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The Effect of Dietary Protein Level on the Growth Performance and Digestive Protease Activity in Juvenile Bluegill (*Lepomis macrochirus*)

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Abstract

A 16-week feeding experiment was conducted in an indoor recirculating aquaculture system (RAS) to determine the effects of dietary protein level on the growth and digestive protease activity in juvenile bluegills. Six isocaloric experimental diets were formulated with 32%, 35%, 38%, 41%, 44% and 47% levels of protein. Menhaden fish meal was used as the sole protein source. Juvenile bluegills of 24.91 ± 0.50 g initial weight were distributed into 24 151 L tanks with 12 fish each. Each diet had four replications. Fish were fed to satiation three times a day by hand. At the termination, there were no mortalities in fish fed 38% through 47% protein diet. Bluegill fed 38% or 47% protein diet had significant higher body weight gain and specific growth rate (SGR) than fish fed 32% or 35% protein diet. No significant differences ($p > 0.05$) were found in weight gain, SGR and FCR among the fish fed 38% or higher protein diets. Protein efficiency ratio decreased with increasing dietary protein level from 32% to 47%. Acid protease activity of complete digestive tract increased in trend with increasing dietary protein level from 32% to 47%. No significant differences were detected in the protease activity at different pH level among the treatments. The optimal dietary protein requirement for juvenile bluegill was about 38.3%.

Introduction

Bluegill (*Lepomis macrochirus*) is a promising aquaculture species in the North Central Region of the United States, due to its status as a popular sport fish, an important forage fish and recently as a food fish (Ali & Aayne, 1987; Brunson & Morris, 2000). Extensive production of bluegill requires the use of artificial diets for good growth. The nutrient requirement of this species, which is essential for designing diets, has not been established (NRC, 2011). There are no commercial diets at this time that are specially designed for bluegill. Protein is the most expensive component of the prepared fish diets and the most important dietary factors affecting fish growth. Thus, dietary protein requirement is always a priority in fish nutrition study. The utilization of ingested protein in fish depends on the activity of protease presenting in its digestive organs (Natalia et al. 2004). Little information is available concerning the dietary protein requirement for bluegill of more than 20 g and how protease activity respond to the change of dietary protein level in bluegills. The objectives of this study were to determine the protein requirement of juvenile bluegill (> 20 g) and the effect of dietary protein level on the protease activity in bluegill.

Materials and Methods

Experimental diets

Six isocaloric diets with graded level of protein 32%, 35%, 38%, 41%, 44% and 47% were used (Table 1).

Experimental condition and fish:

❖ Fish were raised in an indoor water RAS containing 151-L fish tanks supplied with recycled water at 24.2 ± 0.4 °C, a sump tank, a bead filter and a bio-filter.

❖ The water dissolved oxygen was 7.26 ± 0.46 mg/L. Water $\text{NH}_3\text{-N}$ was maintained < 0.1 mg/L. The photoperiod was 14h light /10h dark.

❖ Bluegills with 24.9 ± 0.5 g initial body weight were randomly distributed into 24 tanks at 12 fish per tank. Each treatment had four replications using a completely randomized design.

❖ Fish were fed to apparent satiation three times daily at 8:00 am, 12:00 pm and 4:00 pm by hand six days a week except Sunday.

❖ Experimental duration was 16 weeks.

❖ Body weight gain, FCR, SGR, PER, hepatosomatic index (HSI) and visceral somatic index (VSI) were calculated (Table 2).

Chemical analysis

Proximate analysis: Standard procedure (AOAC, 2000)

Protease activity analysis: Casein hydrolysis method (Walter, 1984) was conducted at a wide range of pH (1.5, 3.0, 4.5, 7.0, 8.5, 9.0, and 10.0).

Data analysis

Data were analyzed by One-way ANOVA. If significant, LSD multiple comparison was performed. $P < 0.05$ was considered significantly difference. A one-slope broken-line regression analysis (Robbins, 1986) with body weight gain was used to determine the dietary protein requirement for juvenile bluegill.

Results

Table 1 Formulation and the proximate compositions of experimental diets

Ingredient (g/100g diet)	Dietary protein level					
	32%	35%	38%	41%	44%	47%
Fish meal ¹	48.08	52.81	57.54	62.27	67.00	71.73
Wheat middlings	4.00	4.00	4.00	4.00	4.00	4.00
Dextrin	23.00	20.00	17.00	14.00	10.90	8.00
Fish oil ¹	9.65	9.24	8.82	8.41	8.00	7.58
Ascorbic acid	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.80	0.80	0.80	0.60	0.80	0.80
Vitamin premix	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
CMC ²	2.00	2.00	2.00	2.00	2.00	2.00
Cellulose	9.32	8.00	6.69	5.37	4.15	2.74
Proximate composition(% dry matter)						
Crude protein	32.16	35.00	38.28	41.28	44.56	46.88
Crude lipid	13.34	13.39	13.56	13.60	13.66	13.94
Crude ash	12.28	13.28	14.56	15.43	16.57	17.59
Moisture	4.96	5.13	5.22	5.48	5.59	5.70
Calculated DE ³ (kcal/100g diet)	346.04	346.02	345.99	346.01	345.64	345.97

1. Menhaden fish meal and fish oil. 2. CMC: Carboxy methyl cellulose (Sodium salt); 3. DE: Digestible energy

Figure 1 One-slope broken-line regression analysis using body weight gain

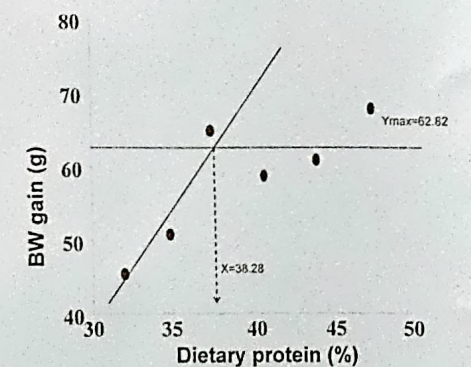


Figure 2 The protease activity (U/mg protein) at different pH response to the dietary protein level. Panel A: protease activity at acidic pH (1.5 & 3.0); Panel B: protease activity at neutral pH (7.0); Panel C: protease activity at basic pH (8.5, 9.0 & 10.0).

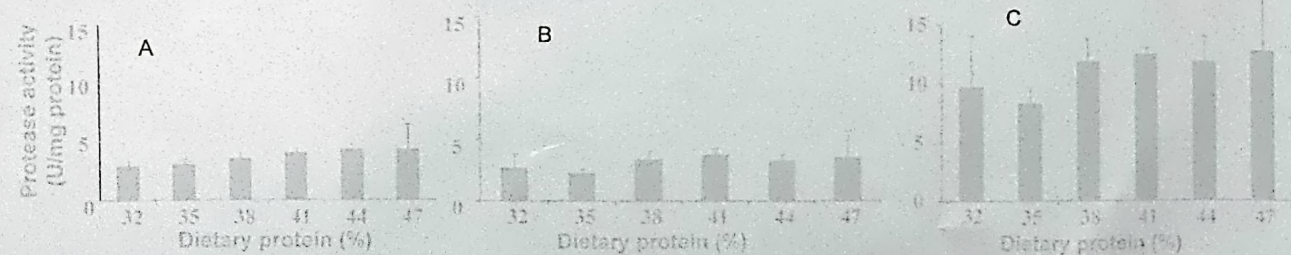


Table 2 Growth and feed utilization of juvenile bluegill after 16-week dietary treatments

Dietary protein	Initial BW (g)	Weight gain(g)	FCR	SGR	HSI	VSI	PER	Survival (%)
32%	25.23±0.67	45.59±11.44 ^c	1.31±0.10 ^a	0.91±0.15 ^c	2.3±0.1 ^a	11.4±0.6 ^a	2.33±0.18 ^a	97.9
35%	24.98±0.70	50.97±9.13 ^{bc}	1.38±0.23 ^a	0.99±0.10 ^{bc}	1.9±0.1 ^b	10.8±0.8 ^{ab}	2.26±0.07 ^{ab}	95.8
38%	24.98±0.44	64.10±5.75 ^a	1.19±0.03 ^b	1.13±0.05 ^a	1.5±0.1 ^c	11.5±0.8 ^a	2.22±0.05 ^{ab}	100.0
41%	24.98±0.24	58.92±4.67 ^{ab}	1.14±0.02 ^b	1.08±0.05 ^{ab}	1.3±0.1 ^d	10.4±0.2 ^b	2.15±0.04 ^{bc}	100.0
44%	24.55±0.21	61.19±5.13 ^{ab}	1.10±0.01 ^b	1.12±0.05 ^{ab}	0.9±0.1 ^e	9.3±0.4 ^c	2.06±0.03 ^{dc}	100.0
47%	24.85±0.64	68.36±11.44 ^a	1.06±0.02 ^b	1.18±0.11 ^a	0.8±0.1 ^e	8.9±0.8 ^c	2.00±0.03 ^d	100.0

Values are means±SD. N=4. Means not sharing same superscript within a column are significantly different ($p < 0.05$). BW: Body weight; FCR: Feed conversion ratio; SGR: Specific growth rate; HSI: Hepatosomatic index; VSI: Visceral somatic index; PER: Protein efficiency ratio.

Discussion

Diet containing 38% or higher protein improved bluegill growth performance and feed utilization in comparison with the diet containing 32% or 35% protein.

PER was decreased with the increase of dietary protein level. This could suggest more dietary protein were used to supply energy instead of being used for growth when higher protein diets were fed to fish.

An increased trend in acidic protease activity existed as dietary protein increased. This may indicate an increased ability of breaking down proteins in stomach when fish fed higher level protein.

Conclusion

The diet containing 35% or lower protein could not support optimal growth in bluegill.

The optimal dietary protein level of juvenile bluegill (> 20 g) was about 38.3% when fish meal was the solely protein source.

The dietary protein level did not affect the activity of digestive protease when a non-specific analysis method was used.

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