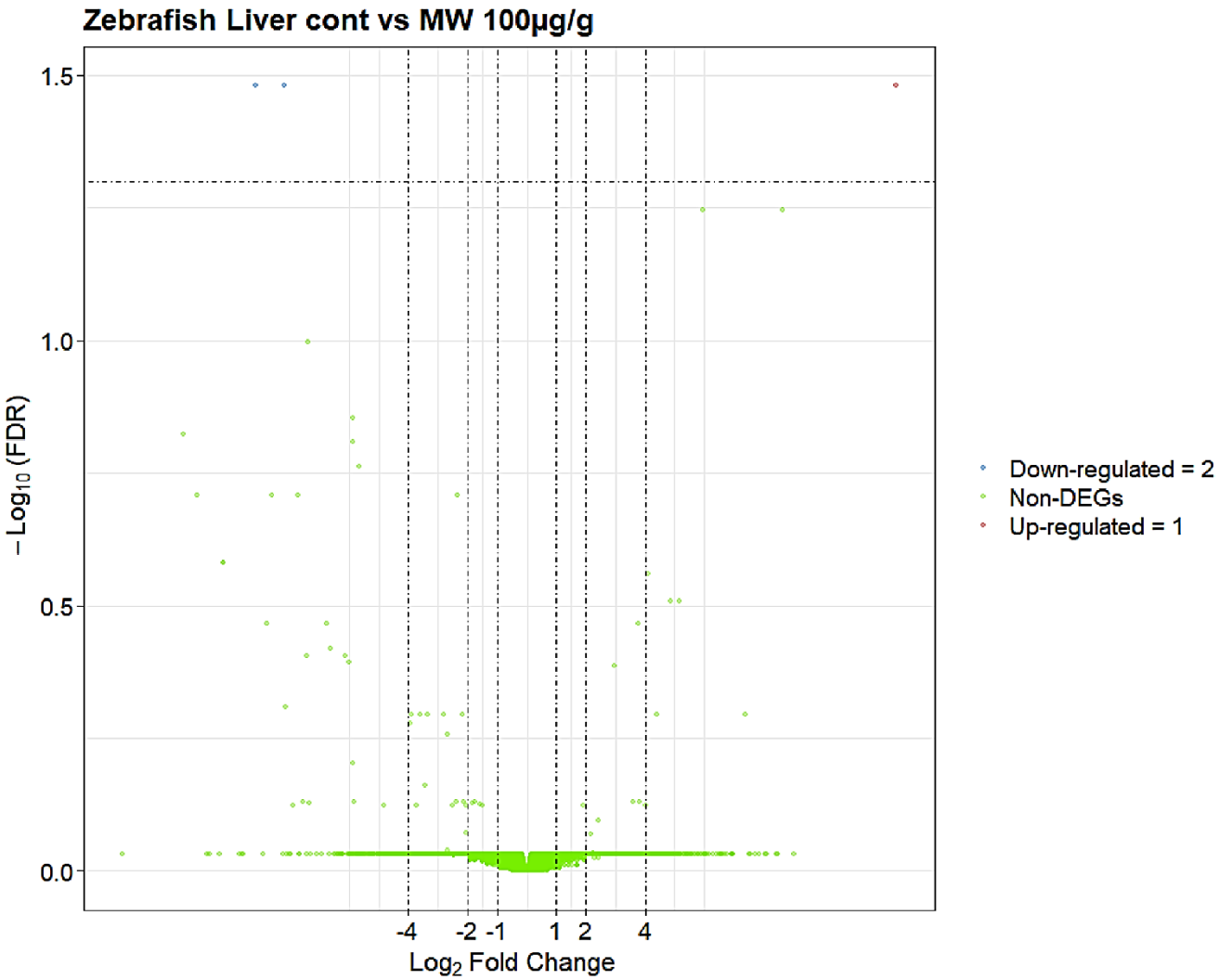


(b)

Figure S4. Transcriptional profiling analysis of the intestine (a) and liver (b) of zebrafish from the control group compared to those fed a diet containing 100 µg/g of mealworm bioactive substance. DEGs were identified using the TCC package in R software



(a)


Zebrafish Liver cont vs MW 100µg/g
1.5
1.0
0.5
0.0
− Log10 (FDR)
-4
-2
-1
1
2
4
Log2 Fold Change
Down-regulated = 2
Non-DEGs
Up-regulated = 1

(b)

Figure S5. Gene Ontology (GO) analysis of cellular components (CC) terms in the zebrafish intestine based on differentially expressed genes (DEGs) identified in the comparison between the mealworm bioactive substance group and the control group

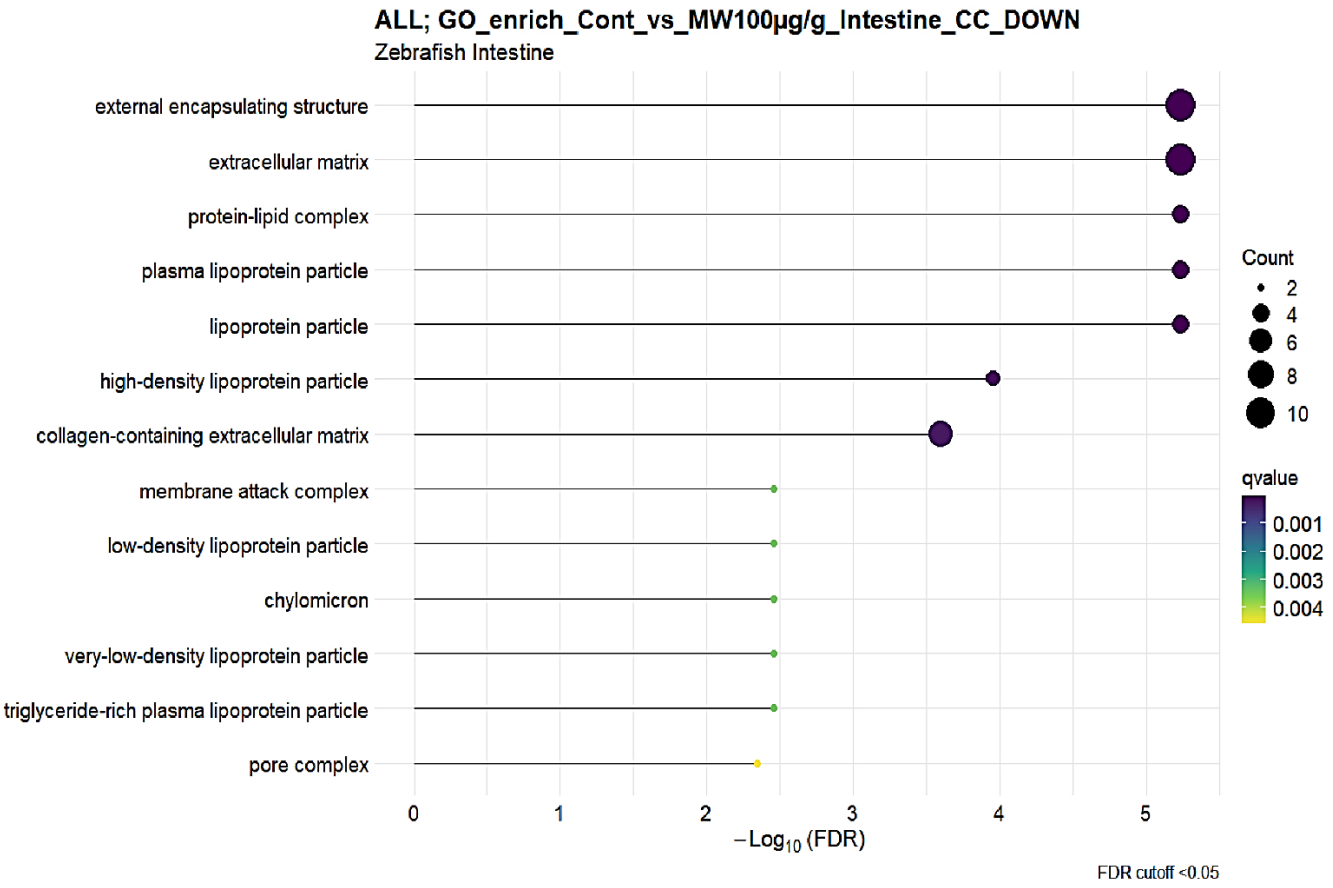
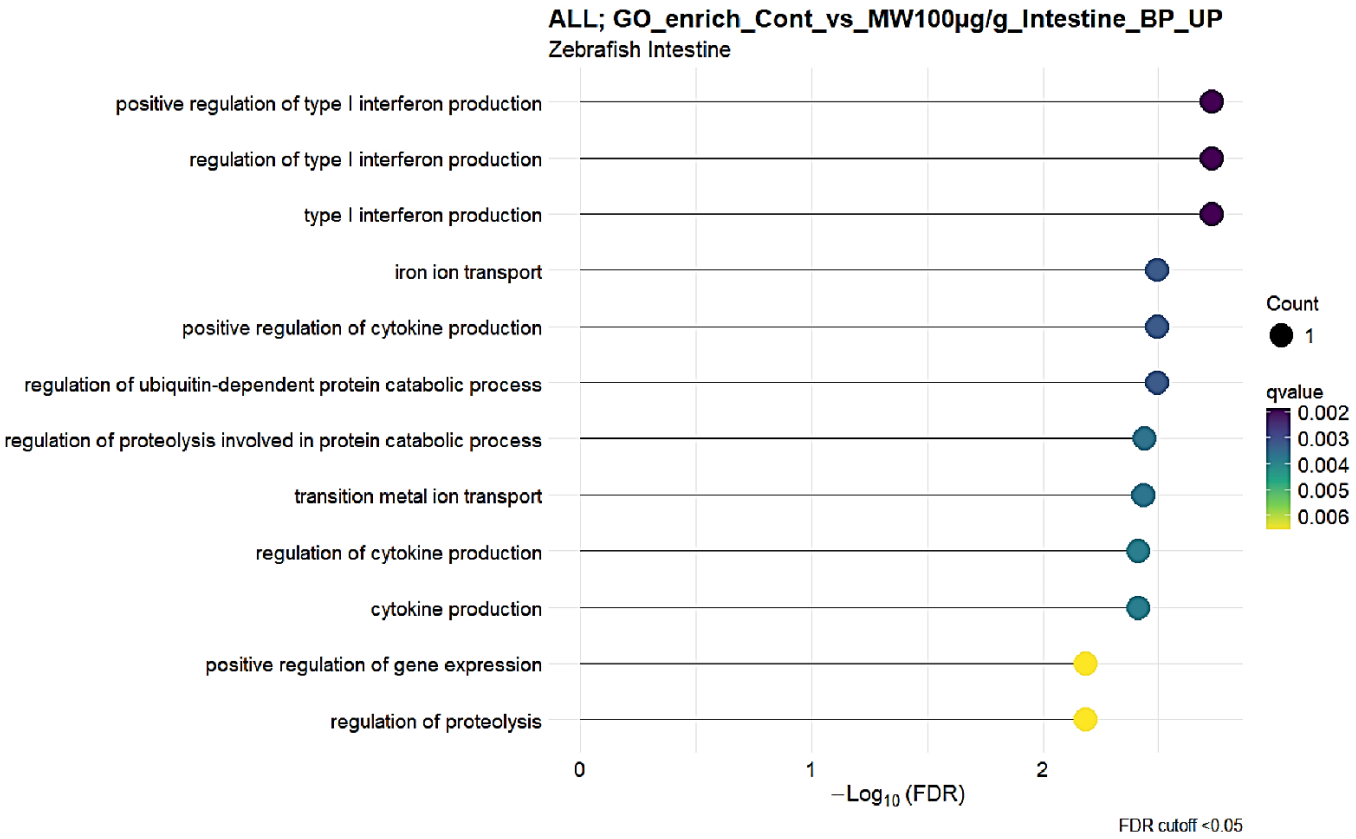
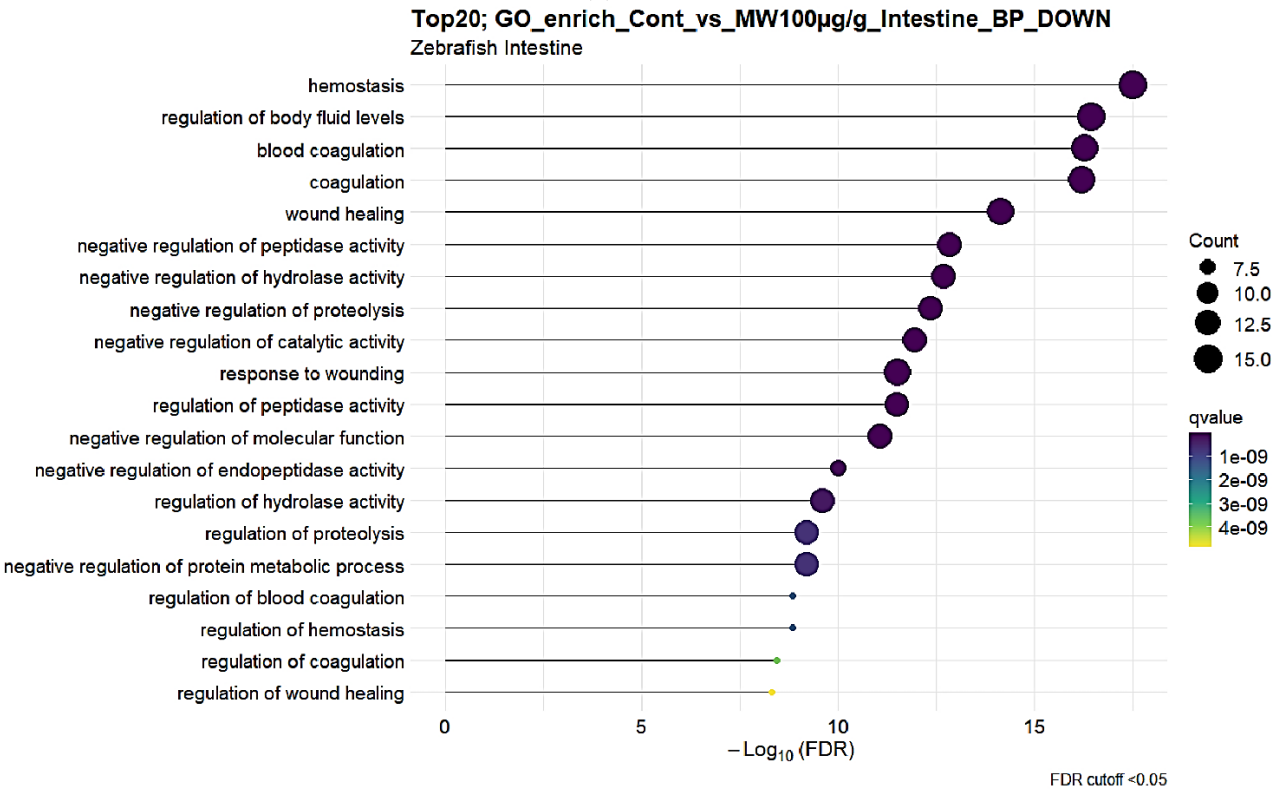


Figure S6. Gene Ontology (GO) analysis of biological process (BP) terms in the zebrafish intestine based on differentially expressed genes (DEGs) identified in the comparison between the mealworm bioactive substance group and the control group. (a) Up-regulated DEGs; (b) Down-regulated DEGs



(a)



(b)

Figure S7. Differentially expressed genes (DEGs) related to protein processing in phenylalanine metabolism (dre00360), identified through the comparison between the mealworm bioactive substance inclusion group and the control (basal diet) group in the zebrafish intestine. Downregulated genes are shown in green, while upregulated genes are shown in red

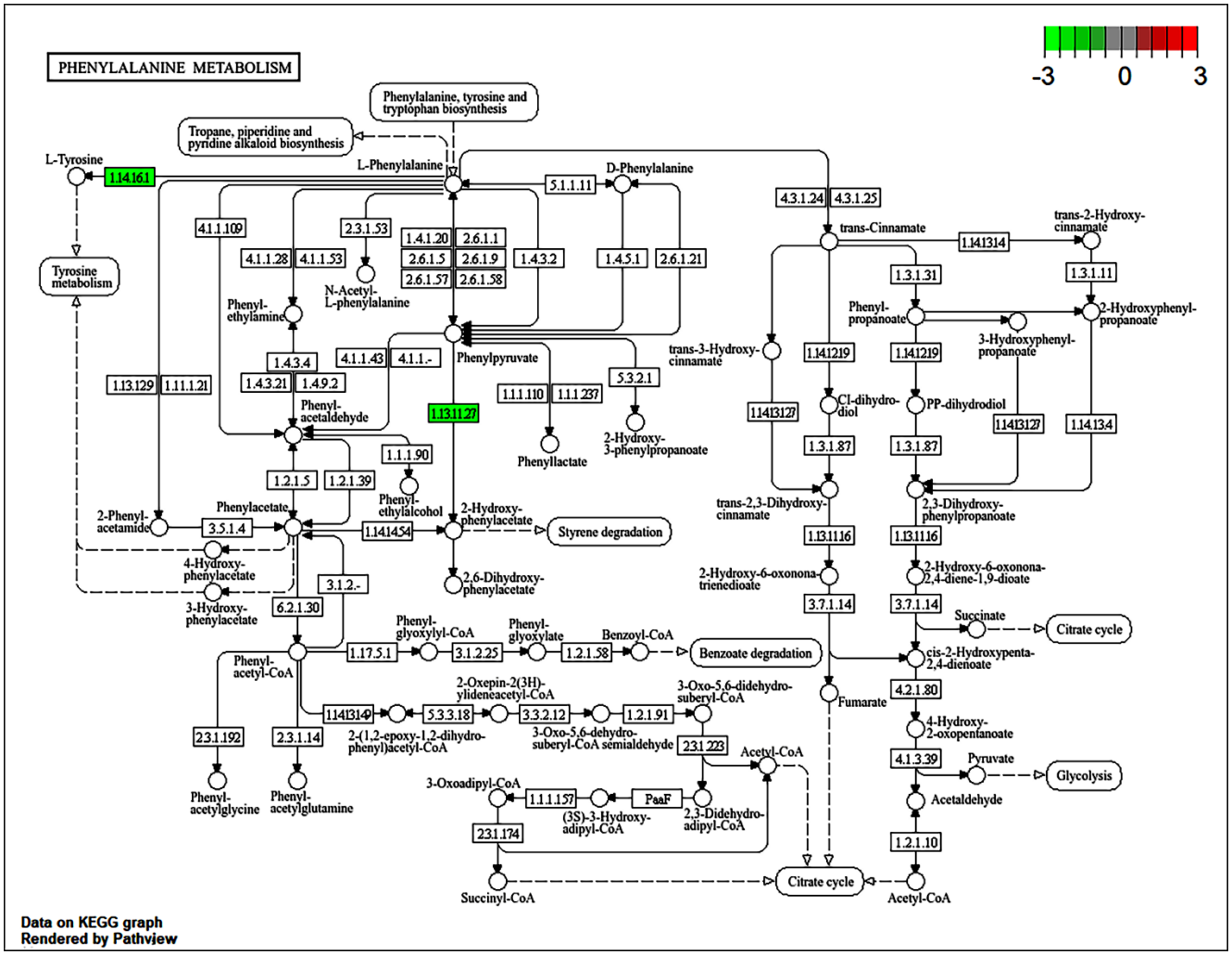


Figure S8. Differentially expressed genes (DEGs) related to protein processing in ferroptosis (dre04216), identified through the comparison between the mealworm bioactive substance inclusion group and the control (basal diet) group in the zebrafish intestine. Downregulated genes are shown in green, while upregulated genes are shown in red

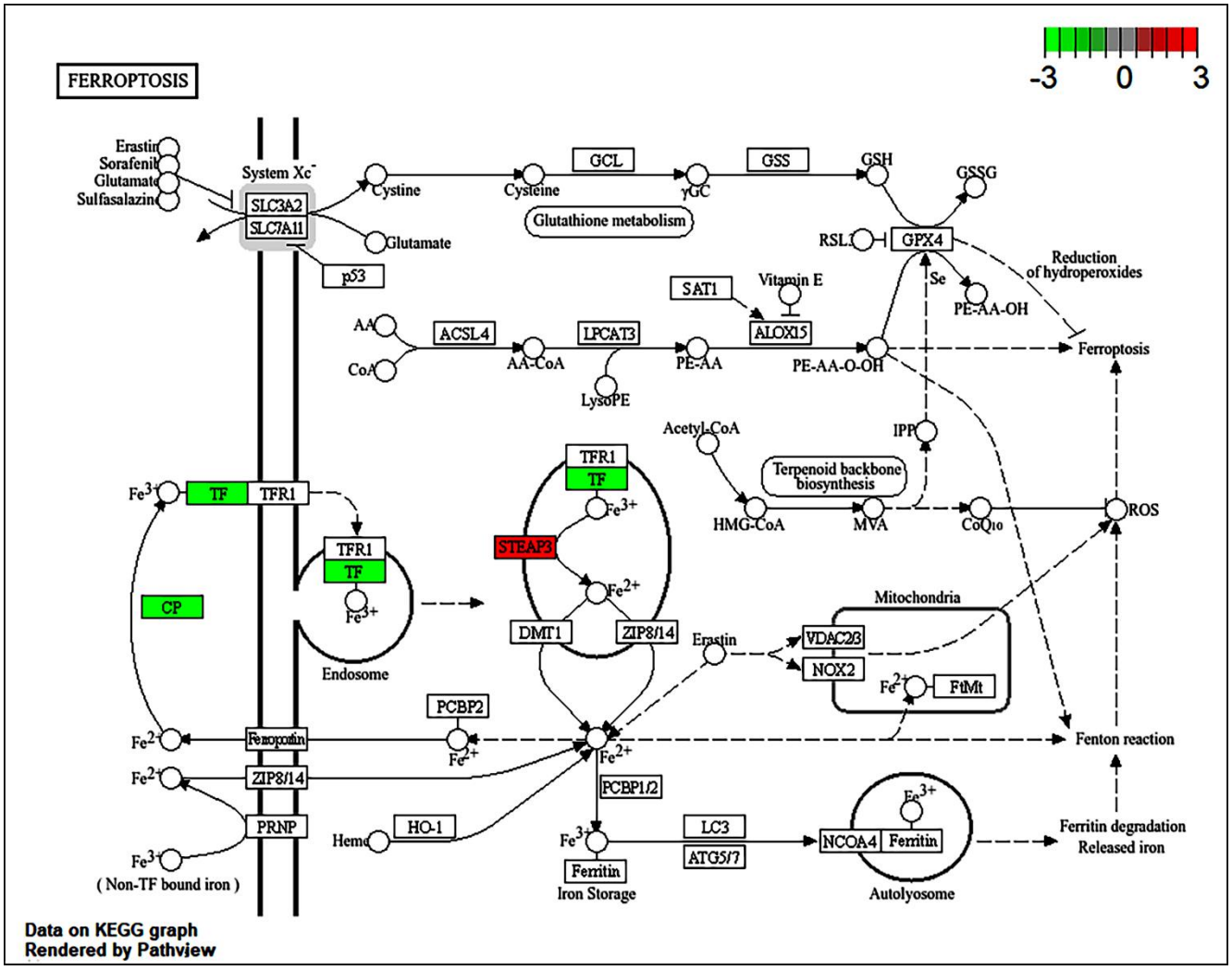


Table S1. Feed Formulation of Zebrafish

Materials	Dry Weight (g)
Fish meal	40
Soybean meal	15
Corn gluten meal	7.5
Wheat flour	6.1
Soybean oil	17.2
Purified cellulose	0.4
Alpha pregelatinized starch	9
Mineral mix*	1
Vitamin mix**	0.6
Choline chloride 50%	0.1
Stabilized vitamin C	0.3
Calcium phosphate	2.8
Total	100

* Mineral mix: Calcium lactate (224.88 mg/kg), ferrous fumarate (65.78 mg/kg), zinc sulphate (50.60 mg/kg), ferrous sulphate (58.82 mg/kg), magnesium sulphate (39.20 mg/kg), copper sulphate (39.80 mg/kg), peptide-iron (30 mg/kg), manganese sulphate (17.46 mg/kg), aluminium hydroxide (0.40 mg/kg), calcium iodate (0.24 mg/kg), and cobalt chloride (0.09 mg/kg).

** Vitamin mix: Vitamin K3 (614.4 mg/g), inositol (78.4 mg/g), vitamin E (64 mg/g), 2-O-α-D-Glucopyranosyl-L-ascorbic acid (42 mg/g), calcium D-pantothenate (15.84 mg/g), nicotinic acid (14.4 mg/g), biotin (0.32 mg/g), folic acid (0.792 mg/g), vitamin B2 (6.4 mg/g), vitamin B1 (6.272 mg/g), vitamin B6 (4.752 mg/g), vitamin B12 (0.064 mg/g), vitamin A (2000 IU/g), and vitamin D3 (480 IU/g).

Table S2. Primers used in qRT-PCR

Primer name	Primer sequence	NCBI Accession Number	Efficiency (%)
EF1A F	TGCGTGACATCAAGGAGAAG	AM422110	110
EF1A R	AGGAAGGAAGGCTGGAAGAG		
IL-10 F	CACTGAACGAAAGTTTGCCTTAAC	NM_001020785	91
IL-10 R	TGGAAATGCATCTGGCTTTG		
TGF-ß F	GATTGGAGGGACGGATGA	NM_199567	111.1
TGF-ß R	GTGACAGGGGCAGTGAAC		
IL-1ß F	TGGACTTCGCAGCACAAAATG	NM_212844	99.4
IL-1ß R	GTTCACTTCACGCTCTTGGATG		
IL-6 F	TCAACTTCTCCAGCGTGATG	JN698962	122.4
IL-6 R	TCTTTCCCTCTTTTCCTCCTG		
IL-8 F	GCTGGATCACACTGCAGAAA	NM_001113651	110
IL-8 R	TGATGAAAGGACAATTCAGTGG		
TNF-α F	GCTGGATCTTCAAAGTCGGGTGTA	NM_212859	127.5
TNF-α R	TGTGAGTCTCAGCACACTTCCATC		

Table S3. Summary of RNA-seq results, including raw reads, clean reads, and Q20 and Q30 quality scores (n = 4)

Sample Name	Raw Reads	Clean Reads	Clean Reads (%)	Q20 (%)	Q30 (%)
C1_Liver-Down	61,117,116	59,906,997	98.02	99.64	98.52
C5-Liver-Down	56,267,396	54,641,268	97.11	99.67	98.67
C3-Liver-Up	55,953,494	53,866,429	96.27	99.71	98.83
C4-Liver-Up	47,806,300	46,510,749	97.29	99.67	98.68
Average	**55,286,077**	**53,731,361**	**97.17**	**99.67**	**98.68**
Std error	**27,643,038**	**26,865,680**	**48.59**	**49.84**	**49.34**
C5-Int-Up	46,974,208	45,518,008	96.90	99.68	98.70
C6-Int-Up	42,561,946	41,327,650	97.10	99.58	98.45
C1-Int-Down	51,464,104	50,522,311	98.17	99.67	98.66
C2-Int-Down	43,573,572	42,545,236	97.64	99.68	98.70
Average	**46,143,458**	**44,978,301**	**97.45**	**99.65**	**98.63**
Std error	**23,071,729**	**22,489,150**	**48.73**	**49.83**	**49.31**
MW100-1-Liver-Down	54,095,568	52,488,930	97.03	99.65	98.61
MW100-3-Liver-Down	45,614,452	44,898,305	98.43	99.48	97.90
MW100-1-Liver-Up	96,331,050	93,922,774	97.50	99.61	98.41
MW100-6-Liver-Up	102,178,578	100,206,531	98.07	99.65	98.59
Average	**74,554,912**	**72,879,135**	**97.76**	**99.60**	**98.38**
Std error	**37,277,456**	**36,439,567**	**48.88**	**49.80**	**49.19**
MW100-1-Int-Down	47,845,782	46,998,912	98.23	99.57	98.27
MW100-2-Int-Down	46,471,722	45,495,816	97.90	99.63	98.52
MW100-4-Int-Down	46,152,776	45,031,264	97.57	99.61	98.42
MW100-4-Int-Up	43,743,180	42,728,338	97.68	99.65	98.59
Average	**46,053,365**	**45,063,582**	**97.85**	**99.62**	**98.45**
Std error	**23,026,683**	**22,531,791**	**48.92**	**49.81**	**49.23**

C represents the control group, while MW100 represents the mealworm bioactive substance (100 μg/g) group. Numbers indicate individual fish, with six fish randomly sampled per group, from which four were selected for RNA sequencing. 'Up' denotes the first repetition, and 'Down' denotes the second repetition.

Table S4. Summary of RNA-seq clean reads and mapping results (n = 4)

Sample Name	Clean Reads	Number of reads mapped to the reference	Number of unmapped reads to the reference	Number of reads mapped to reference (multiple site)
C1-Liver-Down	29,954,340	28,103,163 (93.82%)	1,142,654 (3.81%)	708,523 (2.36%)
C5-Liver-Down	27,320,540	25,643,467 (93.86%)	1,034,135 (3.78%)	642,938 (2.35%)
C3-Liver-Up	26,934,501	25,245,642 (93.73%)	975,430 (3.62%)	713,429 (2.65%)
C4-Liver-Up	23,254,938	21,692,238 (93.28%)	976,310 (4.20%)	586,390 (2.52%)
MW100-1-Liver-Down	26,244,749	24,310,453 (92.63%)	1,319,324 (5.03%)	614,972 (2.34%)
MW100-3-Liver-Down	22,448,952	20,814,702 (92.72%)	1,127,992 (5.03%)	506,258 (2.25%)
MW100-1-Liver-Up	46,963,203	43,475,838 (92.57%)	2,305,205 (4.91%)	1,182,160 (2.51%)
MW100-6-Liver-Up	50,102,771	46,297,284 (92.40%)	2617798 (5.22%)	1,187,689 (2.37%)
C5-Int-Up	22,757,888	20,918,410 (91.92%)	1,230,838 (5.41%)	608,640 (2.67%)
C6-Int-Up	20,663,471	14,259,182 (69.01%)	6,015,963 (29.11%)	388,326 (1.88%)
C1-Int-Down	25,261,811	23,133,037 (91.57%)	1,415,085 (5.60%)	713,689 (2.83%)
C2-Int-Down	21,273,554	19,788,810 (93.02%)	965,322 (4.54%)	519,422 (2.44%)
MW100-1-Int-Down	23,499,595	21,278,047 (90.55%)	1,570,039 (6.68%)	651,509 (2.77%)
MW100-2-Int-Down	22,748,439	20,655,279 (90.80%)	1,455,502 (6.40%)	637,658 (2.80%)
MW100-4-Int-Down	22,515,243	20,406,670 (90.63%)	1,513,267 (6.72%)	595,306 (2.64%)
MW100-4-Int-Up	21,363,617	19,358,726 (90.62%)	1,410,218 (6.60%)	594,673 (2.78%)

C represents the control group, while MW100 represents the mealworm bioactive substance (100 μg/g) group. Numbers indicate individual fish, with six fish randomly sampled per group, from which four were selected for RNA sequencing. 'Up' denotes the first repetition, and 'Down' denotes the second repetition.

Table S5. Lists of immune-related differentially expressed genes (|log2 fold-change|≥1 and FDR < 0.05) and their expression ratios in the liver and intestine of zebrafish after 14 days fed by mealworm bioactive substance

Gene Lists	Gene ID	Gene Symbol	Fold log_2 change	
			Liver	Intestine
Up-regulated genes				
STEAP family member 3, metalloreductase	ENSDARG00000075641	Steap3	NS	5.397
Down-regulated genes				
Nuclear factor, interleukin 3 regulated, member 5	ENSDARG00000094965	nfil3-5	NS	-1.829
Complement C3a, tandem duplicate 3	ENSDARG00000052207	c3a.3	NS	-3.652
Complement component bfb	ENSDARG00000005616	bfb	NS	-4.013
Serpin peptidase inhibitor, clade C (antithrombin), member 1	ENSDARG00000042684	serpinc1	NS	-4.021
Coagulation factor VII	ENSDARG00000034862	f7	NS	-4.155
Coagulation factor V	ENSDARG00000055705	f5	NS	-4.526
Serpin peptidase inhibitor, clade F	ENSDARG00000061383	serpinf2b	NS	-4.531
Coagulation factor II (thrombin)	ENSDARG00000036041	f2	NS	-4.783
Angiotensinogen	ENSDARG00000016412	agt	NS	-4.798
Protein Z, vitamin K-dependent plasma glycoprotein b	ENSDARG00000076900	prozb	NS	-4.848
Serpin peptidase inhibitor, clade A	ENSDARG00000087143	serpina7	NS	-4.934
Chemokine (C-C motif) ligand 39, duplicate 2	ENSDARG00000102945	ccl39.2	NS	-5.041
Protein C	ENSDARG00000093079	proca	NS	-5.241
MBL associated serine protease 2	ENSDARG00000007988	masp2	NS	-5.296
Chemokine (C-C motif) ligand 39, duplicate 1	ENSDARG00000101041	ccl39.1	NS	-5.373
Serpin peptidase inhibitor, clade D	ENSDARG00000021208	serpind1	NS	-5.476
Superoxide dismutase 3, extracellular a	ENSDARG00000070168	sod3a	NS	-5.620
Complement factor H like 4	ENSDARG00000102456	cfhl4	NS	-5.739
Inter-alpha-trypsin inhibitor heavy chain 3a, tandem duplicate 1	ENSDARG00000055036	itih3a.1	NS	-5.901
Complement C3a, tandem duplicate 6	ENSDARG00000043719	c3a.6	NS	-5.918
Coagulation factor VIIi	ENSDARG00000075827	f7i	NS	-5.932
Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1	ENSDARG00000058053	serping1	NS	-6.118
Complement component 9	ENSDARG00000016319	c9	NS	-6.185
Coagulation factor IXb	ENSDARG00000029493	f9b	NS	-6.198
Complement C3a, tandem duplicate 1	ENSDARG00000012694	c3a.1	NS	-6.291
Fibrinogen beta chain	ENSDARG00000008969	fgb	NS	-6.311

Gene Lists	Gene ID	Gene Symbol	Fold log_2 change	
			Liver	Intestine
Complement factor B	ENSDARG00000055278	cfb	NS	-6.321
Fibrinogen gamma chain	ENSDARG00000037281	fgg	NS	-6.368
Inter-alpha-trypsin inhibitor heavy chain 2	ENSDARG00000045516	itih2	NS	-6.378
Complement factor H like 1	ENSDARG00000105212	cfhl1	NS	-6.393
Fibrinogen alpha chain	ENSDARG00000020741	fga	NS	-6.583
Myo-inositol oxygenase	ENSDARG00000002764	miox	NS	-6.592
Complement component 1, r subcomponent	ENSDARG00000100248	c1r	NS	-6.661
Alpha-2-macroglobulin-like 3	ENSDARG00000041645	a2ml3	NS	-6.715
Serpin peptidase inhibitor, clade A	ENSDARG00000090286	serpina1	NS	-6.792
Inter-alpha-trypsin inhibitor heavy chain 3b, tandem duplicate 1	ENSDARG00000102722	itih3b.1	NS	-7.021
Inter-alpha-trypsin inhibitor heavy chain 3b, tandem duplicate 2	ENSDARG00000100406	itih3b.2	NS	-7.064
Complement component 8, alpha polypeptide	ENSDARG00000039516	c8a	NS	-7.156
Haptoglobin	ENSDARG00000051890	HP	NS	-7.392
Chemokine (C-C motif) ligand 39, duplicate 10	ENSDARG00000096060	ccl39a.10	NS	-8.394
Complement factor H like 5	ENSDARG00000105052	cfhl5	NS	-8.658
C-reactive protein 7	ENSDARG00000071456	crp7	NS	-10.908
C-reactive protein 6	ENSDARG00000071457	crp6	NS	-12.274
NLR family, CARD domain containing 11	ENSDARG00000068621	nlrc11	NS	-13.040

*NS = not significantly different

Table S6. KEGG Pathway Enrichment Analysis ($p < 0.05$) of DEGs Between Control and 100 μg/g Mealworm Bioactive Substance Group Using ClusterProfiler (ver. 3.18.1)

No.	KEGG ID	Description	*p-value*	Count
Intestine				
1	dre00360	Phenylalanine metabolism	0.001685	2
2	dre04216	Ferroptosis	0.001704	3
Liver				
1	-	-	-	-