Original article DOI: 10.19027/jai.24.2.158-171

The dynamics glucose on carbohydrate utilization of striped catfish *Pangasianodon hypophthalmus* with yacon leaf *Smallanthus sonchifolius* dietary supplementation

Dinamika glukosa terhadap pemanfaatan karbohidrat ikan patin *Pangasianodon hypophthalmus* dengan suplementasi daun insulin *Smallanthus sonchifolius*

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(Received May 22, 2024; Revised June 7, 2024; Accepted February 17, 2025)

ABSTRACT

The objective of the study was to test dietary supplementation of yacon leaf on the carbohydrate utilization, growth performance, and glucose tolerance of striped catfish during the early-stage period. Fish were fed with five different levels of yacon leaf supplementation (0%, 0.5%, 1%, 1.5%, 2%) for nine weeks. Three hundred striped catfish (initial mean body weight: 14 ± 0.14 g) were maintained in 15 rectangular net cages. The results showed that the specific growth rate of fish with yacon leaf supplementation (3.46-3.79%) was higher than the control fish (3.19%) also the feed efficiency value. The highest protein retention was found on 1% yacon leaf fish diet, while the highest lipid retention was found on 1.5%. The blood glucose post-prandial was rise slower than the control fish. The glucose tolerance test also obtained less time to return to basal level after the glucose load. The increased of enzymatic were also present at the supplemented fish. The liver lipid and glycogen concentration was decreased, and the hepatic somatic index was increased. The blood biochemistry showed the lower level of total protein plasma and albumin in supplemented fish, and higher triglyceride and cholesterol levels due to the replenishment of energy storage in adipose tissues while fasting. Therefore, this study concludes that the yacon leaf dietary supplementation has the potential to improve carbohydrate utilization by promoting glucose use efficiently as the main energy, while protein can be utilized for growth. Our data suggest that the best level of yacon leaf supplementation in fish diets is 1.36%.

Keywords: yacon-leaf, homeostatic, glucose, sparing-effect

ABSTRAK

Tujuan dari penelitian ini adalah untuk mengkaji efektifitas suplementasi daun insulin pada pakan terhadap pemanfaatan karbohidrat, kinerja pertumbuhan, dan toleransi glukosa pada benih ikan patin. Ikan diberi pakan dengan lima tingkat suplementasi daun insulin yang berbeda yaitu 0%, 0,5%, 1%, 1,5%, 2% selama sembilan minggu. Tiga ratus benih ikan patin (rata-rata biomasa awal: 14 ± 0,14 g) dipelihara dalam 15 keramba jaring berbentuk persegi panjang. Hasil penelitian menunjukkan bahwa laju pertumbuhan spesifik ikan yang diberi suplementasi daun insulin (3,46-3,79%) lebih tinggi dibandingkan ikan kontrol (3,19%) linier dengan nilai efisiensi pakan. Retensi protein tertinggi terdapat pada ikan dengan pakan daun insulin 1%, sedangkan retensi lemak tertinggi terdapat pada pakan 1,5%. Kenaikan glukosa darah post-prandial lebih lambat dibandingkan pada ikan kontrol. Kadar toleransi glukosa juga memperoleh waktu yang lebih singkat untuk kembali ke level basal setelah injeksi glukosa. Peningkatan aktivitas enzimatik juga terjadi pada ikan yang diberi suplementasi daun insulin. Konsentrasi lipid dan glikogen hati menurun, dan indeks somatik hati meningkat. Biokimia darah menunjukkan rendahnya kadar total protein plasma dan albumin pada ikan yang diberi suplementasi, serta kadar trigliserida dan kolesterol yang lebih tinggi selama proses pemulihan menggunakan cadangan energi dari jaringan adiposa saat dipuasakan. Oleh karena itu, penelitian ini menyimpulkan bahwa suplementasi daun insulin berpotensi untuk meningkatkan pemanfaatan karbohidrat dengan menstimulasi penggunaan glukosa secara efisien sebagai energi utama, sementara protein dapat dimanfaatkan untuk pertumbuhan. Data kami menunjukkan bahwa tingkat suplementasi daun insulin terbaik dalam pakan ikan adalah 1,36%.

Kata kunci: daun insulin, efek hemat, homeostatis, glukosa

INTRODUCTION

Aquatic organisms, particularly fish, appear to have a limited capacity in utilizing the dietary carbohydrates for energy purposes (Rasal *et al.,* 2020; Li *et al.,* 2019). The precise reason for glucose intolerant in fish is still not fully understood, although several factors can possibly become the main reasons, such as the inability to regulate gluconeogenesis pathway (Polakof & Panserat, 2016; Dai *et al.,* 2024), insulin production shortage (Peres *et al.,* 1999), fewer insulin receptors (Hamann *et al.,* 2015), and absent insulin sensitive glucose transported 4 (Glut4) in peripheral tissues (Zhao *et al.,* 2020). Some studies have been previously conducted to increase the carbohydrate utilization in fish, such as using the digestive enzymes. Yigit & Keser *et al*. (2016) reported that exogenous enzyme could increase the carbohydrate digestibility in Rainbow trout (*Oncorhynchus mykiss*) but had no effect on growth performance and feed efficiency. This condition indicates that glucose absorbed in large quantities remains unutilized due to glucose absorption limit by cells (Li *et al.,* 2019).

The glucose absorption from the blood into cells in the body is regulated by two hormones namely, insulin and glucagon (Halver & Kaushik, 2021). Insulin is a growth hormone that plays a pivotal role in regulating the carbohydrate metabolism and other essential nutrients. Insulin activates the glucose transport system (GLUT) in the bloodstream, which then can utilize glucose when entering the cells (Zhao *et al.,* 2020; Reed *et al.,* 2024). Insulin affects the carbohydrate metabolism of glucose stored in the liver and muscles as glycogen and the glucose conversion to lipid in adipose tissue (Jin *et al.,* 2018). Insulin levels that are too low can cause carbohydrate, protein and lipid metabolism disturbances (Polakof & Panserat, 2016; Zhang *et al.,* 2022). Meanwhile, strategies to overcome the limitations of insulin production in fish have not been widely discovered.

Dietary supplementation is intended as stimulator agents that not only improving the nutrient digestibility, but also increasing the growth performance. One of the natural ingredients that can be used as a dietary supplementation material is yacon leaves (*Smallanthus sonchifolius*). Studies related to the use of yacon leaves had been performed on poultry, which can be used as growth promoters and natural antibiotics (Park & Kim, 2013; Saeed *et al.,* 2017). The supplementation of yacon leaf by-products in diets at 0.5% and 1% doses was effective to increase the feed conversion index and bodyweight of Muscovy ducks during the fattening phase (Fuentes *et al.,* 2012).

The ability of yacon leaves to increase growth is inseparable from the high content of fructooligosaccharides (FOS) at 70-80%, chlorogenic acid, flavonoids, and phenolics, which not only as prebiotics and antioxidant agents (Saeed *et al.,* 2017), but also as an insulin-like biological activity. Increased nutrient metabolism efficiency also occurs after yacon leaf supplementation by stimulating the plasma insulin secretion in pancreatic β-cells (Ong *et al.,* 2013). The FOS in yacon leaves can improve the abundance of microflora in the intestines to produce exogenous enzymes, such as amylase, protease, and lipase (Caetano *et al.,* 2016). Another compound in yacon leaves that plays an active role in nutrient absorption assistance, especially carbohydrates, is quercetin and chlorogenic acid (CGA), which elevates the sensitivity and quantity of insulin receptors on pancreatic β-cells (Ueda *et al.,* 2019).

According to Ong *et al*. (2013), CGA can increase the glucose uptake into muscle by activating the AMPK phosphorylation pathway, thereby stimulating the release of glucose transporter GLUT4 and reducing the activity of glucose-6-phosphatase and acetyl-CoA carboxylase enzymes. Compounds in yacon leaves work synergistically to inhibit gluconeogenesis, glycogenolysis, and fatty acid synthesis process and more optimally utilize carbohydrates as the main energy source (protein-sparing effect) (Oh *et al.,* 2024). Striped catfish (*Pangsianodon hypophthalmus*), one of the most farmed freshwater fish worldwide, has been widely cultured in Indonesia with a total national production in 2022 about 391.656 tons (FAO, 2022). In addition, catfish species can efficiently utilize high levels of dietary carbohydrates in their diets up to 30-50% (Polakof & Panserat, 2016), as the inclusion of protein content in their diets are still relatively high.

The early-stage of catfish culture species requires high protein levels at 28-32% to support growth (BSN, 2018). The use of carbohydrate in feed as the least-cost energy source can improve protein retention, which then spares protein substance to cover the somatic effect (Xiong *et al.,* 2015; Cheng *et al.,* 2017). However, the use of dietary carbohydrates beyond the optimal level is related with the unpleasant effect on growth, metabolism, reproduction and also immune system (Zhou *et al.,* 2016; Asemani *et al.,* 2019). The lack of carbohydrate utilization ability in catfish at an early-stage period becomes a good candidate to study the nutrition regulatory of carbohydrates as protein-sparing effect. Studies regarding the yacon supplementation in freshwater fish have never been conducted. Therefore, this study was performed as a preliminary test to evaluate the effect of yacon leaf dietary supplementation for improving the nutrient digestibility and optimal utilization of carbohydrates as the main energy source of striped catfish.

MATERIALS AND METHODS

The experimental procedures was conducted in accordance to the norms of well-being and ethics in animal experimentation approved by the Animals Ethics Committee of Bogor Agricultural University with certificate No. 237–2022 IPB as an appropriate ethical approval based on legitimate institutional guidelines.

Experimental design and diets

The study used a completely randomized design with five yacon leaf dietary supplementation level at 0%, 0.5%, 1.0%, 1.5%, and 2% with three replications. The doses applied were referred to Fuentes *et al*. (2012); Park & Kim (2013); and Kim (2014). The yacon leaves in powder form were obtained as a commercial product from Wonosobo, Central Java, Indonesia with a composition described in Table 2. The bioactive content analysis of yacon leaf extract used a Gas Chromatography-Mass Spectrometry (GC-MS) instruments.

The experimental diets used commercial catfish feed based on the Indonesian National Standard (SNI) with 30-31% protein content (BSN, 2018). Before feeding, these diets were coated with the yacon leaf powder according to the treatment dose applied per kg of diet. The coating process was carried out by adding fish oil at 10 g/kg diet, 40 g of white egg, then the used of 2 g of egg yolk as a binder, and 0.6% polymethylolcarbamide (PMC) are as a coating agent (Tarmizi, 2016) to improving pellet water stability and avoiding component be leaching out in 100 mL/kg water. After these materials were mixed together and homogeneous, this mixture was then sprayed evenly on the commercial diet and dried in an oven at 40°C. The experimental diet proximate compositions are presented in Table 1.

Experimental fish

Three hundred striped catfish (about 2 inch/fish) purchased from smallholder farmer in Bogor, West Java, Indonesia were reared in an outdoor pond at the Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. Before the feeding trial, fish were first reared to gain a similar size at 14 ± 0.14 g weight and 11 ± 0.81 cm length. The fish were cultured using 2×1×1 m^3 rectangular net cage in a 200 m^2 high density polyethylene (HDPE) concrete pond with 1-1.5 m depth. The reared fish were stocked at 20 fish per cage.

Table 1. The proximate composition analysis of the experimental diets (in wet-weight basis) fed to striped catfish (*P. hypophthalmus*)

Ingredients	Experimental diets (% yacon leaf)[1]				
	0%	0.5%	1%	1.5%	2%
Moisture (%)	9.71	9.66	9.58	9.20	9.17
Protein (%)	31.50	30.78	30.61	30.25	29.55
Lipid (%)	6.15	6.57	6.94	6.46	6.19
Crude fiber (%)	2.65	2.91	2.78	2.99	2.97
Crude carbohydrate[2]	40.93	40.53	40.57	41.37	42.34
Ash (%)	9.07	9.54	9.52	9.73	9.78
Yacon leaf (%)[1]	0.00	0.50	1.00	1.50	2.00
GE[3] (kcal GE/kg)	4020	4003	4030	3997	3973

Note: [1]Doses are referred to Fuentes *et al*. (2012); Park & Kim (2013); and Kim (2014). [2]Based on nitrogen-free extract level. [3]GE = Gross energy (GE protein = 5.41 kcal GE/g; GE lipid = 9.40 kcal GE/g; GE carbohydrate = 4.11 kcal GE/g (Watanabe, 1998).

After gaining a similar size, fish were fed with the experimental diets until apparent satiation manually, three times a day at 08.00, 13.00, and 17.00 of western Indonesian time (WIB) and the feed intake was recorded daily. The water quality maintenance was performed with a flow-through system at an average water flow of 0.8-1 L/s, which enters through the inlet to the outlet of the experimental pond. The water quality were monitored twice weekly during the rearing period at the optimum range for striped catfish, namely temperature of 25-33°C, dissolved oxygen of 5-6 ppm, pH of 7-8.5, and total ammonia nitrogen (TAN) of <0.1 mg/L.

Fish sampling

Sampling activities were carried out at the prior of feeding trial and to provide an estimate initial whole-body nutrient composition, three fish were taken and pooled. At the end of the feeding trial, six fish per cage were randomly selected and anaesthetized by *Ocean Free® Special Arowana Stabilizer* at 0.4 mL/L approximately 24 hours after the last feeding. Three fish were taken and pooled for the whole-body composition proximate analysis. While the other three fish in fasting state about 18-24 hours were first measured for the individual body weight, and their blood were collected from the caudal vein at 2 mL per fish with sterile ethylene diamine tetra-acetic acid (EDTA) syringes.

Blood samples from different fish were preserved separately in another microtubes, and centrifuged immediately (6,000 rpm, 5 minutes) using an *Eppendorf centifuge 5810 R* (*Hamburg, Germany*). After centrifugation process, the separated blood plasma were stored at -20°C for further biochemical analysis (albumin, total protein, triglycerides, and cholesterol). After the blood collection, the three fish were dissected to obtain the liver weight and whole intestine. All livers of these fish were collected for the hepatosomatic index (HSI) calculation, lipid percentage, and glycogen content.

The intestine was collected from the anterior to posterior segment, immediately frozen using a liquid nitrogen, and stored at -80°C for the digestive enzyme activity analysis. After sampling, the remaining fish approximately 42 fish per treatment were redistributed into separate nets and fed for one more week with the experimental diets. The first test was subjected to post-prandial glucose plasma according to Wedemeyer & Yasutake (1977). Fifteen striped catfish per treatment were collected from all fish at the 0, 1, 3, 5 and 8 hours of post-feeding and anaesthetized for taken the blood samples 1.5 mL per fish.

Another fifteen striped catfish were subjected to an acute glucose tolerance test (GTT). After 24 hours of fasting, the fish were injected intramuscularly with 1 g D-glucose per kg body weight. The sterile saline solution of 100 mg glucose per 100 ml was also injected for this test. Blood samples were then collected for post-prandial glucose and GTT. As described above,

Table 2. The bioactive compounds of yacon leaves.

No.	RT[1] (min)	Quality	Compound	Content (%)
1.	30.78	91	1,2-Benzenedicarboxylic Acid, Mono (2-Ethylhexyl) Ester	17.15
2.	30.03	43	Benzoic Acid, 4-(2,5-Dimethyl-phenoxy-carbonylamino)- Ethyl Ester	13.29
3.	31.74	95	Squalene (C30H50)	12.74
4.	30.46	38	3,5-Dimethoxytoluene	9.39
5.	29.33	99	9.12-Octadecadienoic Acid	8.84
6.	31.42	41	Farnesol Isomer A	7.45
7.	28.23	99	Hexadecanoic Acid	5.55
8.	32.73	53	Cis-Decalin, Syn-2-Methoxy	3.65
9.	25.98	93	(2e)-3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	2.69
10.	9.07	98	(2e)-3-Phenyl-2-Propenal	1.49
11.	34.00	93	Vitamin E	1.11
12.	26.55	86	Neophytadiene	1.02

Note: [1]RT = real-time.

the blood plasma samples were prepared and stored at -20°C for further blood glucose content analysis.

Digestive enzyme activity

The digestive enzyme activity test was carried out by isolating the digestive tract of striped catfish at the end of the rearing period. Before the isolation, fish were anaesthetized and dissected to obtain their intestines. This procedure was conducted on an ice plate to avoid tissue damage as the sample was a complete part of intestinal segment. The crude extract was created using a homogenizer with 50 mM Tris-HCl buffer (pH 7.2-8.0) at 1:4 ratio (w/v). The supernatant obtained was used to determine the enzyme activity. The amylase and protease enzyme activities were measured following Bergmeyer & Grassal (1983) method.

Plasma biochemical analysis

Triglycerides, cholesterol, albumin and total protein in the blood plasma were determined by spectrophotometric assay using commercial kits. Triglycerides were determined based on the enzymatic colorimetric test for triglycerides (*triglycerides liquiform[insert], Labtest*) method. Cholesterol content was analyzed using the enzymatic colorimetric test for cholesterol with lipid clearing factor (*cholesterol liquicolour[mono], Human Merck*) method. Albumin concentration was determined by reagent kit for albumin concentration and protein plasma by reagent kit for total protein concentration, *Biomaxima* (AOAC, 2023). Albumin, total protein, triglycerides, and cholesterol were expressed in mg/dL.

Post-prandial blood glucose and glucose tolerance test (GTT)

Blood collection interval time was calculated based on glucose peak of plasma found in several fish after the glucose load (Peres *et al.,* 1999). The plasma glucose content was analyzed spectrophotometrically using an ortho-toluidine/acetic acid (6:94, *v/v*) reagent, following the Wedemeyer & Yasutake (1977) description.

Liver composition

The hepatosomatic index (HSI) was calculated from the liver weight against the fish body weight (Htun-han, 1978). The hepatic glycogen content was determined spectrophotometrically using the KOH/H_2SO_4 method (Watanabe, 1988), while the plasma glucose in glycogen fraction analysis used an ortho-toluidine reagent following the Wedemeyer & Yasutake (1977) method. The liver lipid content was analyzed using chloroform/methanol extraction based on the Folch method (AOAC, 2023).

Proximate composition

The dietary and whole body composition were measured with a proximate analysis based on the Association of Official Analytical Chemist (AOAC, 2023). The moisture content was determined after reaching the constant weight by drying the samples at 110°C. The acid combustion procedure with Kjedahl method was performed to determine the protein content (*Gerhardt, Germany*). Meanwhile, lipid content in the body and liver was analyzed using the chloroform methanol (2:1, *v:v*) extraction process with Folch method, and lipid content in diets was measured using the soxhlet method. Ash content was analyzed using a muffle furnace at 600°C for four hours.

Statistical analysis

In the present study, the entire data are presented as mean ± SD of the three replications. Data were initially tested for the distribution normality (one-sample Kolmogorov-Smirnov test) and homogeneity of variances (Levene´s test) among all treatments. Data were then analyzed using analysis of variance (ANOVA) with yacon leaf supplementation level as a factor. A further test was carried out using the Duncan's multiple range test (DMRT) at a 95% significance level with a *p*-value of .05 and was used to determine significant differences among treatments. The orthogonal Polynomial test was subjected to the data between the supplementation diet and specific growth rate, while the plasma glucose data were subjected to a two-way ANOVA and Repeated-Measured test. All statistical analyses were performed using SPSS 24.0 software for Windows (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2010.

RESULTS AND DISCUSSION

Results

There was an increase of amylase activity in fish fed with yacon leaf supplementation diet compared with the control treatment diet (0%) significantly (P<0.05) (Figure 1). The protease activity was significantly higher than the other treatments (P<0.05) at 1% and 1.5% of yacon leaf dietary supplementation treatment.

After the last feeding, blood plasma glucose levels exhibited a different pattern in all treatment supplementation level (Figure 2). Fish fed with yacon leaf supplemented diets were sluggish in the increase of glucose level, which then reached the peak at three hours after feeding, compared to the control diet (0%) that reached the peak at one hour after feeding (P<0.05). However, all treatment diets are returned to the basal glucose levels at eight hours after feeding. In addition, a significant interaction of plasma glucose level between the experimental diets and repeated time of observation was found on fish fed with 1%, 1.5%, and 2% yacon leaf supplemented diets at one hour and five hours after feeding (P<0.05) (Figure 2).

The blood glucose level of striped catfish after D-glucose injection in all treatments increased at one hour after feeding (Figure 3). Furthermore, a different pattern of decrease was then found in the glucose level for all treatments. Fish fed with yacon leaf supplemented diets exhibited a faster restored blood glucose into basal level at five hours after feeding among all treatments, compared to the fish fed with 0% yacon leaf supplemented diet at eight hours of post-injection (P<0.05). Furthermore, a significant interaction of plasma glucose between experimental diets and repeated time of observance on fish fed with 1.5% and 2% yacon leaf supplemented diets at 0 hours and three hours of post-injection, whereas interactive effects were found in all yacon leaf

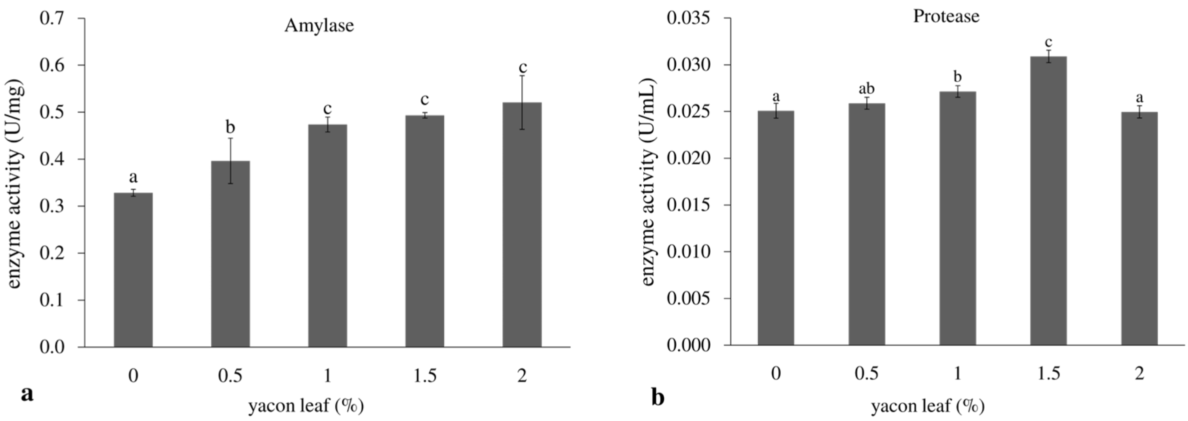
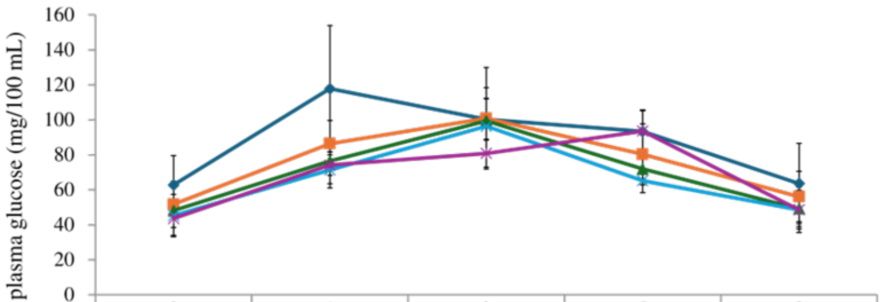


Figure 1. Striped catfish digestive enzyme activities, namely (a) amylase and (b) protease, after feeding with yacon leaf supplemented diets. Different letters denote a significant difference (p<.05) by the DMRT between the treatments within the enzymatic activity. Values are expressed as means ± SD (n=3).



	0	1	3	5	8
0%	62.78^{a}	117.83^{b}	100.28^{ab}	93.33^{ab}	63.61^{a}
0.5%	51.67^{a}	86.39^{ab}	100.83^{b}	80.28^{ab}	56.11^{a}
1%	48.06^{a}	76.39^{*bc}	99.44^{c}	71.94^{*ab}	49.17^{a}
1.5%	45.28^{a}	71.39^{*b}	96.39^{c}	65.28^{*ab}	48.61^{a}
2%	43.61^{a}	74.17^{*b}	80.83^{bc}	93.61^{*c}	48.61^{a}

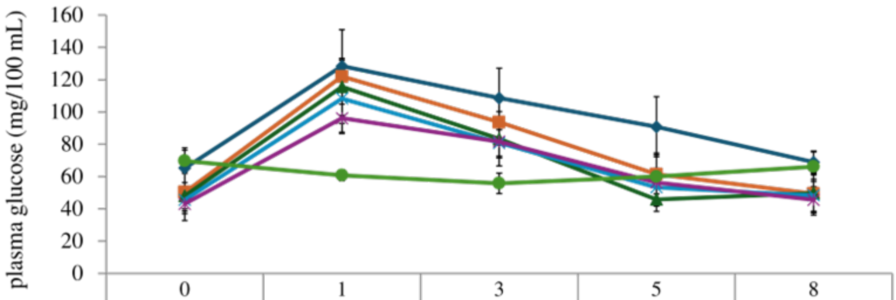
time after feeding (hour)

Figure 2. The post-prandial blood glucose concentration of striped catfish fed with yacon leaf supplemented diets before (0 hours) and after (1, 3, 5, 8 hours) feeding. Diets 0%, 0.5%, 1%, 1.5%, 2% of yacon leaf doses were presented by the different colors and legend symbols for each dose. Values are represented in Means ± SD (n=3). [1]The different uppercase small letters in the same row represent statistical differences (p<.05) between specific times. [2]The asterisk (*) uppercase in the same column represent statistical differences (p<0.5) between the supplemented diets and control diet (0%).

supplemented diet treatments at five hours and eight hours of post-injection (P<0.05).

Fish fed with yacon leaf supplemented diets exhibited a significant increase of triglycerides and cholesterol levels due to fasting state compared to fish fed with 0% yacon leaf supplemented diet (P<0.05). However, no significant effect was observed on the albumin and total protein among all treatments (Table 3). The liver lipid content in fish with dietary yacon leaf supplementation was significantly lower than without dietary yacon leaf supplementation (0%) (Table 4). Meanwhile, the hepatosomatic index (HSI) of striped catfish increased on all dietary yacon leaf supplementation treatments (P<0.05).

The glycogen content in liver decreased significantly at 0.5%, 1% and 1.5% yacon leaf supplementation treatment, however an increased



	0	1	3	5	8
0%	65.28 a	128.33 c	108.61 b	90.83 ab	68.89 a
0.5%	50.28 a	121.94 b	93.61 b	61.39*a	49.44*a
1%	48.06 a	115.56b	83.33 c	45.83*a	49.72 *a
1.5%	45.56 *a	108.33b	80.83*	53.33*a	48.33*a
2%	43.06*a	96.11 b	81.67*b	56.39*a	45.56*a
PBS	69.72 a	60.83 a	55.83 a	60.00a	66.11 a

time after injection (hour)

Figure 3. The blood glucose concentrated in striped catfish fed with yacon leaf supplemented diets before (0 h) and after (1, 3, 5, 8 hours) injection with D-glucose. Diets 0%, 0.5%, 1%, 1.5%, 2% of yacon leaf doses were presented by the different colors and legend symbols for each dose. Values are represented in Means ± SD (n=3). [1]The different uppercase small letters in the same row represent statistical differences (p<.05) between specific times. [2]The asterisk (*) uppercase in the same column represent statistical differences (p<.05) between the supplemented diets and control diet (0%).

Table 3. Biochemical analysis of striped catfish fed with yacon leaf supplemented diets .

Plasma biochemistry (mg/dL)	Experimental diets (g/kg diet)				
	0%	0.5%	1%	1.5%	2%
Albumin	0.99 ± 0.06[a]	1.07 ± 0.06[a]	1.00 ± 0.07[a]	0.96 ± 0.05[a]	0.98 ± 0.07[a]
Triglycerides	185 ± 13.49[a]	229 ± 9.67[b]	235 ± 7.08[b]	222 ± 8.08[b]	239 ± 8.04[b]
Cholesterol	106 ± 2.08[a]	133 ± 2.78[b]	125 ± 6.23[b]	128 ± 12.99[b]	124 ± 7.65[b]
Total Protein	2.37 ± 0.05[a]	2.31 ± 0.13[a]	2.42 ± 0.05[a]	2.45 ± 0.05[a]	2.43 ± 0.10[a]

Note: Data are presented as mean ± standard deviation (n=3); The same uppercase letter on the same line shows a non-significantly different value at 5% confidence level (DMRT).

Table 4. The nutrient utilization in striped catfish fed with yacon leaf supplemented diets.

Parameter	Experimental diets (g/kg diet)				
	0%	0.5%	1%	1.5%	2%
Liver lipid (%)	5.61 ± 0.22[a]	4.77 ± 0.20[b]	4.86 ± 0.56[b]	4.20 ± 0.19[b]	4.28 ± 0.52[b]
Hepatosomatic Index (%)	1.52 ± 0.11[a]	2.06 ± 0.13[b]	1.96 ± 0.13[b]	2.26 ± 0.22[b]	2.04 ± 0.16[b]
Liver glycogen (mg/100 mL)	0.83 ± 0.02[b]	0.78 ± 0.04[ab]	0.78 ± 0.01[a]	0.82 ± 0.04[ab]	0.90 ± 0.02[c]

Note: Data are presented as mean ± standard deviation (n=3); The same uppercase letter on the same line shows a non-significantly different value at 5% confidence level (DMRT).

glycogen content was obtained significantly from the 2% dietary yacon supplementation compared to 0% dietary yacon supplementation (P<0.05). Survival rate was observed had no significant difference (P<0.05) in among all treatments (Table 5). Fish fed with 1.5% yacon supplementation exhibited significantly higher in final weight, specific growth rate and fat retention than other doses and control treatment. Meanwhile, feed efficiency and protein retention were higher in fish fed with 1% yacon leaf supplementation than other treatments (P<0.05).

Determining the best treatment dose of yacon leaf supplementation on striped catfish diet to produce an optimal growth response through orthogonal polynomial tests were presented in Figure 4. The parameters are related to determining the optimal dose based on the specific growth rate value. The correlation coefficient value of 79.42% indicates that the yacon leaf supplementation dose and the increase in the specific growth rate of striped catfish are linear. The relationship between variables produces a regression equation $R^2 = 0.6308$ which shows the optimum point of yacon leaf supplementation dose at 1.36% with optimal growth of 3.65%.

Discussions

In this experiment, fish fed with yacon leaf supplemented diets could significantly increase the amylase activity, while protease activity increased on fish fed with 1% and 1.5% yacon

Table 5. Growth performance of striped catfish fed with yacon leaf supplemented diets.

Parameter	Experimental diets (g/kg diet)				
	0%	0.5%	1%	1.5%	2%
Survival rate (%)	98.33 ± 2.89[a]	96.67 ± 2.89[a]	98.33 ± 2.89[a]	100 ± 0[a]	100 ± 0[a]
Final weight(g)	2360 ± 187[a]	2743 ± 223[ab]	2941 ± 301[b]	3542 ± 284[c]	2931 ± 288[b]
Specific growth rate (%)	3.19 ± 0.10[a]	3.46 ± 0.11[b]	3.53 ± 0.17[b]	3.79 ± 0.12[c]	3.50 ± 0.14[b]
Feed efficiency (%)	50.99 ± 2.82[a]	57.49 ± 3.06[bc]	61.55 ± 3.12[c]	55.74 ± 1.63[ab]	52.32 ± 3.98[ab]
Protein retention (%)	24.58 ± 2.27[a]	32.31 ± 3.39[b]	37.32 ± 0.91[c]	34.87 ± 1.34[bc]	33.37 ± 2.85[bc]
Fat retention (%)	44.61 ± 5.66[a]	58.35 ± 5.79[b]	59.31 ± 1.21[bc]	68.70 ± 3.14[c]	57.59 ± 7.88[b]

Note: Data are presented as mean ± standard deviation (n=3); The same uppercase letter on the same line shows a non-significantly different value at 5% confidence level (DMRT).

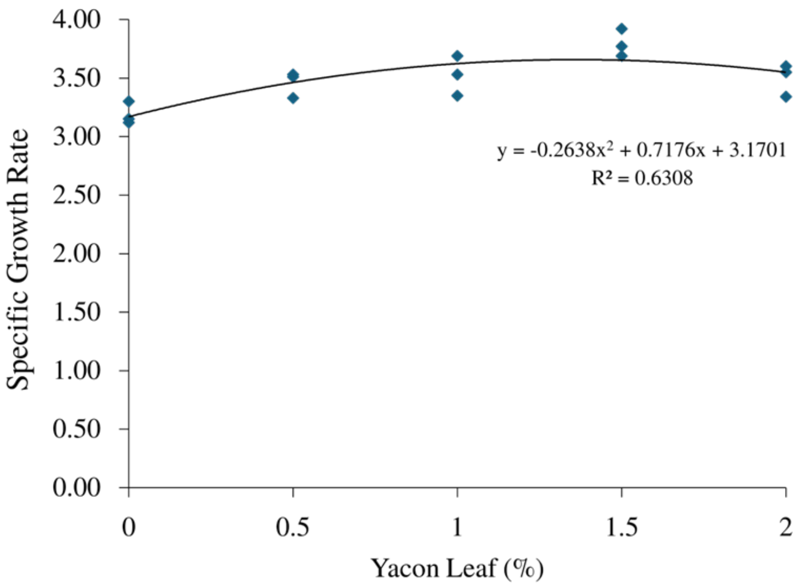


Figure 4. The second-order polynomial regression analysis for specific growth rate (SGR) and different doses of yacon leaf supplementation (%) in diets to striped catfish for nine weeks. The different points represent statistical differences (p<.05) among group supplementation diets.

leaf supplemented diets (Figure 1). This condition occurred due to fructooligosaccharides (FOS) and inulin compounds, which are predominant in yacon plants (Ojansivu *et al.,* 2011; Caetano *et al.,* 2016). The dietary intake of FOS and inulin triggers the bifidogenic effect by selectively exciting the proliferation of beneficial bacteria in gastrointestinal tract and producing numerous short-chain fatty acid (SCFA) and lactic acid that subsequently leads to a lower luminal pH of broiler chicken (Kim, 2014; Liu *et al.,* 2018), mouse (Marcon *et al.,* 2019), and guinea pig (Saeed *et al.,* 2017). Another study also observed the use of yacon as dietary supplementation material in poultry, which induced the microflora presence in pathogen exclusion, immune stimulator, vitamin synthesis, and metabolism assistance by increasing the digestive enzyme secretions, such as amylase and protease (Caetano *et al.,* 2016). Moreover, the abundance of microflora in the intestine can also provide more energy source through the fermentation process and increase the mineral absorptions (Ca^{2+}, K^{+}, Mg^{2+}), besides modulating the metabolic syndrome and dyslipidemia by reducing the cholesterol absorption (Lobo *et al.,* 2007; Honore *et al.,* 2018).

The glucose energy requirement is essential as a major fuel for fish in vital tissues. The utilization of high digestible carbohydrates will increase the glucose levels in blood (Schrama *et al.,* 2018). The present study showed that blood glucose level of striped catfish fed with yacon leaf dietary supplementation were slower to reach the peak at three hours after feeding than the control treatment at one hour after feeding. Furthermore, all treatments returned to basal glucose level at eight hours after feeding, and the duration of hyperglycemia of fish fed with yacon leaf supplemented diets lasted shorter about five hours than fish fed without yacon leaf supplemented diets by about seven hours (Figure 2). This was similar to the previous studies that the blood glucose level reached the peak value at three hours after feeding, and hyperglycemia lasted about seven hours in carp as omnivorous species (Li *et al.,* 2022a).

In herbivorous fish, the hyperglycemia lasted about six hours after feeding with blood glucose reached at three hours (Huang *et al.,* 2015). Therefore, this study suggests that striped catfish as carnivorous fish species have highly control blood glucose level similar to herbivorous fish species even better as the result after adapted with the dietary yacon leaf supplementation, which indicates the efficient used of an overload glucose in the blood. The role of the bioactive compounds in yacon leaf such as phenolic, especially quercetin and chlorogenic-acids, can inhibit the α-glucosidase activity in the intestine to provide a prolonged duration time, so that the glucose absorption is balanced with the availability of insulin hormone secretion (Ueda *et al.,* 2019; Li *et al.,* 2022b). In comparison between fish fed with yacon leaf supplemented diet and control diet, the current study exhibited the peak of blood glucose level in yacon supplemented diet was slightly lower than control diet after feeding.

The peak then increased slowly as the fish could sustain a better certain amount of blood glucose after feeding with yacon leaf supplemented diets. This condition may occur because omnivorous fish had a significant improvement in the carbohydrate digestive mechanism development (Chen *et al.,* 2018). This was also consistent with the results of amylase and protease activities in the present study. In fish, glucose sensing release of insulin is mediated with the hormones that play a pivotal role on glucose homeostasis (Mark, 2021). To ensure the proper of cellular function, blood glucose level were maintain in a narrow normal range around a set point (Dai *et al.,* 2024).

The result of glucose tolerance test exhibited a different pattern in the recovered blood glucose duration level. The fish fed with yacon leaf supplemented diets could return to basal level faster than the control diet, which peaked at one hour after the direct administration of glucose (Figure 3). Similar results have also been observed in tilapia (*Oreochromis niloticus*) (Chen *et al.,* 2018), grass carp (*Ctenophcuyngodon idellus*) (Zhao *et al.,* 2020), and gibel carp (*Carassius gibelio*) (Jin *et al.,* 2018) that spent five to eight hours to clear up the glucose load after direct regulation of glucose. Moreover, diets supplemented with yacon leaf at 6.5% could reduce the blood glucose level in mice (Marcon *et al.,* 2019). This condition is related to insulin action in increasing the glucose from blood into cells to decrease blood glucose level quickly (Polakof & Panserat, 2016).

Decreased glucose level is caused by the increased insulin circulating activity synthesized and secreted by pancreatic beta cells, which inhibits the insulin degradation. Another study demonstrated that 2% yacon tea *ad libitum* could increase the concentration of circulating insulin to diabetic rats (Honore *et al.,* 2018). Benzoic acid in yacon leaves inhibits insulinase and enhances the insulin effect (Lachman *et al.,* 2018. In addition, Ueda *et al*. (2019) reported that caffeoylquinic acid was the active component associated with the reduction of blood glucose levels. The essential role of insulin signaling pathway is to preserved the metabolism of normal glucose (Huang *et al.,* 2018), thus heightening its sensitivity can promote the glucose metabolism (Hamann *et al.,* 2015). This result may become the utmost reason for the limited glucose utilization on fish, which performs not in slow glycolysis process, but only lack of gluconeogenesis inhibition (Zhou *et al.,* 2016; Dai *et al.,* 2024).

Increased triglyceride and cholesterol concentrations in the yacon leaf supplemented diet treatments in fasting state were caused by metabolism changes in the striped catfish digestive system (Table 3). According to de Almeida *et al*. (2015), yacon as a functional consumption ingredient can improve the metabolic disorders such as hyperglycemia dyslipidemia and cholesterol regulation in blood. Also, yacon plays a role in reducing cholesterol level (Ojansivu *et al.,* 2011) Honore *et al.,* 2018) and triglyceride levels (Verediano *et al.,* 2020) without exerting any adverse effect. In this study, blood plasma sampling was carried out when the fish were on fasting state, which means that the increased triglycerides and cholesterol indicates the body's response to convert lipid stores into energy or throughout gluconeogenesis.

According to Schlattner *et al*. (2021), during the fasting state, the insulin hormone is in low, and catecholamine stimulates the release of glucagon to increase the concentration of free fatty acids derived from storage in adipose tissue as energy. During fasting, cholesterol plays a pivotal role as source energy and a precursor in stress hormone synthesis, which can promote gluconeogenesis or combat any stressful condition and thus prolong the longevity of fish (Dai *et al.,* 2024). However, these results may signify that the fish fed with yacon leaf supplemented diets have an excess of energy storage as glycogen in adipose tissue and muscle, which can be used when the blood glucose level is low. Nevertheless, the mechanisms of yacon leaf to affect the hormonal regulation in fish are still remains unclear and need a further investigation right after this preliminary research of yacon.

The digested carbohydrates as glucose in limited quantities are converted into glycogen in muscles, while the smaller fractions will be converted into lipid in liver through lipogenesis (Craig & Helfrich, 2017). The blood glucose homeostasis specifically maintained by the forming of liver glycogen that acts as a universal glucose reservoir (Reed *et al.,* 2024). In our study, we found that 1% dietary yacon leaf supplementation could decrease the hepatic glycogen content in striped catfish, meanwhile 2% dietary yacon leaf supplementation showed an enhancement of glycogen content (Table 4). These results indicate that the glucose-6-phosphate, as an important agent of glycolysis, may be diverted into the pentose phosphate pathway (PPP) for the NADPH production and ribose-5-phosphate, instead of glycogen. NADPH is an important agent that involved in the reduced of glutathione level and *de novo* fatty acid synthesis (Bou *et al.,* 2016).

According to Habib *et al*. (2015), the quercetin and chlorogenic acid in yacon leaves can reduce lipogenesis in the liver by inhibiting the fatty acid formations. From our data, we assume that the optimal dietary yacon leaf supplementation enhances the PPP process. Moreover, the increased liver glycogen may reflect on the limitations of available glucose metabolism efficiency (Li *et al.,* 2022a). The low utilization of glucose at 2% dietary yacon supplementation was described from the glucose tolerance test value, which showed the presence of excess glucose in the bloodstream carried into the liver and muscles and converted into glycogen through the glycogenesis process assisted by the glycogen synthase enzyme (Volkoff, 2016).

In addition, Verediano *et al*. (2020) reported that several bioactive compounds in yacon had various biological activities, one of which is the hypoglycemic effect, which may be due to the lowest blood glucose level in 2% yacon leaf dietary supplementation treatment. However, the hepatosomatic index showed an increased value along with the body biomass, while the lipid liver in recent study on dietary yacon supplementation treatments exhibited the derivation compared with the control group. Honore *et al*. (2018) demonstrated that yacon had another potential role as a biostimulator in reducing the lipid level in rat liver suffered from type 2 diabetic. Therefore, the insulin sensitivity enhancement due to yacon leaf supplementation in fish is still unclear, as the underlying mechanisms remain to be elucidated.

The SCFAs (acetate, propionate, butyrate) produced by the fermentation of FOS contained in yacon is known to repair the intestinal mucosa by providing 70% utilities for metabolism (Caetano *et al.,* 2016). From our data, fish fed with yacon leaf supplemented diets could significantly increase the specific growth rate (Table 5). In other studies, the dietary yacon leaf supplementation showed a positive effect on growth performance in cattle (Wang *et al.,* 2019), calves (Saeed *et al.,* 2017), and broilers (Kim, 2014). According to Lobo *et al*. (2007) and Higashimura *et al*. (2021), the presence of fructans in yacon leaf can increase the digestibility level due to the intestinal wall surface expansion, thereby increasing the nutrient quantity and Ca mineral absorption, which impacts to somatic body through protein retention.

The data above implied that appropriate dietary yacon leaf supplementation (1%) could improve the fish growth by likely improving the nutrient utilizations. The increased final weight in this study is proportionate with the lipid retention. Based on the phytochemical analysis results of yacon leaf, the (Z)-3-Phenyl-2-Propenal is an organic component of cinnamaldehyde (Williamson & Clifford, 2017; Lachman *et al*., 2018). According to Ueda *et al*. (2019), this compound leads to the decreased body lipid retention. In our study, there is a certain level of yacon supplementation, which can trigger the lipogenesis, altering the availability of blood glucose into lipid. The decreased lipogenesis level is correlated with an increase of body lipid consumption.

In conclusion, based on the orthogonal polynomial test results obtained the correlation value of yacon leaf dietary supplementation and specific growth rate of striped catfish at 79.42%, while the gradient value indicates the effect of yacon leaf variable is presented at 63.08% on growth performance. The regression equation results showed that the best dose of dietary yacon leaf supplementation is 1.36% (Figure 4), which can improve the energy utilization from non-protein sources, specifically carbohydrates, which presents a protein sparing-effect condition, based on growth performance values and glucose activity tests and produces the optimal specific growth rate at about 3.65%. Further investigation are needed to describe the detail mechanism of yacon leaf affect carbohydrate digestibility into net energy by using the isolated active ingredient in yacon leaf such as phenolic compound of quercetin and chlorogenic acid.

ACKNOWLEDGMENTS

This article is part of a thesis written by Revita Syefti Palmi, Aquaculture Sciences Study Program, Faculty of Fisheries and Marine Science, IPB University. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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