



Application of pumpkin flour in the diet of Barramundi *Lates calcarifer* fingerling

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ABSTRACT

Barramundi *Lates calcarifer* is a high economic value fish in Indonesia and Southeast Asia countries. However, the aquaculture system still experiences problems, including the high cost of production, specifically from feed, due to its relatively slow growth and low digestibility. Therefore, this study aims to determine alternative ingredients used in the fish diet to improve feed utilization and growth performance using pumpkin flour *Cucurbita moschata*. A randomized design (CRD) with six treatments and three replications was adopted in this study. The tested diet was (A) diet without pumpkin flour (control), (B) with 5% (C) 10% (D) 15% (E) 20%, and (F) 25%. The size of the Barramundi samples was 3-4 cm on average, and the body weight was 1-3 g at the stocking density of 15 fish/container. The fish fed as much as 10% body weight daily, which is divided into two times, namely 08.00 AM and 4.00 PM for 60 days. The results showed adding pumpkin flour significantly affected weight gain, daily and specific growth rate, survival, feed efficiency, conversion ratio, and carcass protein ($P < 0.05$). The diet with 5% pumpkin flour gave the best results over other treatments, with an average weight gain of 0.761 g, feed conversion of 1.578, feed efficiency of 63.455%, and a daily and specific growth rate of 0.012 g and 0.738%, respectively. The highest carcass protein content was found in the control treatment with a value of 18.210%, which was not different from the 5% pumpkin flour treatment. Therefore, it is concluded that pumpkin flour can be added to the Barramundi diet would not exceed 5%.

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Introduction

Barramundi (*Lates calcarife*) is a euryhaline and catadromous fish spawned in brackish waters and grows in freshwaters. It distributed across Persian waters, Asia, and Australia (Rayes *et al.*, 2013; Balston, 2009; McGrouther, 2020). In Indonesia, this fish distributes in all Indonesian coastal waters (Vij *et al.*, 2014; Suman *et al.*, 2014; Suman *et al.*, 2016), and it has high economic value both in local and international markets. Developing Barramundi exports is positive trend; for example, the export volume, which was 7,890 in 2016, has increased significantly to 8,431 tons in 2017 and reached 10,208 tons in 2018 (DJPB, 2019). Therefore, Indonesia has developed the cultivation of Barramundi in response to the markets continuous rising demand. However,

Malaysian and Thailand is still considered superior in terms of the technology used.

There are several problems that hinders fish farming development in Indonesia, including Barramundi culture, is the quality of feed related to its digestibility (Aslamiah *et al.*, 2019). It is a fast-growing fish with a growth rate of approximately 1 kg year⁻¹ and reaching 60 kg (Boonyaratpalin, 1997; Yue *et al.*, 2009). However, Barramundi requires high proteins similar to other carnivorous fish, ranging from 35-45% (Hepher, 1988; Muchlisin, 2019), whereas a diet with high Protein is expensive.

Feed cost, one of the most essential critical components in fish farming, accounts for 40-60% of the total production costs (Craig and Helfrich, 2009; Watters *et al.*, 2012, Muchlisin, 2019). Therefore, its quality and quantity raise deep concerns. A good

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quality feed must contain all the nutrients fish need, namely, protein, fat, carbohydrates, vitamins, and minerals (Halver and Hardy, 2002). Generally, the protein requirements in fish feed differ between species, age, water temperature, and salinity (Pirozzi et al., 2010). Deficiency in Protein causes slow growth, but excesses increase ammonia in the waters, which is inefficient and expensive (NRC, 2011). The ability of fish to digest and utilize feed nutrients depends highly on the composition of the raw materials used and developing of the alimentary tract (Sankar et al., 2014).

Besides protein, another critical component in feed, lipids, which function as a source of energy, essential fatty acids, cell formation, and transporting fat-soluble vitamins (Tocher, 2003; Guyton and Hall, 2007). Carbohydrates also act as an energy source, but fish only require a small amount (Muchlisin, 2019). Antioxidants are also needed in fish feed since they protect the cells from free radical that causes damage through the oxidation process (Ehrlich, 2015). One of the essential antioxidants commonly used in fish feed is Beta-carotene (Bjelakovic et al., 2013), a carotenoid compound with high vitamin A activity. It is converted to retinol by the pancreatic enzymes in the digestive tract (Anam and Handajani, 2010). Pumpkin (*Cucurbita moschata*) is a potential raw material containing these components.

Pumpkin flour contains approximately 0.17% carotenoid, 5-7% crude protein, and is rich in mono-unsaturated fatty acids (Younis et al., 2000; Kim et al., 2012), which are suitable for digestive health (Widayati and Damayanti, 2000). It also contains high fiber and several antinutrients such as polyphenols, flavonoids, and tannins (Del-Vechio et al., 2005); therefore, it must be given in amounts. Based on these reports; knowing the optimal amount of addition in the fish diet is crucial. Knowing the optimal amount of addition in the fish diet is crucial.

Adding pumpkin flour to the diet is intended to increase fish color brightness (Riza et al., 2015; Neny and Sari, 2019), but evaluating their growth is scarcely reported. Therefore, this study aims to determine the optimum concentration of flour *C. moschata* in the *L. calcarifer* fingerling diet.

Materials and Methods

Time and site

This study was conducted from June to August 2021 in the Laboratory of Fish Hatchery, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia.

Experimental design

Every treatment was conducted in three replications, while the tested treatments were (A) Diet without pumpkin flour (control), (B) with 5% of pumpkin flour, (C) with 10% of pumpkin flour, (D) 15% (E) 20% of pumpkin flour, and (F) 25% of pumpkin flour.

Pumpkin flour preparation

Pumpkin skin was peeled, and the flesh was separated into a jug. It was cleaned and chopped to a size of ± 2 mm, then sun-dried for 2-4 days. The dried pumpkin was milled and sieved to form flour. Meanwhile, the proximate analysis of pumpkin flour was 6.49% crude protein, 3.52% crude lipid, and 8.02% fiber (Cerniauskiene et al., 2014)

Diets preparation

Pumpkin flour and other primary raw materials used are fish and soybean meal, corn flour, bran, mineral, and vitamin mix. The experimental diet contains at least 36-37% crude protein, and the raw material composition is shown in Table 1. The raw materials in flour were weighed based on respective doses, mixed evenly, and water was gradually added to become a dough. The dough was molded into pellets, sunrise dried for two days, and stored in the freezer. The diet samples were tested for proximate content.

Experimental fish and medium

The experimental fish was purchased from Brackishwater Aquaculture Center in Ujung Batee, Aceh Besar, Indonesia. Its length and weight range from 30-40 mm and 1-4 g, respectively. Afterward, the fish were distributed into 18 Vol. 25 L plastic jars filled with 15L of seawater with 23 ppt salinity. Every jar was stocked with 15 fish and equipped with aerated continuously.

Feeding

The fish was fed on an experimental diet at a daily ration of 10% body weight twice a day, namely 08.00 AM and 4.00 PM, for 60 days. The weight gain was monitored ten days intervals. The water quality, such as salinity, temperature, oxygen, and pH, was monitored daily.

Protein retention analysis

3 fish samples were taken randomly from each container then weighed, fillet, and dried in a furnace at 70°C for two weeks. The dried fillet was weighed, milled, and sifted to become fishmeal, which was then analyzed for protein content using the Kjeldahl standard method (Muchlisin et al., 2015).

Parameters

Growth performance and survival rate

The weight gain, the daily and specific growth rates, were calculated based on Muchlisin et al. (2016):

- Weight gain (g) = Final weight (g) – Initial weight (g)
- Daily growth rate (g day⁻¹) = weight gain (g)/experimental duration time (days)
- Specific growth rate (% day⁻¹) = $\left[\frac{\ln W_t - \ln W_0}{t} \right] \times 100$, where, W_t= fish body weight at the end of the experiment (g), W₀= fish body weight at the beginning of the experiment (g), t = time (days)
- Survival rate (%) = $\frac{N_t}{N_0} \times 100$, where N₀ is total fish at the beginning of the experiment, N_t is total dead fish during the experiment.

Feed conversion ratio and efficiency

Feed conversion ratio and feed efficiency were calculated based on Muchlisin et al. (2016):

- Feed conversion ratio, where W_t = fish body weight at the end of the experiment (g), W₀= fish body weight at the beginning of the experiment (g), and F = total feed given during the experiment (g).
- Feed efficiency (%) = $\frac{1}{FCR} \times 100$

Data analysis

The data were subjected to a one-way ANOVA analysis to evaluate the effect of the added pumpkin flour. Duncan's multiple range tests were used to determine the best pumpkin flour concentration in the diet of *L. calcarifer* fingerling.

Results

The results showed that the fish experienced a decrease in weight in all treatments from day 1 to 10 probably because they were adapting to the experimental feed. Their weight showed an increase from day 10 to 60, with a rapid rate from day 10 to 30. A slight decline was experienced after day 30 to 50, followed by an increasing trend until day 60. However, the tendency for a rapid increase in weight after day 50 generally occurred in the 5% pumpkin flour treatment (Figure 1).

The results also showed that the range of weight gain, daily, and specific growth rate 0.35 g to 0.76 g, 0.006 g to 0.012 g, and 0.34% to 0.73% per day, respectively. The highest value of these parameters was obtained at the treatment of 5% pumpkin flour. These values differed significantly from other treatments, including controls (P<0.05). Survival percentage ranged from 66.6% to 75.5%, with the highest in 20% pumpkin flour but was not different between treatments except for 25% (Table 1). The feed conversion ratio ranged from 1.57 to 3.72, with the best value in the 5% pumpkin flour treatment, and from others except for D (15% pumpkin flour). In addition, feed efficiency

ranged from 29.64% to 63.45%, with the best value in B (5% pumpkin flour), and from other treatments except for the control. The highest protein retention in carcass was obtained in the control treatment, but it was not different from the 5% pumpkin flour treatment (Table 1).

The ANOVA test showed that applying pumpkin flour in the diet has a significant effect on weight gain, daily and specific growth rates, survival, feed conversion, feed efficiency, and protein retention in the carcass (P<0.05). Duncan's multiple range test showed the highest weight and total length gain, daily and specific growth rates, feed conversion, and feed efficiency were found in the 5% pumpkin flour treatment. However, the weight gain and daily growth rate at 5% pumpkin flour differed significantly from other treatments (P<0.05). The specific growth rate was not different from the control (without pumpkin flour) but different from other treatments. Duncan's multiple range test also showed that the lowest feed conversion ratio, which is the best FCR, was obtained at 5% pumpkin flour treatment and was significantly different from D (15% pumpkin flour), but not from other treatments. In addition, the best feed efficiency value was obtained at 5% pumpkin flour which differed significantly from other treatments, except for the control (without pumpkin flour). The best survival was found at 20% pumpkin flour, but the value was not different from other treatments (P>0.05). Protein retention in the carcass was higher at the control treatment and different from others, except for the 5% pumpkin flour (Table 2).

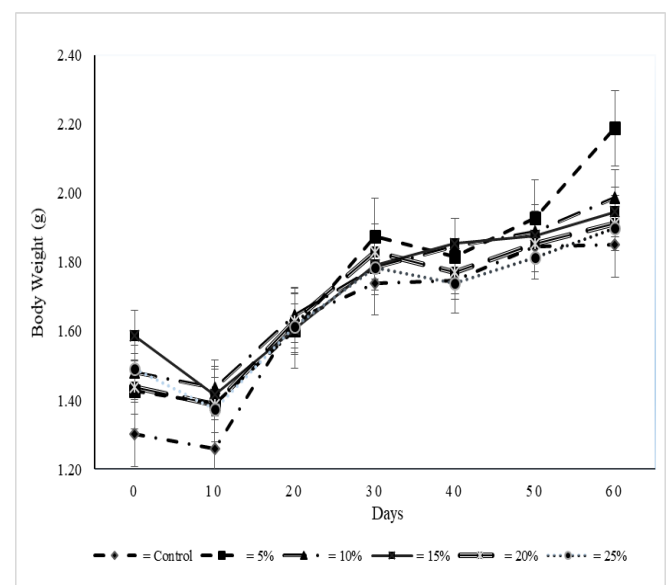


Figure 1. The growth trend of Barramundi *Lates calcarifer* fed on an experimental diet for 60 days

Table 1. The raw materials and composition of the diet.

Raw materials	Crude Protein (%)	Composition (g kg ⁻¹)					
		A	B	C	D	E	F
Pumpkin flour	6.49	0	50	100	150	200	250
Fishmeal	58.97	495	500	500	510	500	500
Soybean meal	35	160	150	130	130	140	140
Starch	4.38	55	46	46	38	38	20
Corn flour	8.78	130	120	70	65	40	25
Bran	11.9	152	127	147	100	75	58
Minerals mix	-	2	2	2	2	2	2
Vitamins mix	-	3	2	2	2	2	2
Squid oil	-	3	3	3	3	3	3
Total (g)		1000	1000	1000	1000	1000	1000
Crude Protein (%)		37.9	37.8	37.2	37.5	37.0	37.0

Table 2. The growth performance, survival, feed utilization, and protein retention of Barramundi were fed on an experimental diet with varying levels of pumpkin flour for 60 days of the experiment. The values ±SD at the same column with different superscripts are different (P<0.05).

Treatment (Proportion of PF)	Weight gain (g)	Daily growth rate (g day ⁻¹)	Specific growth rate (% day ⁻¹)	Survival rate (%)	Feed Conversion Ratio	Feed Efficiency (%)	Protein retention in the carcass (%)
Kontrol (0% PF)	0.513±0.098 ^a	0.008±0.001 ^a	0.559±0.097 ^a	73.33±0.000 ^{ab}	2.253±0.405 ^{ab}	45.470±9,094 ^{ab}	18.210±0.183 ^d
5% PF	0.761±0.046 ^b	0.012±0.0005 ^b	0.738±0.055 ^b	73.33±0.000 ^{ab}	1.578±0.084 ^a	63.455±3,307 ^b	18.135±0.063 ^d
10% PF	0.507±0.176 ^a	0.008±0.002 ^a	0.495±0.183 ^a	73.33±0.000 ^{ab}	2.651±1.170 ^{ab}	42.102±14,916 ^a	16.685±0.360 ^c
15% PF	0.359±0.153 ^a	0.006±0.002 ^a	0.341±0.150 ^a	71.11±3.848 ^{ab}	3.727±1.261 ^b	29.644±12,470 ^a	15.775±0.289 ^b
20% PF	0.475±0.110 ^a	0.008±0.001 ^a	0.480±0.131 ^a	75.55±3.849 ^b	2.598±0.723 ^{ab}	40.278±9,677 ^a	14.655±0.289 ^a
25% PF	0.406±0.122 ^a	0.006±0.002 ^a	0.404±0.131 ^a	66.66±6.666 ^a	3.120±1.172 ^{ab}	34.753±10,784 ^a	14.440±0.593 ^a

The main water quality parameters during the experiment showed that salinity, water temperature, and pH ranged from 23.6 ppt to 25.0 ppt, 27.9°C to 30.3°C, and 7.8 to 8.12, respectively. These values were within limited tolerance for Barramundi.

Discussion

The highest growth performance of Barramundi was obtained in the diet containing 5% pumpkin flour and was different from other treatments, including control (diet without pumpkin flour). However, fish growth decreased when the concentration of pumpkin flour was increased, such that the fingerlings could tolerate only 5% of pumpkin flour in their diet. The high carbohydrates and fiber could, up to 77.65% and 3.84%, respectively, contained in the pumpkin flour, which is difficult to digest by the fingerlings. (Gardjito, 2005; Widowati et al., 2010; Budiman et al., 1984; Usmiati et al., 2005). The flour also contains antinutrients such as polyphenols, flavonoids, and tannins (Del-Vechio et al., 2005). These antinutrients interfere with the metabolism and inhibit the digestion and absorption of nutrients (Jurgens, 1997; Francis et al., 2001).

Fiber influences protein digestibility, such that a higher fiber in the diet results in a lower amount of Protein digested by fish (Cho and Kaushik, 1985; Handajani, 2011). Carnivorous fish can not utilize carbohydrates (Webster and Lim, 2002). According to Ria and Arleston (2021), their optimal carbohydrate and fiber content, including Barramundi, is below 20%. Although pumpkin flour has high carbohydrates and fiber contents, namely 77.65% and 3.84%, respectively, it also contains several enzymes, such as amylase, protease, lipase, and oxidase, which assist the digestion of proteins and lipids (Sufi, 1999). Therefore, the Barramundi in this study tolerated 5% pumpkin flour in their diet, which even resulted in better growth and feed utilization compared to the control (without pumpkin flour).

A diet with adding 5% pumpkin flour, which has an FCR of 1.57, is considered more economical than the control and also saves diet costs by approximately 30% with an FCR of 2.25. It was assumed that the diet price was IDR 17,000/kg, then the control treatment requires a production cost of IDR 38,250 per kg of fish, while adding 5% pumpkin flour only costs IDR 26,690 per kg. Therefore, there is a

difference in IDR 11,560 per kg or the equivalent to 30%.

The utilization of pumpkin in the diet for growth is still rarely reported; it is generally used to increase color brightness in ornamental fish (Riza et al., 2015; Sausan et al., 2017; Neny and Sari, 2019, Sartikawati et al., 2020). It is because pumpkin flour contains high beta-carotene, which plays an important role in fish coloring. However, Teimouri (2013) stated that using carotenoids to increase brightens color can also increase growth in rainbow fish (*Oncorhynchus mykiss*). Sudariono (2013) stated that the carotenoids in the fish diet not only functions as color formation but also plays a role in their reproduction and immune system (Sudariono, 2013). This was shown in this study, adding 5% pumpkin flour significantly increased the growth of *L. calcarifer*. However, Sausan et al. (2017) reported that adding pumpkin flour had no significant effect on the growth of *Carassius auratus* fish.

Conclusion

The application of pumpkin flour in the diet had a significant effect on growth performance, survival, feed efficiency, conversion ratio, and Protein in the carcass of Barramundi (*Lates calcarifer*). The application of 5% pumpkin flour in the diet gave better results than the control treatment, but an increase in proportion of the pumpkin flour followed a decrease in growth performance of fish. Therefore, it was concluded that the addition of pumpkin flour in the Barramundi fish diet would not exceed 5%.

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