Ren H, Cao X, Guo X, Yuan P. Supplementation of Yellow River carp diet with lutein and ferrous fumarate: Growth performance, digestive enzyme activity, skin pigmentation, and intestinal microbiota. *Israeli Journal of Aquaculture - Bamidgeh*. 2025;77(1):10-18. doi:10.46989/001c.127435

Original Research Articles

Supplementation of Yellow River carp diet with lutein and ferrous fumarate: Growth performance, digestive enzyme activity, skin pigmentation, and intestinal microbiota

Hongtao Ren[1a], Xiang Cao[1], Xiangzhi Guo[1], Peng Yuan[1]

[1] Animal Science and Technology College, Henan University of Science and Technology

Keywords: Lutein, ferrous fumarate, intestinal microbiota, pigmentation, Yellow River carp

https://doi.org/10.46989/001c.127435

Israeli Journal of Aquaculture - Bamidgeh

Vol. 77, Issue 1, 2025

In this study, the effect of the diet with lutein and ferrous fumarate on Yellow River carp (*Cyprinus carpio*) was studied, aiming to evaluate skin pigmentation, intestinal digestive enzymes, intestinal microbial diversity, and growth performance. Three experimental diets, including a control group, a lutein group (150mg/kg lutein), and a lutein and ferrous fumarate mixture group (150mg/kg lutein and 100mg/kg ferrous fumarate), were designed. The carp (N=135; 25.0±2.0g) were fed with experimental diets for 42 days. The results showed that the intestosomatic index (ISI) and viscerosomatic index (VSI) of the carp fed with lutein and ferrous fumarate were increased, accompanied by significant changes in body color, with the higher value of blue (b*), color difference (ΔE) and chroma (Ch*) compared with control group ($P < 0.05$). Meanwhile, the higher activity of amylase, lipase, and trypsin were observed in the mixture group ($P<0.05$). High-throughput sequencing and Venn diagrams revealed that lutein or ferrous fumarate has obvious effects on the intestinal microbiota community of carp. The abundance of *Actinobacteria* and *Flavobacterium* was significantly increased in the carp fed with the mixture group compared with the control group. In conclusion, the addition of lutein and ferrous fumarate to the feed can change the skin pigmentation and intestinal microbial composition of Yellow River carp, thereby enhancing the coloring effect and digestive function of the fish. These findings provide valuable insights for optimizing feed formulation and aquaculture management, which can contribute to the improvement of the quality and farming efficiency of Yellow River carp.

INTRODUCTION

For animal-based products, including aquatic products, appearance characteristics play a critical role in influencing consumer preferences.[1,2] The pigments present in the feed serve as the primary source for the development of body coloration in aquatic animals. The main types of feed pigments include carotenoids, pterin, purine, and melanin.[3] Carotenoids are important coloring substances in aquatic products.[4] However, fish cannot biosynthesize carotenoids but rely entirely on natural or synthetic pigments.[5] The use of natural pigments in aquaculture instead of synthetic pigments is beneficial to public health and safety.[6]

The gut microbiota plays a crucial role in the healthy development of fish by regulating gastrointestinal development, supporting nutrient metabolism, and enhancing immune response.[7-9] Feed is the main factor affecting the composition of fish gut microbiota. The active ingredients in the feed can regulate the intestinal flora and improve the balance of gut microecology. Previous studies have shown that lutein or ferrous fumarate can enhance stress resistance, digestive capacity, and promote growth.[10,11] Lutein is an important component of carotenoids, and like other carotenoids, it cannot be synthesized in the human and animal body. Therefore, it needs to be obtained from food. Lutein is widely distributed in natural plants and serves as an important substance for fish coloration. Additionally, lutein also possesses antioxidant activity, which can effectively improve animal health.[12] Ferrous fumarate is a safe and effective organic nutritional iron supplement that can improve the anti-stress and anti-disease ability of animals, has a good synergy with various nutrients, and has the effect of promoting animal growth and enhancing immunity.[10]

To date, little information about the effect of lutein and ferrous fumarate on common carp has been reported. Com-

a Corresponding author. Hong tao Ren, hthn2020@163.com

mon carp (*Cyprinus carpio*) is an important species in global aquaculture, with a global production exceeding 4.1 million tons in 2017 (FAO 2019). The Yellow River carp (*Cyprinus carpio*), as a local variety of carp, is mainly distributed in the Yellow River basin of China and is famous for its tender and tasty meat, long body shape, golden scale, and red tail, and valuable fish resource in China. However, intensive aquaculture practices have negatively impacted the growth performance of Yellow River carp, particularly in terms of coloration, flesh quality, and disease resistance. These factors seriously constrain the production and cultivation of carp. Lutein, being a rich source of bioactive compounds, combined with the significant biological activity of ferrous fumarate, presents a promising candidate for use as a functional feed additive. In the study, the hypothesis was that the supplement of lutein and ferrous fumarate to carp diets would lead to beneficial effects on skin color and other physiological functions of Yellow River carp. In this regard, this study aimed to evaluate the effect of lutein and ferrous fumarate on the pigmentation, digestive enzyme activity, and gut microbial community of Yellow River carp.

MATERIALS AND METHODS

EXPERIMENTAL FISH AND DIET PREPARATION

Yellow River carp was provided from a carp culture farm in Luoyang city, China. The carp were then disinfected with sodium chloride (2%, 15 minutes) and placed in five 500-liter tanks (2 weeks) for acclimation. During the two-week acclimatization, fish were fed with the control diet. At the beginning of the experiment, 135 carps (25.0±2.0g) were weighed and randomly distributed into three experimental treatments with three tanks (15 fish in each tank, 100 × 80 × 60 cm) for the 6-week experiment. During the experiment, carp were fed at 3% of their body weight twice a day at 8:30 and 14:30, respectively, weighed once every two weeks, and then adjusted the feed, and the water in the tank was continuously aerated and changed regularly. Water temperature (22-25℃), dissolved oxygen (DO > 5 mg/L), pH (7.1-7.4), and NH3-N < 0.4 mg/L were monitored every day by a Water quality analyzer (Genesite, China).

Three different diets were designed to feed experimental fish: the basal diet (control group), feed with 150mg/kg lutein, feed with 150mg/kg lutein and 100mg/kg ferrous fumarate. The lutein and ferrous fumarate were both from Chenguang Biology Co., Ltd. (China), the purity of lutein is 90 %, and the purity of ferrous fumarate is 95 %. Commercial carp feeds purchased from Tongwei Co., Ltd. (China) were powdered, the test raw materials were added, mixed with a small mixer, and finally, 2 mm granules were made with a micro granulator. Dried feeds were stored at -20°C.

The fish were fasted for 24 hours before sampling at the end of the experiment. The weight of the experimental fish was measured and recorded, the number of fish was counted, and the survival rate and specific growth rate were calculated. In addition, three randomly selected carp were taken from each replicate tank and treated in a diluted MS-222 solution. Organs were separated and weighed, viscerosomatic index (VSI), hepatosomatic index (HSI) and Intestosomatic index (ISI) were calculated. Then, the small intestine was quickly frozen in liquid nitrogen and stored at -80°C for further examination of intestinal microbiota, which was sent to Shanghai Yuanshen Biotechnology Co., Ltd. for analysis. The foregut was used for the determination of digestive enzyme activity. Digestive enzymes, including intestinal amylase (AMS), lipase (LPS), and trypsin (TPS), were measured using the reagent kit provided by the Nanjing Institute of Bioengineering Research. The kits included an α-amylase assay kit (C016-1-1), a lipase assay kit (A054-1-1), and a trypsin assay kit (A080-2-2). Additionally, the skin and scales were dissected, washed, and drained to determine the content of carotenoids. The determination of carotenoid content in fish followed the method described by Torrissen and Naevdal.[13]

SKIN PIGMENTATION

At 0 d, 15th d, and 42th d of the experiment, the L*, a*, b* of carp skin color were measured near the lateral line and back according to CIE2000 L*a*b* color system with colorimeter of Konica Minolta CR 400. CIE2000 L*a*b* color system according to Hunt[14] and Berns.[15]

INTESTINAL MICROBIOME ANALYSIS

After the experiment, samples of intestinal contents collected from the fish tank were processed using the QIAamp DNA Stool Mini Kit from Qiagen, Germany. Microbial DNA was extracted from the samples. The extracted microbial DNA was then subjected to PCR amplification using universal primers targeting the V3-V4 variable region of the 16S rRNA gene. These primers are known for their high conservation and variability in the V3-V4 region, which allows for identification and description of microbial community composition and structure. The PCR amplicons were analyzed using version 1.17 of the Qiime (Quantitative Insights Into Microbial Ecology) toolkit. By processing and analyzing the raw read data, further insights into the composition, diversity, and functional characteristics of the microbial community in the fish tank can be obtained.

STATISTICAL ANALYSIS

All results are presented as mean ± SEM. Significance was determined using one-way analysis of variance (ANOVA) and Tukey's post hoc test. When the P value was 0.05, the groups were significantly different. Statistical analysis was performed using SPSS v17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

GROWTH PERFORMANCE

During the 6-week experiment, no significant difference in final weight, hepatosomatic index, and specific growth rate was observed among treatments (**Table 1**). In addition,

Table 1. Growth parameters of Yellow River carp fed with experimental diets.

	Control group	Lutein group	Lutein and ferrous fumarate group
Initial weight (g)	25.07±1.13	27.42±2.22	26.36±2.51
Final weight (g)	42.26±0.31	44.24±2.42	45.38±1.28
SGR (% d^{-1})	1.16±0.13	1.14±0.21	1.31±0.12
HSI (%)	2.21±0.36	2.35±0.44	2.30±0.18
VSI (%)	7.72±0.64^{b}	8.15±1.37^{a}	8.32±1.71^{a}
ISI (%)	1.69±0.23^{b}	1.95±0.26^{b}	2.32±0.25^{a}
Survival (%)	100	100	100

Note: In the same row marked with different letters were significantly different ($P < 0.05$). SGR, specific growth rate; HIS, hepatosomatic index; VSI, viscerosomatic index; ISI, intestosomatic index .

Fig. 1. Total carotenoids and colorations of Yellow River carp at the end of experiment. Different letters signify significant difference ($P < 0.05$)

compared with the control group, the viscerosomatic index and the intestosomatic index value were significantly increased in the lutein and ferrous fumarate group compared to the control group ($P < 0.05$). No mortality was observed during the experimental period.

SKIN PIGMENTATION

As can be seen from Fig.1, after feeding the lutein and ferrous fumarate for 42 d, the carp showed different color changes, with the total carotenoid content in carp significantly increased ($P < 0.05$). The L* (light) value of the control group was the highest on the 15th day and the 42nd day compared to the experimental treatments ($P < 0.05$). However, the b* (blue), Ch* (chroma) and ΔE (Color difference) values of carp fed with lutein or ferrous fumarate were significantly higher than that of the control group ($P < 0.05$) (Table 2).

DIGESTIVE ENZYMES ACTIVITIES

The activities of digestive enzymes in the foregut of Yellow River Carp are provided in Table 3. The highest amylase, lipase and trypsin activity were observed in the supplemented diet with lutein and ferrous fumarate. No significant difference was observed among experimental treatments ($P > 0.05$).

INTESTINAL MICROBIOTA

As shown in **Fig.2**, Venn diagrams illustrated the distribution of OTUs in experimental samples. Three groups shared 153 OTUs. Additionally, 99 unique OTUs are found in the lutein group, indicating their sensitivity to lutein. Furthermore, 21 unique OTUs are found only in the lutein and ferrous fumarate mixed group, suggesting their sensitivity to ferrous fumarate.

As shown in **Fig. 3**, after being fed with the lutein and ferrous fumarate, at the phylum level, the dominant phyla of intestinal microbiota in carp are *Proteobacteria, Actinobacteria, and Bacteroidetes*. Compared to the control group, the abundance of *Proteobacteria* is lower, while the abundance of *Actinobacteria* is significantly increased ($P < 0.05$). As illustrated in **Fig. 4**, at the genus level, compared to the control group, the abundance of families such as *Rhodobacteraceae* and *Microbacteriaceae* was significantly decreased in the group with lutein and ferrous fumarate ($P < 0.05$), while the genus *Flavobacterium* is significantly increased ($P < 0.05$).

DISCUSSION

The research findings showed that the addition of lutein and ferrous fumarate in feed can promote the specific growth rate, body weight, and total carotenoid content in the skin and scales of carp. Some intermediate metabolites of ferrous fumarate and lutein in the feed may be the rea-

Table 2. The L*, a*, b*, H°ab, Ch and ΔE values of the skin in Yellow River carp

Item	Periods	Treatments		
		Control group	Lutein group	Lutein and ferrous fumarate group
L*	Initial	53.25±1.15	53.25±1.15	53.25±1.15
	15th day	59.63±1.06[a]	42.98±1.45[b]	31.22±1.46[c]
	42th day	62.65±1.23[a]	31.24±2.53 [b]	23.17±0.88 [b]
a*	Initial	1.42±0.22	1.42±0.22	1.42±0.22
	15th day	-1.16±0.34 [b]	-5.14±0.42 [a]	-3.21±0.31 [b]
	42th day	-2.56±0.47 [b]	-6.62±0.71 [a]	-2.84±0.57 [b]
b*	Initial	13.54±1.48	13.54±1.48	13.54±1.48
	15th day	9.73±1.82 [b]	16.27±1.75 [a]	17.12±1.31 [a]
	42th day	6.47±0.86 [c]	18.87±1.36 [a]	12.51±1.05 [b]
Hab°	Initial	84.01±3.42	84.01±3.42	84.01±3.42
	15th day	97.81±1.87 [b]	107.57±2.51 [a]	100.63±3.12 [b]
	42th day	111.65±4.68	109.34±2.43	102.81±2.49
Ch	Initial	13.61±1.46	13.61±1.46	13.61±1.46
	15th day	9.79±1.31 [b]	17.05±1.42 [a]	17.41±1.62 [a]
	42th day	6.95±1.46 [c]	19.98±1.54 [a]	12.82±1.76 [b]
ΔE	Initial-42th day	12.41±1.72 [c]	24.02±1.21 [b]	30.38±1.43 [a]

Note: In the same row marked with different letters were significantly different ($P < 0.05$). L*(lightness, −100 to +100, black-white,); a* (−128 to +128, green-red), b* (−128 to +128; blue-yellow), chroma (Ch) (0-30, color intensity), Ch=$(a^{*2}+b^{*2})^{1/2}$; Hue (0°-360°, hue angle of the integument): if a* > 0, $H_{ab}°=\tan^{-1}$ (b*/a*) and if a* < 0, $H_{ab}°=180+\tan^{-1}$ (b*/a*); Color difference (ΔE): $\Delta E = [(L2-L1)^2 + (a2-a1)^2 + (b2-b1)^2]^{1/2}$.

Table 3. Digestive enzymes activity in Yellow River carp fed with lutein and ferrous fumarate for 42 days.

	Control group	Lutein group	lutein and ferrous fumarate group
Trypsin (U/gprot)	1.42±0.11[b]	1.79±0.05[a]	2.02±0.11[a]
Lipase (U/gprot)	5.46±0.23[b]	8.45±0.3[5a]	9.19±0.16[a]
Amylase (U/mgprot)	0.44±0.05[b]	1.33±0.07[a]	1.60±0.02[a]

Note: In the same row marked with different letters were significantly different ($P < 0.05$)

sons for the improvement in the growth performance and viscerosomatic and intestosomatic index of the experimental carp. Previous studies have shown that lutein can promote feed utilization and growth performance of fish, while ferrous fumarate can enhance stress resistance, digestive capacity, and promote growth.[10,11,16,17] Additionally, ferrous fumarate is an important trace element for the healthy development and physiological functions of fish. Research has shown that the addition of ferrous fumarate can effectively improve the composition of the intestinal microbiota in Yellow River carp, promoting the reproduction and metabolic activity of beneficial bacteria, thereby enhancing digestive function and immune capacity.[10]

Fish cannot synthesize carotenoids on their own and must obtain them from their diet.[18] Therefore, it is commonly believed that adding carotenoids to feed is widely seen as one way to increase the sales of farmed fish.[19,20] Body color as an important feature of fish can be evaluated by the L*, a*, b* values. In this study, the L* value representing the brightness of skin color was significantly lower in the experimental group compared to the control group at the end of the experiment. The experimental group of carp showed a nearly 2-3 times higher b* value, indicating a significant yellow color. Previous studies have also reported an increase in b* value in ornamental fish fed with natural or synthetic sources of carotenoids, which is consistent with the results of this study.[21-23] Specifically, lutein is a natural compound that regulates the formation of skin pigmentation in fish. It has been found in the study that the addition of an appropriate amount of lutein can enhance the skin color of Yellow River carp, making it more vibrant and beautiful. This is because lutein is ingested by fish through food and accumulates in the skin, thereby altering their pigmentation. Body color is an important aspect of fish quality. It can be seen from the research results that adding a certain amount of lutein and ferrous fumarate to the feed can improve the effect of Yellow River carp, significantly increase the market acceptance of Yellow River carp, and at the same time, improve the breeding efficiency of Yellow River carp, while providing certain guidance for production.

Digestive enzymes in animals are located within the gastrointestinal lumen and are associated with the brush border membrane of intestinal epithelial cells.[24] However, the digestive and metabolic functions of fish are obviously related to the enzyme activity of brush border.[25] Digestive

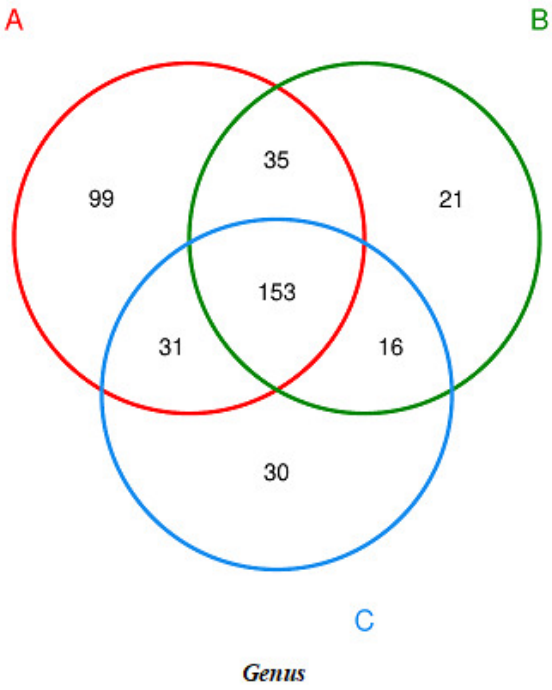

Fig. 2. Venn diagram showing the number of unique and shared OTUs at the genus level among intestinal microbial communities of Yellow River carp fed with different diet. C: control group. A: lutein group; B: lutein and ferrous fumarate group.

enzyme activity and species are critical factors influencing the absorption and utilization of nutrients in the gastrointestinal tract of animals. These parameters serve as valuable indicators for assessing the digestibility of fish.[26] Feed composition can affect the digestive enzyme activity of aquatic animals. In this study, adding an appropriate amount of lutein or a mixture of lutein and ferrous fumarate to the feed effectively improved the activities of digestive enzymes such as amylase, lipase and trypsin in the intestinal tract.

Fish typically possess short digestive tracts, making their gut microbiota highly susceptible to external environmental factors. The composition of the gut microbiota in animals could affect their physiological status, living environment, and feeding habits. In addition, the diversity of the intestinal microbial community can improve the stability of the intestinal environment and enhance stress resistance. In the present study, according to the analysis of the Venn diagram, it was found that there are 99 unique OTUs in the lutein group, which may suggest that these specific OTUs may be sensitive to lutein. On the other hand, 21 unique OTUs were found in the carp fed with lutein and ferrous fumarate, indicating that these 21 OTUs may be sensitive to ferrous fumarate. In summary, the above results suggest that lutein or ferrous fumarate may have a positive impact on the survival and proliferation of intestinal microbiota. At the same time, these research findings indicate that lutein and ferrous fumarate have different mechanisms of action and impact on regulating the gut microbiota. However, the specific mechanisms and details still require further research and exploration for clarification. In this study, the addition of lutein or ferrous fumarate to carp significantly altered the composition of the intestinal microbiota at the phylum and genus levels. The lutein group exhibited a significant increase in the abundance of Bacteroidetes. This bacterial group is known to produce lipopolysaccharides (LPS), which are associated with inflammation and compromised intestinal barrier function. These findings suggest that lutein may exert a beneficial influence in mitigating these effects.[27,28] Furthermore, the study also found that the abundance of *Actinobacteria* in the lutein and ferrous fumarate group was significantly increased. *Actinobacteria* has been shown to improve immune function and enhance disease resistance in fish. Increasing the abundance of Actinobacteria, lutein, and ferrous fumarate may contribute to the improved health status of carp.[29,30] Meantime, after feeding the lutein and ferrous fumarate to carp, the abundance of *Flavobacterium* genus was significantly increased. These results suggest that lutein and ferrous fumarate can alter gut microbiota abundance. This suggests that the active substances in lutein and ferrous fumarate can influence the development of the gut microbiota. Lutein and ferrous fumarate can improve the intestinal microflora of Yellow River carp and increase the number of beneficial bacteria, thereby improving the intestinal environment of Yellow River carp, promoting its healthy growth, enhancing growth, and improving disease resistance. At the same time, it may also promote the coloration of Yellow River carp, which needs further in-depth research. Additionally, the study also documented the presence of some unknown microorganisms in the intestinal tract of the experimental carp. Further in-depth research on these unknown microorganisms can help us better understand their functions and potential impacts.

CONCLUSION

In conclusion, lutein and ferrous fumarate can effectively improve the skin pigmentation and intestinal microbial composition of Yellow River carp, thereby enhancing the coloring effect and digestive function. These findings provide valuable insights for optimizing feed formulation and aquaculture management, which can contribute to improving the quality and farming efficiency of Yellow River carp.

ACKNOWLEDGEMENTS

This work was supported by Henan Natural Science Foundation (182300410032) and Innovation and Entrepreneurship Training Program for college students in Henan Province (S201910464053).

COMPETING OF INTEREST

The authors declare that they have no competing interests.

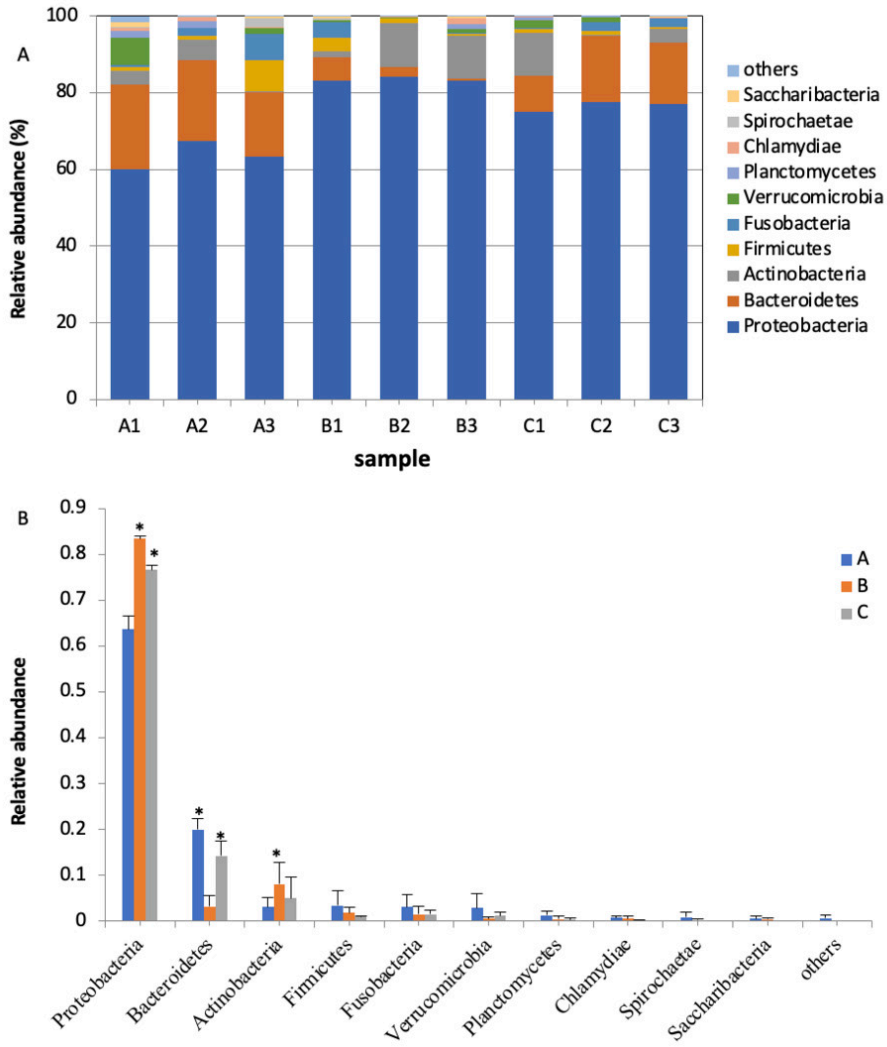

Fig. 3. (A) Relative abundance of intestinal microbiota at the phylum level. (B) Abundance differences of intestinal microbiota in experimental groups at the phylum level. * *P* < 0.05 indicates a significant difference between the groups. A: lutein group; B: lutein and ferrous fumarate group; C: control group.

AUTHORS' CONTRIBUTION

Conceptualization: Hongtao Ren (Lead). Formal Analysis: Hongtao Ren (Lead). Writing – original draft: Hongtao Ren (Lead). Writing – review & editing: Hongtao Ren (Lead). Data curation: Xiang Cao (Equal), Xiangzhi Guo (Equal), Peng Yuan (Equal). Investigation: Xiang Cao (Equal), Xiangzhi Guo (Equal), Peng Yuan (Equal).

ETHICAL CONDUCT APPROVAL

This study was conducted under animal care approved by the Institutional Animal Care and Use Committee of Henan University of Science and Technology (No. 2021002).

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

Submitted: December 09, 2024 CST. Accepted: December 30, 2024 CST. Published: February 05, 2025 CST.

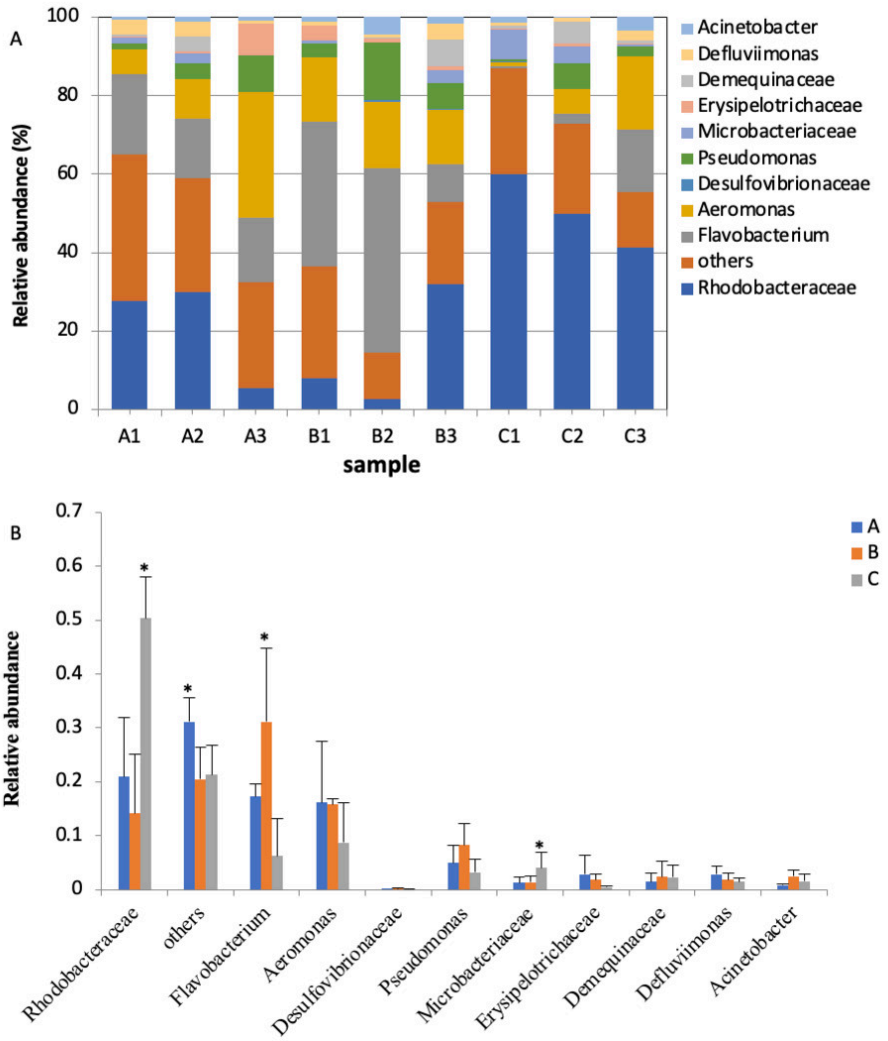

Fig. 4. (A) Relative abundance of intestinal microbiota at the genus level. (B) Abundance differences of intestinal microbiota in experimental groups at the genus level. * $P < 0.05$ indicates a significant difference between the groups. A: lutein group; B: lutein and ferrous fumarate group; C: control group.

REFERENCES

1. Safari O, Atash MMS. The effects of dietary supplement of annatto (*Bixa orellana*) seed meal on blood carotenoid content and fillet color stability in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 2015;437:275-281. doi:10.1016/j.aquaculture.2014.12.012

2. Teimouri M, Amirkolaie AK, Yeganeh S. The effects of Spirulina platensis meal as a feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 2013;396:14-19. doi:10.1016/j.aquaculture.2013.02.009

3. Kop A, Durmaz Y. The effect of synthetic and natural pigments on the color of the cichlids (Cichlasoma severum sp., Heckel 1840). *Aquac Int*. 2008;16:117-122. doi:10.1007/s10499-007-9130-1

4. Gouveia L, Rema P. Effect of microalgal biomass concentration and temperature on ornamental goldfish (*Carassius auratus*) skin pigmentation. *Aquacult Nutr*. 2005;11:19-23. doi:10.1111/j.1365-2095.2004.00319.x

5. Storebakken T, No HK. Pigmentation of rainbow trout. *Aquaculture*. 1992;100:209-229. doi:10.1016/0044-8486(92)90372-R

6. Kim J, Lee KW, Jeong HS, et al. Oral administration effect of yacon, ginger and blueberry on the growth, body composition and plasma chemistry of juvenile olive flounder (*Paralichthys olivaceus*) and immunity test against Streptococcus iniae compared to a commercial probiotic, *Lactobacillus fermentum*. *Aquacult Rep*. 2019;15:100212. doi:10.1016/j.aqrep.2019.100212

7. Jin Y, Wu S, Zeng Z, Fu Z. Effects of environmental pollutants on gut microbiota. *Environ Pollut*. 2017;222:1-9. doi:10.1016/j.envpol.2016.11.045

8. Lee S, Katya K, Park Y, Won S, Seong M, Bai SC. Comparative evaluation of dietary probiotics *Bacillus subtilis* WB60 and *Lactobacillus plantarum* KCTC3928 on the growth performance, immunological parameters, gut morphology and disease resistance in Japanese eel, *Anguilla japonica*. *Fish & Shellfish Immunol*. 2017;61:201-210. doi:10.1016/j.fsi.2016.12.035

9. Zhang Y, Li Z, Kholodkevich S, et al. Microcystin-LR-induced changes of hepatopancreatic transcriptome, intestinal microbiota, and histopathology of freshwater crayfish (*Procambarus clarkii*). *Sci Total Environ*. 2020;711:134549. doi:10.1016/j.scitotenv.2019.134549

10. Guo YL, Feng L, Jiang WD, et al. Dietary iron deficiency impaired intestinal immune function of on-growing grass carp under the infection of *Aeromonas hydrophila*: Regulation of NF-κB and TOR signaling. *Fish & Shellfish Immunol*. 2019;93:669-682. doi:10.1016/j.fsi.2019.08.021

11. Torres Sánchez EG, Fuenmayor CA, Vásquez Mejía SM, Díaz-Moreno C, Suárez Mahecha H. Effect of bee pollen extract as a source of natural carotenoids on the growth performance and pigmentation of rainbow trout (Oncorhynchus mykiss). *Aquaculture*. 2020;514:734490. doi:10.1016/j.aquaculture.2019.734490

12. Dwyer JH, Navab M, Dwyer KM, et al. Oxygenated carotenoid lutein and progression of early atherosclerosis: The Los Angeles atherosclerosis study. *Circulation*. 2001;103(24):2922-2927. doi:10.1161/01.CIR.103.24.2922

13. Torrissen OJ, Naevdal G. Pigmentation of salmonids-genetic variation in carotenoid deposition in rainbow trout. *Aquaculture*. 1984;38:59-66. doi:10.1016/0044-8486(84)90137-6

14. Hunt RWG. The specification of colour appearance. 1. Concepts and terms. *Colour Res Appl*. 1977;2:55-68. doi:10.1002/col.5080020202

15. Berns RS. *Billmeyer and Saltzman's Principles of Color Technology*. Wiley; 2000.

16. Nootash S, Sheikhzadeh N, Baradaran B, et al. Green tea (Camellia sinensis) administration induces expression of immune relevant genes and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunol*. 2013;35:1916-1923. doi:10.1016/j.fsi.2013.09.030

17. Sheikhzadeh N. Influence of dietary vegetable crops on rainbow trout (*Oncorhynchus mykiss*) immune system and growth performance. *Acta Sci Vet*. 2013;41:1-6. doi:10.1186/1746-6148-9-19

18. Goodwin TW. Animals. In: *The Biochemistry of Carotenoids, Volume II*. Chapman and Hall. Press; 1984:64-96. doi:10.1007/978-94-009-5542-4_6

19. Skonberg DI, Hardy RW, Barrows FT, Dong FM. Colour and flavour analyses offillets from farm-raised rainbow trout (*Oncorhychus mykiss*) fed low-phosphorus feeds containing corn or wheat gluten. *Aquaculture*. 1998;166:269-277. doi:10.1016/S0044-8486(98)00294-4

20. Sigurgisladottir S, Torrissen O, Lie Ø, Thomassen M, Hafsteinssn H. Salmon quality: Methods to determine the quality parameters. *Reviews in Fish Sci*. 1997;5:233-252. doi:10.1080/10641269709388599

21. Karadal O, Güroy D, Türkmen G. Effects of feeding frequency and Spirulina on growth performance, skin coloration and seed production on kenyi cichlids (*Maylandia lombardoi*). *Aquac Int*. 2017;25:121-134. doi:10.1007/s10499-016-0017-x

22. Ünver AG. *The Pigmentation Effects of Red Beetroot (Beta Vulgaris Ruba-E162) and Henna (Lawsonia Inermis) as a Pigment Source on Redzebra (Maylandia Estharea)*. Master Thesis. İzmir Kâtip Çelebi University, Graduate School of Natural and Applied Sciences, Department of Fisheries; 2018.

23. Akpınar MD. *Coloring Effects of Natural Pigment Sources on Rusty Cichlids (Iodotropheus Sprengerae)*. Master Thesis. İzmir Kâtip Çelebi University, Graduate School of Natural and Applied Sciences, Department of Fisheries 75; 2018.

24. Bakke AM, Glover C, Krogdahl Å. Feeding, digestion and absorption of nutrients. *The Multifunctional Gut of Fish, Fish physiology*. 2010;30:57-110. doi:10.1016/S1546-5098(10)03002-5

25. Hakim Y, Uni Z, Hulata G, Harpaz S. Relationship between intestinal brush border enzymatic activity and growth rate in tilapias fed diets containing 30% or 48% protein. *Aquaculture*. 2006;257:420-428. doi:10.1016/j.aquaculture.2006.02.034

26. Ribeiro L, Couto A, Olmedo M, Álvarez-Blázquez B, Linares F, Valente LM. Digestive enzyme activity at different developmental stages of blackspot seabream, *Pagellus bogaraveo* (Brunnich 1768). *Aquaculture Research*. 2008;39:339-346. doi:10.1111/j.1365-2109.2007.01684.x

27. Yoshida N, Emoto T, Yamashita T, Watanabe H, Hayashi T, Tabata T. Bacteroides vulgatus and Bacteroides dorei reduce gut microbial lipopolysaccharide production and inhibit atherosclerosis. *Circulation*. 2018;138:2486-2498. doi:10.1161/CIRCULATIONAHA.118.033714

28. Mancuso G, Midiri A, Biondo C, et al. Bacteroides fragilis-derived lipopolysaccharide produces cell activation and lethal toxicity via Toll-like receptor4. *Infect Immun*. 2005;73:5620-5627. doi:10.1128/IAI.73.9.5620-5627.2005

29. Stach JE, Maldonado LA, Ward AC, Goodfellow M, Bull AT. New primers for the class Actinobacteria: application to marine and terrestrial environments. *Environ Microbiol*. 2003;5:828-841. doi:10.1046/j.1462-2920.2003.00483.x

30. Alvarez A, Saez JM, Davila Costa JS, et al. Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. *Chemosphere*. 2017;166:41-62. doi:10.1016/j.chemosphere.2016.09.070