FASTING IN HYBRID GROUPER *EPINEPHELUS FUSCOGUTTATUS* X *EPINEPHELUS LANCEOLATUS* IN RELATION TO PROXIMATE CONTENTS, GROWTH AND FEED EFFICIENCY

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ABSTRACT

Fasting in fish is commonly used to improve the quality of fish product post-slaughtered. This study aims to investigate the alteration in nutrient contents, growth, and feed efficiency of hybrid grouper *Epinephelus fuscoguttatus* x *E. lanceolatus* fasted at different intervals. There were two experiments conducted in this study. The first experiment evaluated alteration in nutrient contents of carcass and liver of hybrid groupers. The fishes were grouped into three feeding regimes: fed daily, fasted at a one-day interval, and fasted at a two-day interval. Each group had 50 fishes with an initial mean weight of 200 ± 11.3 g. The second experiment was to evaluate the growth and feed efficiency of the fishes. Hybrid groupers were subjected to two feeding regimes. The first group (n = 45) was fed daily, and the second group of fish (n = 45) fasted at a two-day interval. The first and second experiments were conducted for one and two months, respectively. The results show that hybrid groupers fed daily have the lowest protein and the highest fat in the carcass compared to those fasted on one- or two-day interval. During the first month, there was no difference between the growth rate of the fishes fed daily and fasted at a two-day interval (32.88 and 32.08%, respectively). However, in the second month, the fishes fed daily have a growth rate of 66.08%, which is 1.4 times higher than those fasted at a two-day interval (48.12%). Altogether, fasting in hybrid groupers could alter proximate contents, slow down growth, and improve feed efficiency.

Keywords: Fasting, hybrid grouper, proximate, growth, feed efficiency.

INTRODUCTION

Seafood is a key source of protein in many parts of the world, in which aquaculture plays a role to meet the growing demand. Capture fisheries production has leveled off over the last decade, while aquaculture production has been increasing (FAO, 2016). As aquaculture becomes pivotal in meeting protein demand from seafood, it is important to ensure that the quality of cultured fishes is maintained to be similar or more than captured fishes.

In aquaculture, quality of cultured fish is assessed from texture, taste, and nutritional contents that could be controlled by feeding (Hardy *et al.*, 2010). Cultured fish often has higher fat content than captured and wild fishes (Poli, 2005), which could lead to decreasing quality of fish product post-slaughtered, particularly texture (Hardy *et al.*, 2010). For example, Alasalvar *et al.* (2002) show that cultured sea bream has lower protein and higher fat compared to captured sea bream that is likely due to a high percentage of fat in the feed given.

Hybrid grouper − a crossbred between tiger grouper *Epinephelus fuscoguttatus* x giant grouper *E. lanceolatus,* is among many fish species cultured in Indonesia. As a hybrid species, hybrid grouper is only supplied from aquaculture. Therefore, its quality depends

on aquaculture techniques to meet the market requirement as a source of protein food. To alternate proximate contents in the carcass of hybrid grouper, fasting or starvation could be an effective technique of feeding (Grikorakis and Alexis, 2005; Zhang *et al*., 2007). Fasting also has another advantage, that it tends to increase feed utilization shown by the feed conversion ratio (FCR) (Ofor and Ukpabi, 2013; Azodi *et al*., 2016). However, the duration and frequency of fasting need to be determined to obtain the best result. More specifically, the influence of fasting on growth should be evaluated to achieve a cost-effective technique in producing market-size hybrid groupers. This study aims to evaluate the alteration of proximate contents in the carcass and livers of hybrid grouper by fasting, as well as to evaluate the growth and feed utilization.

MATERIALS AND METHODS

Experiment I: Investigate the alteration of proximate contents due to fasting

Hybrid groupers were grouped into three feeding regimes. Fishes of group 1 were fed daily, group 2 fasted at a one-day interval (one day feeding, one day fasting) and group 3 fasted at a two-day interval (two days feeding, one day fasting). Each group consisted of 50 fishes with an initial mean weight of 200 ± 11.3 g. Fishes were fed until satiation with commercial feed formulated for groupers, containing crude protein 48%, crude fat 10%, crude fiber 4%, ash content 12%, and 150 ppm vitamin C. Each group was reared in a fiber tank of 5 m^3 with a continuous flow of seawater. After a month, three samples of the carcass were analyzed for protein and fat contents, and three samples of liver were analyzed for fat content only. Both samples of carcasses and livers were pooled for the analysis. The results were then used to develop the experimental design for subsequent experiment II.

Experiment II: Evaluate growth and feed efficiency

Hybrid groupers were subjected to two different feeding regimes, based on the result of proximate analysis of experiment I. Fish of Group IIA (n = 45) were fed daily and fish of Group IIB (n = 45) fasted on a two-day interval. Feeding rate was 2% of body weight for each feeding day. Feed and setting of the experimental unit were similar to experiment I. All the fishes were weighed monthly, and the feed amount was adjusted accordingly. Experiment II was conducted for two months.

Proximate Analysis

Three fish used for proximate analysis were captured randomly from the rearing tanks and dissected soon after. The whole bodies of fish were minced as fine as possible before being dried in the oven at 70°C for 24 hours, blended and processed to powder. Extraction and gravimetric were used to determine fat content using the Bligh and Dyer method. Protein content was analyzed using Kjeldahl. Moisture content was analyzed gravimetrically. The proximate parameters were analyzed at a laboratory of the Institute for Mariculture Research and Fisheries Extension in Gondol, Bali, accredited by the national accreditation committee (KAN). The analysis methods refer to as SNI 01-2891-1992.

Growth and Feed Efficiency Indicators

Data on fish weight and the length were used to calculate growth rate (GR), specific growth rate (SGR), condition factor (CF), and feed conversion ratio (FCR). GR is defined as the percentage of weight compared to the initial weight. The calculation of SGR follows Goda and Chowdhury (2007):

$$SGR = \left(\frac{\text{Ln Final BW} - \text{Ln Initial BW}}{\text{number of day}}\right) x\ 100 \qquad \text{Eq.1}$$

where BW refers to body weight. The calculation for CF and FCR are calculated following (Llmeg and Serrano, 2014):

$$CF = 100\ x\ \frac{\text{body weight}}{\text{body length}^3} \qquad \text{Eq. 2}$$

$$FCR = \frac{\text{total feed (dry) (g)}}{\text{weight gain (g)}} \qquad \text{Eq. 3}$$

Revenue and Profit

Profit from both feeding regimes calculated after sampling and harvesting is based on the price of feed and fish. Here, we report profit in Indonesian Rupiah (IDR) and US Dollar (USD) taking a currency rate of USD 1 = IDR 13,813. Commercial feed costs USD 1.4 (IDR 19,400) per kg, and the price of the fish is USD 7.24 (IDR 100.000) per kg. Only the costs of fish at the beginning of the study and feed are considered as the cost of production. Revenue gained from the sale of fish is assumed as the only benefit herein.

Statistical Analysis

We use R Commander version 2.3-2 to analyze data statistically at a significant level of 95%, and independent samples t-test to determine the differences between the means.

RESULTS

Proximate Contents Alteration

Fasting alters the proximate composition of carcass and the percentage of fat in the liver of hybrid groupers (Table 1). Protein contents of fasted fish increase, while fat contents decreased − both for hybrid groupers that fasted on one- and two-day intervals.

Table 1. Proximate contents of the carcasses and fat content of livers of hybrid grouper *Epinephelus fuscoguttatus* x *Epinephelus lanceolatus* fed daily and fasted after being reared for a month.

	Feeding Regimes		
	Fed daily	Fasted one-day interval	Fasted two-day interval
Carcass			
Moisture (%)	4.42	3.18	2.76
Protein (%)	76.63	77.80	81.29
Fat (%)	11.26	9.92	6.61
Liver			
Fat (%)	22.48	19.24	12.92

The highest level of protein and the lowest fat in the carcass are shown by fishes fasted on a two-day interval at 81.29% and 6.61%, respectively. While fishes that fasted daily show the lowest level of protein and the highest level of fat in the carcass at 76.26% and 11.26%, respectively. Moisture in the carcass is highest in fishes fed daily (4.42%), and lowest in fishes fasted on a two-day interval (2.76%).

Similarly, the fat contents in the liver reflect the finding for fat contents in the carcass. Fishes fed daily have the highest level of fat in the liver (22.48%), while fishes fasted on a two-day interval have the lowest level (12.92%) of fat in the liver compared to the other feeding regimes. Proximate contents of fish fasted on a one-day interval are in between the other two feeding regimes, both in the carcass and in the liver.

Growth and Feed Efficiency

After one month of rearing, we find no significant difference in body weight between hybrid groupers fed daily (452.67 ± 82.28 g) and fasted on a two-day interval (453.78 ± 76.11 g) ($p>0.05$) (Table 2). However, after two months of rearing, hybrid groupers fed daily have significantly higher body weight (565 ± 113.95 g) than those fasted on a two-day interval (508 ± 105.58 g) ($p<0.05$).

Fasting affects the growth of hybrid groupers in the second month of rearing as shown by the calculation of growth rate (GR) and specific growth rate (SGR). Growth rates in the first month of rearing are similar for both fed fishes (32.88%) and fasted fishes (32.08%). In the second month, fishes fed daily have grown 1.4 times higher (66.08%) than those fasted on a two-day interval (48.12%). Moreover, SGR of fishes fed daily (0.94% per day) is similar to those fasted on a two-day interval (0.92% per day) after 1 month of rearing. In the second month, daily growth rates slow down for both feeding regimes. Nevertheless, fishes fed daily have an increase in their body weight (0.82% per day), which is 1.3 times as faster than fishes fasted on a two-day interval (0.63% per day).

Table 2. Body weight, growth rate (GR), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF) of hybrid grouper *Epinephelus fuscoguttatus* x *Epinephelus lanceolatus* fed daily and fasted on a two-day interval after being reared for one and two months.

Parameter	Rearing Time (month)	Feeding Regimes	
		Fed daily	Fasted two-day interval
Initial body weight (g)		340.67 ± 53.91*	343.56 ± 55.89*
Body weight (g)	1	452.67 ± 82.28*	453.78 ± 76.11*
	2	565.78 ± 113.95*	508.09 ± 105.58*
GR (%)	1	32.88	32.08
	2	66.08	48.12
SGR (%/day)	1	0.94	0.92
	2	0.82	0.63
Initial CF (g/cm^3)		1.90 ± 0.19*	1.90 ± 0.25*
CF (g/cm^3)	1	1.97 ± 0.21*	1.99 ± 0.18*
	2	1.85 ± 0.17*	1.80 ± 0.21*
FCR	1	1.79	1.21
	2	2.11	1.88

* Data shown as mean ± standard deviation. Mean values of the same row are significantly different (p<00.05).

Table 3. The costs and revenues of hybrid grouper *Epinephelus fuscoguttatus* x *Epinephelus lanceolatus* fed daily and fasted on a two-day interval after being reared for one and two months. Values reported as IDR (USD).

	Rearing Time (month)	Feeding Regimes	
		Fed daily	Fasted two-day intervalFma
Initial fish cost		1,533,000 (110.98)	1,54,600 (111.92)
Feed cost	1	174,600 (12.6)	116,400 (8.4)
	2	240,560 (17.36)	155,200 (11.2)
Revenue	1	2,037,000 (147.47)	2,042,000 (147.83)
	2	2,546,000 (184.32)	2,290,000 (165.79)
Profit	1	329,400 (23.85)	379,600 (27.48)
	2	597,840 (43.28)	472,400 (34.2)
Profit per kg of fish	1	16,170 (1.17)	18,900 (1.35)
	2	23,480 (1.70)	20,630 (1.49)

Condition factor (CF) of hybrid grouper is not significantly altered by fasting. Hybrid groupers in both feeding regimes have similar CFs at the beginning of study ($p>0.05$). After being fasted for two months, CF of fasted fishes (1.80 ± 0.21) is not significantly different from those fed daily (1.85 ± 0.17). In the first month, CFs from both feeding regimes improve compared to the beginning of the study. However, there is a tendency that CFs of both feeding regimes decrease after two months of rearing.

Our study shows different feed conversion ratios (FCRs) between the two feeding regimes. Based on the experiment, fasted fishes have a better ratio of the amount of feed consumed to weight gain. For every 1 kg of weight gain, fasted fishes need less feed than those fed daily. In the first month, fasted fishes require 1.21 kg of feed to gain a 1 kg of weight, whereas fishes fed daily require 1.79 kg. In the second month, the FCRs both feeding regimes are higher than the previous month. Fasted fishes need 2.11 kg and fed fishes need 1.88 kg to gain 1 kg of weight.

Revenue and Profit

Total revenues of both feeding regimes after one moth of rearing were similar IDR 2,037,000 (USD 147.47) and IDR 2,042,000 (USD 147.83) gained from fish fed daily and fasted on a two-day interval respectively. As expected from the data of final body weight and growth rate above, total revenue after two months from fish fed daily was higher (IDR 2,546,000/USD 184.32) than revenue gained from fish fasted on a two-day interval (IDR 2,290,000/USD 165.79).

DISCUSSION

Our finding on proximate contents of hybrid grouper *E.* and x *E. lanceolatus* from fasting in our study is consistent with previous works (Grikorakis and Alexis, 2005; Zhang *et al*., 2007) showing that fasting alters proximate content of carcass, particularly protein and fat. A study on market-size *Sparusmacrocephalus* by Zhang *et al*. (2007) shows a decrease in both protein and fat contents of the carcass on continuously fasted fish. In that study, the fat content of the carcass decreases earlier on day 3 compared to the protein that significantly decreases from days 14 to 28. In a study of gilthead sea bream, Grikorakis and Alexis (2005) find that protein could be stored up to three weeks of fasting.

Overall, fishes can be grouped into two types based on their metabolic responses to starvation using fat or protein deposit as the main source of energy (Grikorakis and Alexis, 2005; Zhang *et al*., 2007; Azodi *et al*., 2016). In this study, fat content decreases on fasted fishes, while protein content increases. Increasing protein in fasted fishes is also reported in *Chanoschanos* by Llameg and Serrano (2014). Our data suggest that fat deposit may be the first source of energy utilized in the starvation mode of hybrid groupers. Hence, fasting in hybrid groupers with intervals of one or two-day helps to improve the quality of carcass of market-size fish by lowering fat content.

Higher protein content on fasted fishes may relate to a mechanism for survival during fasting. Fishes under a fasting condition need to maintain energy cost to a minimum level to increase chances of survival. Therefore during feeding, there is an increase in feed intake above the normal level to replenish; however, the maintenance cost stays at a minimum. This mechanism results in rapid growth, particularly in muscle tissue form due to higher nutrient intake and lower maintenance cost (Llameg and Serrano, 2014).

The lower contents of fat in the liver of fasted fish is expected as fasting has been reported to immensely affect the liver. For instance, Davis and Gaylord (2011) show that the liver mass of Asian sea bass decreases significantly after a week of fasting and remains low during three weeks of fasting. However, the liver mass increases to the normal size after re-feeding for a week. The study also suggests that the sensitivity of the liver to fasting may be due to the mobilization of nutrients deposition in the liver to sustain maintenance of fasted fish.

Final body weight, growth rate and specific growth rate start to differ after two months of rearing between fed and fasted fishes. In other words, fasting on hybrid groupers on a two-day interval would not affect the weight gain and growth within the first month. The feed given in this study has 10% of fat, which is reported as the optimum dietary fat for growth of grouper *E. luscoioides* (Luo *et al*., 2005). The slower growth of fasted fishes in the second month due to fasting

suggests that the fishes have adapted to the lack of feed by consuming less feed, which resulted in a slower growth. Fasting longer than one month would be a disadvantage for the production by slowing down the growth leading to lower final body weight.

In the second month, both fed and fasted fishes have a lower specific growth rate than the previous month. At this stage, fasting has started to affect the amount of fat or protein deposits shown by lessening of weight gain. Decreased SGR in fed fish also is likely to relate with a high content of fat in the liver. This assumption is in line with a study on *Senegalese sole* juveniles that shows that high content in liver likely decreases growth (Dias *et al*., 2004).

Our results also indicate that fishes fasted at a two-day interval use their energy more effectively than those fed daily as indicated by their FCR values. This result is consistent with Ofor and Ukpabi (2013) that show an improvement in feed utilization of fasted *Clarias gariepinus* as shown by low FCR. Another study by Azodi *et al.* (2016) on Asian sea bass also shows lower FCR in fasted fishes. Even though the growth of fasted fishers is slower after two months, its FCR is still better than the fed fishes. This implies that fasting requires fish to manage their energy efficiently to guarantee survival, as it has been mentioned above.

Higher revenue and profit per kg from fasted fishes is a result of better feed efficiency. Although the increasing weight after a month of rearing is similar, the cost of feed is lower for fasted fished compared to those fed daily. However, better feed efficiency does not result in higher revenue or profit after two months of rearing. Therefore, we recommend that the fasting technique in grow-out of hybrid groupers should be controlled up to a certain time to increase profit. In our study, fasting on a two-day interval is effective for only a month. Some studies have reported that fish could grow faster after starvation or fasting known as compensatory growth (Llameg and Serrano, 2014; Azodi *et al*., 2016) Further works on evaluating the compensatory growth of hybrid groupers after the fasting period are needed.

CONCLUSSION

In summary, fasting on a two-day interval could be used to improve the quality of market-size hybrid grouper *E.* and x *E. lanceolatus* by altering proximate contents. Fasting decreases fat while increases protein within a month. Fasting also improves feed utilization of the fish. However, the duration of fasting should be restricted to no longer than a month in order to retain growth and profit in the production of hybrid grouper.

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