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The Effect of Corn Starch Substitution with *Sargassum* sp. Starch in Diet on Grow-Out of Cultivated Rabbitfish, (*Siganus guttatus*) in Floating Net Cages

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Abstract

Corn starch is a source of carbohydrates for most cultivated fish, including rabbitfish as one of the herbivores with high economic value. Therefore, rabbitfish requires cheaper carbohydrate sources to have a profitable business in its cultivation. This study was aimed to determine the substitution of corn starch with *Sargassum* sp. starch in the diet on the growth, relative feed intake, FCR, and nutrient retention of rabbitfish. The average weight of rabbitfish used was 51.76±0.12 g/fish. Rabbitfish were cultivated in small net cages (1x1x1.5m) with a stocking density of 20 fish/net cage for 90 days reared. This study used an experimental method with a Completely Randomized Design (CRD). Corn starch (CM) substitution with *Sargassum* sp. starch (SM) by 0% (A), 21% (B), 42% (C), 63% (D) and 83% (E) used as the treatments with three replicates each. The measured parameters were weight gain (WG), relative growth (RG), relative feed intake (RFI), feed conversion ratio (FCR), protein retention (PR), energy retention (ER), and condition factor (CF). Data were analyzed using the analysis of variance (ANOVA) and Tuckey's test. The results showed that the lowest FCR in treatment A had no significant effect with treatment B but was significantly different from treatments C, D, and E. The WG, RG, RP, and CF parameters had the same values at all levels of CM substitution with SM, while RFI and FCR increased with the increasing CM substitution by SM. It also indicated that CM in the rabbitfish diet could be substituted with SM by 83%.

INTRODUCTION

Rabbitfish, *Siganus guttatus* are found in a large area in the Indo-Pacific, from the east coast of Africa to Polynesia and from southern Japan to northern Australia. In Indonesia, rabbitfish can be found in almost all coastal areas starting from Sumatra, Java, Sulawesi, Kalimantan, the Maluku Islands to Papua. The condition of the archipelago waters is very suitable for the optimum living needs of rabbitfish (Subandiyono and Hastuti, 2016). Therefore, Indonesia has enormous

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potential and prospects for the development of intensive rabbitfish farming.

In intensive cultivation, rabbitfish as herbivores require high carbohydrate diets. One type of seaweed known as a potential source of carbohydrates and still underutilized as diet material is *Sargassum* sp. *Sargassum* sp. contains sufficient nutrients that meet the nutrition needs of rabbitfish, which are 5.19% crude protein, 1.63% fat, 50.57% carbohydrates, 36.93% ash (Handayani *et al.*, 2004), and has a crude protein content of 7.3%, 0.7% fat, 26.2% crude fiber, 28.5% nitrogen-free extract (NFE), and 37.3% ash (Usman, 2019). Furthermore, rabbitfish can utilize carbohydrates in the diet by 36% optimally (Saade *et al.*, 2019) and 38% (Boonyaratpalin, 1997), while commercial diet generally only contains 24% carbohydrates. Based on those values, *Sargassum* sp. starch contains high enough carbohydrates and other nutrients to be a carbohydrate source and substitute for corn starch. Corn starch is used as a source of carbohydrates in aquaculture, for instance, rabbitfish diet. Corn starch is needed for many kinds of businesses. Aside from being used for aquaculture and livestock needs, corn starch is also needed as a food ingredient for humans. It will increase the price of corn starch and reduce the profits of the aquaculture business. Therefore, it has to be substituted with another ingredient.

Substitution of corn starch with seaweed, *Sargassum* sp. starch in rabbitfish diet should be expected to increase the diversification of the use of *Sargassum* sp., feed efficiency, growth rate, the productivity of the aquaculture business, profit from the aquaculture business, and reduce the feed prices. Therefore, this study was aimed to determine the substitution of corn starch with *Sargassum* sp. starch in the diet on the growth, relative feed intake, FCR, and nutrient retention of rabbitfish.

METHODOLOGY

Place and Time

The research was conducted at floating net cages of IPUW Barru in Awarange Bay, Lawallu Village, Barru Regency of South Sulawesi, precisely at the coordinates of South Latitude 4^{0}23'75" and East Longitude 119^{0}61" 30". It was cultivated for three months from October 2019 until January 2020.

Research Materials

Experimental fish were obtained from the Research Institute for Coastal Aquaculture and Fisheries Extension, Maros, South Sulawesi, Indonesia with an average initial weight of 51,76±0,12 g/fish. The selected fish were distributed to 15 units of 1×1×1.5 m small net that have been mounted on floating net cages with the size of 6×12×13 m. The stocking density used was 20 fish/cage or 863 g/m^3. Fish growth monitoring and changing the nets were done on days 30, 60, and 90. Experimental fish were reared at a temperature of 27.50-29.03°C, 27.00-35.00 g/L salinity, 6.70-7.05 ml/L dissolved oxygen, pH 7.56-8.43, and 0.07-0.27 m/s water current.

The experimental diet was *Sargassum* sp. starch (SM), a substitute for corn starch (CM) as the primary carbohydrate source. The amount and type of raw materials used were the same in each treatment, except for CM and SM. The ingredients and nutrient composition of experimental diets are presented in Table 1. The fish diet used was a dry pellet type artificial diet. The experimental diet was given three times a day in the morning, afternoon, and evening by satiation.

Table 1. Ingredients and nutrient composition of experimental diets.

Ingredients (g/kg)	CM substitution with SM (%)				
	0	21	42	63	83
Corn starch	360	285	210	135	60
Sargassum sp. starch	0	75	150	225	300
Fish starch	220	220	220	220	220
Copra cake starch	100	100	100	100	100
Soybean starch	200	200	200	200	200
Rice bran	80	80	80	80	80
Fish oil	10	10	10	10	10
Vitamin premix[a]	20	20	20	20	20
Mineral premix[b]	10	10	10	10	10
Total	1000	1000	1000	1000	1000
Nutrient's composition (% dry basis)					
Crude protein	29.9	29.0	28.9	28.4	28.4
Crude lipid	10.3	9.9	9.0	9.0	9.0
Crude fiber	7.1	9.4	10.0	11.2	13.3
Ash	10.4	12.0	13.5	15.0	16.9
Nitrogen-free extract	42.3	39.7	38.6	36.4	32.4
Gross energy (kcal/g)[c]	296.63	289.94	281.10	277.10	272.35
C/P ratio	9.92	10.00	9.73	9.76	9.59

[a]Vitamin mix (in 1 kg of diet): Vit. A 60,000 IU; Vit. D 20,000 IU; Vit. K 24 mg; Vit. E 150 mg; Vit B_1 60 mg; Vit B_2 90 mg; Vit B_6 60 mg; Vit B_{12} 60 mg; Vit C 160 mg; Calcium D-Pentathenate 80 mg; Folic acid 30 mg, Biotin 200 mg, Inositol 250 mg, Nicotinamide 400 mg, Cholin chloride 300 mg.

[b] Mineral mix (in 1 kg of diet): Calcium 325 mg; Phosphor 100 mg; Iron 60 mg; Manganese 40 mg; Zinc 73.5; Copper 3 mg; Sodium 1 mg; Cobalt 1 mg; Iodine 0.75 mg; Potassium 0.035 mg.

[c] Calculated from the determined crude protein, crude lipid and carbohydrate of the diet using digestible energy conversion coefficients of 3,00; 8,1 and 2,5 kcal/g, respectively (Furuichi, 1988).

Research Design

This study was done using the experimental method. It was designed using a completely randomized design (CRD) consisting of five treatments and three replicates. Substitution of CM with SM were amounted to 0% (A), 21% (B), 42% (C), 63% (D), and 83% (E) as the treatments in this study. The measured parameters were weight gain (WG), relative growth (RG), relative feed intake (RFI), feed conversion ratio (FCR), protein retention (PR), energy retention (ER), and condition factor (CF).

Work Procedure

Research procedures started with diet and net preparations. The diet was made through steps, namely washed with fresh water, dried, grounded using a hammer mill, and molded into a pellet, while the net was chosen based on its quality. After that, the fish was reared by selecting based on their weight, length, and health condition. They were fed three times a day in the morning, noon, and afternoon by satiation. The feeding was stopped when the fish did not give a response to the feed. The growth monitoring and changing the nets were done once a month.

The relative weight growth of fish is the difference between the weight of fish at the end of the rearing period and the initial weight divided by the initial weight of fish. The calculation of the relative weight growth based on Effendie (2002):

$$RG = \frac{Wt - Wo}{Wo} x100\%$$

Where:
RG = relative growth (%)
Wt = final weight (g)
Wo = initial weight (g)

The relative feed intake can be calculated using the formula (Sun *et al.*, 2016):

$$RFI = 100 \times \frac{F \times 2}{(Wo + Wt)\ T}$$

Where:

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RFI = relative feed intake (%/day)
F = total amount of feed consumed (g)
Wt = final weight (g)
Wo = initial weight (g)
T = rearing period (day)

The feed conversion ratio shows the ratio between the amount of feed consumed and the increase in fish weight, which can be calculated using the following formula (Tacon, 1987):

$$FCR = \frac{F}{Wt - Wo}$$

Where:
FCR = feed conversion ratio
F = amount of dry feed consumed (g)
Wt = final weight (g)
Wo = initial weight (g)

The nutrient retention was calculated by the formula (Buwono, 2000):

$$NR = \frac{\text{final NS} - \text{initial NS}}{N} \times 100\%$$

Where:
NR = nutrient retention (%)
NS = nutrient stored (g)
N = amount of nutrient consumed (g)

The initial and final nutrient stored and the total amount of nutrient consumed can be calculated using the formulas (Buwono, 2000):

$$\text{Final NS} = \frac{Ct - Wt}{100\%}$$

Where:
NS = nutrient stored (g)
Ct = final nutrient content (%)
Wt = final weight (g)

$$\text{Initial NS} = \frac{Co - Wo}{100\%}$$

Where:
NS = nutrient stored (g)
Co = initial nutrient content (%)
Wo = initial weight (g)

$$N = \frac{Cf - F}{100\%}$$

Where:
N = amount of nutrient consumed (g)
Cf = feed nutrient content (%)
F = amount of feed consumed (g)

Condition factor used to compare the species well-being between populations, which also states the physiological fish status. The calculation of condition factor based on Pauly (1983):

$$CF = \frac{100 \times W}{L^3}$$

Where:
CF = condition factor
W = total weight (g)
L = total length (cm)

Data Analysis

The analysis of the nutritional content of the experimental diets and fish were carried out based on AOAC (1999) methods. Data were analyzed using analysis of variance (ANOVA) and followed by Tukey's experimental test.

RESULTS AND DISCUSSION

Performance and feed efficiency of rabbitfish that consumed dry pellet type artificial diet containing CM substituted with various levels of SM are shown in Table 2.

Table 2. Performance and feed efficiency of rabbitfish that consumed artificial diet containing CM substituted with various levels of SM.

Parameters	CM substitution with SM (%)				
	A (0)	B (21)	C (42)	D (63)	E (83)
SR Average (%)	100±0.00[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]
WG Average (g)	152.53±15.19[a]	154.65±6.94[a]	148.93±3.28[a]	158.27±10.67[a]	140.01±7.94[a]
RG Average (%)	294.55±36.34[a]	298.45±14.54[a]	288.71±9.98[a]	305.78±21.42[a]	271.03±16.41[a]
RFI Average (% day^{-1})	1.93 ±0.04[a]	2.09 ±0.08[b]	2.14 ±0.04[b]	2.34 ±0.02[c]	2.47 ±0.01[c]
FCR Average	1.55 ±0.08[a]	1.66 ±0.06[ab]	1.72 ±0.04[bc]	1.85 ±0.06[c]	2.05 ±0.06[d]
PR Average (%)	129.90±12.28[a]	130.56±3.90[a]	122.01±7.83[a]	122.79±5.37[a]	114.83±5.27[a]
ER Average (%)	89.90 ±4.45[c]	85.57 ±4.03[bc]	82.82 ±3.27[ab]	79.31 ±2.09[ab]	70.45 ±1.87[a]
CF Average	2.38 ±0.04[a]	2.36 ±0.09[a]	2.37 ±0.01[a]	2.37 ±0.06[a]	2.36 ±0.06[a]

Notes: Values followed by different letters on the same row indicates significant differences ($p<0.05$); SR=Survival rate; WG=Weight gain; RG = Relative growth; RFI = Relative feed intake; FCR = Feed conversion ratio; PR = Protein retention; ER = Energy retention; and CF = Condition factor.

Rabbitfish that were fed with a dry pellet containing CM substituted with various SM levels obtained average survival rate (SR), weight gain (WG), relative growth (RG), relative feed intake (RFI), feed conversion ratio (FCR), protein retention (PR), energy retention (ER) and condition factor (CF) were 100.00 ± 0.00%; 140.01 ± 7.94–158.27 ± 10.67 g; 271.03 ± 16.41–305.78 ± 21.42 (%); 1.93 ± 0.0–2.47 ± 0.01 (%/day); 1.55 ± 0.08–2.05 ± 0.06; 114.83 ± 5.27–130.56 ± 3.90(%); 70.45 ± 1.87–89.90 ± 4.45 (%); and 2.36 ± 0.06–2.38 ± 0.04 respectively.

ANOVA showed that various levels of CM substitution with SM had a significant effect ($p<0.05$) on the average RFI, FCR, and ER, but were not significantly different ($p>0.05$) with the average WG, RG, PR, and CF. Furthermore, this study also obtained a linear relationship between the CM substitution rate by various SM levels and several measured parameters, where a higher CM substitution level by SM gave a higher average RFI and a lower average ER and FCR (feed efficiency). Otherwise, there is no linear relationship between the experimental diet and the average WG, RG, PR, and CF.

The average RFI of rabbitfish consumed diet A (0% CM substitution with SM) was significantly different ($p<0.05$) from all experimental diets, while no significant effect ($p>0.05$) between diet B (21% CM substitution with SM) and diet C (42% CM substitution with SM), and between diet D (63% CM substitution with SM) and diet E (83% CM substitution with SM). The average FCR of diet E was significantly different ($p<0.05$) from other experimental diets. However, there was no significant effect ($p>0.05$) between diet A and B, diet B and C, and diet C and D on FCR. The average ER in diet A had a significant difference ($p<0.05$) from other experimental diets except for diet B, while there was no significant effect between diet B and C, diet C and D, and diet D and E on ER.

The high average RFI in diet E indicated that rabbitfish as an herbivore are likely to consume plant ingredients in the form of seaweed such as *Sargassum* sp. It is following under the feeding habit of rabbitfish in the sea as a seaweed consumer. The higher the SM content or higher the substitution of CM with SM, the aroma and types of nutrients were dominated increasingly by aroma and types of SM nutrients. It caused the attractiveness and palatability of diet E to be higher compared to other treatments. The diet attractiveness and palatability could increase the fish appetite.

The average RFI was high in diet E but had no positive impact on the FCR and

ER. FCR or feed efficiency and ER values in diet E were the lowest among all treatments. It was thought to be caused by the types of crude fiber contained in SM classified as hard to digest, such as cellulose, hemicellulose, and lectin. Furthermore, the higher the percentage of seaweed in the diet would lead to a higher crude fiber content (Mariana *et al.*, 2019). The crude fiber content could reduce the performance of nutrient metabolism and reduce the rate of fish body weight gain (Nandeesha *et al*., 1991).

The best feed efficiency based on FCR and ER were diet A and B, although diet B was the same as diet C. It was due to the lower crude fiber content compared to other experimental diets. Crude fiber can cause inefficient use of feed nutrients by the experimental fish. Based on this research, the rabbitfish diet was most efficient on CM substitution with SM by 21%. CM substituted up to 83% was also still efficient because the optimal FCR standard for cultured fish is 1.0–2.4 (Fry *et al*., 2018). A low FCR value will result in a better feed efficiency level. If the FCR value is high, so the level of feed efficiency will not be better for fish rearing (Fujaya, 2008; Iskandar and Elrifadah, 2015; Ardita *et al.,* 2015).

Septiana and Asnani (2012) suggested that *Sargassum* sp. also contains fucoidan and phenolic components, which help to make nutrient utilization more efficient. Wijayanti *et al*. (2019) reported that a low FCR value indicated that diet nutrients could be well digested. It means that lower substitution of CM with SM can result in higher nutrient digestibility of the diet, while higher CM substitution with SM results in a lower digestibility of feed nutrients. Digestibility is thought to be the main factor that caused the lower FCR in a diet that contained the higher levels of CM substitution with SM.

The FCR obtained in this study was normal for fish reared in the sea waters. Sih Yang Sim *et al.* (2005) reported that the FCR value for dry pellet for marine fish was 1.67 and 1.54 for humpback grouper, *Cromileptes altivelis* (Suwirya *et al*., 2005). The FCR obtained in this study was better than FCR of rabbitfish using *Ceratophyllum* sp. starch as a source of protein, which was 2.6–3.4 (Laining *et al*., 2016), and also FCR in rohu fish, *Lobeo rohita* of 2.31 with the use of 29% *Sargassum* sp. starch (Bindu and Sobha, 2004). However, it is less efficient when compared to rainbow trout, which was fed with 7.5% *Sargassum ilicifolium* and resulted in an FCR of 1.08 (Zamannejad *et al.*, 2016).

The highest ER values were also obtained in diet A and B, although diet B was the same as diet C. The energy accumulated in diets A and B was higher than in other experimental diets. The energy source of the experimental fish was the macro-nutrient diet which it consumed, including protein, lipid, and carbohydrates. The energy of diet A (296.63 kcal/g) and B (289.94 kcal/g) was the highest among other experimental diets. In this study, the energy content of the diets tended to be lower with higher substitution of CM with SM. It means that the diet energy in the kcal/g unit decreased by the increasing substitution of CM with SM and allowing metabolizing macro-nutrient energy sources to produce higher energy.

The availability of insulin supports on transferring of glucose into cells as an energy source. The lower energy retention with the increasing level of CM substitution with SM in this study was thought to be due to the diet energy consumed and absorbed by the experimental fish body being most used to digest the crude fiber contained in SM. The crude fiber uses much energy to utilize because it is hard to digest. Not all energy consumed can be stored or retained in the body, but firstly it is used for activity, metabolism, and body maintenance needs (Handajani and Widodo, 2010).

The energy retention of the fish body had a linear relationship with the energy content of the diet and feed intake. Diets A and B gave the highest energy retention, the highest digestible diet energy, and the lowest feed intake. The high diet energy caused an increase in energy retention and reduced the appetite of fish. The limited amount of diet consumed by the fish can be due to the high diet energy (Haetami, 2012). Furthermore, it explained that the energy needs of fish are expected to be met most by non-protein nutrients such as fats and carbohydrates. Therefore, the use of *Sargassum* sp. starch as the primary source of carbohydrates in the experimental diets was expected to be a source of non-protein energy. If the energy derived from these non-protein nutrients is sufficiently available, most of the protein will be used for growth. However, if the energy and non-protein nutrients are insufficient, the protein will be used as an energy source and reduce its function as a bodybuilder. In general, energy is needed for metabolic processes, physical activity, growth, and reproduction (NRC, 1993).

Based on the statistical experimental test, the SR, WG, or production, RG and CF were the same at all levels of CM substitution with SM. It indicated that CM could be substituted for SM up to 83%. The survival rate of 100% in all treatments showed that the diet nutrient contents and the environmental conditions of the culture met the minimum proper living conditions for rabbitfish. There were several supporting factors used in obtaining the maximum SR in this study, namely (i) the rabbitfish as experimental fish in this study were about six months old and had an average weight of 51.76±0.12 g/fish, which means that the age and size of fish was already in the adult stage which had a high ability to adapt to environmental changes, (ii) the experimental fish used was the result of sorting with healthy fish standards, (iii) the quality of water in the rearing media was with a temperature of 27.50-29.03°C, 27.00-35.00 g/L salinity, 6.70-7.05 ml/L dissolved oxygen, pH of 7.56-8.43 and water current of 0.07-0.27 m/s, which were optimal range for rabbitfish cultivation, (iv) a density of 17 fish/m^3 or 863 g/m^3 was feasible for fish as they are able to live under pressure, (v) there were no predators and pathogenic microbes that entered the net cages during the study, (vi) the quantity and quality of the experimental diets met the optimal needs of the experimental fish, and (vii) avoidance of stress in handling experimental fish during the study includes transportation, stocking, feeding, weighing or growth monitoring.

The survival of fish is affected by internal and external factors (Yusup *et al*., 2015). Internal factors that affect fish survival are age, size, quality of fish, and the ability to adapt to the environment, while external factors are water quality, density, predators, pathogenic microbes, quality and quantity of diet, and handling methods. The survival rate of rabbitfish obtained in this study was higher than saline tilapia fed with 2% *Sargassum cristaefolium* starch (Nugraha *et al.,* 2018) and catfish with 3% *Sargassum* starch (Sahara *et al.,* 2015).

WG or production, RG, and CF are parameters that were closely related to the role of diet nutrition, especially protein. The protein content of the experimental diets was almost the same (28-30% dry basis) in all treatments. Protein is one of the macronutrients that plays a role in the growth, the largest body constituent in meat that is about 65–75% of the total body dry weight and is a building block for body tissues (Halver and Hardy, 2002). Pangkey (2011) added that protein is one of the elements in the diet that directly affects weight gain and the growth of fish tissues and organs and can also generate energy.

The growth-supporting factors in diet B to E that were the same as diet A (control diet) were essential amino acids and "growth promoter" compound that contained in SM (Asha *et al*., 2004; Bindu and Sobha, 2004), which can increase the absorption of feed nutrients. Furthermore, the highest feed intake obtained in diet E could support the growth of the experimental fish, which remained comparable to diet A (non CM substitution with SM). Sunarto and Sabariah (2009) and Barakat *et al.* (2011) reported that the level of feed intake is one of the supporting factors for the high growth of fish and its ability to absorb the given diet nutrients (Subekti *et al.*, 2011). SM contained crude fiber that is hard to digest. However, other nutrients such as macronutrients in the form of protein, lipids, and NFE, as well as micronutrients in the form of vitamins and minerals, were still well utilized, which could give the similar growth (WG, RG, and CF) in all experimental diets from 0 to 83% CM substitution.

Another factor that supports the same average WG, RG, and CF in all treatments is the balance between protein and energy (C/P ratio) of experimental diets. The C/P ratio of all experimental diets was ±10. This balance caused the experimental fish to normally grow without negative effects for 90 days of rearing. A low C/P ratio or higher protein content than energy will make the fish body shorter and fatter due to the high protein utilization. While if the energy content is higher than protein, it will produce a flatter and longer fish because of reduced feed intake and nutrient intake from the diet. Both of these are not profitable from the economic and business aspects. It means that SM can be substituted for CM up to 83% in rabbitfish rearing in floating net cages. The fulfillment of protein and energy sources must be balanced (Tacon, 1987). Excess or lack of energy can cause a decrease in the growth rate. Feeding with low energy content causes the use of some proteins to meet energy needs. It causes the amount of protein for fish growth to decrease, resulting in decreased growth.

CONCLUSION

The WG, RG, RP, and CF parameters had the same values at all levels of CM substitution with SM. The RFI and FCR parameters increased with the increasing CM substitution by SM. It also indicated that CM in the rabbitfish diet could be substituted with SM by 83%.

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