Comparative Study on the Effects of 80% Lysine Sulfate and 98% Lysine Hydrochloride on Growth Performance, Digestive and Absorptive Capacity, and Digestive Organ Development in Mid-Growth Stage Grass Carp (Postprint)

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Abstract

This experiment compared the effects of 80% L-lysine sulfate [80% L-Lys · H2SO4, abbreviated as 80-Lys] and 98% L-lysine hydrochloride [98% L-Lys · HCl, abbreviated as 98-Lys] on growth performance, digestive and absorptive capacity, and digestive organ development in mid-growth stage grass carp, to explore the biological efficacy of 80-Lys and 98-Lys in grass carp and determine the optimal dietary lysine level when using 80-Lys as the lysine supplementation form. A total of 540 healthy grass carp (Ctenopharyngodon idella) with an initial body weight of approximately 275.80 g were selected and randomly divided into 6 groups (3 replicates per group, 30 fish per replicate), and fed diets containing 0.8% lysine (basal diet), 1.0%, 1.2%, 1.4%, and 1.6% lysine from 80-Lys supplementation, and 1.2% lysine from 98-Lys supplementation for 60 days. The results showed that: compared with the basal diet, supplementation with appropriate levels of 80-Lys to achieve 1.2% dietary lysine significantly increased weight gain rate (WGR), specific growth rate (SGR), feed intake (FI), whole intestine lipase and amylase activities, hepatopancreas glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities, anterior, mid, and posterior intestine alkaline phosphatase (AKP) and creatine kinase (CK) activities, hepatosomatic and intestosomatic indices, and anterior and posterior intestine villus heights ($P<0.05$), significantly decreased serum GOT and GPT activities ($P<0.05$), and the effects of 80-Lys on these parameters were significantly superior to those of 98-Lys ($P<0.05$); furthermore, it also significantly increased feed efficiency (FE), whole intestine trypsin activity, anterior, mid, and posterior intestine Na+,K+-ATPase and γ-glutamyl transpeptidase (γ-GT) activities, intestinal length and intestinal length index,

and mid-intestine villus height (P<0.05), but the effects of 80-Lys on these indicators were not significantly different from those of 98-Lys (P>0.05). It was concluded that compared with 98-Lys, 80-Lys can more effectively improve the digestive and absorptive capacity of mid-growth stage grass carp, thereby promoting their growth. When using 80-Lys as the lysine supplementation form, based on SGR and FE as criteria, the optimal dietary lysine levels for mid-growth stage grass carp (276–667 g) were 1.31% (4.68% of dietary protein) and 1.27% (4.54% of dietary protein), respectively.

Full Text

A Comparative Study: Effects of 80% L-lysine · H_2SO_4 and 98% L-lysine · HCl on Growth Performance, Digestive and Absorptive Capacity, and Development of Digestive Organs in Young Grass Carp (Ctenopharyngodon idella)

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Abstract: This study compared the effects of 80% L-lysine · H_2SO_4 (80-Lys) and 98% L-lysine·HCl (98-Lys) on growth performance, digestive and absorptive capacity, and development of digestive organs in young grass carp (Ctenopharyngodon idella). The objective was to evaluate the biological efficacy of these two lysine forms and determine the optimal dietary lysine content when using 80-Lys as the supplemental form. A total of 540 healthy grass carp with an initial body weight of approximately 275.80 g were randomly allocated into six groups (three replicates per group, 30 fish per replicate). The fish were fed experimental diets for 60 days: five diets supplemented with 80-Lys to achieve lysine levels of 0.8% (basal diet), 1.0%, 1.2%, 1.4%, and 1.6%, and one diet supplemented with 98-Lys to achieve 1.2% lysine.

The results demonstrated that compared with the basal diet, supplementation with appropriate levels of 80-Lys to reach 1.2% dietary lysine significantly improved weight gain rate (WGR), specific growth rate (SGR), feed intake (FI), activities of intestinal lipase and amylase, hepatopancreatic glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT), and intestinal alkaline phosphatase (AKP) and creatine kinase (CK) activities in the proximal, mid, and distal intestine (P<0.05). Additionally, it significantly increased

hepatosomatic index (HSI), intestosomatic index (ISI), and fold heights in the proximal and distal intestine ($P<0.05$), while significantly decreasing serum GOT and GPT activities ($P<0.05$). The effects of 80-Lys on these parameters were significantly superior to those of 98-Lys ($P<0.05$).

Furthermore, appropriate 80-Lys supplementation significantly enhanced feed efficiency (FE), total intestinal trypsin activity, Na^+,K^+-ATPase and γ-glutamyltransferase (γ-GT) activities in all intestinal segments, intestinal length, relative gut length (RGL), and mid-intestinal fold height ($P<0.05$). However, no significant differences were observed between 80-Lys and 98-Lys for these particular parameters ($P>0.05$). In conclusion, 80-Lys was more effective than 98-Lys in improving digestive and absorptive capacity, thereby promoting growth in young grass carp. Using 80-Lys as the supplemental form, the optimal dietary lysine contents for young grass carp (276–667 g) were 1.31% (4.68% of dietary protein) based on SGR and 1.27% (4.54% of dietary protein) based on FE.

Keywords: 80% L-lysine·H_2SO_4; 98% L-lysine·HCl; grass carp (Ctenopharyngodon idella); growth performance; digestive and absorptive capacity

Introduction

Lysine is an essential amino acid for many economically important cultured fish species, including grass carp (Ctenopharyngodon idella), and its deficiency significantly reduces production performance. Exogenous amino acid supplementation is an effective approach to compensate for lysine deficiency in plant protein sources, ensure amino acid balance in feeds, maintain normal animal growth, and expand protein feed resources. However, the utilization of synthetic amino acids varies depending on fish species and the supplemental form of the amino acid.

Feed-grade commercial lysine is primarily available in two forms: lysine hydrochloride [L-Lys·HCl, such as 98% L-Lys·HCl (98-Lys, $ 78*{2}SO{4}, *suchas*65*{2}SO{4}$ ($ 51*{2}SO{4}$ ($ 55 46.8 50.7*{2}SO{4}$ and L-Lys · HCl in animals have yielded inconsistent results. These two forms showed equivalent biological efficacy in channel catfish, rainbow trout, pigs, and broiler chickens, yet L-Lys · HCl promoted better growth than L-Lys · H_2SO_4 in tiger shrimp. These findings suggest that different animal species may utilize L-Lys · H_2SO_4 and L-Lys · HCl differently.

Recently developed in China, 80% L-Lys · H_2SO_4 (80-Lys, $ $63% lysine) offers a more environmentally friendly and cost-effective production process compared to L-Lys · HCl. However, research on 80-Lys in animals is limited to a single report in pigs. Given that fish live in aquatic environments and exhibit substantial differences in amino acid absorption and metabolism compared to terrestrial animals, it remains unclear whether 80-Lys affects fish similarly to pigs. For in-

stance, while lysine absorption antagonizes arginine in pig diets and black sea bream, arginine did not significantly affect lysine utilization in Japanese flounder. Therefore, the effects of 80-Lys on fish require investigation.

Grass carp is one of the most widely cultured freshwater species in China, accounting for over 20% of total aquaculture production. To date, no studies have reported the effects of 80-Lys (the novel L-Lys·H_2SO_4) on growth performance, digestive and absorptive capacity, or digestive organ development in grass carp, nor have any compared its efficacy with 98-Lys. This study aimed to investigate the effects of dietary 80-Lys supplementation, determine optimal inclusion levels, elucidate potential mechanisms of action, compare the biological efficacy of 80-Lys and 98-Lys, and provide reference data for the application of novel lysine forms in freshwater fish culture.

1.1 Experimental Design and Diet Formulation

The 80-Lys and 98-Lys used in this experiment were provided by Changchun Dacheng Industrial Group Co., Ltd. A basal diet was formulated using rice gluten meal, soybean meal, cottonseed meal, and rapeseed meal as primary protein sources. The basal diet was supplemented with 80-Lys to achieve dietary lysine levels of 0.8% (basal), 1.0%, 1.2%, 1.4%, and 1.6%, or with 98-Lys to achieve 1.2% lysine (meeting the growth requirement of grass carp). All diets were balanced for protein level by adding glycine. Dietary compositions and nutrient levels are presented in Table 1 . Amino acid compositions of the experimental diets were analyzed using the method of Feng et al. and are listed in Table 2 .

1.2 Feeding Trial

The feeding trial was conducted at the Daya Research Base of the Aquatic Animal Nutrition Laboratory at Sichuan Agricultural University using cage culture. Grass carp were acclimated for four weeks before the formal experiment began. A total of 540 healthy grass carp with an initial body weight of (275.80\$±\$0.64) g were randomly divided into six groups with three replicates each (30 fish per replicate). Each replicate was stocked in a 1.4 m × 1.4 m × 1.4 m cage, with no significant difference in initial body weight among groups (P>0.05). Fish were fed the experimental diets for 60 days. Feeding management followed the protocol established in our previous studies: fish were hand-fed four times daily, and uneaten feed was collected 30 minutes after each feeding, dried, and weighed. Fish health was monitored regularly, water was exchanged periodically, and routine parasite and bacterial control measures were implemented. Water temperature was maintained at $(28\$\pm2)°C, pHat7.2\pm\$0.2$, and dissolved oxygen concentration remained above 6 mg/L throughout the experiment.

1.3 Sample Collection and Parameter Determination

At the beginning and end of the growth trial, fish in each cage were weighed to calculate weight gain (WG), weight gain rate (WGR), and specific growth rate (SGR). Daily feed intake was recorded, and residual feed was collected and weighed to determine feed intake (FI) and feed efficiency (FE). Mortality was recorded during the experiment to calculate survival rate (SR). At the end of the trial, 15 fish per group were randomly selected and anesthetized with p-aminobenzoic acid according to Geraylou et al. Fish were then weighed, measured for body length, and bled following Huang et al. The hepatopancreas and intestine were rapidly dissected and weighed. Intestinal length was measured before the proximal (PI), mid (MI), and distal (DI) intestine segments were isolated, snap-frozen in liquid nitrogen, and stored at -80°C for laboratory analysis. An additional six fish per group were randomly selected to measure intestinal fold height following Lin et al.

Activities of trypsin, chymotrypsin, lipase, and amylase in the whole intestine; AKP, CK, Na^+,K^+-ATPase, and γ-GT activities in PI, MI, and DI; and GOT and GPT activities in serum and hepatopancreas were determined using the methods described by Li et al. and Tang et al., respectively.

1.4 Calculation Formulas

WGR = [WG (g) / initial body weight (g)] × 100
SGR = {[ln(final body weight (g)) - ln(initial body weight (g))] / experimental days (d)} × 100
FI = total feed offered (g) - total residual feed (g)
FE = [WG (g) / feed intake (g)] × 100
Survival rate = {[initial fish number - dead fish number] / initial fish number} × 100
Hepatosomatic index (HSI) = [hepatopancreas weight (g) / body weight (g)] × 100
Intestosomatic index (ISI) = [intestinal weight (g) / body weight (g)] × 100
Relative gut length (RGL) = [intestinal length (cm) / body length (cm)] × 100

1.5 Data Processing and Statistical Analysis

Data are expressed as means ± standard deviation. Statistical analysis was performed using SPSS 18.0 software. One-way ANOVA combined with Duncan' s multiple comparison test was used to analyze data from different lysine levels in the 80-Lys groups, with $P<0.05$ considered statistically significant. Regression analysis was performed for parameters showing significant differences.

Independent t-tests were used to compare the 80-Lys (1.2% lysine) and 98-Lys (1.2% lysine) groups, with $P<0.05$ indicating significant differences between treatments.

2.1 Effects of 80-Lys and 98-Lys on Growth Performance of Young Grass Carp

As shown in Table 3 , survival rate was 100% in all groups. Final body weight, WG, WGR, SGR, FI, and FE increased significantly with increasing dietary 80-Lys levels ($P<0.05$), reaching maximum values at 1.2% dietary lysine, then decreased significantly with further 80-Lys supplementation ($P<0.05$). At 1.2% dietary lysine, the 80-Lys group showed significantly higher final body weight, WG, WGR, SGR, and FI compared with the 98-Lys group ($P<0.05$), while FE and survival rate did not differ significantly ($P>0.05$).

Using 80-Lys as the supplemental form, quadratic regression equations were established between dietary lysine content (x) and growth parameters:
WG: $yWG = -431.56x^2 + 1132.06x - 367.88$ ($R^2 = 0.869$, $P<0.01$)
WGR: $yWGR = -157.13x^2 + 412.08x - 134.38$ ($R^2 = 0.867$, $P<0.01$)
SGR: $ySGR = -1.2x^2 + 3.15x - 0.64$ ($R^2 = 0.884$, $P<0.01$)
FI: $yFI = -370.6x^2 + 1009.99x - 33.3$ ($R^2 = 0.895$, $P<0.01$)
FE: $yFE = -0.38x^2 + 0.97x - 0.05$ ($R^2 = 0.795$, $P<0.01$)

Based on quadratic curve analysis using SGR and FE as criteria, the optimal dietary lysine contents for young grass carp (276–667 g) were 1.31% and 1.27%, respectively (Figure 1 [Figure 1: see original paper]).

2.2 Effects of 80-Lys and 98-Lys on Intestinal Digestive Enzyme Activities

As shown in Table 4 , dietary 80-Lys supplementation did not significantly affect chymotrypsin activity ($P>0.05$). Trypsin activity increased significantly with 80-Lys supplementation ($P<0.05$), peaking at 1.2% dietary lysine, then decreased significantly with higher supplementation levels ($P<0.05$). Lipase and amylase activities showed similar trends. At 1.2% dietary lysine, the 80-Lys group exhibited significantly higher lipase and amylase activities but lower chymotrypsin activity compared with the 98-Lys group ($P<0.05$), while trypsin activity did not differ significantly ($P>0.05$).

2.3 Effects of 80-Lys and 98-Lys on Intestinal Brush-Border Enzyme Activities

AKP activities in PI, MI, and DI increased significantly with 80-Lys supplementation ($P<0.05$), reaching maximum values at 1.2% dietary lysine before decreasing significantly ($P<0.05$). CK activities in all intestinal segments and γ-GT activities in MI and DI showed similar trends. At 1.2% dietary lysine, Na^+,K^+-ATPase activities in PI and MI were significantly higher than in the 0.8%, 1.4%, and 1.6% groups ($P<0.05$) but did not differ from the 1.0% group ($P>0.05$). Na^+,K^+-ATPase activity in DI and γ-GT activity in PI were significantly higher than in the 0.8%, 1.0%, and 1.6% groups ($P<0.05$) but similar to the 1.4% group ($P>0.05$). At 1.2% dietary lysine, the 80-Lys group showed significantly higher AKP and CK activities in all intestinal segments compared with the 98-Lys group ($P<0.05$), while Na^+,K^+-ATPase and γ-GT activities did not differ significantly ($P>0.05$).

Quadratic regression equations using 80-Lys as the supplemental form were:
AKP-PI: $yAKPPI = -453.81x^2 + 1077.45x - 487.36$ ($R^2 = 0.910$, $P<0.01$)
AKP-MI: $yAKPMI = -612.86x^2 + 1499.27x - 765.68$ ($R^2 = 0.971$, $P<0.01$)
AKP-DI: $yAKPDI = -285.55x^2 + 692.07x - 352.23$ ($R^2 = 0.740$, $P<0.01$)
CK-DI: $yCKDI = -154.54x^2 + 353.64x - 96.88$ ($R^2 = 0.709$, $P<0.01$)
γ-GT-MI: $y\gamma\text{-}GTMI = -185.1x^2 + 469.02x - 201.25$ ($R^2 = 0.748$, $P<0.01$)
γ-GT-DI: $y\gamma\text{-}GTDI = -225.14x^2 + 574.93x - 275.62$ ($R^2 = 0.716$, $P<0.01$)

2.4 Effects of 80-Lys and 98-Lys on Serum and Hepatopancreatic GOT and GPT Activities

Serum GOT and GPT activities decreased then increased with 80-Lys supplementation, reaching minimum values at 1.2% dietary lysine. Serum GOT activity at 1.2% lysine was significantly lower than all other groups ($P<0.05$), while serum GPT activity was significantly lower than all groups except the 1.0% group ($P<0.05$). Hepatopancreatic GPT activity showed the opposite trend, with the 1.2% group being significantly higher than all other groups ($P<0.05$). Hepatopancreatic GOT activities in the 1.2% and 1.4% groups were significantly higher than in the 0.8%, 1.0%, and 1.6% groups ($P<0.05$), with no significant difference between the 1.2% and 1.4% groups ($P>0.05$). At 1.2% dietary lysine, the 80-Lys group showed significantly lower serum GOT and GPT activities but significantly higher hepatopancreatic GOT and GPT activities compared with the 98-Lys group ($P<0.05$).

Quadratic regression equations were:
Hepatopancreatic GOT: $y = -9.077x^2 + 22.851x - 7.002$ ($R^2 = 0.751$, $P<0.01$)
Serum GOT: $y = 5.119x^2 - 12.069x + 8.289$ ($R^2 = 0.786$, $P<0.01$)

2.5 Effects of 80-Lys and 98-Lys on Hepatopancreatic and Intestinal Development

Hepatopancreatic weight increased then decreased with 80-Lys supplementation, peaking at 1.2% dietary lysine and being significantly higher than all other groups ($P<0.05$). Intestinal length, RGL, intestinal weight, ISI, and fold heights in all segments showed similar trends. HSI in the 1.2% group was significantly higher than in the 0.8%, 1.4%, and 1.6% groups ($P<0.05$) but did not differ from the 1.0% group ($P>0.05$). At 1.2% dietary lysine, the 80-Lys group showed significantly higher hepatopancreatic weight, HSI, intestinal weight, ISI, and fold heights in all intestinal segments compared with the 98-Lys group ($P<0.05$), while intestinal length and RGL did not differ significantly ($P>0.05$).

Quadratic regression equations were:
Hepatopancreatic weight: $y = -33.67x^2 + 86.87x - 35.32$ ($R^2 = 0.769$, $P<0.01$)
Intestinal weight: $y = -40.92x^2 + 101.02x - 45.91$ ($R^2 = 0.795$, $P<0.01$)
Proximal intestinal fold height: $y = -2041.28x^2 + 4946.26x - 1691.77$ ($R^2 = 0.771$, $P<0.01$)
Mid-intestinal fold height: $y = -1070.57x^2 + 2616.35x - 462.67$ ($R^2 = 0.790$, $P<0.01$)
Distal intestinal fold height: $y = -1451.31x^2 + 3558.72x - 1128.55$ ($R^2 = 0.753$, $P<0.01$)

3.1 Effects of 80-Lys on Growth Performance and Comparison with 98-Lys

Lysine is an important essential amino acid for fish. This study demonstrated that dietary 80-Lys supplementation significantly improved final body weight, WG, WGR, and SGR in young grass carp, with significant quadratic relationships between dietary lysine content and growth parameters, indicating that appropriate 80-Lys levels promote growth. Moreover, at 1.2% dietary lysine, 80-Lys showed superior growth-promoting effects compared with 98-Lys. Although no previous studies on 80-Lys in fish exist, these results align with findings on other lysine forms in grass carp at different life stages and in other fish species. However, inconsistent results have been reported regarding crystalline lysine supplementation in grass carp, possibly due to differences in feeding frequency affecting amino acid absorption synchrony and utilization efficiency. Fish growth depends on nutrient intake, which is closely related to dietary essential amino acid content. This study showed that FI and FE increased then decreased with 80-Lys supplementation, peaking at 1.2% lysine. At 1.2% dietary lysine, the 80-Lys group showed significantly higher FI than the 98-Lys group. These results suggest that 80-Lys promotes growth by improving FI and FE more effectively than 98-Lys. Using 80-Lys as the supplemental form, the optimal dietary lysine contents for young grass carp (276–667 g) were 1.31% (4.68% of dietary protein) based on SGR and 1.27% (4.54% of dietary protein) based on FE.

3.2 Effects of 80-Lys on Digestive and Absorptive Capacity and Amino Acid Metabolism and Comparison with 98-Lys

Digestive and absorptive capacity significantly influences FI and FE in fish. Intestinal digestive enzyme activities are important indicators of digestive capacity. This study showed that appropriate 80-Lys supplementation significantly increased trypsin, lipase, and amylase activities in the whole intestine. At 1.2% dietary lysine, 80-Lys produced significantly higher lipase and amylase activities but lower chymotrypsin activity compared with 98-Lys, while trypsin activity did not differ. These results align with previous studies on L-Lys · H_2SO_4 in later-stage grass carp and L-Lys · HCl in other species. The lack of significant effect on chymotrypsin may be because the basal diet' s lysine content (0.8%) already met the requirements for chymotrypsin synthesis, secretion, and activation. Lysine may affect digestive enzyme activity through secretion and release mechanisms, as it stimulates pancreatic trypsinogen release in fish.

The intestine is the primary site for nutrient absorption, which is closely related to brush-border enzyme activities. AKP is a membrane-bound protein that provides phosphate groups for phosphorus and nucleic acid metabolism. Intestinal alkaline phosphatase (IAKP) on the brush-border surface is involved in absorption of vitamin D, calcium, amino acids, cholesterol, lipids, and glucose. CK catalyzes ATP generation to provide energy for nutrient absorption. Na^+,K^+-ATPase directly affects energy supply for active nutrient transport and participates in amino acid and glucose absorption. γ-GT is a key enzyme in the glutamate cycle that promotes amino acid transport into cells for protein synthesis. Therefore, these enzyme activities reflect nutrient absorptive capacity. This study demonstrated that appropriate 80-Lys supplementation significantly increased AKP, CK, Na^+,K^+-ATPase, and γ-GT activities in all intestinal segments, consistent with previous research. At 1.2% dietary lysine, 80-Lys significantly increased AKP and CK activities compared with 98-Lys, suggesting it may enhance absorptive capacity more effectively.

GOT and GPT are crucial enzymes for amino acid metabolism in fish, with hepatic activities reflecting liver amino acid metabolism. This study showed that hepatopancreatic GOT and GPT activities increased with 80-Lys supplementation, peaking at 1.4% and 1.2% lysine, respectively, indicating enhanced hepatic amino acid metabolism. At 1.2% dietary lysine, 80-Lys increased hepatic amino acid metabolism more effectively than 98-Lys. Previous studies have shown that appropriate L-Lys · H_2SO_4 and L-Lys supplementation increased GOT and GPT activities in grass carp and swimming crab, respectively. Hepatic GOT activity correlates positively with protein retention rate in fish. Therefore, 80-Lys may enhance amino acid metabolism and dietary protein utilization more effectively than 98-Lys, thereby promoting growth, though the specific mechanisms require further investigation.

3.3 Effects of 80-Lys on Digestive Organ Development and Comparison with 98-Lys

The hepatopancreas and intestine are the most important organs for digestion, absorption, and nutrient metabolism in fish. Their development is closely related to digestive capacity and amino acid metabolism. This study showed that appropriate 80-Lys supplementation significantly increased hepatopancreatic weight, HSI, intestinal weight, ISI, and RGL. At 1.2% dietary lysine, the 80-Lys group showed significantly higher hepatopancreatic weight, HSI, intestinal weight, and ISI compared with the 98-Lys group. These results align with findings in juvenile grass carp where crystalline L-Lys increased HSI.

Intestinal fold height is another important indicator of intestinal development and absorptive surface area. This study demonstrated that appropriate 80-Lys supplementation significantly increased fold heights in all intestinal segments, with the 80-Lys group showing significantly higher proximal and distal intestinal fold heights than the 98-Lys group at 1.2% lysine. Normal hepatic cellular structure and function are essential for digestion and nutrient metabolism. Changes in cell permeability indicate cellular damage, and release of cytoplasmic enzymes GOT and GPT reflects cell membrane leakage. This study showed that appropriate 80-Lys supplementation significantly reduced serum GOT and GPT activities, with the 80-Lys group showing significantly lower values than the 98-Lys group at 1.2% lysine. Similar results have been reported in mandarin fish fed L-Lys · HCl. These findings suggest that 80-Lys may more effectively promote digestive organ development, increase intestinal absorptive surface area, and maintain normal hepatic cellular structure and function compared with 98-Lys, though the specific mechanisms warrant further investigation.

Conclusions

1. Compared with 98-Lys, 80-Lys more effectively improved growth performance in young grass carp.
2. Compared with 98-Lys, 80-Lys more effectively protected hepatopancreatic structural integrity, increased intestinal absorptive surface area, promoted digestive organ development, and enhanced digestive and absorptive capacity, thereby increasing feed intake and promoting growth.
3. Using 80-Lys as the supplemental form, the optimal dietary lysine contents for young grass carp (276–667 g) were 1.31% (4.68% of dietary protein) based on SGR and 1.27% (4.54% of dietary protein) based on FE.

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Note: Figure translations are in progress. See original paper for figures.