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Fermented Golden Apple Snails as an Alternative Protein Source in Sex-Reversed Red Tilapia (*Oreochromis niloticus x O. mossambicus*) Diets

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Abstract

The main purpose of this study is to find out the possibility of using fermented golden apple snail (FGAS) as an alternative protein source in sex-reversed red tilapia (*Oreochromis niloticus x O. mosambicus*) diets. FGAS was prepared by incubating minced golden apple snail (GAS) with locally screened lactic acid bacteria and using molasses as carbon sources at ambient temperature for 10 days. Five experimental diets were formulated by replacing fishmeal with FGAS at level of 0 (control), 25, 50, 75 and 100 % of fishmeal protein. Another diet with minced GAS was also included in the experiment. Each diet was randomly fed to triplicate groups of fish with an initial weight of 2.73 ± 0.09 g for 8 weeks. The results showed that growth performance and feed utilization of fish fed 75 % fishmeal replacement diet were significantly better ($p < 0.05$) than those fed the control diet. The fish fed 100 % replacement diet showed lower growth rates compared with the control diet but were not significantly different ($p > 0.05$). In addition, fish fed a diet containing minced GAS showed better growth performance and feed utilization than those of the control diet. The digestibility study indicated that fish utilized protein from snail meal either in a minced or fermented form much better than protein from fishmeal. It was concluded that snail meal is a potential protein source in sex-reversed red tilapia diets. Replacement of fishmeal protein with minced snail meal could be at 50 % and FGAS could be up to 100 % but 75 % is recommended.

Keywords: Golden apple snail, fermented golden apple snail, fish meal replacement, lactic acid bacteria, sex-reversed red tilapia

Introduction

Golden apple snails (GAS) (*Pomacea* sp.) are a significant pest in many rice fields in Thailand. There have been attempts to utilize this snail as food for animals because of its high nutritional value [1,2]. Snail meal has been used for broilers, laying hens and pigs' diets [2]. For aquatic animal diets, Norachan *et al*. [3] found a significant improvement in growth and feed efficiency of juvenile Nile tilapia (*Oreochromis niloticus*) when dried GAS meal was substituted for 47.7 % of fishmeal protein or 12.2 % of diet. The total fishmeal replacement with ground sundried GAS in diet of the striped catfish (*Pangasianodon hypophthalmus*) has been reported [4]. In shrimp diet, Bombeo-Tuburan *et al*. [1] reported the use of chopped fresh GAS mixed with cooked cassava or cooked maize in the proportion of 60 %:40 % of total weight to feed black tiger shrimp (*Penaeus monodon*) for the 4-month culture period. The recommended level of GAS meal in the diet for giant freshwater prawn (*Macrobrachium rosenbergii*) was not more than 50 % of fish meal protein or 17.5 % of diet [5].

The limitation of GAS in aquatic animal diet when incorporated at a high level is the low digestibility coefficient [3,5]. In addition, GAS is easily spoiled. An earlier study by Rattanaporn *et al*. [6] indicated that the lactic fermentation processes is an efficient method for preserving and improving GAS meal quality. They produced the snail silage by incubating minced GAS with locally screened lactic acid bacteria at ambient temperature using molasses as the carbon source for bacterial growth. The results showed that the ensilation of GAS helped improve the quality of the meal by increasing free amino acids, which can readily be used by aquatic animals. The recommended fermentation period was 10 days. The application of ensiled GAS in aquatic animal diet has been reported by Phonekhampheng *et al*. [7]. They replaced fishmeal with ensiled GAS in the diet of African catfish (*Clarias gariepinus*). The growth performance and feed consumption was recorded for a period of 6 weeks. The results indicated that ensiled GAS has a high nutritive value and could be used to completely replace fish meal in African catfish diet.

In this study, a fermentation technique according to Rattanaporn *et al*. [6] was used either to predigest fibrous protein in GAS meat or to preserve the GAS nutritional profile. The fermented golden apple snails (FGAS) were then used as an alternative protein source in diets and the optimum level of FGAS in sex-reversed red tilapia diet was investigated.

Materials and methods

Preparation of minced GAS

GAS was collected from rice fields in Nakhon Si Thammarat Province, Southern Thailand. They were then dipped into boiling water for 2 min. After that, the shells were removed and the meat was then ground using a Hobart mincer. Minced GAS was kept at −20 °C until used.

Preparation of fermentation inoculum

Fermentation inoculum was prepared according to the method of Rattanaporn *et al*. [6]. Local screened lactic acid bacteria (L1/2) cells were obtained from the Biotechnology Laboratory, Walailak University. To prepare a starter culture, a loopful of lactic acid bacteria from a stab of MRS agar was transferred into 10 mL of inoculum medium containing 50 g/L of sucrose, 5 g/L of peptone, 5 g/L of yeast extract, K_2HPO_4 at 5 g/L and $MnSO_4$ at 0.03 g/L and incubated at 37 °C for 24 h. To prepare the fermentation inoculum, the starter culture was transferred to a 90 mL sterile inoculum medium and was incubated at 37 °C for 24 h.

Preparation of FGAS

FGAS was prepared following the method of Rattanaporn *et al*. [6]. Briefly, frozen minced GAS was thawed and then incubated with the fermentation inoculum at 1 kg of minced GAS per 1 L of inoculum medium with the addition of 0.15 L of molasses as a carbon source. The mixture was incubated at ambient temperature (27.0 - 31.0 °C) for 10 days.

Analysis of feed ingredients

The chemical composition of feed ingredients was determined using the AOAC method [8]. The total acidity and pH of minced GAS and FGAS were measured. The pH of the sample was measured using an Orion pH meter model 420 A. Total acidity was measured by titrating against 0.1 N NaOH to a final pH of 8.4 [9]. The composition of feed ingredients is presented in **Table 1**.

Table 1 Proximate analysis of feed ingredients.

Ingredients	Moisture[1] (%)	Protein[1] (%)	Fat[1] (%)	Ash[1] (%)	Fiber[1] (%)
Fish meal	4.70±0.03	63.14±1.12	7.30±0.07	30.50±0.33	0.99±0.07
Shrimp head meal	4.60±0.14	56.95±1.29	4.51±0.11	24.46±0.18	13.18±0.19
Soybean meal	7.41±0.02	44.77±1.91	19.28±1.14	5.76±0.16	4.30±0.20
Palm kernel meal	5.25±0.14	18.27±0.94	12.05±0.10	3.56±1.26	12.56±0.22
Rice bran	8.58±0.02	15.68±0.20	15.07±0.19	12.97±0.22	5.22±0.13
Cassava meal	9.63±0.10	2.54±0.15	0.54±0.04	4.08±0.27	1.30±0.18

[1]mean±SD, n = 3

Preparation of experimental diets

Six practical diets were formulated to contain 30 % protein and 9 % lipid. Diet compositions are presented in **Table 2**. FGAS was used as a fishmeal protein substitute. Chromic oxide was added at 1.0 % of diet as an inert indicator. All ingredients were mixed using a Hobart mixer and then cooked cassava meal was added as a binder. The diet was pelleted using an extruder with a 2 mm diameter die. The extruded pellets were dried at 60 °C overnight, packed and stored at −20 °C until used. Proximate analyses of diets were determined using the AOAC method [8].

Table 2 Composition of experimental diets (g/kg diet).

Ingredient	Diet (Ratio of fish meal protein : FGAS or GAS protein)					
	1(100:0)	2(75:25)	3(50:50)	4(25:75)	5(0:100)	6(50:50)
Fish meal	250	188	125	63	0	125
GAS	0	0	0	0	0	152
FGAS	0	96	192	288	384	0
Soybean meal	280	280	280	280	280	280
Palm kernel meal	150	160	160	160	160	160
Rice bran	100	100	100	100	100	100
Cassava meal	180	136	103	69	36	143
Cr_2O_3	10	10	10	10	10	10
Mineral premix[1]	20	20	20	20	20	20
Vitamin premix[2]	10	10	10	10	10	10

[1]Mineral premix (g/kg diet) : NaCl 0.25; MgO 1.1; KC1 4; $Ca(H_2PO_4)_2$ 9; $FeSO_4$ 0.72; Calcium lactate 0.88; $ZnSO_4.7H_2O$ 0.088; $MnSO_4.7H_2O$ 0.04; $CuSO_4.5H_2O$ 0.088; $CoSO_4$ 0.0002; Kl 0.0008; cellulose 1.183

[2]Vitamin premix (mg/kg diet) : Thiamine (B_1) 10: Riboflavin (B_2) 20; Pyridoxine (B_6) 10; Cobalamin (B_{12}) 2; Retinal (A) 4; Choleccalciferal (D_3) 0.4; Philloquinone (K_1) 80; Folic acid 5; Calcium pantothenate 40; Inositol 400; Niacin 150; Tocopherol (E) 60; Choline 6,000; Ascorbic (C) 500; Cellulose 2.717

Fish rearing and culture conditions

Sex-reversed red tilapia juveniles were obtained from Nakhon Si Thammarat Inland Fisheries Station, Department of Fisheries and acclimatized to laboratory conditions for 2 weeks in 70 L aquaria equipped with continuous aeration and 50 % water discharge daily. Six experimental diets were randomly fed to triplicate groups of fish with an initial weight of 2.73 ± 0.09 g. A hundred grams of fish in the same population were randomly sampled for initial carcass analysis. The diet was fed to satiation twice daily at 9.00 and 16.00 h for 8 weeks. Feed intake and mortality were recorded daily. Fish weight in each experimental unit was measured every 2 weeks for growth data. At the end of the experiment, 6 fish were randomly taken from experimental unit for carcass analysis.

Protein digestibility study

During the last 3 weeks of the experiment, daily faeces collection was done 1 h after the evening meal [10]. Before collection, the tanks were cleaned of uneaten food and 50 % water volume was replaced, then the siphon method was used to collect faeces from the bottom of the tank. In each treatment, collected faeces were pooled and frozen at −20 °C. Pooled samples were dried at 60°C in a hot air oven and blended using a porcelain mortar. Protein content of faeces was determined according to AOAC [8]. Acid digestion was used to determine chromic oxide content in diets and faeces [11]. Apparent protein digestibility coefficients were calculated according to De Silva and Anderson [12].

Water quality measurement

Water temperature was recorded daily and water samples were collected weekly for analysis of dissolved oxygen using a DO meter model WTWOxi 330i/SET, pH using a pH meter model HACH Sension 3, and ammonia and total alkalinity following the method of Boyd and Tucker [13].

Statistical analysis

All analyses were conducted in triplicate and data reported as the mean±SD. Statistical analyses were performed according to Steel and Torrie [14] using one-way analysis of variance, ANOVA (SPSS v.11) with a 5 % level of probability ($p < 0.05$) selected in advance to sufficiently demonstrate a statistically significant difference. Where significant differences were observed, treatment means were differentiated using pairwise multiple comparison procedures (Duncan's new multiple range test).

Results and discussion

Generally, fermentation is a wildly used technique for preserving and improving nutritive values of raw materials before use as protein sources in animal diet such as shrimp waste [15], fish waste [16] or even golden apple snail [6,7]. Various methods were used to produce fermented feed stuff. Fagbenro and Fasakin [17] reported that poultry viscera was preserved by adding 4.5 % (v/w) citric and 0.5 % (v/w) propionic acids for autolysis for 30 days then the product was used as protein source for catfish (*Clarias gariepinus*) up to 30 % of replacement for poultry by product meal without apparent differences in pellet quality or diet acceptability. Moist fermented fish silage prepared using *Lactobacillus plantarum* as a starter culture and fermented under anaerobic conditions at 30 °C for 7 days had highly digestible and suitable protein which could be used as a supplement for tilapia (*Oreochromis niloticus*) [16].

In this study, the fermentation technique according to Rattanaporn *et al*. [6] was applied for preparation of the FGAS before adding into the diet of sex-reversed red tilapia (*Oreochromis niloticus x O. mossambicus*) as a fishmeal protein replacement. FGAS was prepared by incubating minced GAS with locally screened lactic acid bacteria and using molasses as carbon sources. The ingredient mix (1 kg minced snail per 1 L culture medium and 0.15 L of molasses) was incubated at ambient temperature (27.0 - 31.0 °C) for 10 days. The proximate composition of 10 days fermented snail compared with the minced golden apple snail (GAS) is presented in **Table 3**. The moisture content of FGAS was higher than GAS due to the liquid (inoculum medium and molasses) added and the autolysis process during the incubation period. The protein and ash content of FGAS was lower than for the GAS. In addition, the acidity of the FGAS increased which related with the lower pH. The range of pH and acidity of the fermented product corresponded to preparation of lactic acid fermented snail prepared by Rattanaporn *et al*. [6]. From their results, the 10 days incubation period gave the highest free amino acid content in the FGAS.

Table 3 The proximate composition of GAS and FGAS (as fed basis).

Ingredients	Moisture[1] (%)	Protein[1] (%)	Fat[1] (%)	Ash[1] (%)	pH[1]	Acidity[1] (% lactic acid)
GAS	66.01±1.35	49.54±0.40	0.83±0.15	13.98±0.21	8.18±0.05	0
FGAS	75.23±1.72	39.11±0.38	0.75±0.04	3.62±0.03	4.97±0.67	1.47±0.74

[1]mean±SD, n = 3

The feeding trial performed using the GAS meal either the fermented or minced form added to the diet at different levels of fishmeal protein replacement. FGAS was replaced at 25, 50, 75 and 100 % of fishmeal protein while replacing 50 % fishmeal protein with minced GAS was tested. The proximate composition of six experimental diets is presented in **Table 4**. The crude protein and lipid contents of these diets ranged from 30.11 to 34.98 % and 8.78 to 9.59 %, respectively. The average moisture ranged from 0.80 to 2.26 %. Ash content was highest in the control diet (diet 1) which contained the highest amount of fishmeal. Dietary fiber ranged from 4.57 to 5.46 %.

Table 4 Proximate analysis of experimental diets.

Diet[1]	Moisture[2] (%)	Ash[2] (%)	Protein[2] (%)	Lipid[2] (%)	Fiber[2] (%)
1	2.16±0.02	13.61±0.06	30.11±0.34	9.59±0.05	5.46±0.23
2	1.59±0.02	12.52±0.16	31.19±0.15	9.92±0.11	4.57±0.16
3	2.25±0.02	11.48±0.06	31.76±0.53	9.31±0.22	4.97±0.41
4	0.80±0.03	10.76±0.08	32.32±0.10	8.71±0.06	4.93±0.12
5	1.97±0.03	9.18±0.01	32.31±0.33	9.20±0.36	4.85±0.88
6	1.67±0.01	10.99±0.11	34.98±0.04	8.78±0.35	5.23±0.16

[1]Diet 1, control diet; Diet 2, 25 % fishmeal protein replacement with FGAS; Diet 3, 50 % fishmeal protein replacement with FGAS; Diet 4, 75 % fishmeal protein replacement with FGAS; Diet 5, 100 % fishmeal protein replacement with FGAS and Diet 6, 50 % fishmeal protein replacement with minced GAS
[2]mean±SD, n = 3

The average body weight every 2 weeks of the fish in the feeding trial is shown in **Figure 1**. Body weight of fish fed the control diet (diet 1) compared with those fed the snail meal (diet 2 - 6) appeared significantly different ($p < 0.05$) after 4 weeks of study. The results indicated that snail meal could be a growth enhancement. At the end of study, fish fed the diet replacing fishmeal protein with 50 % of protein either as FGAS or minced snail meal had a higher body weight ($p < 0.05$) than those fed the control diet. The body weight of fish fed the diet which totally replaced fishmeal protein with FGAS was not significantly different ($p > 0.05$) with the fish fed control diet.

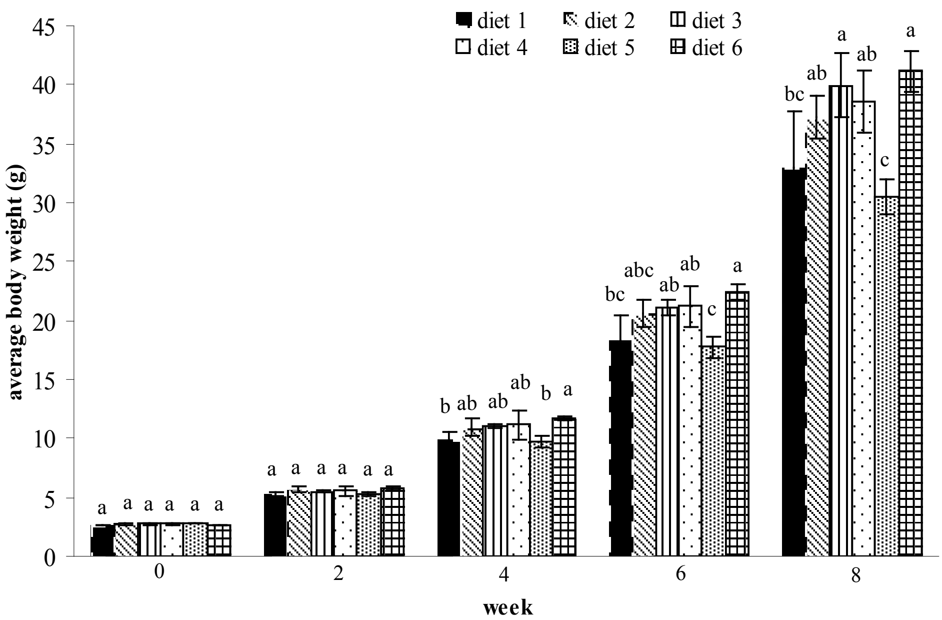

Figure 1 Average body weight of fish fed experimental diets every 2 weeks.
(Diet 1, control diet; Diet 2, 25 % fishmeal protein replacement with FGAS; Diet 3, 50 % fishmeal protein replacement with FGAS; Diet 4, 75 % fishmeal protein replacement with FGAS; Diet 5, 100 % fishmeal protein replacement with FGAS and Diet 6, 50 % fishmeal protein replacement with minced GAS; mean ± SD, n = 3; values not sharing a common superscript are significantly different ($p < 0.05$))

Fish survival rate ranged from 90.74 % to 100 % (**Table 5**). There was no significant difference ($p > 0.05$) between treatments. The substitution of fishmeal with GAS meal either minced or fermented benefited fish growth. The data of growth performance; final weight, weight gain and specific growth rate are presented in **Table 5**. Fish fed diets with 50 % fishmeal replaced with either minced GAS or FGAS showed significantly higher growth performance than those of fish fed the control diet ($p < 0.05$).

Fish fed diets containing snail meal, both minced and fermented, at 50 % fishmeal replacement showed no differences in growth performance. However, the group of fish fed the diet which totally replaced fishmeal protein with FGAS grew less than other groups but similar to those fed the control diet.

Table 5 Initial weight, final weight, weight gain, specific growth rate (SGR) and survival rate of sex-reversed red tilapia fed the experimental diets for 8 weeks.

Diet[1]	Initial weight[2] (g/fish)	Final weight[2] (g/fish)	Weight gain[2] (g/fish)	SGR[2] (%/day)	Survival rate[2] (%)
1	2.68±0.03[a]	33.03±0.66[bc]	30.34±4.63[bc]	4.47±0.24[bc]	100.00±0.00[a]
2	2.74±0.05[a]	37.26±1.81[ab]	34.52±1.85[ab]	4.66±0.12[ab]	90.74±11.56[a]
3	2.74±0.11[a]	39.96±2.70[a]	37.23±2.69[a]	4.79±0.14[a]	98.15±3.21[a]
4	2.78±0.07[a]	38.59±2.58[ab]	35.81±2.54[ab]	4.69±0.10[ab]	94.44±0.00[a]
5	2.79±0.13[a]	30.52±4.10[c]	27.73±3.99[c]	4.26±0.18[c]	98.15±3.21[a]
6	2.66±0.14[a]	41.13±3.96[a]	38.47.73±3.91[a]	4.88±0.16[a]	98.15±3.21[a]

[1]Diet 1, control diet; Diet 2, 25 % fishmeal protein replacement with FGAS; Diet 3, 50 % fishmeal protein replacement with FGAS; Diet 4, 75 % fishmeal protein replacement with FGAS; Diet 5, 100 % fishmeal protein replacement with FGAS and Diet 6, 50 % fishmeal protein replacement with minced GAS
[2]mean±SD, n = 3
Means within each column not sharing a common superscript are significantly different ($p < 0.05$)

Feed efficiency of fish fed different diets is presented in **Table 6**. Feed intake (FI) of fish fed different diets ranged from 31.87 to 39.80 g/fish. The substitution of fishmeal protein with 100 % FGAS protein caused lower FI. Fish fed diets with 50 % fishmeal protein as either minced GAS or FGAS protein showed higher FI than those of fish fed the control diet. However, there was no significant difference in FI among treatments ($p > 0.05$).

Feed conversion ratio (FCR) ranged from 1.02 to 1.19. It was found that the optimal snail meal substitution improved FCR. The results of fish fed the diet containing snail meal (diets 3, 4 and 6) showed a significantly better FCR than fish fed the fishmeal diet ($p < 0.05$). While FCR of the 100 % snail meal diet showed no significant difference with the control diet. For protein efficiency ratio (PER) and productive protein value (PPV) of fish fed different diets, it was found that there were no significant difference in PER and PPV among treatments ($p > 0.05$).

Table 6 Feed intake (Fl), feed conversion ratio (FCR), protein efficiency ratio (PER) and productive protein value (PPV) of sex-reversed red tilapia fed the experimental diets.

Diet[1]	FI[2] (g/fish)	FCR[2]	PER[2]	PPV[2] (%)
1	35.80±3.09[ab]	1.19±0.08[a]	2.80±0.19[a]	41.04±2.88[ab]
2	38.07±3.92[a]	1.10±0.06[abc]	2.92±0.16[a]	43.41±2.29[ab]
3	39.80±2.57[a]	1.07±0.03[bc]	2.94±0.07[a]	43.91±1.08[ab]
4	37.03±2.36[ab]	1.03±0.03[c]	2.99±0.07[a]	45.38±1.13[a]
5	31.87±3.11[ab]	1.15±0.05[ab]	2.68±0.13[a]	39.53±1.88[b]
6	39.02±3.22[a]	1.02±0.02[c]	2.81±0.07[ab]	44.01±1.03[ab]

[1]Diet 1, control diet; Diet 2, 25 % fishmeal protein replacement with FGAS; Diet 3, 50 % fishmeal protein replacement with FGAS; Diet 4, 75 % fishmeal protein replacement with FGAS; Diet 5, 100 % fishmeal protein replacement with FGAS and Diet 6, 50 % fishmeal protein replacement with minced GAS
[2]mean±SD, n = 3
Means within each column not sharing a common superscript are significantly different ($p < 0.05$)

In this study, fish growth performance and feed utilization data indicated that protein from raw and fermented GAS can replace fishmeal in diets for sex-reversed red tilapia. Replacing 50 % fishmeal protein in diet with raw GAS showed benefit in tilapia diet similar with the results found by Norachan *et al*. [3]. They also recommended that using dried GAS in juvenile Nile tilapia (*O. niloticus*) feed should not be more than 47.7 % of fishmeal protein or 12.2 % of feed weight. The fermentation applied to produce FGAS can modify snail protein to make it easy to digest which allows a higher level of GAS in the diets as compared with other studies in fish [3] or shrimp [5]. The fermentation of GAS with lactic acid bacteria with the addition of molasses as a carbon source was successful in stabilizing and preventing it from spoilage and helped improve the quality of snail meal by increasing the free amino acids, which can readily be used by aquatic animals [6].

Predigested snail meal with acid digestion gave free amino acids which may be readily absorbed in the animal intestinal tract. The digestibility study was carried out in the last period of this study and the results are presented in **Table 7** which indicated that the FGAS diet had higher digestibility and protein was better utilized than the control diet ($p < 0.05$). However, increased FGAS amount in diet does not improve protein digestibility with the data showing that no significant difference found in fish fed diets 2 to 5. The highest protein digestibility was found in fish fed diet 6 in which 50 % of the fishmeal was replaced with minced GAS. This was due to higher proportion of predigested protein in the FGAS in the diet.

Table 7 Apparent protein digestibility of the experimental diets.

Diet[1]	Protein digestibility (%)[2]
1	84.42 ± 0.02^{c}
2	88.42 ± 0.03^{ab}
3	87.46 ± 1.88^{b}
4	87.50 ± 0.70^{b}
5	87.59 ± 1.54^{b}
6	90.10 ± 1.01^{a}

[1]Diet 1, control diet; Diet 2, 25 % fishmeal protein replacement with FGAS; Diet 3, 50 % fishmeal protein replacement with FGAS; Diet 4, 75 % fishmeal protein replacement with FGAS; Diet 5, 100 % fishmeal protein replacement with FGAS and Diet 6, 50 % fishmeal protein replacement with minced GAS
[2]mean±SD, n = 3
Means within each column not sharing a common superscript are significantly different ($p < 0.05$)

Water quality measured during the feeding trial indicated that the mean value of any parameter was in the acceptable range. Daily temperature ranged from 28.29 to 28.52 °C, pH ranged from 6.43 to 6.53. The average alkalinity, and dissolved oxygen was 25.20 ppm and 5.60 ppm, respectively. Although the total ammonium reached 2.37 ppm the low water pH caused a less toxic ammonium form.

Conclusions

It was concluded that FGAS is a potential protein source in sex-reversed red tilapia diets. FGAS can be used to replace fishmeal in diets up to 100 % however, 75 % is recommended. In addition, replacing fishmeal protein with GAS protein at 50 % is also successful.

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