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## Effect of cyclic feeding on compensatory growth in milkfish *Chanos chanos* juveniles

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Abstract. This study aims to evaluate various cyclic feeding regimes in milkfish (Chanos chanos) juveniles whether or not compensatory growth (CG) could be elicited that could improve growth, feed efficiency, body composition or any combination of these three responses. Fish were assigned randomly to five feeding regimes, namely, a control (C), fed continuously, one day fasting followed by 6 days refeeding (1:6, days starved: days fed), 2:5, 5:23, or 7:21 cyclic feeding. Fish in 1:6 and 2:5 groups were exposed to 8 cycles while 5:23 and 7:21 groups for 2 cycles throughout the 56 days of experiment period. Milkfish juveniles fed continuously without fasting (control group) exhibited statistically similar mean weight with those offered the 1:6 and 2:5 feeding regimens while those in the 7:21 group significantly exhibited the lowest mean weight and consistently sustained the lowest values until the termination of the experiment. Specific growth rate (SGR) of milkfish fed the control regimen and those fed under 1:6 and 2:5 feeding regimes exhibited significantly higher than in those fasted for 5 or 7 days and refed (i.e. 5:23 and 7:21, respectively). Food conversion ratio (FCR) and Protein efficiency ratio (PER) values were significantly the best in milkfish offered the 2:5 feeding regimen while those of fish offered the control regimen and the rest of the cycled fed fish exhibited lower values but were statistically similar to each other. Survival rate, condition factor (CF), hepatosomatic index (HIS), and viscero-somatic index (VSI) were not influenced by the cyclic feeding regimes. In conclusion: cyclic feeding was successful in eliciting a CG response in milkfish fingerling under the experimental conditions of the present study. Fish offered the 2:5 cyclic feeding regimen resulted in statistically similar SGR with those in the control group and better overall FCR compared to other treatment and control groups of fish. Based on the results of this study, complete growth compensation could be elicited in 1:6 and 2:5 cyclic feeding regimen.

Key Words: compensatory growth, cyclic feeding, Chanos chanos, refeeding, starvation.

**Introduction**. Milkfish - *Chanos chanos* (Forsskal, 1775) is a popular finfish cultured in the Philippines, Indonesia, and Taiwan. Its global annual aquaculture production has increased every year since 1997; by 2005 it had risen to nearly 595 000 tonnes, with a value of almost USD 616 million. The most important producers at this time were the Philippines (289 000 tonnes), Indonesia (254 000 tonnes) and Taiwan Province of China (50 000 tonnes)(FAO 2007).

A substantial number of studies have already been undertaken in many aspects of milkfish culture resulting in the development of technologies that led to intensive production. Considering that feed contributes 60 to 75% of the total cost of production in intensive culture, strategies to reduce feed inputs is of prime importance to make the industry more profitable at a sustainable level. The present study utilized cyclic feeding strategy as management tool to reduce production cost, enhance feed efficiency and minimize waste loading into the water environment. Cyclic feeding, a feeding scheme that involves feed deprivation followed by a period of refeeding, can elicit compensatory growth. Compensatory growth (CG), or "catch-up" growth, has been defined as a physiological process whereby an organism accelerates its growth after a period of restriction, to attain the weight of cohorts whose growth was never hampered (Hornick et al 2000). Compensatory growth has been reported in several terrestrial vertebrates (Wilson & Osborne 1960) and is very promising in its potential to improve growth rate

and feed efficiency. Many aquatic species have also been shown to exhibit CG (Ali et al 2003) with similar improvements.

Rapid growth following food restriction has been observed in several cold and warm water fish (Cho et al 2006a; Kankanen & Pirhonen 2009; Nikki et al 2004; Oh et al 2007; Tian & Qin 2003). The response of fish to food limitation differs among species and is influenced by the duration of food deprivation and refeeding. The scheme of application that is either making use of single or cyclic feeding may also affect the degree of growth recovery of the fish. Extent of recovery can either be (1) overcompensatory, in which several cycles of feed deprivation and re-feeding result in a weight gain higher than that of fish fed continuously; or (2) complete compensation, in which fish previously subjected to food restriction achieve the same body mass as fish continuously fed; or (3) partial compensation, in which food-restricted fish exhibit accelerated growth after resumption of normal feeding, but do not achieve the same body mass as fish continuously fed (Jobling & Johansen 1999).

Although different feed restriction-refeeding cycles have been tested on several fish species, practically no study has been done yet on the growth response of milkfish to cyclic feeding. The only work on record is on the effect of starvation and subsequent feeding on the hepatocytes of milkfish fingerlings and fry (Storch & Juario 1983). Varying responses to different restriction-refeeding protocol of different species of fish have been observed not only in growth indices but also in feed efficiency and body composition. Thus, there is a necessity to establish appropriate cyclic feeding scheme for a given species (Cho et al 2006b; Nikki et al 2004; Tian & Qin 2003).

A study on the effect of restricted feeding on red sea bream, *Pagrus major*, shows a decrease in the crude protein, crude lipid, and energy content and the ratio of lipid to lean body mass with the increased duration of feed deprivation (Oh et al 2008). At the end of refeeding period the protein, ash and energy content of those starved fish are significantly higher of those under the control feeding regime but not the moisture content. Black rockfish, *Sebastes schlegeli* starved for 5 days and refed for 15 days have similar protein, lipid and ratio of lipid to lean body mass with the control but lower in energy content.

The results of many studies show that cyclic feeding that elicit CG in fish may have some potential application to commercial fish production in improving production rate (Ali et al 2003). Some experiments have applied different periods of feed deprivation to elicit a CG response in fish followed by periods of refeeding (i.e. cyclic feeding). Some studies report short-term improvements in growth and feed efficiency for an initial period following realimentation, then returning to normal levels thereafter (Johansen et al 2001; Kim & Lovell 1995; Wang et al 2000; Xie et al 2001). Other studies report increases in overall feed efficiency (FE) (Chatakondi & Yant 2001; Quinton & Blake 1990). Improved growth may decrease time to attain marketable size in the production process while increased FE could reduce costs by enhancing nutrient utilization and minimize wastes in aquaculture effluents.

Modification of feeding protocol as in a cyclic feeding of fish may alter growth rates and consequently may change the nutritional demand of the fish. These improvements may be due to decreased energetics demand or improved rates of retention, but improved hormonal conditions could also be a metabolic basis.

The present study aims to evaluate various cyclic feeding regimes in milkfish juveniles whether or not CG is effected that could improve growth, feed efficiency, body composition or any combination of these three responses.

## Material and Method

**Experimental fish and set up**. The experiment was conducted at the Institute of Aquaculture Multispecies Hatchery, University of the Philippines Visayas, Miagao, Iloilo. A pond nursery grown milkfish juveniles (average body weight, ABW of  $3.2\pm0.1$  g) were used in the experiment. A 2-ton capacity rectangular fiber glass tank served as holding tank where the fish were conditioned for at least one day prior to randomly distributing these to the experimental aquaria.

An 8-week (December 2009 to February 2010) growth trial was conducted with approximately 15 milkfish juveniles per aquarium. Fish were allowed to acclimate for two weeks during which time they were fed with commercial pellet twice daily at 10% body weight. Following the acclimation period, each aquarium was randomly assigned to one of 5 cyclic feeding regimes, namely, a control (C), fed continuously, one day fasting followed by 6 days refeeding (1:6, days starved: days fed), two days fasting followed by 5 days refeeding (2:5), 5 days fasting followed by 23 days refeeding (5:23) or 7 days fasting followed by 21 days refeeding (7:21). Also, after the two-week acclimation period, fish were sampled to obtain an initial weight. Fish in 1:6 and 2:5 groups were exposed to 8 cycles while 5:23 and 7:21 groups for 2 cycles throughout the 56 days of experimental period.

**Feeding management and growth monitoring**. Commercial feed for milkfish juvenile was used as feed during the acclimatization period and during the entire duration of the cyclic feeding experiment (Table 1). In all experimental feeding regimes, feeding/refeeding was done three times daily at 07:00, 11:00 and 16:00 h using 2-8% of body weight feeding rate during the entire experiment.

Bulk sampling of fish per replicate were carried out every two-week interval and individual length measurement were taken at the termination of the experiment. Immediately following sampling, survival rate was determined to adjust the feeding ration.

Table 1

Proximate composition (% dry matter) of the commercial diet used in the cyclic				
fasting/feeding experiment				

Proximate composition	% dry weight basis
Crude protein	39.09
Crude fat	6.82
Ash	21.16
Fiber	3.77
Nitrogen-free extract (NFE)	29.16
Gross energy (GE)	326.69 kcal g <sup>-1</sup>

*Chemical analysis*. Proximate composition of the experimental diet, initial and terminal fish carcass were analyzed following standard methods (AOAC 2002).

Crude protein (N x 6.25) was determined using Kjeldahl distillation apparatus, crude lipid with Soxhlet ether extraction, crude fiber with Fibertic system 1010 heat extractor, moisture content by drying to constant weight in an oven at  $100^{\circ}$ C while ash content were determined by incinerating the samples in muffle furnace at 550–600°C for 6 h. The digestible energy of the diet and of fish carcass was estimated by using the physiological values of 4.5, 8 and 3.3 kcal g<sup>-1</sup> for protein, fat and carbohydrates respectively.

*Growth performance indices*. The following growth and feed utilization parameters were calculated:

Specific growth rate (SGR, % day<sup>-1</sup>) = 100 X (InWf-InWi)/t; Feed conversion ratio (FCR) = amount of feed fed/weight gain, survival rate (%) = 100 X final count/initial count; Condition factor (CF) = 100 X body weight/body length<sup>3</sup>; Protein efficiency ratio (PER) = weight gain/protein intake; Lipid deposition (LD) (%) = 100 X lipid gain/lipid intake; Hepatosomatic index (HIS) = liver weight x 100/fish wet weight; Viscerasomatic index (VSI) = viscera weight x 100/fish wet weight.

**Physico-chemical monitoring of water**. Dissolved oxygen and temperature of the water in the experimental aquaria were monitored daily (06:00-07:00) using oxygen meter. Ammonia and phosphorus content of the water were checked twice a week following standard methods (AOAC 2002).

**Statistical analysis**. Data were tested for uniformity in variance and normality of distribution before they were analyzed using Analysis of Variance (ANOVA) at p = 0.05 to determine significant differences among treatments. Once significance was detected, data were subjected to post hoc analysis, specifically, Tukey's Test.

**Results**. Water quality parameters during the experimental period were as follows: pH 7.4-8.2; dissolved oxygen 5.0-6.0 mg L<sup>-1</sup> and temperature  $26-32^{\circ}$ C. Mean temperature, pH and dissolved oxygen levels were not affected by feeding scheme and very well within the acceptable limits for fish growth and health (Boyd 1979).

After two weeks of the feeding trial, milkfish in the 7:21 group significantly exhibited the lowest mean weight and consistently sustained the lowest values until the termination of the experiment (Table 2). In contrast, milkfish fed continuously without fasting (control group) exhibited statistically similar mean weight with those offered the 1:6 and 2:5 feeding regimens.

Table 2

Feeding	Mean body weight (g)				
regimen	0 d	14 d	28 d	42 d	56 d
Control	3.12 <u>+</u> 0.02	4.02 <u>+</u> 0.36 <sup>a</sup>	5.27 <u>+</u> 0.32 <sup>ab</sup>	7.09 <u>+</u> 0.38 <sup>a</sup>	8.65 <u>+</u> 0.72 <sup>a</sup>
1:6	3.15 <u>+</u> 0.02	4.13 <u>+</u> 0.06 <sup>a</sup>	5.92 <u>+</u> 0.29 <sup>a</sup>	6.30 <u>+</u> 0.31 <sup>a</sup>	7.77 <u>+</u> 0.58 <sup>a</sup>
2:5	3.21 <u>+</u> 0.05	4.11 <u>+</u> 0.21 <sup>a</sup>	5.26 <u>+</u> 0.29 <sup>ab</sup>	5.85 <u>+</u> 0.41 <sup>ab</sup>	7.72 <u>+</u> 0.63 <sup>a</sup>
5:23	3.12 <u>+</u> 0.02	3.89 <u>+</u> 0.02 <sup>a</sup>	4.88 <u>+</u> 0.16 <sup>b</sup>	5.73 <u>+</u> 0.21 <sup>ab</sup>	7.15 <u>+</u> 0.14 <sup>b</sup>
7:21	3.20 <u>+</u> 0.03	3.14 <u>+</u> 0.10 <sup>b</sup>	4.77 <u>+</u> 0.18 <sup>b</sup>	4.55 <u>+</u> 0.30 <sup>b</sup>	6.68 <u>+</u> 0.47 <sup>c</sup>

Periodic mean weight of milkfish subjected to different cyclic feeding regimes

Mean values ( $\pm$  SEM) with different letters in the same column are significantly different (p < 0.05).

SGR of milkfish fed continuously without fasting and those fed under 1:6 and 2:5 feeding regimes exhibited significantly higher specific growth rate (SGR) than those fasted for 5 or 7 days and refed (i.e. 5:23 and 7:21, respectively) (Table 3). However, values of efficiencies of conversion (i.e. FCR and PER) were significantly the best in milkfish offered the 2:5 feeding regimen while those of fish offered the control regimen and the rest of the cycled fed fish exhibited lower values and were statistically similar with each other. Survival rate, CF, HIS, VSI were not influenced by the cyclic feeding regimes.

Table 3

Growth performance and feed utilization of *milkfish* fingerlings at various cyclic feeding regime

Parameter	Control	1:6	2:5	5:23	7:21
Init. Wt (g)	3.21 <u>+</u> 0.09	3.15 <u>+</u> 0.02	3.20 <u>+</u> 0.05	3.12 <u>+</u> 0.15	3.21 <u>+</u> 0.03
Final Wt.(g)	8.65 <u>+</u> 0.72 <sup>a</sup>	7.77 <u>+</u> 0.58 <sup>a</sup>	7.72 <u>+</u> 0.63 <sup>a</sup>	7.15 <u>+</u> 0.15 <sup>b</sup>	6.68 <u>+</u> 0.47 <sup>c</sup>
SGR(%day)	1.76 <u>+</u> 0.95 <sup>a</sup>	1.60 <u>+</u> 0.13 <sup>a</sup>	1.56 <u>+</u> 0.16 <sup>a</sup>	1.30 <u>+</u> 0.03 <sup>b</sup>	1.47 <u>+</u> 0.75 <sup>b</sup>
FCR	3.19 <u>+</u> 0.14 <sup>b</sup>	3.03 <u>+</u> 0.25 <sup>b</sup>	2.50 <u>+</u> 0.22 <sup>a</sup>	2.90 <u>+</u> 0.03 <sup>b</sup>	3.28 <u>+</u> 0.42 <sup>b</sup>
PER	0.86 <u>+</u> 0.04 <sup>b</sup>	0.91 <u>+</u> 0.08 <sup>b</sup>	1.07 <u>+</u> 0.10 <sup>a</sup>	0.92 <u>+</u> 0.02 <sup>b</sup>	0.86 <u>+</u> 0.12b
LD	41.1 <u>+</u> 1.3 <sup>b</sup>	40.6 <u>+</u> 2.7 <sup>b</sup>	62.1 <u>+</u> 3.6 <sup>a</sup>	39.6 <u>+</u> 0.6 <sup>b</sup>	42.5 <u>+</u> 4.9 <sup>b</sup>
Survival (%)	80 <u>+</u> 4	82 <u>+</u> 9	82 <u>+</u> 4	85 <u>+</u> 2	78 <u>+</u> 6
CF	1.30 <u>+</u> 0.05	1.25 <u>+</u> 0.08	1.32 <u>+</u> 0.03	1.33 <u>+</u> 0.02	1.29 <u>+</u> 0.09
HIS	1.36 <u>+</u> 0.07	1.47 <u>+</u> 0.06	1.55 <u>+</u> 0.09	1.47 <u>+</u> 0.05	1.62 <u>+</u> 0.10
VSI	7.41 <u>+</u> 0.32	7.76 <u>+</u> 0.49	8.34 <u>+</u> 0.14	8.21 <u>+</u> 0.32	7.33 <u>+</u> 1.86
PER	0.86 <u>+</u> 0.04	0.91 <u>+</u> 0.08	1.07 <u>+</u> 0.10	0.92 <u>+</u> 0.02	0.86 <u>+</u> 0.12

Mean values ( $\pm$  SEM) with different letters in the same row are significantly different (p < 0.05).

After eight weeks of feeding trial, body crude protein was significantly the highest in milkfish fed under a 1 day starvation cycle while those fed continuously (control) exhibited significantly the lowest (Table 4). All other milkfish that had starvation as a cycle gave higher body protein than did those fed continuously. Fat content of milkfish fed under the 2:5 feeding scheme was significantly the highest and those fed other feeding regimes exhibited lower but similar fat content with each other. Ash and

nitrogen-free extract contents in all groups were not significantly different from each other.

Table 4

Carcass composition of milkfish juveniles fed continuously (control) or under various cyclic feeding regime

Feeding scheme	Crude protein	Crude lipid	Ash	NFE
Control	51.87 <u>+</u> 0.71 <sup>c</sup>	24.66 <u>+</u> 0.16 <sup>b</sup>	10.65 <u>+</u> 0.13	11.73 <u>+</u> 1.13 <sup>a</sup>
1:6	56.86 <u>+</u> 0.75 <sup>a</sup>	24.90 <u>+</u> 0.40 <sup>ab</sup>	9.98 <u>+</u> 0.05	6.96 <u>+</u> 0.92 <sup>b</sup>
2:5	55.16 <u>+</u> 0.28 <sup>ab</sup>	27.13 <u>+</u> 0.46 <sup>a</sup>	9.97 <u>+</u> 0.22	6.18 <u>+</u> 0.11 <sup>b</sup>
5:23	56.10 <u>+</u> 0.19 <sup>b</sup>	22.66 <u>+</u> 0.6 <sup>b</sup>	10.65 <u>+</u> 0.14	7.91 <u>+</u> 0.83 <sup>b</sup>
7:21	55.86 <u>+</u> 1.56 <sup>ab</sup>	23.71 <u>+</u> 0.13 <sup>b</sup>	10.49 <u>+</u> 0.14	6.61 <u>+</u> 1.46 <sup>b</sup>
Mean values $(\pm SEM)$ with different letters in the same column are significantly different (p < 0.05).				

**Discussion**. Feed deprivation results in a response in fish by increasing SGR and improving FCR or both during the refeeding period (reviewed by Ali et al 2003). These responses in various fish species resulted in partial or complete growth compensation, with one study reporting overcompensation (Hayward et al 1997). Following the eight weeks of feeding trial in the present study, significantly lower ABW values were observed in milkfish offered the 5:23 and 7:21 feeding cycles while the rest of the control and cycled fish (i.e. 1:6, 2:5) exhibited significantly higher ABW values. However, significantly the best mean FCR and PER for all treatments was exhibited by milkfish juveniles offered the 2:5 feeding regimen while the rest of the treatments exhibited poorer FCR and PER values and were statistically similar which each other including those of the control group. FCR and FCE but not the SGR results following the eight weeks appeared to have resulted from a CG response.

The improved FCR has important cost-saving implications, savings as a result of improved FCR with additional benefit of (statistically) no lost growth, as final weights of fish in the control regimen were not significantly different from all cycled fish. It was apparent that there was complete growth compensation which indicated that the CG response, as indicated by statistically similar SGR was not temporary and could overcome (numeric) lost weight.

Shorter feed deprivation periods and longer refeeding periods results in full growth compensation (Chatakondi & Yant 2001; Hayward & Wang 2001; Johansen et al 2001; Kim & Lovell 1995; Miglavs & Jobling 1989). In the present study, the shorter period of feed deprivation (1:6 or 2:5 cyclic feeding) resulted in a statistically similar SGR with that of the control treatment. Also, fish offered the 2:5 cyclic regimen displayed both similar SGR and significantly better FCR at the end of the trial. Hence, 2 days of feed deprivation was sufficient to trigger a measurable CG response that led to improved FCR in milkfish fingerling. Since the final weight of fish in the 2:5 feeding regimen statistically reached those in the control treatment, the increase in growth rate was of sufficient magnitude and duration to compensate for numeric lost growth.

Studies show that CG of various fishes suggest the response is species-specific (Hayward & Wang 2001) and highly dependent on the life history of the species being examined. However, detailed mechanisms underlying the CG response as of yet have not been elucidated (Ali et al 2003). Broekhuizen et al (1994) describe a two-part model in which there is an attainment of an optimal ratio between reserve and structural tissue. In starved fish, Turano et al (2008) explains, the ratio falls below an ideal level in which appetite increases, but maintenance is kept constant. Under prolonged deprivation, fish keeps maintenance costs to a minimum that could result in increase chances of survival. Upon locating food, consumption is increased above the normal maintenance level to replenish the ratio; however, the maintenance cost stays at a minimum. The resulting increase in nutrient intake and decreased maintenance costs allow for more rapid growth, particularly in the form of muscle tissue. This could be the reason why the cycled fish exhibited higher carcass protein and lipid than those fish fed the control regimen. In the present study, HSI and VSI levels were monitored to provide an indication of sufficient feed deprivation (reserve tissue). Hepatic tissue responded rapidly to feed deprivation

and were not significantly different among all treatments. The observation of Turano et al (2008) that a reduction in liver size (or perhaps the viscera) may be an important indicator for a potential CG response was not observed in the present study. The same author states that since CF measurements show a pattern that follows feed deprivation and refeeding, it may be used as an indicator of a potential CG response. This was not the case in the present study since there was no significant difference in the CF of milkfish fingerlings in all treatments.

The improvement in FCR observed in cyclic fed milkfish fingerlings in the present study would not only reduce feed costs, but may have positive impacts on water quality. Only 25 to 30% of the nitrogen and phosphorus applied to ponds in feeds is recovered during the harvest (Boyd & Tucker 1998). Hence, a large amount of potential waste remains in the pond and must be assimilated. However, with only the milkfish fed the 2:5 cycle being significantly superior, significant differences in water quality variables were not observed in the present study.

**Conclusions**. Cyclic feeding was successful in eliciting a CG response in milkfish fingerling under the experimental conditions of the present study. Fish offered the 2:5 cyclic feeding regimen had a statistically similar SGR with those under the normal continuous feeding and better overall FCR compared to other treatment and control groups of fish. Based on the results of the present study, complete growth compensation was observed in 1:6 and 2:5 cyclic feeding regimen. Practical application of these regimens is possible if farmers are willing to sacrifice weight gain for improved FCR and could lead to improved water quality of aquaculture effluents.

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