

## Comparative Analysis of Muscle Quality of Grass Carp Fed Two Diets (Postprint)

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### Abstract

In this experiment, Pennisetum sinense powder feed served as the experimental diet and commercial compound feed as the control diet, which were fed to grass carp with an average body weight of  $(300.00 \pm 10)$  g for 2 months. Growth performance, morphological indices, muscle pH, water-holding capacity, textural properties, conventional nutrient content, trace element content, amino acid composition, and nutritional value were compared and analyzed between the two groups. The results indicated that the visceral weight, liver weight, abdominal fat weight, viscerosomatic index, hepatosomatic index, and abdominal fat index of grass carp in the experimental group were all extremely significantly lower than those in the control group ( $P < 0.01$ ), the condition factor was significantly lower than that in the control group ( $P < 0.05$ ), whereas the empty shell rate was extremely significantly higher than that in the control group ( $P < 0.01$ ). The muscle springiness, chewiness, gumminess, and hardness in the experimental group were all extremely significantly higher than those in the control group ( $P < 0.01$ ), and the cohesiveness was significantly higher than that in the control group ( $P < 0.05$ ). The muscle pH, drip loss, water loss rate, and frozen exudate rate of grass carp in the experimental group were all extremely significantly lower than those in the control group ( $P < 0.01$ ). The crude fat content in muscle of grass carp in the experimental group was significantly lower than that in the control group ( $P < 0.05$ ), while the iron (Fe) content was extremely significantly higher than that in the control group ( $P < 0.01$ ). Both groups contained 16 amino acids in grass carp muscle, with total amino acid contents of 17.43% and 17.23% in the experimental and control groups, respectively. There were no significant differences in the content of various amino acids between the two groups ( $P > 0.05$ ), and the essential amino acid indices were 84.66 and 84.94, respectively. It can be concluded that, compared with commercial compound feed, feeding Pennisetum sinense powder feed can improve the muscle quality of

grass carp.

## Full Text

### Comparative Analysis of Muscle Quality in Grass Carp (*Ctenopharyngodon idellus*) Fed Two Different Diets

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**Abstract:** This experiment compared grass carp (*Ctenopharyngodon idellus*) with an average body weight of (300.00±10.00) g fed a *Pennisetum sinense* Roxb. meal diet (trial group) versus a commercial formulated diet (control group) for two months. Growth performance, physical indicators, muscle pH, water-holding capacity, textural properties, proximate nutrient composition, trace element content, amino acid profile, and nutritional value were analyzed. The results demonstrated that the trial group exhibited extremely significantly lower viscera weight, liver weight, intraperitoneal fat weight, viscera somatic index, hepatosomatic index, and intraperitoneal fat index compared to the control group (P<0.01), with significantly lower condition factor (P<0.05) and extremely significantly higher carcass rate (P<0.01). Muscle springiness, chewiness, gumminess, and hardness were all extremely significantly higher in the trial group (P<0.01), while cohesiveness was significantly higher (P<0.05). Muscle pH, drip loss, thawing loss rate, and liquid loss rate were all extremely significantly lower in the trial group (P<0.01). Crude fat content in muscle was significantly lower in the trial group (P<0.05), while iron (Fe) content was extremely significantly higher (P<0.01). Both groups contained 16 amino acids, with total amino acid contents of 17.43% and 17.23%, respectively, showing no significant differences in individual amino acid concentrations (P>0.05). The essential amino acid index was 84.66 for the trial group and 84.94 for the control group. These findings indicate that *Pennisetum sinense* Roxb. meal diet improves grass carp muscle quality compared to commercial formulated feed.

**Keywords:** grass carp (*Ctenopharyngodon idellus*); *Pennisetum sinense* Roxb. meal; textural properties; water-holding capacity; nutritional value

## 1 Introduction

Grass carp (*Ctenopharyngodon idellus*), belonging to Osteichthyes, Cypriniformes, Cyprinidae, and *Ctenopharyngodon*, is a typical herbivorous fish commonly known as wan, wan fish, or grass wan. As an important economically

farmed species in China, grass carp reached a national production of 5.6762 million tons in 2015 [1], representing the world's largest freshwater aquaculture species. However, with increasing production, muscle quality has gradually declined [2]. Previous studies have reported that long-term feeding with formulated diets causes excessive intraperitoneal fat deposition, abnormal body shape, and deteriorated flavor, ultimately reducing flesh quality [3]. Consequently, researchers have attempted to improve grass carp muscle quality by supplementing diets with various forages, including hybrid napier grass (*Pennisetum americanum* × *P. purpureum*) [4], dwarf elephant grass (*Pennisetum purpureum*) [5], Sudan grass (*Sorghum sudanense*) [6], and ryegrass (*Lolium perenne*) [7], achieving favorable results. The use of grass meal as a feed ingredient for herbivorous or omnivorous fish represents an active research direction. Zhang et al. [8] demonstrated that supplementing common carp diets with alfalfa meal improved flesh quality.

*Pennisetum sinense* Roxb., a perennial gramineous herb, is rich in nutrients including crude protein, minerals, and vitamins, offering high utilization value [9-10]. Li [11] reported that combining fresh *P. sinense* with formulated feed improved grass carp muscle quality. However, no studies have investigated the effects of dietary *P. sinense* meal supplementation on grass carp muscle quality improvement.

Therefore, this study examined grass carp (approximately 300 g) fed either *P. sinense* meal diet or commercial formulated diet for two months, comparing muscle nutrient composition and quality characteristics to provide fundamental data and theoretical basis for grass carp flesh quality improvement.

### 1.1 Experimental Diets

The *Pennisetum sinense* meal diet was processed by Guangfo Feed Mill in Heshan, Jiangmen, Guangdong Province. While the *P. sinense* meal was provided by the Precision Aquaculture Base of Pearl River Fisheries Research Institute, all other feed ingredients and the commercial formulated diet were supplied by the same mill. According to Huang et al. [12], grass carp exhibit optimal growth when dietary crude fiber content is 12.0%. Therefore, this experiment incorporated 25% *P. sinense* meal to replace rice bran and partial soybean meal in the commercial formulated diet, yielding a final crude fiber content of 12.1%. Proximate nutrient composition and gross energy of the two diets are presented in Table 1.

### 1.2 Experimental Animals and Culture Management

The experiment was conducted at Zutan Xingying Aquaculture Cooperative in Longjiang Town, Shunde District, Foshan City. Two groups were established: a trial group fed *Pennisetum sinense* meal diet and a control group fed commercial formulated diet. Each group comprised three ponds (six ponds total), with each pond covering 0.5 hm<sup>2</sup>. Approximately 10,000 healthy grass carp of uniform

size and source, averaging  $(300.00 \pm 10.00)$  g, were stocked per pond. The trial commenced on July 1, 2017, and lasted for two months. Fish were fed twice daily at 09:30 and 17:30 at 3-5% of body weight. During the experiment, pond water temperature (WT) ranged from 28.0 to 33.0 °C, dissolved oxygen (DO) concentration was 5.6-6.4 mg/L, and pH was 6.8-7.1.

### 1.3 Sample Collection

After culture, six healthy grass carp were randomly selected from each pond (36 fish total: 18 control, 18 trial) on September 1, 2017. Following 24-hour starvation, fish were anesthetized. From each group, nine fish were randomly chosen for measurement of body weight and length (length defined as vertical distance from snout tip to caudal fin base). Fish were then dissected to weigh viscera, hepatopancreas, and intraperitoneal fat. Dorsal muscle above the lateral line was collected and stored at -20 °C for subsequent analysis of proximate nutrient composition, mineral element content, and amino acid profile. The remaining nine fish per group were dissected to obtain dorsal muscle above the lateral line for immediate determination of muscle pH, water-holding capacity, and textural properties.

#### 1.4.1 Physical Indicator Calculations

Viscera somatic index (%) =  $(W1/W) \times 100$

Hepatosomatic index (%) =  $(W2/W) \times 100$

Carcass rate (%) =  $(W3/W) \times 100$

Intraperitoneal fat index (%) =  $(W4/W) \times 100$

Condition factor (CF, %) =  $(W/L^3) \times 100$

Where: W1 = viscera weight; W2 = liver weight; W3 = carcass weight; W4 = intraperitoneal fat weight; W = final body weight; L = body length.

#### 1.4.2 Muscle pH and Water-Holding Capacity Determination

pH measurement: A lateral incision was made in the dorsal muscle above the lateral line, and the glass probe of a muscle pH meter (German Meites pH-Star) was fully inserted to measure pH.

Water-holding capacity determination: Following the method of Cheng et al. [7], water-holding capacity was assessed through drip loss, thawing loss rate, and liquid loss rate.

#### 1.4.3 Muscle Textural Properties Determination

Following the method of Xia et al. [13], dorsal muscle above the lateral line was collected and cut into 2.0 cm × 2.0 cm × 1.0 cm blocks. A TA.XT2i texture analyzer (Stable Micro Systems, UK) with a flat-bottom cylindrical probe P/35R was used for two-cycle compression, i.e., Texture Profile Analysis

(TPA). Test conditions: pre-test speed 1.0 mm/s, test speed 1.0 mm/s, post-test speed 1.0 mm/s, compression degree 30%, and interval between cycles 5.0 s. Data acquisition rate was 400.0 pps with automatic trigger. TPA tests were conducted at room temperature.

#### 1.4.4 Proximate Nutrient Content Determination

Muscle crude ash, moisture, crude protein, and crude fat contents were determined according to GB 5009.4-2010 (muffle furnace at 550 °C), GB 5009.3-2010 (drying at 105 °C), GB 5009.5-2010 (Kjeldahl method), and GB/T 5009.6-2003 (Soxhlet extraction method), respectively.

#### 1.4.5 Mineral Element Content Determination

Prior to measuring magnesium (Mg), phosphorus (P), iron (Fe), zinc (Zn), and selenium (Se) contents, muscle samples were processed according to GB/T 5009.90-2003, Method 1 of GB/T 5009.87-2003, GB/T 5009.90-2003, Method 1 of GB/T 5009.14-2003, and Method 1 of GB 5009.93-2010, respectively. A Model 300 atomic absorption spectrophotometer was used for Mg, Fe, and Zn determination, an atomic fluorescence photometer for Se, and a Model 721 spectrophotometer for P.

#### 1.4.6 Amino Acid Composition

Amino acid composition and content were determined by high-performance liquid chromatography according to GB/T 5009.124-2003.

#### 1.4.7 Amino Acid Nutritional Value Evaluation

Based on the FAO/WHO amino acid scoring pattern [14] and whole egg protein pattern [15], amino acid score (AAS), chemical score (CS), essential amino acid index (EAAI), and F value [16] were calculated to evaluate nutritional value. Formulas were as follows:

$$\text{AAS} = (\text{Content of amino acid in test protein (mg/g prot)}) / (\text{Content of same amino acid in FAO/WHO scoring pattern (mg/g prot)})$$

$$\text{CS} = (\text{Content of amino acid in test protein (mg/g prot)}) / (\text{Content of same amino acid in whole egg protein (mg/g prot)})$$

$$\text{EAAI} = 100 \times [(A1/AE1) \times (A2/AE2) \times \dots \times (An/AEn)]^{1/n}$$

Where:  $n$  = number of compared essential amino acids;  $A1, A2, \dots, An$  = essential amino acid contents in grass carp muscle protein (mg/g prot);  $AE1, AE2, \dots, AEn$  = essential amino acid contents in whole egg protein (mg/g prot).

F value = ratio of branched-chain to aromatic amino acids, where branched-chain amino acids = valine (Val) + leucine (Leu) + isoleucine (Ile), and aromatic amino acids = phenylalanine (Phe) + tyrosine (Tyr).

## 1.5 Data Processing and Analysis

Data were organized using Excel 2016 and analyzed by independent samples t-test using SPSS 21.0. Results are expressed as mean  $\pm$  standard deviation. Statistical significance was set at  $P < 0.05$  and extreme significance at  $P < 0.01$ .

## 2 Results

### 2.1 Effects on Growth Performance and Physical Indicators

Effects of the two diets on grass carp growth performance and physical indicators are shown in Table 2. No significant differences were observed in final body weight or length between groups ( $P > 0.05$ ). However, the trial group showed extremely significantly lower viscera weight, liver weight, intraperitoneal fat weight, hepatosomatic index, viscera somatic index, and intraperitoneal fat index ( $P < 0.01$ ), significantly lower condition factor ( $P < 0.05$ ), and extremely significantly higher carcass rate ( $P < 0.01$ ) compared to the control group.

### 2.2 Muscle Textural Properties

Regarding textural properties (Table 3), no significant difference in resilience was observed between groups ( $P > 0.05$ ). However, the trial group exhibited significantly higher cohesiveness ( $P < 0.05$ ) and extremely significantly higher springiness, chewiness, gumminess, and hardness ( $P < 0.01$ ) compared to the control group. For physical characteristics (Table 4), muscle pH was extremely significantly higher in the trial group ( $P < 0.01$ ), while drip loss, liquid loss rate, and thawing loss rate were all extremely significantly lower ( $P < 0.01$ ).

### 2.3 Proximate Nutrient and Mineral Element Contents

For proximate nutrient composition (Table 5), crude fat content in muscle was significantly lower in the trial group ( $P < 0.05$ ), while crude ash, crude protein, and moisture contents showed no significant differences from the control group ( $P > 0.05$ ). Regarding mineral element contents (Table 6), no significant differences were observed in Mg, P, Zn, or Se between groups ( $P > 0.05$ ), but Fe content was extremely significantly higher in the trial group ( $P < 0.01$ ).

### 2.4 Amino Acid Composition

As shown in Table 7, amino acid profiles were similar between groups, with both containing 16 amino acids and showing no significant differences in individual amino acid contents ( $P > 0.05$ ). Total amino acid (TAA) contents were 17.43% and 17.23% for trial and control groups, respectively. The 16 amino acids comprised 7 essential amino acids (EAA), 2 semi-essential amino acids (SEAA), and 7 non-essential amino acids (NEAA). Total delicious amino acids (TDAA) were 6.88% and 6.78%, representing 39.45% and 39.35% of total essential amino acids (TEAA), respectively. Glutamic acid was the most abundant in both groups (3.02% and 2.96%), while histidine was the least abundant (0.39% and 0.41%).

Lysine contents were 1.80% and 1.76%, respectively. The percentage of total semi-essential amino acids (TSEAA) to TAA was significantly lower in the trial group ( $P < 0.05$ ), while TAA, TDAA, TEAA, TSEAA, total non-essential amino acids (TNEAA), TEAA/TAA ratio, TEAA/TNEAA ratio, TNEAA/TAA ratio, and TDAA/TAA ratio showed no significant differences ( $P > 0.05$ ).

## 2.5 Amino Acid Nutritional Value Evaluation

Comparison of AAS and CS between groups revealed methionine + cysteine as the first limiting amino acid and valine as the second limiting amino acid in both groups. EAAI and F value serve as nutritional evaluation indices. In this study, EAAI values were 84.66 and 84.94, and F values were 2.33 and 2.32 for trial and control groups, respectively (Table 8).

## 3 Discussion

### 3.1 Analysis of Growth Performance and Physical Indicators

Results showed no significant effects on final body length or weight between diets. Huang et al. [12] reported that 12.0% dietary crude fiber does not affect grass carp growth; the *P. sinense* meal diet in this study contained 12.1% crude fiber. Torrissen et al. [17] demonstrated that dietary fiber stimulates gastrointestinal motility and digestive enzyme secretion, promoting nutrient digestion and absorption. Thus, appropriate *P. sinense* meal supplementation does not impair grass carp growth.

Body shape constitutes an important commercial quality indicator, with well-shaped fish gaining consumer preference. The trial group exhibited extremely significantly lower viscera weight, liver weight, and intraperitoneal fat weight, with significantly lower condition factor, consistent with Huang et al. [3] who used fresh forage. Hepatosomatic index, viscera somatic index, and intraperitoneal fat index are sensitive indicators of nutritional changes [18-20]. Their extreme significant reduction in the trial group indicates that *P. sinense* meal diet effectively resolves abnormal body shape caused by excessive fat accumulation. Excessive fat deposition increases fatty acid oxidation and peroxidation, compromising fish health and flesh quality [21-22]. The reduced intraperitoneal fat and improved body shape in the trial group meet commercial market requirements.

### 3.2 Analysis of Muscle Textural Properties and Physical Characteristics

Muscle texture evaluation has become a consumer focus, necessitating analysis of grass carp textural properties. The TPA method is currently predominant, offering quantifiable indicators, simple operation, minimal human error, and objective quality assessment [23].

Multiple factors influence muscle textural properties, including feed composition, rearing environment, and culture mode, with feed composition exerting substantial effects [24-25]. Studies have shown that dietary supplementation with broad beans, plant proteins, and glutamic acid significantly improves grass carp muscle hardness, springiness, chewiness, and gumminess, enhancing textural characteristics.

In this study, muscle chewiness and hardness were extremely significantly higher in the trial group. Johnston et al. [26] reported that higher chewiness and hardness correlate with better flesh texture, indicating superior quality in the trial group.

Muscle pH affects flesh quality and serves as an evaluation indicator. Lower pH causes protein denaturation, while higher pH improves tenderness [27]. The trial group' s extremely significantly higher muscle pH demonstrates that *P. sinense* meal diet substantially enhances tenderness. Water-holding capacity reflects muscle' s ability to retain water during physical and chemical changes, correlating with juiciness and overall quality [28-29]. The trial group' s extremely significantly lower drip loss, liquid loss rate, and thawing loss rate indicate stronger water-holding capacity. These results demonstrate that *P. sinense* meal diet effectively improves grass carp muscle quality.

### 3.3 Analysis of Proximate Nutrient and Trace Element Contents

Muscle constitutes the primary edible portion, with proximate nutrients including crude ash, crude fat, crude protein, and moisture serving as important quality indicators. The trial group' s significantly lower muscle crude fat content aligns with findings from Zhang et al. [8] in common carp fed alfalfa meal. Grigorakis et al. [30] demonstrated that reduced crude fat increases friction between muscle bundles, enhancing chewiness and texture [25]. These results indicate that *P. sinense* meal diet reduces muscle fat content, thereby improving chewiness and overall quality.

Fish muscle is rich in essential mineral elements, particularly trace elements crucial for growth and development, including Fe, Zn, and Se. Mineral composition and content thus evaluate nutritional value. Mg and P promote growth and reduce fracture risk in elderly populations; Zn enhances children' s immunity and intellectual development; Se prevents cardiovascular disease and offers antioxidant, anti-aging, and anti-cancer benefits [31]. Fe, a key myoglobin component, prevents lipid oxidation and preserves flavor, improving muscle quality [32]. The trial group' s extremely significantly higher Fe content (more than double the control) demonstrates richer nutrient composition and higher edible value.

### 3.4 Amino Acid Composition Analysis and Nutritional Value Evaluation

Amino acid type, quantity, and composition are important quality indicators [33-34]. This study found no significant differences in individual amino acid contents between groups, with only the percentage of TSEAA to TAA showing significant difference.

Delicious amino acids (DAA) determine flavor intensity. The non-significant difference in TDAA between groups indicates similar flavor profiles. Amino acid balance is crucial for human health. According to FAO/WHO optimal patterns, protein is considered high-quality when TEAA/TAA is approximately 40% and TEAA/TNEAA exceeds 60% [35]. Both groups met these criteria, with TEAA/TAA >40% and TEAA/TNEAA >60% (87.18% in the trial group). EAAI values of 84.66 (trial) and 84.94 (control) indicate balanced amino acid profiles that meet human requirements according to FAO/WHO standards.

## 4 Conclusion

Compared to grass carp fed commercial formulated diet, those fed *Pennisetum sinense* meal diet exhibited higher cohesiveness, hardness, springiness, chewiness, and gumminess, along with elevated pH, water-holding capacity, and Fe content. These results demonstrate that *P. sinense* meal diet improves grass carp muscle quality.

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