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Utilization of fermented fruit processing wastes as a protein source in the diet of Gangetic koi (*Anabas cobojius*) FRY

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Abstract
A 64 day feeding trial was conducted to evaluate the growth performance of *Anabas cobojius* by feeding fermented fruit processing wastes. Grapes, Pineapple and Papaya wastes were used for enrichment by Solid state fermentation employing Brewer's yeast, *Saccharomyces cerevisiae*. Four Iso-nitrogenous diets containing 40% crude protein in which fish meal was replaced by fermented fruits at 0%(T_o), 5% Grapes (T_1), 5% Pineapple (T_2), 5% Papaya (T_3) were formulated. Fry of *Anabas* were fed @ 5% of their body weight twice daily. Weight gain, FCR, FCE, SGR and % survival was compared against diet with fish meal (T_o) as the sole protein source. Most important water quality parameters were analyzed every week. It was observed at the end of the study that the weight of the fish showed significant difference ($P<0.05$) between Days and Treatment of fish fed the various diet. There was also significant difference in the SGR, FCR and FCE between treatments ($p>0.05$). Based on the results, it could be recommended that in practices, 5% inclusion of fermented grapes, pineapple and papaya in the diet is optimal for *Anabas cobojius* fingerlings without necessarily compromising growth rate or causing deleterious effects on the fish performance.

Keywords: fruit processing waste, *Saccharomyces cerevisiae, Anabas cobojius,* fermentation, growth parameters, feeding efficiency

1. Introduction

Fish meal is considered as one of the most important ingredients of fish feeds, due to its adequate amino acid, essential fatty acid and mineral profile [1, 2]. The increasing demand and progressive scarcity of fishmeal in the international market boosted its price and launched the quest for the reduction of fishmeal in fish diets and the consequent search for alternative, acceptable and digestible protein sources [3]. Nonconventional feed resources (NCFRs) are feeds that are not commonly in use as traditional ingredients used for commercial fish feed production. But, NCFRs are credited for being non-competitive in terms of human consumption, relatively inexpensive, and are often by-products or waste products from agriculture/ animal husbandry/ processing industries. Animal by-product meals are good protein alternatives in fish diets [4, 5], but health related issues limit their use in feeds for farm animals. Though plant materials are relatively safe protein sources, the utilisation of nonconventional feedstuffs of plant origin had been limited due to amino acid imbalances, as also the presence of anti-nutritional factors.

Agricultural production and further processing can become one of the most serious sources of pollution [6]. The treatment and environmentally friendly disposal and distribution of the wastes generated were not the main interests of the industry. This paradox could be handled by directing wastes back into production. Fruit processing industries generate considerable amount of waste during processing industry and is a rich source of sugars and proteins. This untapped source of energy and protein when dumped as waste, causes sizeable environmental hazard. The development of fermentation technologies has been lost in the mist of history and has been regarded as one of the oldest methods of food processing as well as preservation for decades and virtually every culture has fermented products as part of its diet [7]. Fermentation increases the nutrient contents of food through the biosynthesis of vitamins, essential amino acids and proteins. It improves protein quality and fiber digestibility. It also enhances the availability of micronutrients to organisms for utilization and aids in the degradation of antinutritional factors.

The bioconversion of agricultural and industrial wastes to chemical feedstock using solid state fermentation, has led to extensive studies. The Single Cell Protein (SCP) thus produced are being focussed due to their nutritional richness, and also work as immuno stimulants. *Saccharomyces cerevisiae* has proved to be a very promising source of single cell proteins due to its large size, cost effectiveness and desirable protein content with a good blend of amino acids. *S. cerevisiae* has been investigated for its protein yield on various substrates such as papaya extracts, banana skin, mango waste, sweet orange peels, apples and pomegranate rind in combination with each other and with many other fruit wastes. Despite its appropriate characteristics, the use of yeast as a protein source has not been extensive, and has been limited largely to the feeding of livestocks.

Among various production inputs, the choice of fast growing species with desirable aquaculture traits is a prerequisite for augmenting fish production in culture based fisheries. In this regard Gangetic koi (*Anabas cobojius*) is an excellent fish for culture. *Anabas cobojius* is a promising culture species, but being a relatively new entrant in the culture scenario, hardly any information is available on the feeds and feeding of this fish. The present study evaluates the growth performance of Gangetic koi (*Anabas cobojius)* by feeding with fermented fruit processing wastes as protein source, as a substitute for fish meal.

2. Materials and Methods

2.1 Preparation of the experimental diet

Four iso-nitrogenous diets (40% protein) were prepared following the procedure of Varghese *et al.*, [8] as follows namely, 1) Fishmeal based control feed (T_o), 2) Fermented Grape waste based feed (T_1), 3) Fermented Pineapple waste feed (T_2), 4) Fermented Papaya waste based feed (T_3). The fruit processing wastes were fermented with 1gm of *S. cerevisiae* inoculums containing 2.5 x 10^6 cfu/g for a period of 4 days until the $_p$H reaches 4. The fish meal was replaced with grape, pineapple and papaya at 5% inclusion levels in T_1, T_2, T_3 and fish meal as the sole protein source in T_0. The quantity of individual ingredients required to formulate a kg of diet was worked out using Pearson's square method to balance protein and energy levels are summarized in (Table 1). The required quantity of ingredients were weighed out and mixed into smooth dough. The dough was cooked under pressure for 20 minutes and cooled before adding vitamin and mineral mix. The dough was extruded using hand extruder and the pellets were then dried in a hot air oven at a temperature of 60^{o}C to a moisture content of less than 10%, cooled and stored in air tight containers.

2.2 Proximate composition

The proximate composition of the feed ingredients and feeds (Table 2) were analysed following standard methods [9].

$$\text{Moisture \%} = \frac{\text{Initial weight of sample - Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

$$\text{Nitrogen content of the sample \%} = \frac{\text{Titre value} \times \text{Normality of standard sulfuric acid} \times 0.14 \times 100 \times 100}{\text{Weight of the sample}}$$

$$\text{Crude protein (\%)} = \text{Nitrogen content x 6.25}$$

$$\text{Crude fat \%} = \frac{\text{Weight of fat}}{\text{Weight of the sample}} \times 100$$

$$\text{Crude fibre (g/100g)} = \frac{100 - \{\text{\% Moisture+\% Fat}\} \times \{w_2\text{-}w_3\}}{\text{Weight of sample}}$$

$$\text{Ash \%} = \frac{\text{Weight of ash}}{\text{Weight of the dried sample}} \times 100$$

2.3 Experimental design

The study was conducted outdoors in cement cisterns of 500 l capacity over a period of 64 days. The fry of *Anabas cobojius* were procured locally and transported to the wet lab of Kerala University of Fisheries and Ocean Studies, Panangad in oxygen packing. The fry were acclimatized to the culture conditions for a period of two weeks prior to commencement of the experiment. The fishes were kept in a circular, flat bottom cement cisterns of 500 l capacity for acclimatization. The tank was half filled with fresh water and provided with gentle aeration. The fry were fed with commercially available pelleted feed. No water exchange was done during acclimatization. After 14 days, the fry were transferred to experimental tanks. Fry of Anabas were stocked @ 10 nos per cistern. Feeding was done twice @ 5 % of the bodyweight on a daily basis. Each feed was tried in triplicate. Sampling was done at weekly intervals to assess fish growth as well as variation in water quality parameters over the experimental period. Standard procedures were followed for assessment of water quality parameters on termination of the experiment.

2.4 Nutritional evaluation of experimental diet

The growth rate in terms of specific growth rate (%), food conversion ratio (FCR), Feed conversion efficiency (FCE) and Survival were calculated by following the standard procedures as follows:

$$\text{SGR\%} = \frac{\text{(ln Final body weight - ln Initial body weight)}}{\text{Total no. of experimental days}} \times 100$$

$$\text{FCR} = \frac{\text{Total feed intake(g)}}{\text{Total live weight gain(g)}}$$

$$\text{FCE \%} = \frac{\text{Gain in weight of fish(g)}}{\text{Weight of feed given (g)}} \times 100$$

$$\text{Survival \%} = \frac{\text{Final Number surviving}}{\text{Initial Number stocked}} \times 100$$

2.5 Statistical methods

The experiment was conducted in a Completely Randomised Design (CRD) with 3 replications. Growth and feed efficiency results were subjected to a two way analysis of variance (ANOVA) and the comparison of means were done employing the Post Hoc Test, Duncan's Multiple Range Test (DMRT) with $P = 0.05$. All analyses were carried out using IBM SPSS 19.0 software.

3. Results and Discussion

3.1 Water quality parameters

Average of the water quality parameters from the experimental units like temperature, pH, dissolved oxygen, total alkalinity, ammonia, nitrite and nitrate were analysed adopting the standard procedures as described by APHA [10]. The pH values recorded in the present study in all the treatments range between 6.5 to 7.4 and were within the acceptable range and hence ideal for fish growth as described by Boyd [11] who opined that the waters with a pH range of 6.5 – 9, are the most suitable for fish production.

McKee and Wolf [12] assessed the dissolved oxygen requirement of pond fish and stated that the dissolved oxygen content of warm water fish habitats shall be not less than 5mg/l during at least 16 hr, may be less than 5mg/l for a period not to exceed 8 hr period, but at no time shall the oxygen content be less than 3mg/l. The present study agrees with the hypotheses showing mean dissolved oxygen ranging from 4.2 to 6.1ppm.

The temperature of the culture water in the present experiment was maintained between 25.5ºC to 28ºC which is in agreement with the observation made by Peetabas *et al.,* [13] who studied the temperature tolerance of air-breathing fishes such as *Anabas, Heteropneustes and Amphipnous* and revealed that the fishes could survive 72h of exposure to a higher temperature of 40ºC, 36.5ºC and 36ºC, whereas, none of the fishes survived at 15ºC in low lethal temperature in the environment.

The mean total alkalinity of the study ranged between 77 to 121 mg/l, which was within the acceptable limits, as stated by Wurts and Durborow [14] that the alkalinity between 75 to 200 mg/L, but not less than 20 mg/L is ideal for an aquaculture pond. The ammonia, nitrite and nitrate concentrations in the investigation fluctuated between 0.0 to 2.5mg/L, 0.0 – 1.5 mg/L and 0.0 – 5.0 mg/L which was in conformity with the research findings of Stone and Thomforde [15] that the total NH_3-N: less than 4 mg/L, Un-ionized NH_3-N: less than 0.4 mg/L, NO_2 less than 4 mg/L and NO_3 is relatively non-toxic except at exceedingly high levels (above 90 mg/L).

Alam *et al.,* [16] studied the physico-chemical parameters of water in different cemented tanks under different treatments during the trial of *Anabas testudineus* and found out that the temperature, pH and dissolved oxygen of water in different cemented tanks under different treatments ranged from 28.8 to 29.8 ºC, 7.6 to 8.1 and 5.5 to 6.2 mg/L. The water quality parameters measured in different treatments in laboratory conditions are presented in (Table 3) during the experimental period registered a more or less similar trend and all of them were in the acceptable range for the growth and survival of *Anabas.*

3.2 Growth performance of Anabas cobojius

Anabas cobojius recorded the highest total average weight of 23.22gms in treatment T_2 (Fermented pineapple waste feed) followed by 22.95gms in T_1 (Fermented grape waste feed), 22.56gms in T_3 (Fermented papaya waste feed) and lowest total average weight of 14.48gms in T_0 (Fish meal based feed) which are depicted in (Table 4). The results of ANOVA showed that the recorded weight is significantly different between days and between treatments. *Anabas cobojius* recorded the highest specific growth rate of 3.84% in the treatment T_3 (Fermented papaya waste feed) and lowest of 3.00% in the treatment T_1 (Fermented grape waste feed). Li and Gatlin [17] also observed that including dietary brewer's yeast (1%- 2% of the total diet) increases growth and weight gain of sunshine bass, despite the presence of *Mycobacterium marinum*. Tilapia fry grown on diets where 65% of the protein was derived from a blend of plant proteins and yeast, with 30% of the protein from yeast, exhibited higher growth rates than fry grown on a diet with 100% of the protein from fish meal (FM) [18]. The results of Manuel and Ockiyo [19] showed that 50% of the fishmeal in a practical diet for *O. niloticus* could be effectively replaced by yeast SCP and was better utilized by the fish than the 100% fishmeal diet, at the end of the feeding trial, without any significant reduction, in the growth performance and carcass composition.

3.3 Survival rate

Survival is an important factor in fish production. The survival of fish depends on availability and type of feed, physio-chemical conditions of water etc. The survival rate of *Anabas* was 100% in all the experimental treatments are shown in the (Table 5) which agrees with Doolgindachabaporn [20] who observed that 38.6% protein, to be the best feed formula, in terms of growth and survival, for *Anabas* fry.

3.4 Feed utilisation parameters

The best FCR of 1.62 in the treatment T_3 (fermented papaya waste feed) and lowest of 2.45 in the treatment T_o (Fish meal based feed). The highest FCE of 61.86% was recorded in the treatment T_3 (Fermented papaya waste feed) while the lowest FCE of 40.69% was recorded in the treatment T_o (Fish meal based feed). The feed utilisations were shown in the (Table 5). Doolgindachabaporn [20] stated that the FCR value of *A. testudineus* ranges from 1.8-3.0. Potongkam [21] reported that FCR of *A. testudineus* fed on trash fish and pellet were 2.07 and 1.89, respectively.

Table 1: Proportion of the ingredients in various test diets (in%)

Ingredients	(T_o)	(T_1)	(T_2)	(T_3)
Fish meal	30.44	23.68	23.68	23.68
Ground nut oil cake	26.35	28.35	28.35	28.53
Wheat bran	17.85	15.0	13.8	13.9
Tapioca	25.00	12.56	16.55	16.30
Fermented grape waste	-	20.26	-	-
Fermented pineapple waste	-	-	17.53	-
Fermented papaya waste	-	-	-	16.58
Vitamin & mineral mix	1.00	1.00	1.00	1.00

Table 2: Proximate composition (%) of feed ingredients

Ingredients	Moisture (%)	Dry Matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Ash (%)
Fish meal	8.42	91.58	73.90	8.67	12.83	8.55
GNOC	10.48	89.52	51.19	13.13	7.57	1.77
Wheat bran	10.91	89.09	18.01	2.46	13.29	5.96
Tapioca	6.21	93.78	2.61	0.50	13.69	4.33
Fermented grape	95.93	4.07	24.68	6.83	15.10	3.21
Fermented pineapple	95.74	4.26	28.53	2.14	15.12	3.98
Fermented papaya	96.33	3.67	30.15	6.37	8.19	2.12

Table 3: Range of water quality parameters in the experimental tanks over the period of study

Temperature (ºC)	pH value	Dissolved oxygen (ppm)	Alkalinity (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)
25.5 - 28ºC	6.5 - 7.4	4.2 -6.1	77 - 121	0.0 - 2.5	0.0 – 1.5	0.0 – 5.0

Table 4: Statistical analysis of weight gain of Anabas cobojius (P<0.05)

Total average	(T_o)	(T_1)	(T_2)	(T_3)
Weight	14.4756^a	22.9485^c	23.2207^d	22.5604^b

Table 5: Growth parameters of Anabas cobojius fed with various diets

Mean values	(T_o)	(T_1)	(T_2)	(T_3)
Specific growth rate (%)	3.05±0.11	3.01±0.04	3.25±0.03	3.84±0.18
Feed conversion ratio (FCR)	2.45±0.40	1.73±0.14	1.79±0.07	1.62±0.09
Feed conversion efficiency (%)	40.70±0.62	58.05±4.51	54.59±1.94	61.86±3.44
Survival %	100±0.00	100±0.00	100±0.00	100±0.00

4. Conclusion

The investigation reported that the effect of some bioconverted fruit processing wastes on growth and survival of the fry of *A. testudineus*. Different growth parameters, such as fish weight, survival rate, feed conversion ratio (FCR), feed conversion efficiency (FCE) and specific growth rate (SGR) were used to see the growth performance and feed utilization during the study period. It can be concluded that the three protein sources tested namely bioconverted fruit wastes of grapes, pineapple and papaya were found to be well utilized by *Anabas cobojius* at 5% inclusion level. The feed ingredients selected for this study are less costly than fishmeal and mass production of fermented fruit wastes would pave way for replacing high cost fishmeal in fish feeds. Therefore, the bioconversion of fruit processing wastes, and utilization of the harvested biomass of bioconversion, for the production of fish feed, would be economically advantageous. Although many efforts have been made with the utilization of these bioconverted fruit wastes, digestibility of the components by the fish is an area which needs further inputs in terms of research and development. Research must be focused to develop simple and self sustainable technologies for the efficient utilization of indigenous processing wastes as substrates in bioconversion.

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