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Original Research Articles

A Preliminary Study on the Effect of Replacing Part of Fishmeal by Brewer's Yeast in Feeding Taiwan Loach

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In this experiment, brewer's yeast was used to replace part of the fish meal in the feed to formulate a high-fat and low-protein feed for Taiwan loach. The effects of brewer's yeast on the growth performance, muscle quality, hepatopancreas and pancreas carnitine content, intestinal flora, immunity, and antioxidant ability of Taiwan loach were preliminarily investigated. In experiment 1, 600 Taiwan loaches were randomly divided into 4 groups, and 1% (group A), 4% (group B), 8% (group C), and 12% (group D) of brewer's yeast was substituted for an equal amount of fishmeal in the basal diets. The loaches in each group were fed for 60 d. In experiment 2, 60 Taiwan loaches were selected and randomly divided into a control group (Group E) and a test group (Group F). The loaches were fed the basal diet and the high-fat and low-protein diet supplemented with 8% fishmeal, replaced by brewer's yeast. The experiment lasted for 60 days, and the growth performance, hepatopancreas carnitine content, muscle quality, intestinal flora, plasma antioxidant, and immune capacity indices of loaches in each group were determined. The results were as follows: the weight gain rate and specific growth rate were significantly higher in group C than those in groups A, B, and D ($P < 0.05$); the intestinal length ratio, muscle hardness, hepatopancreatic carnitine content, plasma superoxide dismutase activity, catalase activity, total antioxidant capacity, and lysozyme activity were significantly greater in group F than those in group E ($P < 0.05$); the Lactobacillus and Bifidobacterium counts in Group F were considerably higher than those in Group E ($P < 0.05$), and the Salmonella counts in Group F was significantly lower than that in Group E ($P < 0.05$); the survival rate, weight gain rate, bait coefficient, muscle crude protein, crude fat, viscosity, elasticity, cohesion, chewability, and restorative capacity were not significantly different between Group F and Group E ($P > 0.05$). The above results showed that adding 8% brewer's yeast to feed Taiwan loach instead of fish meal could enhance its hepatopancreatic carnitine synthesis, optimize the intestinal flora, improve the body's immune and antioxidant ability, and have a certain promotional effect on the intestinal development and muscle quality improvement.

INTRODUCTION

In recent years, the shortage of fishmeal has led to soaring feed costs, seriously affecting the sustainable development of aquaculture. Finding high-quality protein sources to replace fishmeal has become a hotspot in aquatic animal nutrition research.[1-3] Animal bodies can use lysine, methionine, VC, VB3, VB6, VB9, and iron ions to synthesize carnitine in the body.[4] Adding carnitine to high-fat feeds can promote the utilization of animal fat.[4-8] Brewer's yeast is rich in proteins, B vitamins, and biologically active substances such as β-glucan, mannan oligosaccharides, and nucleotides, which have been used to replace part of fishmeal in aquaculture.[9-17] In addition, due to the abundance of vitamin B in brewer's yeast, it can also be used as one of the ingredients for synthesizing L-carnitine. Taiwan loach has excellent characteristics such as high adaptability, large size, rapid growth, strong disease resistance, and high yield, but the muscle texture of adult Taiwan loach is poor. Improving the meat quality of Taiwan loach and reducing the feed cost is significant for the healthy development of Taiwan loach aquaculture.

Until now, no reports have been on using brewer's yeast to formulate high-fat, low-protein feeds to rear Taiwan loaches. This approach aims to leverage the abundant vitamin B present in brewer's yeast to facilitate the synthesis

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of carnitine, thereby enhancing the efficient utilization of fat by the loaches. In this experiment, we first explored the suitable ratio of brewer's yeast in replacing fishmeal in the feed of the Taiwan loach and then formulated high-fat and low-protein feeds to feed the Taiwan loach for 60 d. We investigated the growth performance, muscle quality, intestinal flora, hepatopancreas carnitine content, plasma antioxidant, and immune capacity indices of loaches, to provide the basis for the in-depth development of the resources of brewer's yeast and its application in the practice of Taiwan loach aquaculture.

MATERIALS AND METHODS

EXPERIMENTAL DIETS

Brewer's yeast was purchased from Xuzhou Saifu Biotechnology Co., Ltd (measured crude protein 52.4%, crude fat 0.4%, VB3 500 mg/kg, VB6 40 mg/kg, VB9 30 mg/kg). The multivitamin and mineral premix were purchased from Beijing Fishing Economy Bio-Technology Limited Liability Company. The remaining feed ingredients were purchased from the Animal Nutrition Research Institute of Sichuan Agricultural University. The raw materials were crushed, sieved, mixed following a step-by-step premixing method, and made into pellets for drying. Four kinds of test feeds were prepared by replacing 1% (Group A), 4% (Group B), 8% (Group C) and 12% (Group D) of fish meal with brewer's yeast in the basal feed in Test 1. In Test 2, a high-fat and low-protein feed was formulated by replacing fishmeal with 8% brewer's yeast meal (group F), using a brewer's yeast-free basal feed as a control (group E) (**Table 1**).

EXPERIMENTAL ANIMALS AND MANAGEMENT

Taiwan loaches were purchased from Meishan Mai-Lu Fisheries Co. Ltd, Meishan, Sichuan Province, and treated with 1% NaCl after purchase. In experiment 1, 600 healthy Taiwan loaches weighing (17.92±1.42) g were selected and randomly divided into 4 groups (A-D) with 6 replicates in each group and 25 loaches in each replicate. The loaches were kept in a cement pond (Length: Width: height =7 m: 2m: 1m) in the teaching and research base of Sichuan Agricultural University, and were fed regularly at 2%~3% of their body weight daily. The loaches were fed daily for 60 days, and the natural water temperature was 24.5 ~ 30.5℃.

In experiment 2, 60 healthy loaches (13.32±1.51) g were randomly divided into the control group (Group E) and experimental group (Group F), with 6 replicates in each group and 5 loaches in each replicate, and kept in the fish tank (Length: Width: height =40.6 cm: 26.6cm: 38.5cm) of Zoology Laboratory of Sichuan Agricultural University. The fish were fed at 2%~3% of their body weight every day, and the amount of feed was adjusted according to the remaining bait. The loaches were fed daily for 60 days, and the natural water temperature was 23.5~31.8℃. The water quality was kept in good condition by microfluidic water.

SAMPLE COLLECTION

At the end of the feeding test, the loaches were fasted for 24 h. The loaches in each group were collected and weighed. The length and weight of loaches in groups E and F were measured, and blood was collected from the tail vein and placed in sodium heparin tubes, centrifuged at 4000 r/min for 10 min at 4℃, and the upper layer of plasma was aspirated. After blood sampling, the loach were dissected, the intestines were separated, and the length and weight of the intestines were measured, and the hepatopancreas and back muscles were collected. The samples were frozen at -20℃ for further analysis.

INDICATOR MEASUREMENT

GROWTH PERFORMANCE PARAMETERS

Survival rate (SR, %) = 100 × final number of loaches / initial number of loaches;

Weight gain rate (WGR, %) = 100× [W_t - W_0] / W_0

Special Growth Rate (SGR,%/d)=100× (InW_t - InW_0) / t

Feed conversion ratio (FCR) = W_f / [W_t-W_0]

Plumpness (g/cm3) = 100×W /L3

Intestinosomatic index (ISI, %) = W_I /W

Intestinal length ratio (ILI,%) =L_I /L

W_0 is the first weight (g), W_t is the last weight (g), W is the body weight (g), W_I is the intestinal weight (g), L is the body length (cm), L_I is the intestinal length (cm), W_f is the total amount of feed ingested (g), and t is the number of days of experimental breeding (d).

CARNITINE CONTENT IN THE HEPATOPANCREAS

The hepatopancreas of 5 loaches from each replicate of group E and F were mixed into one sample to be tested, and the free carnitine content was separated and detected by derivatization HPLC.

The chromatography conditions were as follows: column: ZORBAX SB-C18 (4.6 × 150 mm); column temperature: 35℃; detection wavelength: 263 nm; mobile phase: 0.6% triethylamine (pH = 6.20)/methanol = 40/60 (V/V); flow rate: 0.6 mL/min; injection volume: 20 μL; detection period: 15 min.

MUSCLE QUALITY

Muscle moisture content was determined by 105 ℃ oven drying method (GB/T 6435-1986), crude protein content by Kjeldahl method (GB /T 6432-1994), crude fat content by ether extraction method (GB /T 6433-1994), and crude ash content by 550℃ cauterization method (GB/T 6438-1992).

The hardness, viscosity, elasticity, cohesion, chewability, and restorative capacity of the dorsal muscle tissue of loach were measured by a TA. XT. plus tester (Stable MicroSystem, UK). The TPA measurement mode was used, and the measuring probe was P/36R. The measurement speed was divided into three steps, namely, the premeasurement rate of 1 mm/s, the mid-measurement rate of 0.5 mm/s and the post-measurement rate of 1 mm/s, with a measuring dis-

Table 1. Experimental feed composition and nutrient levels (DM basis).

Ingredients (%)	Experimental diets					
	Group A	Group B	Group C	Group D	Group E	Group F
Fishmeal	21.00	18.00	14.00	10.00	22.00	12.00
Brewer's yeast	1.00	4.00	8.00	12.00	0.00	8.00
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00
Rapeseed meal	8.00	8.00	8.00	8.00	8.00	8.00
Flours	24.00	24.00	24.00	24.00	24.00	24.00
Bran	13.50	13.50	13.50	13.50	13.50	13.50
Maize	5.00	5.00	5.00	5.00	5.00	5.00
Rapeseed oil	2.00	2.00	2.00	2.00	2.00	4.00
Ball milling rice bran	4.00	4.00	4.00	4.00	4.00	4.00
Calcium dihydrogen phosphate	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride 50%	0.25	0.25	0.25	0.25	0.25	0.25
Betaine hydrochloride 98%	0.20	0.20	0.20	0.20	0.20	0.20
premix	0.05	0.05	0.05	0.05	0.05	0.05
Crude protein (%)	32.26	31.80	31.17	30.54	32.42	29.81
Crude fat (%)	5.85	5.61	5.29	4.97	5.93	7.11
Crude ash (%)	6.23	5.99	5.60	5.20	6.39	5.30
Crude fiber (%)	6.47	6.46	6.45	6.43	6.48	6.43
P (%)	1.36	1.29	1.20	1.10	1.38	1.13
Ca (%)	1.48	1.33	1.11	0.90	1.54	1.00
Lys (%)	1.81	1.77	1.70	1.64	1.83	1.60
Met (%)	0.61	0.58	0.53	0.49	0.62	0.50

*Lys (lysin) 337.66 g, Met (methionine) 337.66 g, Vc 21.86 g, and FeSO4 17.24 g were added per 100 kg of Group F feed prepared.

tance of 2 mm and a dwell interval of 5 s. The measurement was performed in the same way as that of the TPA, with a 2-mm distance and a 5-s dwell interval.

INTESTINAL FLORA

To determine the intestinal flora, 0.8 g of the whole intestine of loaches from each group was weighed in a sterile environment, 7.2 mL of saline was added according to a ratio of 1:9 (mass: volume), and ground the mixture into a pulp under aseptic conditions, followed by shaking and centrifugation to obtain a 10-1 stock solution. Subsequently, 0.4 mL of the supernatant was placed into 3.6 mL of saline for 10-fold dilution, followed by dilution to obtain a concentration of 10-5; for each dilution gradient, three parallels were set up. We took 100 μL of the bacterial solution, inoculated it onto the corresponding selection medium by the plate spreading method, and then inverted the Petri dish after the agar had slightly absorbed the water, marked it, and placed into the incubator at 37℃ (**Table 2**). After incubation, the Petri dishes with 30–300 colonies were selected for plate counting. The counts were expressed by the logarithmic value of the colony-forming unit per gram of intestine, i.e., lg (CFU/g).

ANTIOXIDATIVE PARAMETERS IN HEMOLYMPH

Total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), alkaline phosphatase (AKP), and lysozyme (LZM) were determined using the Nanjing Jianjian Bioengineering Research Institute kits, following the manufacturer's instructions.

STATISTICAL ANALYSIS

The results are expressed as "mean values ± standard error of means", and the SPSS 20.0 software was used for data analysis and statistics. First, the data were subjected to one-way analysis of variance (ANOVA), and subsequently, Duncan's' multiple comparisons were used if the differences among the groups were significant, with $P < 0.05$ indicating statistically significant differences.

RESULTS

EFFECTS OF BREWER'S YEAST ON GROWTH PERFORMANCE, BODY COMPOSITION AND MUSCLE TEXTURE CHARACTERISTICS OF TAIWAN LOACH

There was no significant difference in survival rate among group A, group B and group C, but their survival rates were all significantly higher than that in group D (*P<0.05*). The weight gain rate and specific growth rate of Taiwan loach

Table 2. Media and culture conditions for investigating the intestinal flora.

Strain	Culture medium	Culture conditions	Dilution
Salmonella	Bismuth sulfite agar medium	37℃, aerobic condition, 24 h	10^{-3}, 10^{-4}, 10^{-5}
Escherichia coli	Erythromycin blue agar medium	37℃, anaerobic conditions, 48 h	10^{-3}, 10^{-4}, 10^{-5}
Bifidobacterium	*Bifidobacterium* bifidum BS medium	37℃, anaerobic conditions, 72 h	10^{-3}, 10^{-4}, 10^{-5}
Lactic acid bacteria	*Lactobacillus* MRS agar medium	37℃, anaerobic conditions, 72 h	10^{-3}, 10^{-4}, 10^{-5}
Bacillus sp.	*Bacillus* selective medium	37℃, anaerobic conditions, 72 h	10^{-3}, 10^{-4}, 10^{-5}

Table 3. Effects of different levels of brewer's yeast replacing equal amounts of fishmeal on the growth performance of Taiwan loach

Item	Dietary curcumin level /%			
	Group A	Group B	Group C	Group D
IBW (g)	18.45 ± 1.24	17.01 ± 1.83	17.31 ± 0.92	18.89 ± 1.67
FBW (g)	33.95 ± 2.68	30.62 ± 3.82	39.12 ± 2.24	33.81 ± 3.87
SR/%	92.17 ± 3.49^{a}	96.22 ± 2.86^{a}	93.51 ± 1.84^{a}	88.76 ± 0.45^{b}
WGR (%)	84.01 ± 2.17^{b}	80.01 ± 3.31^{b}	126.00 ± 0.93^{a}	78.98 ± 4.71^{b}
SGR/%	1.02 ± 0.02^{b}	0.98 ± 0.03^{b}	1.37 ± 0.01^{a}	0.97 ± 0.04^{b}

* Different letters on the shoulder label indicate significant differences ($P<0.05$) , while the same letter on the shoulder label indicates no significant differences ($P>0.05$) , the same below.

in group C were significantly higher than those of group A, group B and group D ($P<0.05$), and there was no significant difference in the weight gain rate and specific growth rate among group A, group B and group C. The above results showed that the replacement of fish meal by brewer's yeast, with a replacement ratio of 8%, could significantly improve the weight gain rate and specific growth rate of Taiwan loach (**Table 3**).

The survival rate of Taiwan loach in group F (experimental group) was lower than that of group E (control group), the weight gain rate was higher than that of group E and the bait coefficient was lower than that of group E, but none of them reached the level of significant difference ($P>0.05$). The specific growth rate of Taiwan loach in group F (experimental group) was higher than that of group E (control group)($P<0.05$). The plumpness, intestinal body ratio and intestinal length ratio of Taiwan loach in group F were higher than those of group E and the difference of the intestinal length ratio was significant ($P<0.05$). The muscle moisture, crude protein, crude fat and crude ash of Taiwan loach in group F did not differ significantly from those of Taiwan loach in group E (**Table 4**).

The muscle viscosity, elasticity, cohesion, chewability, and restorative capacity of group F were not significantly different from group E, but the muscle hardness was significantly higher than that of group E ($P<0.05$). It showed that replacing fish meal with 8% brewer's yeast in the feed had a positive effect on the growth performance of Taiwan loach, promoting the development of its intestinal tract, significantly enhancing muscle hardness and improving meat quality, but the effect on the body composition of Taiwan loach was not obvious (**Table 4**).

EFFECTS OF BREWER'S YEAST ON IMMUNE AND ANTIOXIDANT FUNCTIONS, INTESTINAL FLORA, AND HEPATOPANCREAS' ABILITY TO SYNTHESIZE CARNITINE IN TAIWAN LOACH

The liver carnitine content was significantly higher in group F than that in group E ($P<0.05$), and there was no significant difference in the number of Escherichia coli and Bacillus spp. in the intestinal tract of the two groups of Taiwan loaches ($P>0.05$), but the number of Salmonella spp. in group F was significantly lower than that in group E ($P<0.05$) and the number of Lactobacillus spp. and Bifidobacterium spp. was significantly higher than that in group E ($P<0.05$). Plasma alkaline phosphatase (AKP) activity and malondialdehyde (MDA) content were not significantly different between the two groups of Taiwan loach ($P>0.05$), but plasma superoxide dismutase (SOD) activity, catalase (CAT) activity, total antioxidant capacity (T-AOC), and lysozyme content (LZM) of Taiwan loach in group F were significantly greater than those in group E ($P<0.05$). The above results indicated that replacing fish meal with 8% brewer's yeast in the feed could promote the synthesis of carnitine in the liver of Taiwan loach, optimize the intestinal flora, and enhance the immune and antioxidant capacity of Taiwan loach (**Table 5**).

DISCUSSION

EFFECTS OF BREWER'S YEAST ON GROWTH PERFORMANCE, INTESTINAL FLORA, IMMUNE FUNCTION AND MUSCLE QUALITY OF FISH

Brewer's yeast is rich in amino acids, nucleotides, β-glucan, mannan oligosaccharides and B vitamins, etc. Adding

Table 4. Effects of the inclusion of brewer's yeast in the diet of Taiwan loach on growth performance, body composition, and muscle texture characteristics.

Item	Growth performance		Item	Body composition		Item	Muscle texture characteristics	
	Group E	Group F		Group E	Group F		Group E	Group F
Survival rate (%)	100.00 ± 0.00	96.67 ± 8.16	Crude protein (%)	18.68±0.37	19.17±0.23	Hardness	713.83±161.89^{b}	767.16±194.33^{a}
WGR (%)	113.48±3.39	126.24±6.48	Crude fat (%)	1.22 ± 0.10	1.29 ± 0.21	Elasticity	0.80 ± 0.07	0.81 ± 0.07
SGR (%)	1.06±0.04^{b}	1.24±0.02^{a}	Crude ash (%)	1.29 ± 0.03	1.32± 0.05	Cohesion	0.54 ± 0.04	0.54 ± 0.04
Feed conversion ratio	1.89 ± 0.79	1.83 ± 0.85	Moisture content	78.75±0.04	78.24±0.05	Chewability	312.10± 85.25	314.89±96.45
Plumpness	0.53 ± 0.04	0.55 ± 0.03				Restorative capacity	0.24 ± 0.02	0.24 ± 0.03
Gut-to-body ratio	0.025 ± 0.001	0.027 ± 0.002				Viscosity	387.06± 96.01	418.39±113.47
Intestinal length ratio	0.48±0.05^{b}	0.53±0.03^{a}						

Table 5. Effects of the inclusion of brewer's yeast in the diet of Taiwan loach on liver carnitine content, intestinal flora, antioxidant activity, and immune capacity.

Item	Intestinal flora and hepatopancreatic carnitine content		Item	Immunity and antioxidant activity	
	Group E	Group F		Group E	Group F
Hepatopancreas carnitine content (μg/g)	216.80±16.79^{b}	234.24±6.5^{a}	SOD(U/ml)	66.86±9.31^{b}	70.10±8.71^{a}
Escherichia coli (lg (CFU/g))	6.93±0.07	6.90±0.04	CAT(U/ml)	4.39±1.94^{b}	9.93±2.56^{a}
Salmonella (lg (CFU/g))	6.11±0.02^{a}	5.64±0.19^{b}	T-AOC(U/ml)	7.99±1.89^{b}	9.92±4.92^{a}
Bacillus sp. (lg (CFU/g))	6.98±0.05	6.97±0.01	MDA(nmol/ml)	4.17±0.41	3.99±0.23
Lactic acid bacteria (lg (CFU/g))	6.84±0.06^{b}	7.24±0.05^{a}	AKP（U/100 ml）	39.84±0.28	39.87±0.20
Bifidobacterium (lg (CFU/g))	6.33±0.19^{b}	6.89±0.14^{a}	Lysozyme（μg/ml）	2.94±0.20^{b}	3.06±0.18^{a}

brewer's yeast to feeds can improve the growth performance of fish, and also play an important role in fish immunity and antioxidant. Studies by Amir et al.,[10] Huyben et al.,[11] Peterson et al.,[12] Gabriel et al.,[13] Silvia et al.,[14] Gultepe et al.,[15] Xiao et al.,[16] Yunfeng et al.,[17] and Kai et al.[18] showed that the addition of moderate amounts of brewer's yeast or its extracts to feeds could promote the growth of wild pangasius, rainbow trout, catfish, sharp-toothed houmous catfish, European seabass, gilthead seabream, bullfrogs, Japanese swamp shrimp, and seabass through the improvement of hepatic and intestinal tissue development, optimization of the intestinal micro-ecological environment, or enhancement of immunity and antioxidant capacity. In this study, feeding 8% brewer's yeast instead of fish meal to Taiwan loaches improved the development of their intestinal tract, promoted the growth of beneficial bacteria such as Lactic acid bacteria and Bifidobacterium, inhibited the proliferation of Salmonella, and improved the antioxidant and immune ability of Taiwan loaches, which positively affected the growth performance, and this is consistent with the findings of Amir et al..The weight gain rate of the 8% brewer's yeast replacement group (Group C) was significantly higher than that of the other groups (Groups A, B, and D) in Trial 1. In contrast, the weight gain rate of the 8% brewer's yeast replacement group (Group F) did not reach the level of significant difference between Trial 2 (Group E) and the control group (Group E) (P>0.05), which might be related to the fact that Trial 1 was conducted in the outdoor cement pond. In contrast, Trial 2 was conducted in the laboratory glass tank. Of course, the difference in feed formulation (group F was fed a high-fat diet while group C was fed a normal diet) was also an important reason for this difference.

Studies on the effects of adding brewer's yeast to high-fat and low-protein feeds on Taiwan loach's body composition and muscle textural properties have not been reported. The contents of moisture, crude protein, crude fat and crude ash in the muscles of Taiwan loach in the test group (Group F) did not differ significantly (*P>0.05*) from those of the control group (Group E). Na[6] tested the effect of adding 9% brewer's yeast to high-fat and low-protein feeds on the body composition of carp and found that muscle crude protein and crude ash were significantly higher than those of the control group. In contrast, the differences in moisture and crude fat between the two groups were not significant. The different eating habits of the subjects examined may be an important factor contributing to the differences in the experimental results. There is a direct relationship between the textural properties of fish meat and its texture. The higher the hardness, viscosity, elasticity, cohesion, chewability, and restorative capacity of fish meat, the better the texture.[19] Although the differences in viscosity, elasticity, cohesion, chewability, and restorative capacity between the two groups of Taiwan loach muscles were not significant (*P>0.05*), the hardness of Taiwan loach muscles in the test group (Group F) was significantly higher than that in the control group (Group E). It showed that the muscle texture of loach in the test group was improved compared to the control group.

EFFECT OF BREWER'S YEAST ON CARNITINE SYNTHESIS IN FISH AND ITS APPLICATION TO HIGH-FAT, LOW-PROTEIN FEEDS

High-fat and low-protein feeds can save higher-cost protein raw materials,[20] but when the fat content in the feed is too high, it is easy to lead to the hazards of excessive fat deposition and decreased immunity in fish. The studies of Xueli[7] and Tao[8] showed that the addition of L-carnitine to high-fat feeds can promote fat utilization and effectively reduce the fat content of fish. Lysine, methionine, VC, VB3, VB6 and VB9 with iron ions are the components required for the synthesis of carnitine in animals.[4] Hongping[5] pointed out that the addition of carnitine synthesizing ingredients to high-fat diets significantly promoted the synthesis of carnitine by the hepatopancreas of carp, thus achieving similar effects as exogenous addition of L-carnitine. In this study, we utilized the abundant B vitamins in brewer's yeast to formulate a high-fat diet with 8% brewer's yeast instead of fishmeal, and additionally added lysine and methionine to promote the synthesis of carnitine to utilize fat in Taiwan loach. The results showed that replacing fishmeal with

brewer's yeast promoted the synthesis of carnitine in the hepatopancreas of Taiwan loach, which was similar to the results of the study conducted by Na[6] on adding brewer's yeast to high-fat diets for feeding carp, and this further supported the findings of Hongping.

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AUTHORS' CONTRIBUTION

Conceptualization: Wen Anxiang (Lead), Zhou Maoyuan, Pu Liu; Data curation: Zhou Maoyuan (Lead), Wen Anxiang, Li Zongzhang, Xu Jiahao; Formal Analysis: Wen Anxiang (Lead); Funding acquisition: Wen Anxiang (Lead); Methodology: Wen Anxiang (Lead), Zhou Maoyuan; Supervision: Wen Anxiang (Lead); Writing-original draft: Zhou Maoyuan (Lead); Writing-review & editing: Zhou Maoyuan (Equal), Wen Anxiang (Equal).

COMPETING OF INTEREST – COPE

The authors of this article declare that they have no competing interests.

ETHICAL CONDUCT APPROVAL – IACUC

All Taiwan loaches followed the guidelines for the care and use of animals for scientific purposes established by the Institutional Animal Care and Use Committee (IACUC) of Sichuan Agricultural University.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

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