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The effects of garlic, banana, and onion as prebiotic supplementation on growth performances, feed utilization, and survival rate of *Anabas testudineus*

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Abstract

The aim of this research was to investigate the influences of local plants as prebiotic supplementary feeds on growth performances, feed utilization, and survival of climbing perch (*Anabas testudineus*). Economics of feeding in terms of the cost and return of climbing perch culture was also investigated. Three plants including garlic, banana, and onion at 20 g/kg feed were added to the feed and compared with a basal diet (control). The average initial weight of climbing perch was 5.47 – 5.51 g/fish. The research was carried out for 150 days. The results that showed there were no significant differences in the effects of chosen local plant supplementation on growth performances ($p>0.05$). However, fish fed with banana powder supplementation displayed significantly higher in feed utilization and survival rates ($p<0.05$) than other groups. The percentage of edible flesh was inversely proportional to protein in fish flesh. Results showed that control fish groups had the highest edible flesh ($p<0.05$) but lowest in protein in fish flesh. On the other hand, climbing perch fed with banana and onion feed additives had lower percentage of edible flesh, but higher percentages of protein in edible flesh. In summary, local plant additive feeds were not able to improve growth performances. However, a banana powder supplementary diet possibly enhances fish immunity and improves fish health leading to a higher survival rate which is able to provide a better return investment and higher protein in fish flesh.

Keywords: climbing perch, prebiotic supplement, garlic, banana, onion

1. Introduction

The climbing perch, *Anabas testudineus* is one of the most commercially important cultured species in Thailand. Department of Fisheries reported in 2016 that the total production of climbing perch was 8,000 ton and was valued at 434 million baht. However, commercially climbing perch farming have faced the high operating cost problem, about 50 – 60 % from feed [1]. Feed and feeding are major factors affecting on growth performances, disease resistance, and survival rate leading to final yield and profit. In addition, there is now more concern about food safety, the chemical and antibiotic usage trends to be prohibited. Therefore, prebiotic supplementation is the alternative for growth and health enhancement. In addition, it could promote the enzymatic activity in intestine affecting on the digestibility and nutrient absorption. The health of cultured climbing perch and other fish species in Thailand has improved resulting in use of dietary supplements of prebiotic. Prebiotic are non-digestible carbohydrates that involve in fish digestion and metabolism by boosting beneficial bacteria in gut [2] have an antagonistic activity against bacteria in the gut [3]. The characteristics are needed to be used as a prebiotic including hydrolysis resistance by digestive enzymes, growth improvement, and stimulation bacteria activity in gastrointestinal tract to enhance immune responses [4]. Oligo-fructose is commonly used as prebiotic feed ingredients in various animals. They usually appear in banana, onion, asparagus, tomato, wheat and garlic; especially found in onion, banana, brewer's yeast and garlic which have been used in aquaculture industry. In addition, the application of garlic had a positive effect on the rainbow trout's growth [5], improved the feed

conversion rate in Nile tilapia [6], and enhanced disease resistance against *Streptococcus iniae* in Cobia [7]. Moreover, an improvement in growth, feed utilization, and some hematological parameters was observed when garlic or onion powder was added in sea bass feeds [8]. Also, the dietary banana peel extract administration at 6.0 g/ kg promoted growth performances and enhanced immunity in the giant freshwater prawn [9]. Thus, the objective of this study was to determine the growth performances, feed utilization, and survival of climbing perch (*Anabas testudineus*) that affected from the supplementation of garlic, banana, and onion as a prebiotic in climbing perch feeding compared to basal diet group.

2. Materials and Methods

2.1 Fish

Climbing perch with initial average weight of 5.47 – 5.51 g/fish were obtained from commercial fish farm (Phetchaburi province, Thailand) and acclimated for 1 week in a 2000 liter cement tank. During the acclimation period, fish were given with control diet (no supplemented prebiotic) at 5 % body weight two times a day at 9.00 am and 16.00 pm.

2.2 Feed preparation

The commercial pellet feed contained 32 % protein and 3000 Kcal/kg. Garlic, banana and onion were bought from a local market in raw forms in Phetchaburi province, Thailand. They were cut to small pieces, oven – dried at 80 ^{o}C for 24 h, and crushed to small grains. Then, they were ground to fine powder with household electric grinder. After that, they were mixed with fish pellet feed at 20 g of prebiotic per Kg of feed while a control was without prebiotic supplement. Coated feed trials with albumin at 100 ml/Kg of feed, dried at room temperature for 24 hours, and kept at room temperature.

2.3 Experimental system

Juvenile climbing perch were randomly allocated to 12 experimental net cages (1m x 1m x 1.5m), 25 fish in each net cage with three replicates per diet. The net cages were set up in 800 m^2 earth pond. Continuous aeration was provided at the bottom center of earth pond. During the trial, all groups were hand – fed 2 times daily (9.00 and 16.00) for period of 150 days. Fish in each net cage were evaluated at day 30, 60, 90, 120 and 150 of being fed with trial diets (basal diet (control), 20g Kg/diet garlic, 20g Kg/diet banana and 20g Kg/diet onion) in order to measure growth and feed utilization including B/C ratio, cost and yield. The growth performance were calculated follow standard formulas: Specific growth rate (SGR%/d), Weight gain (WG%), Survival rate (%), Average daily gain (ADG), Protein efficiency ratio Daily feed intake (%/day), and Feed conversion ratio (FCR) [5]. Water quality was maintained to standard values throughout the trial as an example, Temperature remained at 23 – 32 ^{o}C, pH and Dissolve Oxygen (DO) maintained at 6 – 9 about 3 mg/ml. For instance, ammonia – nitrogen remained at lower than 1 mg/l and nitrite – nitrogen not higher than 0.6 – 1 mg/ml.

2.4 Chemical analysis

The proximate chemical analysis of experimental diets and flesh were carried out at the beginning and the end of experiment. Briefly, moisture content was determined in an oven (105 ^{o}C for 4 h until constant weight). Protein contents were calculated as 6.25 × % N analyzed via the kjeldahl method after acid digestion [10]. The crude fat content was determined by ether extraction using Soxhlet method [10], crude fiber by glass crucible method, and ash content by combustion in a furnace muffle (550 ^{o}C for 5 h) [10].

2.5 Data Analysis

The data were subjected to Statistical Package for the Social Sciences (SPSS). Duncan's multiple range tests were used to determine the differences among treatment means at $p > 0.05$, and each data was the mean± SD.

3. Results and Discussion

3.1 Growth performances and cost

The effect of natural prebiotic (banana, onion and garlic) on growth performance in juvenile climbing for 150 days is given in Table 1. The result showed that final weight, weight gain, percent weight gain, mean daily gain, and specific growth rate including cost among groups were not significantly different ($p>0.05$). However, fish fed

banana prebiotic diet group significantly increased ($p<0.05$) survival rate compared to other groups. On the other hand, it was significantly lowest in yield ($p<0.05$). It was not significant difference in B /C ratio but it displayed trend on fish fed banana prebiotic diet group which was higher than other groups ($p=0.0512$).

Table 1 Growth performance, survival rate, cost and B/C ratio of climbing perch fed with experimental diet for 150 days (mean ± SD, N = 10)

Parameter	Natural prebiotic source				p – value
	Basal diet	banana	Onion	Garlic	
Initial weight (g/fish)	5.48 ± 0.06	5.51 ± 0.06	5.48 ± 0.08	5.47 ± 0.05	0.9250
Final weight (g/fish)	32.34 ± 3.46	26.45 ± 1.41	28.53 ± 0.40	32.15 ± 3.92	0.2075
Weight gain (g/fish)	26.86 ± 3.51	20.97 ± 1.36	23.09 ± 0.44	26.67 ±3.87	0.2082
Average daily gain (g/fish/day)	0.22 ± 0.02	0.17 ± 0.00	0.20 ± 0.02	0.22 ± 0.03	0.2140
Percent weight gain (%)	450.42 ± 0.91	382.74 ± 21.79	424.36 ± 11.34	486.69 ± 66.39	0.1801
Specific growth rate (%/day)	1.47 ± 0.09	1.31± 0.04	1.41± 0.05	1.47 ± 0.09	0.1931
Survival rate (%)	50.00 ± 2.82^{bc}	84.00 ± 5.65^{a}	64.00 ± 5.65^{b}	46.00 ± 8.48^{c}	0.0104
Yield (g/ net cage)	731.49 ± 2.53^{a}	590.08 ± 18.58^{b}	628.33 ± 12.67^{a}	748.38 ± 68.24^{a}	0.0284
Cost (baht)	44.26 ± 1.16	42.41 ±2.33	42.70 ± 1.03	43.63 ± 3.32	0.7191
B/C ratio	0.73 ± 0.15	0.91 ± 0.05	0.84 ± 0.07	0.60 ± 0.09	0.0512

Note: Mean ± SD with the different superscripts are significantly differences ($p<0.05$)

Table 2 shows feed utilization of fish fed with natural prebiotic diets. The protein efficiency ratio (PER) was significantly higher ($P<0.05$) in fish fed with banana prebiotic diet group than other ones. However, daily feed intake and feed conversion ratio were not significantly different ($P>0.05$).

Table 2 Feed utilization of climbing perch fed with experimental diet for 150 days (mean ± SD, N = 10)

Parameter	Prebiotic source				P - value
	Basal diet	banana	Onion	Garlic	
Daily feed intake (%/day)	55.08 ±16.60	45.53 ± 0.00	59.75 ± 0.52	64.30 ± 20.71	0.5850
Feed conversion ratio	1.52 ± 0.33	1.69 ± 0.07	1.49 ± 0.06	1.42 ± 0.05	0.5482
Protein efficiency ratio	0.57 ± 0.13^{c}	1.52 ± 0.06^{a}	1.02 ± 0.16^{b}	0.46 ± 0.16^{c}	0.0050

Note: Mean ± SD with the different superscripts are significantly differences ($P<0.05$)

3.2 Body and carcass composition

Body and carcass composition was displayed in Table 3. The percentage of bone and viscera of fish fed with diet trial were not significantly different ($P>0.05$). In contrast, fish fed with basal diet test had higher percentage of edible flesh that those fed with the trial feed contain natural prebiotic, banana, onion and garlic ($P<0.05$).

Table 3 Carcass composition of climbing perch fed with experimental diet for 150 days (mean ± SD, N = 10)

Composition (%)	Prebiotic source				P - value
	Basal	banana	Onion	Garlic	
Edible flesh	71.88 ± 1.76^{a}	59.03 ± 3.04^{b}	57.90 ± 2.84^{c}	61.08 ± 1.35^{b}	0.0019
bone	24.25 ± 2.00	27.79 ±2.07	24.83 ±0.30	26.67 ± 1.26	0.1620
Viscera	5.61 ± 1.69	6.08 ± 0.40	5.52 ± 0.24	6.20 ± 0.35	0.8064

Note: Mean ± SD with the different superscripts are significantly differences ($P<0.05$)

Proximate composition of climbing perch's edible flesh is given in Table 4. There were significantly different in percentage of moisture, crude protein and crude lipid ($P<0.05$) while percentage of ash was not significantly different ($P>0.05$) among experimental groups. Percentage of moisture was highest in banana prebiotic diet group while percentage of crude protein was higher in fish fed with prebiotic banana and onion diet group than other groups. Moreover, fish fed with garlic prebiotic diet and basal diet group were higher percentage of crude lipid than other experimental groups.

Table 4 Body composition of climbing perch fed with experimental diet for 150 days (mean ± SD, N = 10)

Composition (%)	Initial	Prebiotic source				P - value
		Basal	banana	Onion	Garlic	
Moisture		5.30 ± 0.03 [b]	6.50 ± 0.12[a]	3.49 ± 0.01[c]	3.49 ± 0.23[c]	0.0001
Crude protein		62.12 ± 0.09[c]	63.71 ±0.04[a]	63.87 ±0.09[a]	62.81 ± 0.17[b]	0.0003
Crude lipid		11.97 ±0.07[a]	10.40 ± 0.21[c]	10.99 ± 0.10[b]	12.05 ± 0.14[a]	0.0008
Ash		17.08 ± 0.05	17.47 ± 0.01	17.37 ± 0.07	17.32 ± 0.26	0.1684

Note: Mean ± SD with the different superscripts are significantly differences (P<0.05)

3.3 Discussion

This study examined the influence of natural prebiotic supplementary feeds namely banana, onion and garlic powder on growth performance and feed utilization of juvenile climbing perch. From the research pointed that natural prebiotic did not clearly show positive impacts on growth performances including specific growth rate, weight gain, and mean daily gain. The research on natural prebiotics in fish is limited [11] while Oslen et al. [12] stated that Arctic charr fed with 15% inulin supplemented diet resulted in an intestinal damage. The several research are agreement with result from this current experiment for instance the research on Gulf sturgeon [13] and tilapia [14]. The properties or the main purpose for using of prebiotic emphasize for disease resistance and immune response improvement [3] including on disease control and immunostimulants [4&15]. Prebiotic positively influences on competition pathogen for nutrients and stimulation of the immune system. In addition, it stimulates the beneficial bacteria activity in the gut and improves the intestinal balance of microorganisms [3; 16]. From previous reasons, in most case indicated that prebiotic affected on health – promoting more than feed utilization and natural prebiotic (banana, onion and garlic) are not appropriate for supplementation in the climbing perch diet which is agreement with Reza [2] who observed that inulin supplementation in beluga diet could not improve the growth performance and feed utilization as well. Additionally, Gatesoupe [16] stated that dominant bacteria in aquatic animal intestine were different from those found in mammals. The previous and current studies showed no growth response to the natural prebiotic was in concordance with results from studies on Atlantic salmon, which fed with dietary supplement of the prebiotic mannanoligosacharide (MOS) and fructooligosacharide (FOS) from inulin and galactooligosacharide (GOS) at 10 g/kg; there was no significant effect on growth performance and feed utilization but it provided positive effects for salmon production [17]. In this study, 20 g prebiotic/kg of feed were applied since it is not synthetic prebiotic; however, various amounts of banana will be a further investigation. Reza et al. [2] founded that inulin supplementation at 1, 2 and 3% in Beluga's (*Huso huso*) diet did not have positive effects on growth rate. Moreover, the application of Grobiotic^{tm} AE commercial prebiotic and brewer yeast at 1 and 2% on hybrid strip bass which fed for 4 weeks were not able to improve the growth and feed efficiency of hybrid strip bass (19.7 g/fish) diet but the growth and feed efficiency was significant increase when using for 7 weeks [8]. The using immunostimulant, prebiotic, probiotic at slightly volume played on increasing of immune system, feed utilization and growth performance in fish and crustacean [18]. Otherwise, enzyme supplementation clearly enhance growth performance more than using prebiotic or probiotic [3] that were in line with this trial pointed that natural prebiotic showed not clearly the difference growth rate although it's affected on protein efficiency ratio and survival rate. The reasons for the imprecise results, it may be due to the different raw ingredient, supplementary level, chemical structure, fructant type and acclimation period, and fish characteristics (age and species) [2&15] origin of fructooligosacharide, types or strains of natural prebiotic, source of natural prebiotic, palatability of diet and prebiotic's diet stability. However, there are many research found that prebiotic can improve the growth performances, survival rate, and feed utilization depending on the types of prebiotic ingredients [15&19].

Daily feed intake and feed conversion ratio were not affected by natural prebiotic while protein efficiency ratio (PER) highly increase in prebiotic banana group. From these reasons pointed natural prebiotic have no effected on palatability of diet that agreement with Reza et al. [2] reported that beluga fed with 2% inulin supplementary diets were not significant difference in feed intake. The feed palatability did not influence on beluga growth performances. Moreover, the prebiotic banana group had the highest PER because of the composition of banana such as resistant starch (RS) and inulin that highly resistance for digestion and cannot be absorbed at intestine. These properties were similar with other dietary fibers which can be used as prebiotic. From this observation, banana displayed the best ingredient that suitable for climbing perch diet.

The carcass and body composition displayed the inversely related data of edible fresh percentage and edible flesh protein concentration especially in prebiotic banana group which shown in Table 3 and 4 that related with protein efficiency ratio. This group, moreover, showed the high concentrate of protein and lowest lipid in edible flesh (P<0.05) that gave the similar results with studies on hybrid tilapia and rainbow trout which the flesh protein concentration was increased since the mannanoligosacharide (MOS) levels were included in the feed from 1.5 – 84.5 g/Kg. [14,16,20]. On the other hand, 10 g/Kg MOS or GOS supplementary feed for salmon [16] and 18.5 g/ Kg MOS supplementary feed for *Peneaus semisalcatus* [21] resulted in reduction in the flesh protein

concentration. In this study, banana as a prebiotic supplement affected on protein and nutrient digestibility because banana is outstanding on prebiotic property. In addition, the low lipid concentration of banana supplementation group was related with the nutrient utilization because the high feed utilization can decrease the lipid concentration.

4. Conclusion

In conclusion, banana, onion and garlic supplementation as prebiotics in climbing perch feeding had no clearly effect on growth performance and feed utilization of juvenile climbing perch. However, banana as a prebiotic supplement seems affect on protein efficiency ratio increasing that improved the high percentage of protein in edible flesh and survival rate. In addition, the B/C ratio displayed the trend better than other groups. Therefore, the supplementation of banana should be suitable prebiotic supplement in feeding of climbing perch.

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