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Dietary Protein Level Affects Compensatory Growth and Feed Efficiency in Milkfish *Chanos chanos* Juveniles Under Cyclic Feeding

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An experiment was conducted to determine whether or not changes in dietary crude protein (CP) level could impact compensatory growth (CG) in milkfish juveniles under short-term fasting and refeeding cycle. Four experimental groups of milkfish were fed diets containing graded levels of CP; 30% CP (C30), 35% CP (C35), 40% CP (C40), and 45% CP (C45) fasted for 2 days and fed for 5 days in one week for a total of 8 weeks. A fifth diet containing 40% CP continually fed to the fish served as the control treatment (C). After 8 weeks of feeding trial, CG in terms of compensation coefficient calculated in terms of weight gain was observed only in the C40 group (CC_{WG}=1.14). However, CG in terms of total length (CC_{FTL}) was observed in all cycled milkfish with the peak at C40 (CC=1.48). Final weight, final total length, and specific growth rate increased as dietary CP level increased from 30% to 40% and decreased at 45% CP. The CP level that elicited maximum values of these responses was estimated using a quadratic regression analysis to be 38.5%. Results show that when dietary CP level was close to the optimum level of 40%, CG was observed under the cyclic feeding used (2:5).

Key words: coefficient of compensatory growth, optimum dietary protein, refeeding, short-term fasting

INTRODUCTION

Milkfish *Chanos chanos* (Forsskal, 1775) is one of the most important food fish in Asia particularly Philippines, Indonesia, and Taiwan. Intensification of its culture requires high stocking densities and concomitantly, increased input of formulated feeds as well as increased expenditure on labor cost of feeding the fish. There is a need to reduce these costs to make milkfish culture more profitable at a sustainable level. Wu & Gatlin III (2014) reviewed three approaches used to minimize the protein input to fish without negatively affecting their growth performances. These are (1) reducing the use of protein for energy (2) starvation and subsequent feeding (Heide et al. 2006) that elicits compensatory growth (CG) (termed cyclic feeding), and (3) lastly the use of protein restriction-realimentation (Wu & Dong 2002; Sevgili et al. 2012).

Previously, the researchers showed that cyclic feeding was successful in eliciting a CG response in milkfish juvenile (Llameg & Serrano, Jr. 2014). Fish offered the two-day deprivation and five-day feeding cycle (2:5) resulted in statistically similar specific growth rate (SGR) with those in the control group and higher overall feed efficiency (FE)

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than the control and other groups of fish. Results showed that complete growth compensation could be elicited in 1:6 and 2:5 cyclic feeding regimens. Various works to elicit CG in aquatic animals have already been documented at various fasting and refeeding cycles. Rainbow trout *Onchorhynchus mykiss* was fed diets containing protein restriction for 3 weeks and then realimented for 9 weeks (Sevgili et al. 2012). Red drum *Sciaenopsis ocellatus* were fed diets containing optimal and restricted protein alternated in one day or these diets were cycled weekly (Wu & Gatlin III 2014). In gilthead sea bream *Sparus aurata*, four groups of fish were subjected to feeding levels ranging from starvation to satiation for the first half of the experiment and were fed to satiation during the second half (Bavcevic et al. 2010). Also, duration of starvation tested were 28 days (Rodgers et al. 2003), 9 days (Perez-Jimenez et al. 2007), 3 days (Gaylord & Gatlin III 2001), or even one day (Ofor & Ukpabi 2013).

Information on the effects of dietary protein level on cycled milkfish is necessary to reduce its level, thus increasing profitability of culture as well as reduce pollution (i.e., nitrogenous waste) from effluents. As far as the researchers know, this study has never been done on milkfish; thus the aim to determine the effects of graded dietary protein levels on CG, FE, and body composition of cycled milkfish.

MATERIALS AND METHODS

Experimental Diet and Design

Four isoenergetic practical diets with 30% (C30), 35% (C35), 40% (C40), and 45% (C45) crude protein levels were formulated (Table 1) and fed to juvenile milkfish under a weekly cycle of 2 days starvation and 5 days feeding. This regime was previously demonstrated to elicit CG in juvenile milkfish (Llameg & Serrano, Jr. 2014). A fifth diet similar to the composition of C40 was fed continually three times daily and served as control (C).

Diets were prepared following the practical formulation for milkfish juveniles developed by the Southeast Fisheries Development Center - Aquaculture Department (SEAFDEC-AQD) (Table 1). All dry ingredients were passed through a 150 µm sieve, mixed thoroughly before adding the oil containing the vitamin and mineral premixes. Gelatinized starch was added to make a dough, extruded through a grinder (2mm), steamed for 3-5 min, and oven dried for 8-12 h at 50-60°C. The spaghetti-like diets were broken into appropriate sizes, packaged, and stored in a cool place until use.

Experimental Procedures

Two hundred twenty-five (225) juvenile milkfish (*Chanos chanos*) (initial weight, IW = 2.26 g) were divided into 15, 80-L aquaria (15 milkfish each) connected in a closed recirculating system with a common filter. Water quality was monitored daily for temperature using mercury thermometer, dissolved oxygen using dissolved oxygen meter, and salinity with refractometer. Weekly monitoring of ammonia was done using phenol hypochlorite method; salinity was maintained at a range of 28 to 30 o/00. Groups C30, C35, C40, and C45 were fed three times daily for 5 days following a starvation period of 2 days in a weekly cycle for a total of 8 weeks. The C group was fed continually three times daily for the duration of the experiment; this diet-feeding scheme served as the positive control. The feeding rate was 8% of body weight (BW) for 2 weeks and reduced to about 6% thereafter. These feeding rates were preliminary established to approach apparent satiation without overfeeding, as described by Wu and Gatlin III (2014) in red drum *Sciaenops ocellatus*. At the

Table 1. Composition and proximate composition of the experimental diets (g $100g^{-1}$ dry diet).

Ingredient	C	C30	C35	C40	C45
	% CP				
	40	30	35	40	45
Fish meal	33.3	19.78	26.74	33.3	38
Soybean meal	34.3	19.66	26.74	34.3	40
Wheat pollard	10.75	23.3	18.1	10.75	3.7
Rice bran	7.55	23.2	14.32	7.55	4.2
Bread flour	5.0	5.0	5.0	5.0	5.0
Soybean oil	2.0	2.0	2.0	2.0	2.0
Cod liver oil	2.0	2.0	2.0	2.0	2.0
Vitamins[1]	2.0	2.0	2.0	2.0	2.0
Minerals[2]	2.0	2.0	2.0	2.0	2.0
Lecithin	1.0	1.0	1.0	1.0	1.0
Analyzed proximate composition, g 100 g^{-1} dry diet					
Crude protein	38.48	28.8	32.89	38.48	43.46
Crude fat	4.32	4.95	4.79	4.32	3.00
Ash	18.1	17.7	16.12	18.1	18.19
Crude fiber	3.56	6	4.95	3.56	3.05
GE[3]	330	340	335	330	330

[1]Vitamin mixture contain the following vitamins for every 25 kg feed: Vit. A, 10,000 IU; Vit. D3, 1,500,000 IU; Vit. E, 7,000 IU; Vit. K, mg; Vit. B1, 500 mg; Vit. B2, 1,100 mg; Vit. B6, 1,100 mg; Vit. B12, 500 mg; niacin, 15,000 mg; calcium paniothenate, 6,000 mg; chloride, 15,000 mg; folic acid, 100 mg; lysine, 13,200 mg
[2]Mineral mixture contain the following minerals for every 400 g diet: calcium (Ca), 0.02; chloride (Cl), 54.71; copper (Cu), 0.44; iodine (I), 0.01; iron (Fe), 3.97; magnesium (Mg), 0.04; manganese (Mn), 0.44; selenium (Se), 0.01; zinc (Zn); 4.41; sodium (Na), 35.95
[3]GE = estimated gross energy (kcal kg^{-1}) from physiological fuel values of 4.0 kcal g^{-1} for protein and carbohydrate and 9.0 kcal g^{-1} fat

start and every 14 days, all fish were anesthetized with phenoxyethanol (150 mg L^{-1}) and individually weighed. Carcass analyses were done at the start and end of the feeding trial (AOAC 2002).

Estimation of Optimum Crude Protein

Growth and feed efficiency data (FBW, WG, SGR, and FE) were fitted into a quadratic regression model. The regression equation used was:

$$R = a + bI + cI^2 \quad (1)$$

where

R = measured response;

I = dietary nutrient concentration; and

a, b, and c are constants that are calculated to provide the best fit of the data.

The value of I that produces the maximum response I_{max} is calculated as follows:

$$I_{max} = -0.5\ (b/c) \quad (2)$$

Calculation and Statistical Analysis

The growth and feed utilization parameters were calculated as follows:

Weight gain (WG, g) = final body weight (FBW, g) – initial average body weight (IBW) (3)

Final total length (FTL) = the length of a fish measured from the tip of the snout to the posterior end of the caudal fin in mm; (4)

Specific growth rate (SGR, % day^{-1}) = 100 * (ln FBW – ln IBW) / t (5)

where: InWf - natural log of final weight
InWi - natural log of initial weight
T - culture period (in days)

Feed efficiency (FE) = 100 * WG (g) / food intake FI (g) (6)

Protein gain (PG, g) = (FBW * final carcass CP in decimal) – (IBW * initial carcass CP in decimal) (7)

Protein efficiency ratio (PER) = WG (g) / FI * dietary CP in decimal (8)

Protein or lipid retention (PR or LR) = 100 * [final carcass CP or CL in decimal x FBW (g)] – [initial carcass CP or CL in decimal * IBW (g)] / FI (g) * dietary CP or CL intake in decimal (9)

Compensation coefficient (CC) = $\Delta T \times \Delta C^{-1}$ (10)

where:

ΔT = average weight gain (g) in the treatment group tanks divided by the number of feeding days, and

ΔC = average weight gain (g) in the control group tanks divided by the number of feeding days; thus, CC>1.0 would indicate compensation)

Survival (%) = 100 * (final number of fish / initial number of fish stocked); (11)

Hepatosomatic index (HSI) = liver weight (g) x 100 / fish weight (g) (12)

Viscerasomatic index (VSI) = viscera weight (g) x 100 / fish weight (g) (13)

Data were presented as mean ± standard error of the mean (SEM) prior to testing for normality using Shapiro-Wilk test and variance homogeneity using Levene's test. All data that passed the tests and were subjected to one-way Analysis of Variance (ANOVA). Growth (FBW, WG, SGR), feed efficiency (FE, protein, lipid, and energy retention rates), body composition, and survival rates were subjected to ANOVA at α=0.05. Percentage data were first transformed by arcsine transformation prior to analysis. Tukey's test was performed to rank the mean values of the performance parameters.

RESULTS

Growth Performance and Feed Utilization

The total number of feeding days in the control group and the cycled groups in the present study were 56 and 40 days, respectively. In terms of weight gain wise compensatory coefficient (i.e., CC_{WG}), only group C40 exhibited CG. In contrast, total length wise CC (i.e., CC_{FTL}) in all cycled treatments exhibited CG (CC>1.0), which increased with increasing dietary protein level peaking in the C40 group and decreased in the C45 group.

Dietary CP level significantly affected the growth performance of the cycled milkfish (p<0.05, Table 2). Final average body weight (FABW), final average total length (FATL), and SGR values increased as dietary CP level increased from 30% to 40% but decreased at 45% CP. Based on the quadratic regression analysis of WG, FABW, and FE of the cycled milkfish, the estimated optimum dietary CP level of *Chanos chanos* was 38.3%, 38.3%, and 39.0% (average = 38.5%), respectively (Fig. 1). Condition factor (CF) was higher in the C group than

Table 2. Growth performance and feed utilization of cyclic fed juvenile milkfish (*Chanos chanos*) fed with varying protein levels.

	C	C30	C35	C40	C45
IBW	2.30±0.04	2.23±0.02	2.23±0.02	2.29±0.08	2.24±0.09
FBW	7.49±0.23^{c}	4.40±0.28^{a}	5.11±0.37^{ab}	6.54±0.30^{bc}	5.43±0.30^{b}
FTL	7.49±0.23^{b}	7.01±0.14^{a}	7.23±0.12^{a}	7.91±0.19^{c}	7.40±0.04^{b}
CF	1.78±0.05^{c}	1.28±0.06^{a}	1.35±0.07^{b}	1.32±0.05^{ab}	1.34±0.06^{b}
WG	5.18±0.19^{d}	2.16±0.26^{a}	2.88±0.19^{b}	4.23±0.13^{c}	3.19±0.13^{c}
SGR	2.10±0.03^{d}	1.19±0.09^{a}	1.47±0.06^{ab}	1.87±0.06^{cd}	1.58±0.05^{bc}
FI	14.10±0.12^{c}	7.74±0.18^{a}	7.61±0.10^{a}	8.80±0.46^{b}	8.81±0.50^{b}
FE	37.0±0.00^{ab}	33.0±0.01^{a}	38.0±0.00^{ab}	48.0±0.01^{b}	39.0±0.00^{ab}
PER	1.47±0.05^{a}	2.10±0.17^{b}	2.17±0.09^{b}	2.02±0.07^{b}	2.017±0.07^{b}
Survival	84.4±5.9	84.4±2.2	95.6±2.2	82.2±2.2	88.9±5.9
CC_{WG}	1.0	0.58	0.78	1.14	0.86
CC_{FATL}	1.0	1.31	1.35	1.48	1.38

Mean values (±SEM) in the same row with different superscript are significantly different (p<0.05). Abbreviations used: IBW = initial weight (g); FBW = final weight (g); FTL = final total length (mm); condition factor (CF) = 100 * FBW (kg) / FTL (cm)^3; WG = weight gain (g); compensation coefficient for WG (CC_{WG}) = [FTW in the cycled fish (g) / number of feeding days (day)] / [FABW in the control group (g) / number of feeding days (day)]; compensation coefficient for FTL (CC_{FTL}) = [FTL in the cycled fish (g) / number of feeding days (day)] / [FATL in the control group (g) / number of feeding days (day)]; SGR = specific growth rate (% body weight day^{-1}); FI = feed intake (g); FE = feed efficiency; PER = protein efficiency ratio; Survival in %; GE = estimated gross energy (kcal kg^{-1}) from physiological fuel values of 4.0 kcal g^{-1} for protein and carbohydrate and 9.0 kcal g^{-1} fat; protein to energy ratio (P/E) = mg protein kcal^{-1}

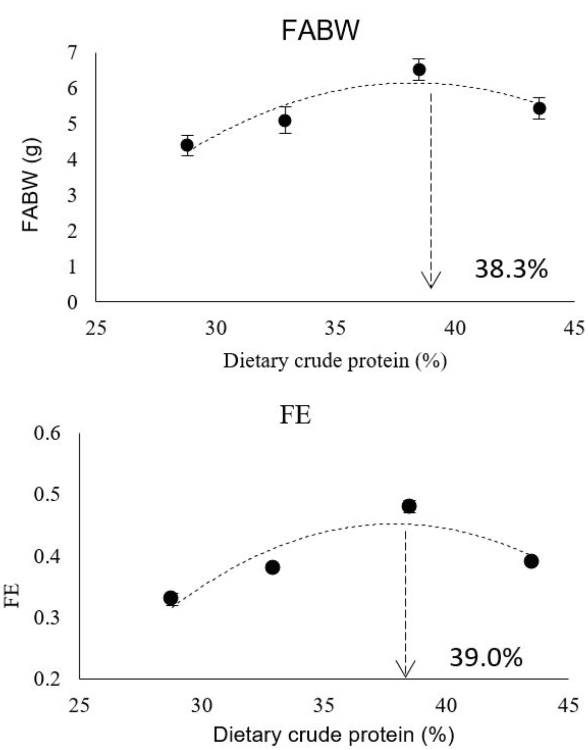

Figure 1. FBW and FE of milkfish juveniles to graded dietary crude protein levels fitted into a quadratic model. The dietary crude protein values that elicited the maximum response (i.e., I_{max}) was estimated to be 38.3% and 39.0% weight dietary crude protein using final average body using FE, respectively.

any cycled groups. FE increased with increasing dietary CP level in the cycled milkfish up to C40, which was the highest FE value observed. Dietary CP level did not significantly affect survival (p>0.05), which ranged from 82.2% to 95.6%.

Nutrient Utilization and Organ Indices

Protein retention (PR) rate in cycled fish increased from the C30 group reaching the highest values in the C35 and C40 groups declining significantly (p<0.05, Table 3) to the lowest level in the C45 group. The highest PR was observed in C35 and C40 (20.17 in both groups), which were statistically similar with each other. Lipid retention (LR) of all cycled fish were significantly higher (p<0.05) than those in control groups. There were no significant differences in HSI and VSI values observed among the treatments.

The temperature (25.9-31.0°C), dissolved oxygen (4.9-5.8 ppm), and pH (7.9-8.2) values observed during the entire duration of the experiment were within the required range for normal growth of fish (Boyd 1979).

DISCUSSION

This study demonstrated that milkfish juvenile fasted for 2 days and fed for 5 days in a week exhibited CG, a confirmation of the researchers' earlier study (Llameg & Serrano, Jr. 2014). CG manifested through increased FE

Table 3. Nutrient utilization and body indices of control and cycled milkfish juveniles *(Chanos chanos)* fed varying dietary protein levels.

Group	C	30	35	40	45
PR	17.48±0.80[ab]	16.01±1.44[ab]	20.17±0.37[a]	20.17±0.98[a]	14.86±0.57[b]
LR	53.52±2.02[c]	79.54±0.64[a]	69.51±2.43[ab]	60.75±2.61[bc]	71.57±0.26[ab]
HSI	1.28±0.07	1.57±0.30	1.52±0.16	1.36±0.18	1.24±0.09
VSI	10.03±0.13	9.68±0.71	10.91±0.41	9.29±0.63	9.04±0.32

Mean values (±SEM) in the same column with different superscript are significantly different ($p<0.05$)

and PER apparently required that the diet contain optimal level of protein. This was also the observation of other authors (Wang et al. 2000; Gaylord & Gatlin III, 2000; Cho et al. 2006; Oh et al. 2007). Full CG under short-term food deprivation has also been observed in some fish species (Chatakondi & Yant 2001; Hayward & Wang 2001; Johansen et al. 2001).

Cycled milkfish in the present study exhibited compensation in total length, the intensity of which increased with increasing dietary CP level up to C40 and decreased in C45. This was also observed in juvenile whitefish (*Coregonus lavaretus*) that were not fed during weekends (Kankanen & Pirhonen 2009), but not in gilthead sea bream *(Sparus aurata*) that were subjected to restriction and refeeding cycles for 60 days (Bavcevic et al. 2010). The proportion between weight and total length (i.e., CF) was not affected by the dietary protein level except in the C30 group.

Under standard methods of nutrient requirement study, the estimated optimal protein requirement of *Chanos chanos* was 40% (Lim et al. 1979), 30-40% CP for 0.5-0.8 g milkfish (Pieded-Pascual 1989), 42.8% for 2.8 g milkfish (Coloso et al. 1988) and 40 % (Jana et al. 2006). These levels were close to the estimated 38.5% CP in the present study. Results of the present study showed that as long as the CP content of the feed was close to the optimum protein requirement of milkfish, a short period of fasting and refeeding did not impact on the growth or feed/nutrient efficiency. This is in agreement with the finding in channel catfish that feeding with diets containing 37% CP either to satiation or cyclic feeding (3 days starvation and 11 days feeding) both resulted in improved WG and FE over those fed the diet containing 32% CP (Gaylord & Gatlin III 2001).

The PR of C35 and C40 were statistically similar, with C30 and the control having intermediate results and C45 groups having the lowest PR values. The LR, on the other hand, were all significantly higher in cycled fish than in the control group; this could be the major effect of short period of starvation leading to CG mainly reflected as higher body lipid.

CONCLUSION

In conclusion, CG in milkfish could be elicited under short-term deprivation (2 days) and refeeding (5 days) similar to continually fed fish if the protein level was at optimum (~40% CP). CG in terms of total length was elicited from all the treatments but was highest in milkfish fed containing 40% CP. Estimated level of optimal dietary protein using the quadratic regression model was 38.5% in cycled milkfish.

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