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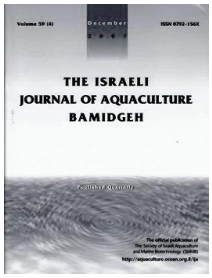

Effect of Different Protein Source Diets on Growth, Sensory Parameters and Flesh texture of On-Growing Grass *Carp (Ctenopharyngodon idellus)*

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Keywords: protein source; growth performance; flesh quality; grass carp

Abstract

The introduction of plant protein (PP) sources in aquafeed is regarded as an economic and environmental-friendly strategy for aquaculture. Our objective was to investigate the effects of pure PP diets on the growth performance and to analyze their effects on flesh quality of grass carp (357.2±0.7g). The study was performed using and comparing a control fish meal diet and two PP diets. Fish meal (FM), soybean meal (SBM), and rapeseed meal (RM) were served as sole protein source of corresponding feeds, respectively. The results suggested that the PP diets significantly impaired the growth performance of grass carp. In regard to flesh quality, the SBM and RM diets decreased the muscle lipid content, n-3 series of PUFA, EPA, DHA, and n-3/n-6 ratio at the nutritional level. However, from the consumer's perspective SBM significantly improved flesh firmness, and RM diets, and PP diets had no adverse effects on flesh flavor, amino acids composition, and sensory characteristics. Fish fed the RM diet displayed a higher water holding capacity (WHC) in the fillets than in the other groups. In summary, compared to the FM diet, both SBM and RM diets reduced fish growth and flesh lipid nutritive values, but improved the fillet firmness. Plant ingredient-rich diets do not have detrimental effects on muscle flavor because of the free amino acids composition and organoleptic attributes.

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Introduction

Global fish consumption has increased rapidly. In 1961, the per capita consumption was 9.0 kg, preliminary estimates for 2015 displayed a further growth in per capita consumption to nearly 20.3 kg (FAO, 2017). However, deterioration of fish flesh quality affects consumer's acceptance and causes severe economic losses for producers (Hosseini et al. 2010). More attention must be aimed at improving flesh quality of marketed fish.

Fish quality is a complex trait. It includes nutritive composition, physical-chemical parameters, texture, and sensory properties (Fuentes et al. 2010; Brinker and Reiter 2011). From a consumer perspective, fish quality is more often related to nutritive values and organoleptic characteristics of the flesh. Flesh protein, amino acids, lipid and polyunsaturated fatty acids (PUFA) content are usually used to evaluate the nutritive values of flesh (Jiang et al. 2016). Fish are rich in polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) (20:5n-3), and docosahexaenoic acid (DHA) (22:6n-3) that are beneficial for human health. Amino acids and lipids contribute directly or indirectly to the fish taste and flavor. Flesh water holding capacity (WHC) and firmness, are considered crucial indexes to evaluate fish flesh quality (Einen and Skrede 1998; Brinker and Reiter 2011). Better WHC and fillet firmness contribute to flesh firmness which is more appealing to consumers. Flesh firmness is also associated with lipid content and/or collagen in flesh fillet (Rørå et al. 1998;Johnston et al. 2006). It is therefore necessary to analyze these as well. Sensory evaluation and instrumental measurements are usually combined in the assessment of flesh quality. However, very few studies focus on the effects of single protein source diets although there are systematic evaluation indices of flesh quality; the apparent changes in flesh quality induced by pure protein sources have apparently not yet to be detected.

Fish meal (FM) is widely recognized as the best protein source in fish feeds due to its balanced nutritional level. However, a growing shortage, and rising prices of FM have severely restricted the sustainable development of aquaculture. Thus, the use of sustainable and eco-friendly plant protein (PP) sources has become a major trend in aquaculture feeds. Among the PP sources, soybean meal (SBM) and rapeseed meal (RM) have received wide attention because they are cheaper and easily available. At present, SBM and RM seem to be promising alternatives for fish meal. However amino acid imbalance and the presence of anti-nutritional factors of PP ingredients may restrict fish growth. In the present study, we examined different dietary treatments to investigate their impact on fish quality as well as on growth performances. Previous studies have demonstrated that fish fed diets containing high levels of PP sources were in some cases associated flesh properties (De Francesco et al. 2004; Cabral et al. 2013). In the present study we tested pure protein diets and evaluated their effects on quality aspects (nutritive values and sensory characteristics).

Grass carp (*Ctenopharyngodon idellus*), is an herbivorous freshwater fish that is widely cultured worldwide. Given the lack of information on the effects of dietary protein sources on the flesh quality of growinggrass carp, the aim of the present study was to evaluate the effects of pure FM, SBM, and RM diets on growth, nutritional value, textural parameters, as well as the sensory characteristics of grass carp, and combine the knowledge to formulate well-balanced feeds and improve flesh quality.

Materials and methods

Experimental diets. The trial comprised three extruded isonitrogenous (30% crude protein), isolipidic (5% crude lipid) and isoenergetic (17.5 MJ/kg) dietary treatments. A control fish meal-based diet (FM) was compared with two PP diets containing soybean meal (SBM) and rapeseed meal (RM) as single protein sources, respectively. Details on experimental diet formulation and chemical composition are shown in Table 1. The amino acid and fatty acids compositions of the diets are listed in Table 2 and Table 3, respectively. All ingredients were sieved through a 550 μm sieve and mixed thoroughly. Diets were prepared with a laboratory extruder (SLP-45, Fishery Mechanical Facility Research Institute, Shanghai, China) with which 3.6 mm pellets were prepared. The extruded wet pellets were oven-dried at 70°C and stored at 4°C until use.

Table 1. Diet formulation and chemical composition of experimental diets (% dry matter)

Ingredients	*FM*	*SBM*	*RM*
White fish meal[1]	39.58	0.00	0.00
Soybean meal[2]	0.00	56.20	0.00
Rapeseed meal[3]	0.00	0.00	69.62
Oil mixture[4]	2.05	4.48	4.44
Corn starch	35.81	13.95	2.68
α-Starch	5.00	5.00	5.00
Vitamin premix[5]	0.39	0.39	0.39
Minerals premix[6]	5.00	5.00	5.00
Choline chloride	0.11	0.11	0.11
Carboxymethyl cellulose	3.00	3.00	3.00
Cellulose	9.06	11.87	9.76
Chemical composition			
Moisture	7.26	11.62	11.55
Crude protein	30.65	30.34	30.92
Crude lipid	4.53	3.96	4.10
Gross energy (MJ/kg)	17.84	18.79	19.14

[1] White fish meal was imported from American Seafood Company, Seattle, Washington, USA.
[2] Soybean meal are purchased from Coland Feed Co. Ltd., Wuhan, Hubei, China.
[3] Rapeseed meal are purchased from Coland Feed Co. Ltd., Wuhan, Hubei, China.
[4] Fish oil: soybean oil=1:1 (W/W). Fish oil are from Peru, purchased from Coland Feed Co. Ltd., Wuhan, Hubei, China. Soybean oil was purchased from the Zhong Bai supermarket, Wuhan, Hubei, China.
[5] Vitamin premix (mg kg^{-1} diet): Thiamin, 20; Riboflavin, 20; Pyridoxine, 20; Cyanocobalamine, 0.02; Folic acid, 5; Calcium pantothenate, 50; Inositol, 100; Niacin, 100; Biotin, 0.1; Cellulose, 3412; Ascorbic acid, 100; Vitamin A, 11; Vitamin D, 2; Vitamin E, 50; Vitamin K, 10.
[6] Mineral premix (mg/kg diet): NaCl, 500; $MgSO_4 \cdot 7H_2O$, 8155.6; $NaH_2PO_4 \cdot 2H_2O$, 12500; KH_2PO_4, 16000; Ca $(H_2PO_4) \cdot 2H_2O$,7650.6; $FeSO_4 \cdot 7H_2O$, 2286.2; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1750; $ZnSO_4 \cdot 7H_2O$,177.9; $MnSO_4 \cdot H_2O$, 61.4; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 6H_2O$, 0.91; KI, 1.5; Na_2SeO_3,0.6; starch, 899.7.

Experimental fish and feeding trial.

Grass carp were obtained from Shishou Original Seed Stock Farm of Four Major carp, Hubei, China and the growth trial was conducted in net cages (2×2×2m, water depth 1.7m) in the same farm. Prior to onset of the experiment, all fish were reared and fed a commercial feed (Catalogue No. 108, Tongwei Group Co., Ltd., Chengdu, Sichuan, China) twice a day (9:00, 16:00) for 3 weeks. Once acclimated they were divided into triplicate groups of 18 homogeneous fish (mean initial weight 357.2±0.7g) per dietary treatment were randomly distributed among 9 net cages. Fish were hand-fed to apparent satiation four times daily (8:30, 12:30, 16:00 and 18:30). The feeding trial started on July 4th. Water temperature was recorded daily and ranged from 26-35°C. Dissolved oxygen and ammonia-N were kept >6 and <0.40 mg/L throughout the trial. There was natural photoperiod (July 4th to September 1th, 2014) throughout the feeding trial.

Table 2. Amino acids composition of experimental diets (% dry matter).

Amino	*FM*	*SBM*	*RM*
Asp	2.63	2.96	2.01
Thr	1.22	1.01	1.25
Ser	1.36	1.34	1.24
Glu	3.90	4.93	5.10
Gly	2.15	1.13	1.45
Ala	1.77	1.15	1.30
Val	1.39	1.27	1.45
Met	0.74	0.20	0.20
Ile	1.15	1.19	1.14
Leu	2.09	2.03	2.04
Tyr	0.49	0.53	0.55
Phe	1.10	1.34	1.14
His	0.60	0.58	0.62
Lys	2.01	1.57	1.59
Arg	1.62	1.72	1.63
Pro	1.28	1.29	1.73
∑EAA	11.93	10.90	11.07
∑AA	25.45	24.21	24.42

Table 3. Fatty acids composition of the experimental diets (mg/g dry feed)

Fatty acids	*Diets*		
	FM	*SBM*	*RM*
14:00	2.08	0.79	0.65
16:00	8.80	7.90	6.58
18:00	1.73	2.06	1.64
20:00	0.12	0.21	0.22
22:00	ND	0.13	0.14
24:00	ND	0.10	0.19
SFA[1]	12.73	11.18	9.42
16:1n-7	2.70	0.92	1.11
18:1n-9	5.42	10.92	16.56
20:1n-9	1.29	0.46	0.47
22:1n-9	0.25	0.09	ND
24:1n-9	0.65	ND	0.11
MUFA[2]	10.30	12.38	18.25
18:2n-6	5.56	21.29	18.39
18:3n-6	ND	0.09	0.09
20:3n-3	0.09	ND	ND
20:3n-6	ND	ND	ND
20:4n-6	0.40	0.12	0.10
18:3n-3	0.65	3.03	3.18
20:5n-3 (EPA)	5.96	1.13	0.77
22:6n-3 (DHA)	6.79	1.49	1.00
PUFA[3]	19.45	27.14	23.54
n-3	13.49	5.64	4.96
n-6	5.96	21.50	18.58
n-3/n-6	2.26	0.26	0.27

1SFA: saturated fatty acid
2MUFA: monounsaturated fatty acid
3PUFA: polyunsaturated fatty acid
ND: Not detected

Sampling.

At the beginning of the experiment, six fish were randomly sampled for initial body composition analysis. At the end of the growth trial and after a 24 h starvation period, the fish in each cage were batch-weighted. One fish from each cage was sampled and stored at -20 °C for final body composition analysis. Three fish per cage were sampled condition factor (CF) measurements, body weight, length and viscera weight. In addition, samples of the dorsal and abdominal flesh were taken to determine viscerosomatic index (VSI), muscle free amino acid, fatty acids, and proximate chemical compositions. Two fish per cage were sampled for analysis of WHC and total collagen content, and three fish per treatment were sampled for texture analysis and sensory evaluation, respectively.

Chemical analysis.

Proximate composition analysis of all samples including diets, whole fish, dorsal, and abdominal muscle were performed according to the methods described by AOAC (2003). Dry matter was determined by oven drying a portion of the samples at 105°C to constant weight. Crude protein content (N × 6.25) was determined by Kjeldahl method after acid digestion using 2300 Kjeltec Analyzer Unit (FOSS Tecator, Haganas, Sweden). Crude lipid content was determined by ether extraction in a Soxtec system (Soxtec SystemHT6, Tecator, Haganas, Sweden) with diethyl ether as extraction liquid. Crude ash content was obtained by incineration in a muffle furnace at 550°C for 12 h. Gross energy was determined by an automatic oxygen bomb calorimeter (Parr Isoperibol Calorimeter 6200, Moline, Illinois, USA). All analyses were conducted in duplicate.

Amino acid and fatty acid analysis.

The analysis of amino acids composition of experimental diets was conducted using the method described by GB/T 18246-2000, China. The free amino acid composition of the dorsal muscle was determined according to a previous study (Yang, 2002) after acid hydrolysis in 6 N HCl for 22 hs at 110°C, then separated by ion-exchange chromatography. The amino acid concentration was dilution to 50 nM with 0.2 N sodium citrate buffer which pH at 2.2. Finally, the pH-adjusted samples were measured by using Hitachi L-8800 Amino Acid Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). The dorsal muscle was freeze-dried and comminuted for fatty acids analysis. In addition, all experimental diets were thoroughly ground and well mixed before analysis. Fatty acid methyl esters (FAME) of total lipids were carried out according to a previous study (Folch et al. 1957). The analyses were conducted in a 14-C gas chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a flame ionization detector (ID) and a capillary column (60 m × 0.25 mm ID). Nitrogen was used as carrier gas at a constant linear velocity of 20 cm/s and column temperature was programmed to increase (130°C:1min; 130-170°C:6.5°C/min; 170°C-215°C:2.75°C/min; 215°C:12 min; 215°C-230°C:4°C/min; 230°C:3 min). Injection and detector temperatures were set at 270°C and 280°C respectively. The individual fatty acids were identified by comparing their retention times with standards of fatty acid methyl esters supplied by Sigma Chemical Company (St. Louis, MO, USA).

Total collagen analysis.

Muscle hydroxyproline content was determined according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nan jing, China). Total collagen content of the dorsal muscle was obtained on the basis of hydroxyproline by using a conversion factor to convert (figure 1).

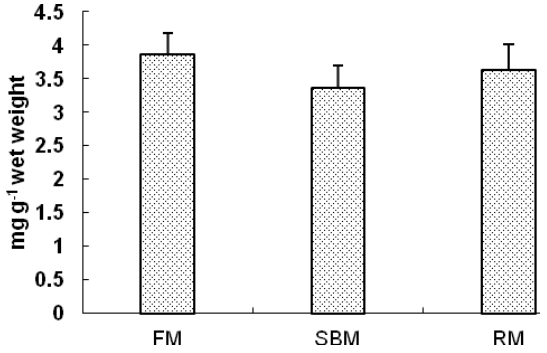

Figure 1. The total collagen content of dorsal muscle of grass carp fed with different experimental diets (mg/g wet weight). Columns represented the means ± SE (n=6).

WHC analysis.

WHC analysis was a modification of method used in previous studies conducted by Pastoriza and collaborators (Pastoriza et al. 1998); this method was especially designed for fish. The samples were weighed (initial weight) and hung on a string vertically in a plastic bag without touching the bag, and the water released; they were then kept in a refrigerator at 4°C for 24 h before determining and recording their final weight. The amount of water extracted from the muscle portions is represented as a percentage of the initial weight.

Instrumental texture analysis.

Firmness and springiness of flesh were determined by using a TA.XT.Plus texture Analyser (Stable Micro Systems Ltd., Godalming, UK) equipped with a flat-ended cylindrical probe (P/36R). The compression site is in front of the dorsal fin, above the scale of the lateral line. The probe was pressed downwards at a test speed of 1mm/s on the fillets until a penetration depth 60% of the fillet height. The force was recorded on the time-force curves. Defining traits value of texture profile analysis (TPA) was according to the previous study (Bourne 1978).

Sensory analysis.

Sensory evaluation was performed as previously described by Zhou and collaborators (Zhou et al. 2016). The test group was composed of nine semi-trained individuals who were selected from the Institute of Hydrobiology, Chines Academy of Sciences. Preparation of specimens involved the preparation of 3 1.0-cm-thick cutlets of each fish followed by vacuum packing. The fish fillets were heated in a water bath for 1 h at 70°C. The samples were then randomly served to the panelists. Their scores were recorded on a scale from 0 to 5 (0 = lowest intensity, 5 = highest). The panelists individually ranked the samples at their own speed.

Statistical analysis.

Data are showed as the means ± S.E. All results were subjected to one-way ANOVA after normality and homogeneity were tested using SPSS Statistic 21.0. If one-way analysis identified that overall differences were significant at $P<0.05$, Duncan's multiple range test was used to test the difference between groups.

Results

Growth performance and feed utilization.

During the entire feeding period, all diets were well accepted and no fish died. After 60 days, the SBM and RM diet fish groups had a significantly lower final body weight (FBW), specific growth rate (SGR) and protein retention efficiency (PRE) compared to the FM treatment group (Table 4) ($P<0.05$). No differences in feeding rates (FR), feeding efficiency (FE), CF, VSI, and gutted yield emerged among the three treatments ($P>0.05$).

Proximate composition and total collagen.

As shown in Table 5, fish fed the SBM and RM diets exhibited significantly higher moisture content and lower crude lipid levels of dorsal muscle compared to FM treatments ($P<0.05$). The dietary treatments displayed no effects on crude protein and ash contents in dorsal muscle. No statistical differences were observed among dietary treatments in proximate composition of the whole body and abdominal muscle. There were no significant differences in the muscle collagen concentration among the different feeds ($P>0.05$).

Table 4. Growth performance and morphological measurements of grass carp fed with different experimental diets

Diets	*FM*	*SBM*	*RM*
IBW[1]	357±1.2	357±1.1	358±1.8
FBW[2]	1004±23.7[a]	942±7.9[b]	926±15.9[b]
FR[3]	2.28±0.03	2.28±0.01	2.32±0.03
SGR[4]	1.66±0.03[a]	1.62±0.02[ab]	1.55±0.01[b]
FE[5]	69.47±2.56	65.75±1.00	63.78±1.59
CF[6]	2.24±0.05	2.08±0.04	2.18±0.06
Gutted yield[7]	90.41±0.27	91.08±0.27	90.43±0.26
VSI[8]	9.59±0.27	8.92±0.27	9.57±0.26
PRE[9]	39.43±0.74[a]	36.13±0.43[b]	34.35±1.36[b]

Values in the same row with different superscripts are significantly different (P<0.05).
[1]IBW (g): initial body weight.
[2]FBW (g): final body weight.
[3]Feeding rate (FR) (% BW/d) = 100 × dry feed intake / [feeding days × (FBW+IBW) / 2], n=3.
[4]Specific growth rate (SGR) (%/d) =100 × [Ln (FBW) −Ln (IBW)]/days, n=3.
[5]Feed efficiency (FE) (%) = 100 × fresh body weight gain / dry feed intake, n=3.
[6]Condition factor (CF) (g cm^{-3}) =100×body weight / body length^3, n=9.
[7]Gutted yield (%) =100×gutted weight/body weight, n=9.
[8]Viscerasomatic index (VSI) (%) =100×viscera weight / body weight, n=9.
[9]Protein retention efficiency (PRE) (%) = 100 × body protein gain / protein intake, n=3.

Table 5. Chemical composition of whole fish, dorsal muscle and abdominal muscle of
Values in the same row with different superscripts are significantly different(P<0.05).

Diets	*FM*	*SBM*	*RM*
Whole fish composition of grass carp (% wet weight)			
Moisture	73.93±0.66	74.30±0.47	74.27±0.56
Crude protein	17.02±0.03	17.22±0.25	16.75±0.11
Crude lipid	5.76±0.44	6.52±0.49	6.06±0.86
Ash	3.57±0.12	3.60±0.08	3.53±0.24
Dorsal muscle composition of grass carp (% wet weight)			
Moisture	76.22±0.28[b]	77.42±0.12[a]	77.66±0.08[a]
Crude protein	20.10±0.06	20.63±0.94	20.67±0.32
Crude lipid	1.96±0.15[a]	1.39±0.10[b]	1.24±0.15[b]
Ash	1.27±0.10	1.31±0.004	1.19±0.08
Abdominal muscle composition of grass carp (% wet weight)			
Moisture	77.80±0.29	77.69±0.31	77.20±1.85
Crude protein	19.26±0.20	19.50±0.20	19.24±0.24
Crude lipid	2.40±0.24	2.50±0.26	1.46±0.29
Ash	1.36±0.13	1.10±0.08	2.01±0.37

Amino acid and fatty acids.

The free amino acid content of the dorsal muscle is given in Table 6, there were no significant differences in free amino acids including the flavor amino acids among all treatments (P>0.05). Fatty acids of the dorsal muscle are presented in Table 7. Compared to FM diet, a small amount of saturated fatty acid (SFA) was found in 14:0 and 16:0 in fish fed with SBM and RM diets (P<0.05). The monounsaturated fatty acids (MUFA) contents in the dorsal muscle namely 16:1n-7, 20:1n-9 and 24:1n-9, were significantly lower in PP diets compared to those in the control (P<0.05). The total PUFA and linoleic acid (18:2n-6) contents in the dorsal muscle were significantly lower in fish fed with the FM and RM diets than in those subjected to the SBM diet (P<0.05). The

PUFA levels of arachidonic fish fed acid (20:4n-6), linolenic acid (18:3n-3) and dihomo-γ-linolenic (20:3n-3), had a markedly decreased DHA (22:6n-3), EPA (20:5n-3) and n-3/n-6 ratio in the dorsal muscle compared with those fed the FM diet ($P<0.05$).

Table 6. Free amino acids composition of dorsal muscle of grass carp fed with different experimental diets (n=3) (mg/100g wet weight).

Amino acids	*FM*	*SBM*	*RM*
Essential amino acids (EAA)			
Thr	1.03±0.24	1.26±0.05	2.41±1.23
Val	1.69±1.14	1.90±0.44	3.14±1.37
Met	3.57±0.80	2.83±1.22	2.65±1.60
Phe	0.77±0.54	1.00±0.30	3.12±1.70
Ile	1.05±0.59	1.38±0.44	3.17±1.56
Leu	1.16±0.74	1.53±0.55	3.24±1.17
Lys	19.15±3.98	14.55±1.36	16.07±2.95
ΣEAA	28.43±0.42	24.50±1.20	33.80±11.30
No-essential amino acids (NEAA)			
Asp	8.75±2.04	5.26±0.55	5.88±1.25
Glu	34.19±1.81	33.31±1.73	29.92±6.49
Ala	4.55±0.85	7.45±0.98	9.76±2.43
Ser	1.82±0.12	2.52±0.06	3.15±1.13
Gly	6.45±0.02	7.27±1.67	4.58±1.66
Tyr	1.89±0.94	2.93±1.05	3.99±2.26
Pro	1.60±0.12	2.49±0.83	3.04±0.74
Arg	11.58±4.38	7.27±1.17	7.49±3.30
His	58.86±7.98	94.69±5.56	65.00±12.91
EAA/DEAA	0.22±0.02	0.15±0.01	0.25±0.07
ΣFAA	53.94±4.67	53.30±1.72	50.15±11.50
ΣAA	158.13±8.80	187.66±5.04	166.64±22.70

The muscle essential amino acids: Thr, Val, Met, Phe, Ile, Leu, Lys (for human being).
*Trp was not analysed
FAA: flavor amino acids, including Glu, Gly, Asp, Ala.

Table 7. Dorsal muscle fatty acids composition of grass carp fed with different experimental diets (n=3) (mg/g wet weight)

Fatty acids	*FM*	*SBM*	*RM*
14:00	0.30±0.03[a]	0.20±0.02[b]	0.10±0.01[c]
16:00	3.27±0.35[a]	3.10±0.25[b]	1.73±0.04[b]
18:00	0.62±0.04	0.70±0.04	0.50±0.04
20:00	0.02±0.001	0.02±0.001	0.02±0.0001
22:00	0.07±0.01	0.08±0.008	0.05±0.005
SFA[1]	4.28±0.44[a]	4.10±0.32[a]	2.38±0.09[b]
16:1n-7	1.19±0.22[a]	0.74±0.09[ab]	0.24±0.001[b]
18:1n-9	3.75±0.54	4.13±0.46	2.01±0.24
20:1n-9	0.16±0.02[a]	0.13±0.02[ab]	0.07±0.01[b]
24:1n-9	0.05±0.005[a]	0.02±0.00[b]	0.02±0.001[b]
MUFA[2]	5.15±0.78	5.03±0.57	2.35±0.25
18:2n-6	0.69±0.06[b]	2.51±0.27[a]	1.39±0.23[b]
18:3n-6	ND	0.02±0.001	0.01±0.001
20:3n-6	0.07±0.002[c]	0.17±0.006[a]	0.12±0.001[b]
20:3n-3	0.02±0.001	0.03±0.005	0.02±0.002
20:4n-6	0.38±0.01[b]	0.63±0.04[a]	0.70±0.03[a]
18:3n-3	0.09±0.01[c]	0.31±0.03[a]	0.17±0.02[b]
20:5n-3(EPA)	0.71±0.02[a]	0.22±0.007[b]	0.20±0.03[b]
22:6n-3(DHA)	1.63±0.03[a]	1.06±0.01[b]	0.87±0.05[c]
PUFA[3]	3.57±0.08[b]	4.94±0.34[a]	3.49±0.37[b]
n-3	2.43±0.05[a]	1.61±0.05[b]	1.27±0.11[b]
n-6	1.14±0.08[c]	3.32±0.29[a]	2.22±0.27[b]
n-3/n-6	2.16±0.16[a]	0.49±0.03[b]	0.57±0.02[b]

Values in the same row with different superscripts are significantly different (P<0.05).
1SFA: saturated fatty acid
2MUFA: monounsaturated fatty acid
3PUFA: polyunsaturated fatty acid
ND: Not detected

Water holding Capacity.

Drip loss is shown in Figure 2, no differences in drip loss were found between fish fed with the FM and SBM diets, but those were significant higher than in fish fed the RM diet (P<0.05). Fish in the FM and SBM groups showed lower WHC compared to fish with the RM treatment.

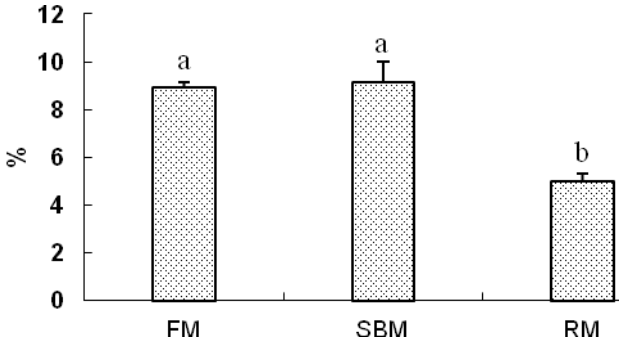

Figure 2. The water holding capacity of dorsal muscle of grass carp fed with different experimental diets (%). Columns represented the means ± SE (n=6). Columns sharing different superscript letters are significantly different (P<0.05).

Texture

Compared to FM diet, SBM and RM diets significantly improved the flesh firmness (Figure 3) (P<0.05). However, no differences were observed in flesh springiness regardless of dietary treatments (Figure 4) (P>0.05).

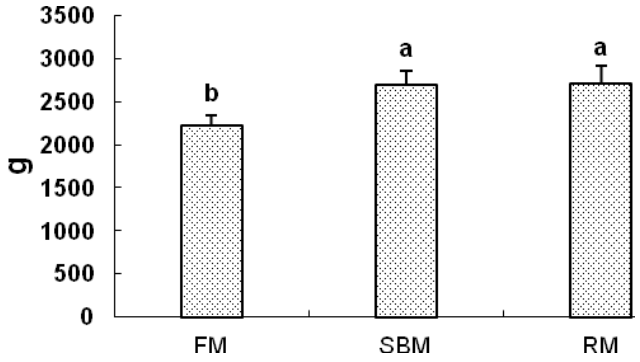

Figure 3. Hardness of dorsal muscle of grass carp fed with different experimental diets (g). Columns represented the means ± SE (n=3). Columns sharing different superscript letters are significantly different (P<0.05).

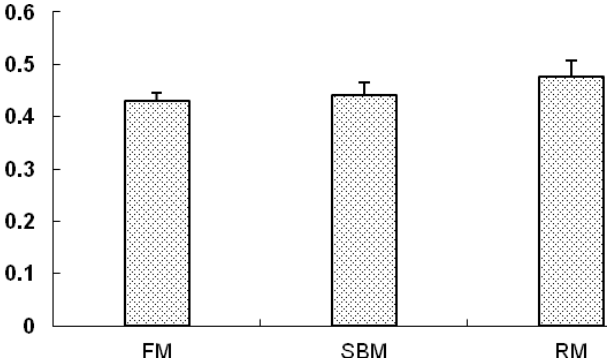

Figure 4. Springiness of dorsal muscle of grass carp fed with different experimental diets. Columns represented the means ± SE (n=3).

Sensory evaluation.

As shown in Figure 5, dietary protein sources showed no effects on the sensory properties including appearance, intensity of odor, off odor, flavor, off flavor, firmness, juiciness, and fattiness of fish fillets (P>0.05).

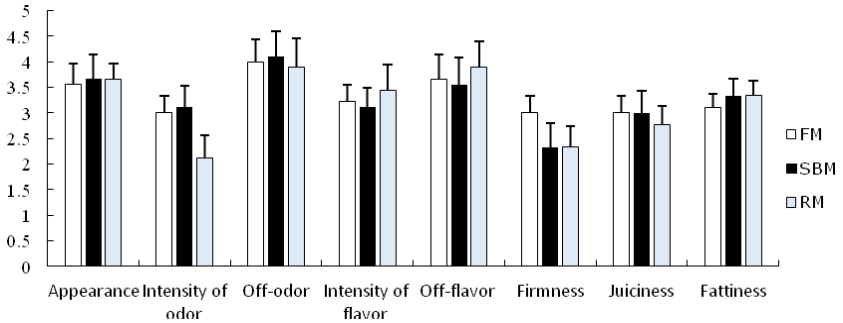

Figure 5. Organoleptic evaluate of grass carp fillet by sensory test panel. Scores from 0 to 5 are used, and 0 represent for low intensity, 5 represent for high intensity. Columns represented the means ± SE (n=3).

Discussion

To ensure the future development of sustainable aquaculture, it is important to expand the use of vegetable feed ingredients. Several studies have proved that grass carp can well utilize PP sources when substituted for a certain proportion of fish meal (Ehsani et al. 2014). Even though numerous PP substitutive experiments had previously conducted, some questions remain: 1) Previous studies mainly focused on the effects of PP sources replacing different proportions of fish meal on grass carp and the potential effects of PP sources alone need to be further discussed; 2) Differences in fish quality between grass carp fed with whole PP sources and FM diets. To answer these questions, we fed grass carp with two PP diets and FM diet respectively, with special focus on flesh quality.

Growth performance of grass carp

In the present experiment, the fish readily accepted all experimental diets. No diet-related mortalities or observable macroscopic irregularities attributable to any of the dietary treatments were observed during the entire experiment. Some studies showed that extremely high dietary inclusion of PP sources had adverse effects on fish growth performance (Slawski et al. 2011; Wang, 2014). As expected, the present trial found that the pure PP diets resulted in impaired growth performance in terms of FBW and SGR values. Previous studies demonstrated that suppressed growth parameters of fish fed the PP diets was possibly attributable to the presence of anti-nutritional factors and the reduced palatability of the feeds (Francis et al. 2001), as well as the imbalanced dietary amino acids (Espe et al. 2006). In the present study, the SBM and RM diets had no negative impact on FE of growing grass carp. A previous study found that an all-vegetable diet significantly decreased the FE of smaller-sized grass carp, but not the larger ones (Wang, 2014). Thus, we suspected that for growing grass carp, the palatability of feed is apparently not a limiting growth factor. Furthermore, methionine (Met) and lysine (Lys) are two limiting amino acids in feeds of grass carp (Yang et al., 2010), and they are significantly lower in the SBM and RM diets than those in the FM diet (Table 2).

Protein is the main organic substance in fish tissues (65%-75% of fish dry weight), and fish growth depends on the deposition of protein in the body. However, the purpose of this work was evaluate the effects of dietary protein sources on fillet nutritional and sensory quality, from a consumer perspective.

SBM and RM diets have essential implications on flesh quality of grass carp.

Our results revealed that the chemical composition of the dorsal muscle was affected by the dietary treatments. This meant that there was a decrease in muscle crude lipid content in fish fed the all-vegetable diets. Different and contrasting results can be found in the literature concerning the effect of PP diets on muscle lipid concentration. Augmentation of muscle lipid content occurred in fish fed with PP diets (De Francesco, et al., 2007). However, the high inclusion level of plant ingredients had no effects on the muscle crude lipid content of gibel carp (Zhou, 2015). The SBM and RM diets significantly increased the muscle moisture content, nevertheless the higher muscle moisture content suggested that the muscles were less nutritional (lower in dry matter). Furthermore, the present results showed that the fatty acid profile in the muscle was significantly affected by dietary plant ingredients. Although all diets were supplemented with mixture oil (soybean oil: fish oil=1:1), significant differences in fatty acids profiles were found between all-vegetable and FM diets. Since vegetable protein sources are devoid of n−3 series of PUFA its inclusion at high levels can detrimentally affect the fillet fatty acids composition and especially the EPA and DHA in muscle are responsible for the high health benefits attributed to fish consumption. Grass carp fed the SBM and RM diets showed a conspicuous reduction in n-3 series of PUFA, EPA, DHA as well as n-3/n-6 ratio, indicating that the PP diets had adverse effects on muscle lipid. These findings highlighted that for growing grass carp, a total substitution of single PP sources was not recommended.

In the present study, we also observed an increase in the firmness of the dorsal muscle when the fish were fed SBM and RM. These results agreed with previous observations which also showed that the flesh firmness was negatively correlated with muscle lipid content (Johnston et al., 2006). Collagen with its amino acids is a protein involves intramuscular connective tissues that play an important role in the quality of flesh texture. In the present trial, no remarkable differences were found in relation to collagen among the different treatments and thus didn’t play a role in the variations of flesh hardness.

Several flavor amino acids (such as Asp, Glu, Gly and Ala) are known to contribute to the flavor characteristic of fish (Ruiz-Capillas and Moral, 2004). In the present trial, our results showed that SBM and RM diets did not compromise the flavor amino acid composition of muscle. Similarly, the organoleptic evaluation which was related to fillet flavor properties did not differ between the treatments. The sensory evaluation was conducted by a tasting panel, but panelist did not find any differences between the

samples they tested, despite the observed differences in the proximate composition, texture firmness, fatty acids contents, and flesh WHC. This may have been due to the individual preferences of the different evaluators, and the apparent changes in flesh quality did not seem to cause obvious changes in sensory response in the cooked fillet. Moreover, the pure vegetable diets did not have detrimental effects on sensory characteristics.

WHC is a key flesh quality trait, which affects the acceptability of consumer as well as food processing companies. In the present study, RM diet improved the flesh WHC of grass carp compared to that in FM and SBM fed fish. Further studies are needed to further elucidate related information gaps.

Conclusion

Our results demonstrated that the PP diets had adverse effects on growth performance, and also affected the flesh quality of growing grass carp. The SBM and RM diets decreased the muscle lipid content, n-3 series of PUFA, EPA, DHA and n-3/n-6 ratio at the nutritional level, whereas the SBM and RM diets significantly improved the flesh firmness. Moreover, all vegetable diets had no any adverse impacts on muscle flavor. Further studies should be conducted to find the optimal inclusion levels of PP sources in diet formulation so as to improve the fish growth and feed utilization, reduce the feed costs, and also improve fish quality.

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