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A New Culture Method of High Ecological Efficiency of Grass Carp (*Ctenopharyngodon idellus*)

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ABSTRACT

To explore new culture methods to save aquaculture water and reduce the emission of aquaculture wastewater in current culture of grass carp mainly feeding with compound feed now, in this study, grass carp were respectively fed with hybrid napier grass (grass group), compound feed (feed group), and a mixture of hybrid napier grass and compound feed (mixture group). Growth rate, aquaculture water quality, and sediment indexes in these three groups were analysed. The results revealed that mixture group had the highest growth rate and grass group had the lowest rate ($P < 0.05$). The lowest levels of water TN, NH_4^+-N, TP, SP, CODcr, *Cyanobacteria* and sediment available N, P, K were observed in the grass group, and the highest were observed in the feed group ($P < 0.05$). Overall, the feeding approach of compound feed mixed with hybrid napier grass might be a better model for grass carp. In the future, if hybrid napier grass can be formed into a palatable diet combined with compound feed, it will perhaps reduce the accumulation of sediments brought by the hybrid napier grass, and further enhance the utilization efficiency of the hybrid napier grass in grass carp aquaculture.

INTRODUCTION

Improvement in living standard has shifted fish consumption from quantity-oriented to quality-oriented, and excellent flesh quality of fish is now more preferred by consumers. It has been studied that flesh quality of fish was affected by different feeds (Quillet et al. 2007) and farming methods (Johnston et al. 2006, Kriton 2007). Grass carp, as a typical herbivorous fish, is one of China's major commercial freshwater fish species which are preferred by consumers. In China, with the continuous expansion of farming scale, compound feed has become the most important source of food for grass carp because of its many advantages, such as high nutritional content, digestibility, and growth-promoting effect. However, long-term intake of compound fed by grass carp has led to decreased flesh quality and abnormal body shape (Huang & Huang 1992, Guo et al. 2012). It has been pointed out that as a natural food, green forage not only has a wide range of sources and low cost (Pandit et al. 2004), but also can provide a variety of vitamins and amino acids necessary for the growth of grass carp (Raa et al. 1982). So, addition of green forage to the compound feed may improve the flesh quality and body shape of grass carp.

It was demonstrated that the mixture of green forage and compound feed could reduce body cavity fat, improve body shape, and promote the growth of grass carp (Huang & Huang 1992, Guo et al. 2012, Feng et al. 2008). Compared with compound feed, the mixture feed could reduce the body cavity and liver fat, serum triglycerides, and cholesterol of grass carp (Huang & Huang 1992). An experiment comparing the addition of hybrid *Pennisetum* (*Pennisetum americanum* × *P. purpureum*) to compound feed in the ratio of 1:1 (dry matter) and compound feed alone demonstrated that hybrid *Pennisetum* significantly increased the muscle content of n-3 polyunsaturated fatty acids (PUFA) of the grass carp (Feng et al. 2008). In the study of the feeding of grass carp with mixtures of duckweed and compound feed in the different ratios (dry matter), it has been found that the addition of duckweed to compound feed in the ratio of 1:3 was not only associated with a high growth rate, but also maintenance of an appropriate body shape and a high muscle nutritional content (Guo et al. 2012).

Unlike aquatic and other terrestrial plants, hybrid napier grass (*Pennisetum sinese* Roxb.), as a perennial terrestrial plant, has an annual output of up to 30,000 kg/hm^2, and is rich in crude protein, crude fibre, crude fat, and nitrogenfree extract (Lowe 2011). It has been demonstrated that grass carp fed with hybrid napier grass had growth rates three and five times higher than those fed with *Hydrilla* and *Ceratophyllum*, respectively (Venkatesh & Shetty 1978). Therefore, hybrid napier grass has great potential for adding to compound feed in the diet of grass carp. However, to date, analysis of meat amino acids, aquaculture water quality, sediments, and net incomes of grass carp feeding a mixture of hybrid napier grass and compound feed have rarely been reported.

To study the effects of the mixture of hybrid napier grass and compound feed on grass carp aquaculture, in the present study, grass carp were fed with three different diets, hybrid napier grass alone (grass group), compound feed mixed with hybrid napier grass (mixture group), and compound feed alone (feed group). The effects of the three different diets on the growth, muscle amino acids, and fatty acids composition of grass carp were assessed, and the physical and chemical indicators and algae in the aquaculture water and sediment were investigated. The results of this study will provide a scientific basis for the application of hybrid napier grass in the aquaculture of grass carp.

MATERIALS AND METHODS

Aquaculture experiment: The experiment was conducted in concrete ponds (length × width × height: 2.5 × 2.5 × 2 m) at a precise aquaculture site at the Pearl River Fisheries Research Institute of *CAFS*. Before fingerling stocking, the pond walls and bottom were washed with bleach and tap water. The ponds were then filled with water to depth of 1.5 m and kept aerated for two days. The farming period was from July 15, 2013 to December 15, 2013. The fingerlings were stocked at a density of 3029.44 kg/ha, and an average weight of 94.67±10.41g. There was no significant difference in fingerling weight between the treatment groups. The fingerlings used in the experiment were acclimated to feed voluntarily on compound feed and hybrid napier grass.

The hybrid napier grass used was planted at the Pearl River Fisheries Research Institute, and typically harvested at a height of 0.5 m, containing 71.96% moisture, 18.46% crude protein, 17.78% crude fat, and 1.74% crude fiber. The compound feed used was expanded feed, containing 10.1% moisture, 28% crude protein, 3% crude fat, and 10% crude fiber.

The same amount of crude protein was fed to each treatment group. The feed group was fed with compound feed only; daily ration = 3% of the total weight of the grass carp. The grass group was fed with hybrid napier grass only; daily ration = [ration of feed group × (1-10.1%) × 28% (protein content of compound feed)] / [18.46% (protein content of hybrid napier grass) × (1-71.96%)]. The mixture group was fed with 50% of the feed group ration and 50% of the grass group ration. Two replicates were performed for each treatment group. Five fish were randomly selected from each pond each month and weighed to estimate the total weight. The ration was adjusted according to the newly estimated total weight.

Analysis of biological characteristics: At the end of the feeding experiment, all fish from each treatment group were starved for 24 h and then weighed. Ten fish were also randomly sampled from each pond to measure body height, intestinal length, and weight of internal organs.

Determination of conventional nutrients: Nutrients were determined according to the national standards of China, such as Determination of Moisture in Foods (GB5009.3-2010), Determination of Ash in Foods (GB5009. 4-2010), Determination of Protein in Foods (GB5009.5-2010), and Determination of Fat in Foods (GB/T5009.6-2010).

Determination of aquaculture water quality: Pond water was sampled once a month, including the initial water to determine the water TN, NH_4^+-N, TP, SP, COD according to the operating manual of Spectroquant NOVA multi-parameter water quality analyser (Merck, Germany). Dissolved oxygen and temperature were determined *in situ* using a dissolved oxygen meter (Pro2030, YSI, USA). pH was measured by a digital pH meter (PSH-25, Shanghai Jingke Leici Factory, China).

Determination of algae in aquaculture water: In total, 0.1 mL of the concentrated water sample was transferred into a 0.1 mL count box, covered with a coverslip, and counted at 10×40 magnification, with care taken to avoid spilling or bubbles. Three to four slides from each bottle were prepared, with 50 fields of vision per slide. When the difference of a count from the average count was not greater than ± 15% of the average count, it was considered a valid count.

Analysis of physical and chemical properties of pond sediment: At the end of the experiment, sediments from all ponds were sampled. Three mixed samples, each from four sampling points, were collected from each pond. The sediment samples were air-dried, smashed with a wooden tool, and filtered through a 20-mesh sieve to measure pH, available nitrogen, phosphorus and potassium; the samples were also filtered through a 100-mesh sieve for organic matter analysis. The physical and chemical parameters were determined as follows: pH by potentiometry, organic matter by potassium dichromate volumetric method, available nitrogen by alkaline hydrolysis diffusion method, available phosphorus by sodium bicarbonate method, and available potassium by ammonium acetate flame photometry method.

Data analysis: Data are presented as mean ± standard deviation (X ± SD). Statistical differences were determined by one-way ANOVA followed by Duncan's multiple range test ($P < 0.05$). All statistical analyses were performed using software SPSS 19.0 (IBM Company, Chicago, IL, USA).

RESULTS

Effects of Different Diets on Growth

In terms of appearance, the feed group had a short and fat body, whitish body colour, and less pigment contained in scales. By contrast, the grass group had a relatively long and thin body, dark body colour with light dark green gloss, and more pigment contained in scales. The different diets also had a significant impact on the growth of grass carp. The average weight of the mixture group was 78.9% higher than that of the grass group ($P < 0.05$), and was slightly higher than that of the feed group ($P > 0.05$) (Table 1).

Effects of Different Diets on Water Quality Indicators

In all the three treatment groups, temperature dropped from approximately 30°C in summer to approximately 16°C in the late period, and the pH fluctuated between 7.34 and 8.27, with an average of 7.51. Since the same number of oxygenators was added at the bottom of each pond during the farming period, the dissolved oxygen level in each treatment group fluctuated in a small range of 5.38-6.24, with an average of 5.81. Because all treatments were performed under "zero-water exchange" conditions, the contents of nutrients in the aquaculture water of different treatment groups changed significantly over time during the farming period.

The trends of TN and NH_4^+-N contents in aquaculture water over time were roughly the same in the different treatment groups (Fig. 1-A and 1-B). However, TN and NH_4^+-N contents in the feed group were always significantly higher than those of the other two groups ($P < 0.05$). TN and NH_4^+-

Table 1: Growth and morphological characteristics of grass carp feeding different diets.

Item	Grass group	Mixture group	Feed group
Initial body weight (g)	95.69±8.20^a	97.20±5.51^a	98.59±6.47 ^{a}
Final body weight (g)	275.95±49.17^b	493.69±93.38^a	480.83±78.79^a
Final gross weight (g)	11038.2	19747.4	19233.2
Weight gain (%)	188.38±5.13^b	407.91±17.57^a	387.71±12.34^a
Purtenance/body weight (%)	5.19±1.34^b	11.22±1.49^a	11.28±2.34^a
The intestine ratio	1.98±0.27^a	2.07±0.74^a	2.03±0.86^a

Note: Values in the same row with different letters are significantly different (*P* < 0.05).

Table 2: The composition of algae in culture water of grass carp feeding different diets.

	September			December		
	Composition	Dominant species	Amount (cell/L)	Composition	Dominant species	Amount (cell/L)
Grass group	4 of phylum (Cyanobacteria, Chlorophyta, Chrysophyta, Bacillariophyta), 16 of species	Oscillatoriales, *Aulacoseira granulata*	2.1×10^7	5 of phylum (Cyanobacteria, Chlorophyta, *Chrysophyta,* Bacillariophyta, Diatom), 18 of species	*Aphanizomenon flos aquae,* Oscillatoriales, *Spirulina*	1.5×10^7
Mixture group	4 of phylum (Cyanobacteria, Chlorophyta, Chrysophyta, Diatom), 26 of species	*Hyalotheca dissiliens, Lynbya*	2.5×10^7	5 of phylum (Cyanobacteria, Chlorophyta, Chrysophyta, Dinoflagellates, Diatom), 24 of species	*Aphanizomenon flos aquae, Lynbya*	6.9×10^7
Feed group	5 of phylum (Cyanobacteria, Chlorophyta, Chrysophyta, Cryptophyta, Diatom), 24 of species	*Lynbya,* Oscillatoriales	3.2×10^7	5 of phylum (Cyanobacteria, Chlorophyta, Chrysophyta, Cryptophyta, Diatom), 21 of species	*Aphanizomenon flos aquae,* Oscillatoriales	4.6×10^7

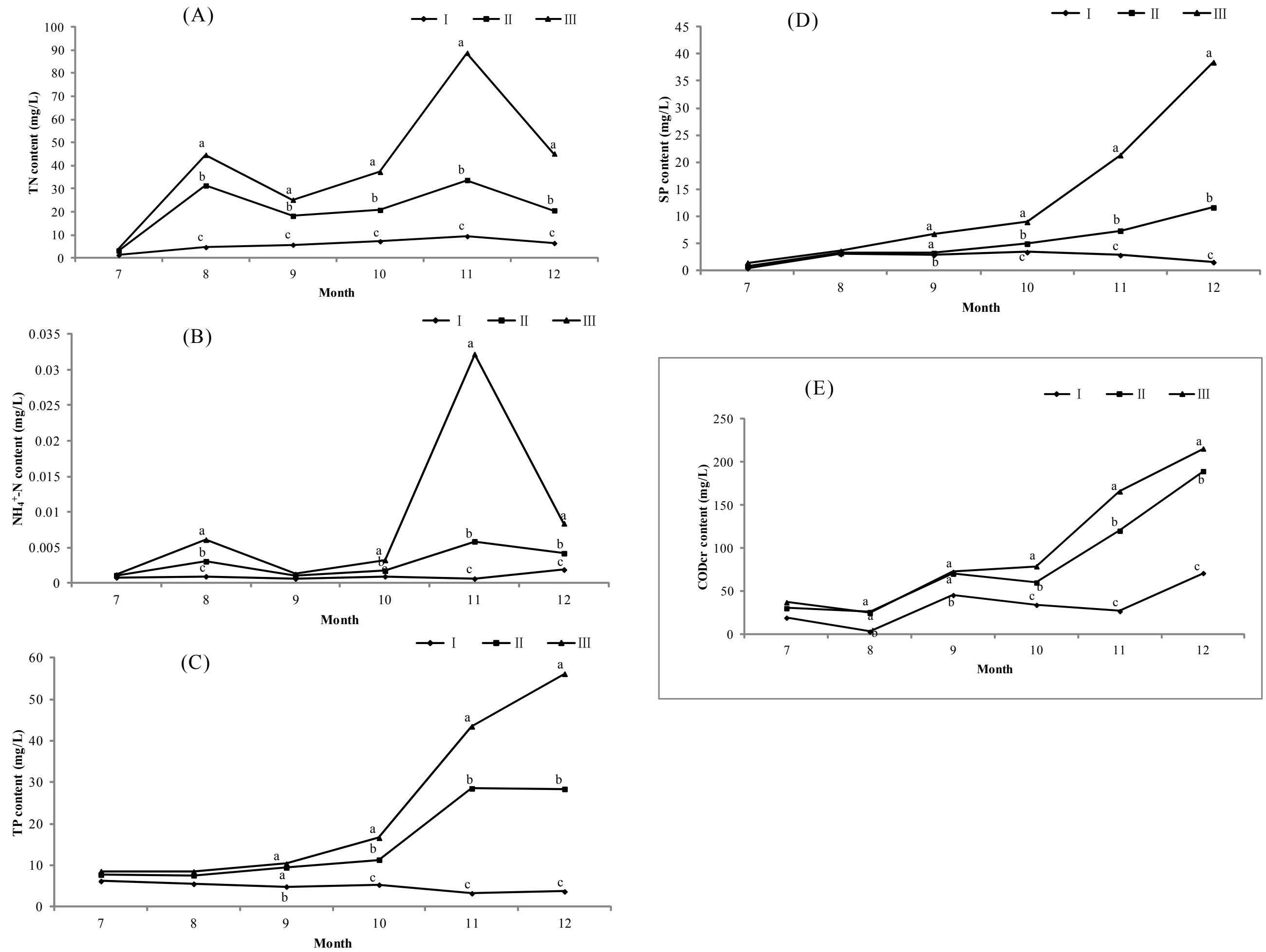

Fig. 1: Changes in the water quality of grass carp feeding different diets. I: Grass group; II: Mixture group; III: Feed group. Values in the same row with different letters are significantly different (P < 0.05).

N contents maintained a relatively low level in the grass group throughout the farming period, and were significantly different from the other two groups (P < 0.05) (except September), indicating that the best water quality was found in the grass group. The trends of TP and SP contents in aquaculture water over time were similar in the different treatment groups (Fig. 1-C and 1-D). The TP and SP contents in the feed group were higher and increased over time. Those in the mixture and grass groups, fluctuated in a relatively small range. The water TP content showed no significant difference among the three treatment groups in July, August and September, while it was significantly higher in the feed group than in the other two groups in the last three months (P < 0.05). Significant differences in water SP content began to appear among the three treatment groups in October. The trend of CODcr content in aquaculture water over time was broadly similar in the three groups (Fig. 1-E). The CODcr content was always significantly lower in the grass group than in the other two groups (P < 0.05). Overall, the highest water TN, TP, SP, NH_4^+-N and CODcr contents were noted in the feed group, and the lowest in the grass group.

Effects of Different Diets on Algae in Aquaculture Water

Qualitative detection of algae: Changes in algae composition in different treatment groups in September (autumn) and December (winter) are shown in Table 2. Algal species richness increased to varying degrees over time in all three treatment groups, and great changes were also seen in the dominant species. Large differences were also noted among the different treatments. The mixture group was richest in algae species and quantity, and the grass group was the least rich. The composition of dominant species was also not the same in each group. The dominant algal species in

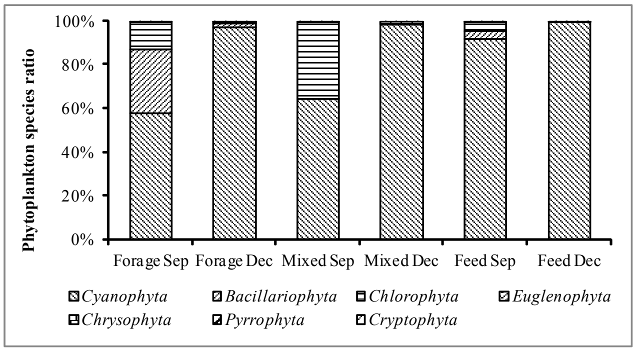

Fig. 2: Ratio change of phytoplankton species during the cultivation time.

the grass group were Oscillatoriales from Cyanobacteria and *Aulacoseira granulata* from Bacillariophyta in September, and the three dominant algal species were all from Cyanobacteria in December. The dominant species in the mixture group were *Lyngbya* from Cyanobacteria and *Hyalotheca dissiliens* from Chlorophyta in September, and all from Cyanobacteria in December. The dominant species in the feed group were all from Cyanobacteria and included Oscillatoriales throughout the farming period.

Changes in quantitative composition of algae in different treatments: The quantitative composition of algae was different in the two periods among different treatment groups. Although most species were from Chlorophyta, Cyanobacteria accounted for the largest quantitative proportion. Moreover, the number of Cyanobacteria increased over time in all the treatment groups (Fig. 2). The number of Cyanobacteria increased from 57.65% in September to 96.58% in December in the grass group, representing an increase of 67.53%. In the mixture group, this number increased from 64% to 97.99%, representing an increase of 53.11%. In the feed group, the number of Cyanobacteria increased from 91.29% to 98.55%, representing an increase of 7.96%. In both September and December, the ranking of the three treatment groups was grass group < mixture group < feed group in terms of the number of Cyanobacteria. A significant difference was noted among the three groups in September, but not in December.

Effects of Different Diets on Physical and Chemical Properties of Sediments

Sediments from the ponds of all treatment groups were slightly alkaline in the late period (Table 3). Although the sedimentary organic matter content was higher in the grass group than those in the feed and mixture groups, the sedimentary nitrogen, phosphorus, and potassium contents were the lowest in the grass group ($P < 0.05$).

DISCUSSION

The growth rate of grass carp in the mixture group was significantly higher than that of the grass group, and was slightly higher than that of the feed group. Among the three groups, the benefit of the mixture group was adequate and balanced nutrition for the growth of grass carp in this study. A previous study demonstrated that grass carp fed with compound feed alone developed significantly fatty livers mainly because of deficiency of vitamin C and other biologically active substances of compound feed, and this trial suggested that mixture feeding of compound feed and green fodder could control the development of fatty livers and accelerate the growth of fish (Huang & Huang 1992). It also had been suggested that appropriate amounts of dietary fibre were important for digestion in fishes because fibre can enhance the peristaltic movements of the intestine, stimulate the secretion of digestive enzymes and enhance the contact surface between food and enzymes (He et al. 2009). Therefore, the mixture of hybrid napier grass and compound feed took advantage of hybrid napier grass resources to reduce production costs, as well as possibly promoted more efficient digestion and utilization of compound feed. In this study, the lowest TN, NH_4^+-N, TP, SP and CODcr levels in aquaculture water were detected in the grass group, and the highest TN, NH_4^+-N, TP, SP and CODcr levels were found in the feed group. In terms of number of Cyanobacteria, the ranking of three treatment groups was grass group < mixture group < feed group, with a significant difference in September, but not in December.

There are two exogenous sources of nutrients, i.e., nitrogen and phosphorus, in aquaculture water. One of these is waste entering the water during farming (uneaten feed, faeces, excreta from aquatic organisms, etc.), and the other source is fertilizers and poultry feces washed into the aquaculture water (Lazzari & Baldisserotto 2008). It had been pointed out that 25% of nitrogen of compound feed input was retained by fish, and 75% of nitrogen input was excreted into the aquaculture system (Hargreaves 1998). It had been reported that production of 1,000 kg of salmon resulted in the discharge of 92-102 kg of nitrogen and 9.0-9.5 kg of phosphorus into the water in a variety of forms (Hall et al. 1992, Folke et al. 1997). As a fresh forage grass, the hybrid napier grass contains nitrogen and phosphorus as compounds that can be ingested by grass carp without direct contamination. Moreover, the hybrid napier grass has lower nitrogen and phosphorus contents than the compound feed, which leads to significantly reduced exogenous nitrogen and phosphorus sources. In our study, the lowest nutrient contents were observed in the grass group, and the highest in the feed group. Since all treatments were performed

Table 3: The properties of ponds sediments for grass carp feeding different diets.

Group	pH	Organic matter (%)	Available N(mg/kg)	Available P(mg/kg)	Available K(mg/kg)
Grass group	7.32±0.03[a]	15.49±0.87[a]	13.45±0.43[c]	5.67±0.22[b]	5.13±0.13[c]
Mixture group	7.58±0.04[a]	10.31±0.46[b]	15.83±0.52[b]	6.75±0.28[a]	5.86±0.25[b]
Feed group	7.87±0.05[a]	8.84±0.38[c]	17.47±0.48[a]	6.98±0.32[a]	6.75±0.34[a]

Note: Values in the same row with different letters are significantly different ($P < 0.05$).

under “zero water exchange” conditions, which enhanced the accumulation of nutrients, the water nutrient contents increased over time in all groups.

There are also endogenous sources of nutrients, such as residual feeds, feces, and other organic matter deposited at the bottom, which will be re-released into the water under certain environmental conditions and become an important endogenous source of water pollution (Lazzari & Baldisserotto 2008). Aure & Stigebrand (1990) found that large amounts of carbon, nitrogen and phosphorus deposited at the bottom of a cage salmon farming area, and only approximately 10% of organic matter was decomposed each year; most was deposited in the sediment and continuously released into the water. In the present experiment, the increased intake of grass carp would lead to increased residual feeds and feces deposited at the bottom, and possibly increase the release of endogenous nutrients. The highest levels of nitrogen, phosphorus and potassium sediments were measured in the feed group. Nutrients deposited at the bottom might be re-released into the water, which may be an endogenous reason for the high water trophic levels in the feed group.

In short, poor water quality, manifested as serious eutrophication, dark brown colour, and low transparency, was observed in the feed group throughout the farming period, especially in the late period. By contrast, a better water quality was observed in both the mixture and grass groups.

During the farming period, as residual feeds, feces and excreta continue to enter the water, part of the organic matter will be dissolved in the water, and part will be deposited at the bottom due to gravity, thus increasing nutrients in sediments. In an experiment about nitrogen transformations and balance in channel catfish ponds, 22.6% of nitrogen was accumulated in the bottom soils (Gross et al. 2000). It was also found that approximately 10% of organic matter was decomposed each year; most was still deposited in the sediment (Aure & Stigebrandt 1990). These findings indicate that, although most of the feed will be ingested by aquatic animals, only a small proportion can be utilized and retained in the animals, while most is excreted via feces into the water or accumulates at the bottom of the farming system. In the present experiment, the higher sedimentary organic matter content in the grass group than in the compound and mixture groups was presumably related to the low digestion and utilization efficiency of the hybrid napier grass. Therefore, the digestion and utilization efficiency of the hybrid napier grass needs to be further improved in future studies of grass carp aquaculture. Processing hybrid napier grass into a palatable feed and adding it to the conventional compound feed may be a solution to improve the utilization efficiency of the hybrid napier grass, and may help reduce the accumulation of organic matter.

The growth rate of grass carp in the mixture group was not significantly different from that of the feed group, but was much higher than that of the grass group. Moreover, the mixture group had a higher grass carp muscle crude protein content and a lower crude fat content compared with the feed group. The highest muscle contents of protein, total amino acids, essential amino acids, semi-essential amino acids, and flavour amino acids in grass carp were observed in the grass group, indicating that the hybrid napier grass was a high-quality protein for grass carp. The nutrient concentrations and the number of Cyanobacteria in aquaculture water were lower in the grass and mixture groups than in the feed group. Moreover, the value of input cost/net income was significantly higher in the mixture group than in the other two groups. Hence, the mixture of hybrid napier grass and compound feed was presumably a good pattern for grass carp aquaculture. However, feeding with hybrid napier grass lead to a high accumulation of sedimentary organic matter. Therefore, the digestion and utilization efficiency of hybrid napier grass needs to be further improved in future studies. Processing hybrid napier grass into a palatable feed and adding to the conventional feed may be a solution and development direction to improve the utilization efficiency of hybrid napier grass and reduce the accumulation of organic matter.

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