# The effect of diets suplemented with different natural foods on growth and feed utilization of snakehead (*Channa striata*)

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**Abstract**. A sixty day feeding study was performed to determine the impact of diets enriched with various natural foods on growth and feed utilization of snakehead. Six hundred fingerlings weighing 4.33 to 4.71 g each fish were randomly stocked into 4 triplicate plastic tanks (1x1x1.5 m), fifty fish each tank. Four isoproteic and isoenergetic diets containing 45% crude protein and 18.5 KJ $g^{-1}$gross energy were formulated. Control diet was formulated using fishmeal, salted trash fish, tofu by-product meal, rice bran, vitamin and mineral mix. The three diets were prepared with the same ingredients as control diet but were supplemented with 15% fresh earth worm (W), golden snail (S) and frog (F), respectively. The diets were fed to the fish at 6% body weight, twice daily for 60 days. Feeding the fish with diet F and S did not influence fish survival rate, weight gain, specific growth rate, feed intake, feed efficiency ratio, protein efficiency ratio and protein retention. However, feeding the fish with diet W increased weight gain and feed intake. It can be concluded that the supplementation of snakehead diet with fresh worm can improve growth performance and feed intake by the fish.

## 1 Introduction

Snakehead (*Channa striatus,* Bloch, 1793) is a freshwater food fish, which are usually found in streams , lakes, oxbow, , rice fields, agricultural canal, swamps and marshes [1,2, 3, 4]. It is a carnivorous species, which consumes frogs, fish, insects, tadpole, snail and earthworms [5, 6, 7]. The fish is capable of breathing atmospheric air, so that it can tolerate the waters of low dissolved oxygen and high ammonia concentration [6, 8, 9, 10]. The fish is known for its good flesh quality with delicate taste [11; 12; 13], and its flesh extract is believed to contain pharmaceutical compounds that are usefull for post-operative wound healing [12], anti-inflammation [14; 15; 16], anti-hypertension [17], and anti-hyperglicemia [18;19]. For these reasons, snakehead is a desirable aquaculture species.

The demand of the fish is increasing particularly in the health supplement industries,

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which use the fish as a raw material for their products [19]. The bulk of fish production comes from the wild, however, the supply is now decreasing due to overfishing and environmental destruction [20; 21]. It is envisioned that the future supply of this fish, will rely significantly on aquaculture. Farming of this species has been practiced in Riau. Seed supply is available, and the fish farmer feeds the growing fish with natural food, including golden snail, frog and earthworm [7, 22; 23]. Some researchers reported that total substitution of natural food by artificial feed reduced growth, feed utization and biochemical composition of the muscle [19;24]. Dietary mixture of natural and artificial feed therefore needs to be investigated in order to assess whether this would result in the improvement of growth and biochemical composition of the fish.

Snakehead naturally feeds on frog, earth worm and snail; thus, supplementation of these food items in the diet may improve odor and palatability of the diets. This is in turn improves growth and feed utilization. This research therefore was to investigate the impacts of diets fortified with fresh frog, snail and earth worm on growth and feed utilization of snakehead.

## 2 Materials and Methods

### 2.1. Ingredients and diet formulation

Fishmeal, salted trash fish meal, tofu by product meal, rice bran, vitamin and mineral mix were purchased from a local feedstuff supplier. All ingredients were finely ground and analyzed for proximate composition. Natural food including fresh frog (*Fejervarya cancrivora*), golden snail (*Pomacea sp*.) and earthworm (*Lumbricus rubellus*) were purchased from local farmer in Kampar district. These were kept frozen before being used in the dietary formulation.

Four experimental diets were prepared to compose 45% crude protein and 18.5 KJ $g^{-1}$ gross energy as the optimum dietary protein and energy for snakehead [25]. Diet C (control) was formulated using fishmeal, salted trash fish meal, tofu by product meal, rice bran, vitamin and mineral mix. Diets F, S and W were formulated diets supplemented with 15% fresh frog, golden snail and earthworm, respectively (Table 1). The diets were made into dry pellets (3 mm in diameter) using a pelleting machine; and the pellets were analyzed for proximate and amino acid composition. Pelleted diets were stored in the refrigerator (5$^{o}$-10$^{o}$C) until fed to the fish.

### 2.2. Feeding trials

Seven hundred snakehead fingerlings, weighing 4.33-4.71 g each fish were purchased from private hatchery in Pekanbaru. The fish were acclimatized in plastic lined tanks and fed commercial diet for 2 weeks before feeding trial. Ten fishes were randomly collected for initial body proximate and amino acid analysis. Four groups of fifty fishes were weighed and randomly distributed to each of the four triplicate plastic lined tanks (1 x 1 x 1.5 m, water depth 80 cm). Triplicate tank was randomly allocated to each diet, and feeding was performed at 6% of fish body weight (26), twice daily at 08.00 and 16.00 for 60 days. Uneaten feed that remained in the bottom of the tanks was siphoned out, 25% of the water was changed and water quality was monitored every 3 days.

**Table 1.** Diet formulation and analysis

| Ingredients | Diet (%) | | | |
|---|---|---|---|---|
| | C | F | S | W |
| Fishmeal | 41 | 27 | 28 | 27 |
| Salted trash fish | 24 | 22 | 24 | 22 |
| Earth worm | - | 15 | - | - |
| Golden snail | - | - | 15 | - |
| Frog | - | - | - | 15 |
| Tofu by-product | 17 | 17 | 16 | 17 |
| Rice bran | 17 | 18 | 16 | 18 |
| Vitamin and mineral mix[a] | 1 | 1 | 1 | 1 |
| **Proximate composition by analysis (%)** | | | | |
| Crude protein | 45.36±0.18 | 45.31±0.09 | 45.37±0.16 | 45.12±0.15 |
| Crude lipid | 6.69±0.26 | 6.79±0.23 | 6.74±0.23 | 6.89±0.23 |
| Ash | 15.23±0.18[a] | 13.70±0.13[b] | 13.52±0.18[b] | 15.08±0,30[a] |
| NFE | 32.72±0.22[a] | 34.20±1.32[b] | 34.37±0.26[b] | 32.91±0.21[a] |
| Gross Energy (KJ $g^{-1}$)[b] | 18.81±0.06 | 18.81±0.16 | 18.97±0.05 | 18.75±0.05 |

[a]Hasan et al., 2022

[b]Gross energy was estimated as 16.7 KJ $g^{-1}$ protein, 16.7 KJ $g^{-1}$ carbohydrate and 37.7 KJ $g^{-1}$ lipid.

Values (triplicate±S.D) in the same row marked with the same superscript are not significantly different (P>0.05).

## 2.3. Growth parameters

All fish were weighed at the end of feeding trial, and five fishes each tank were randomly taken and kept in the freezer (-18°C) for analysis of proximate and amino acid composition. Zootechnical parameters were assessed including survival rate, weight gain, specific growth rate, feed intake, feed efficiency ratio, protein efficiency ratio and protein retention. The values were calculated by the following formulae:

Survival rate (SR, %) = 100 × (Number of fish at final/ number of fish at initial)

Weight gain (WG, g) = Life weight of fish at the beginning – life weight of fish at the end of experiment

Specific growth rate (SGR, %/day) = 100 × [Ln (Fish weight at the beginning) – Ln (Fish weight at the end)]/days

Feed intake (FI, %/day) =100 × total feed consumed/[(Fish weight at initial + fish weight at final)/2]/days

Feed efficiency ratio (FER) = Gain in wet weight (g)/total feed consumed (g)

Protein efficiency ratio (PER) = Gain in wet weight (g)/total protein consumed (g)

Protein retention (PR, %) =100 × Gain in wet body protein (g)/protein consumed (g)

Proximate analyses were carried out following standard methods [27]. Determination of moisture was made after oven-drying the sample at 105°C for 24 h until its weight became constant. Determination of ash was conducted after muffle-incinerating the sample at 500°C for 5 h. Micro-Kjeldahl procedure was used for crude protein analysis, and the value was calculated as N × 6.25. Determination of crude lipid was performed by Soxhlet-extracting

the sample with petroleum ether. The NFE value was estimated by difference: 100 – (crude protein + crude lipid + ash + moisture) [28]. Amino acid analysis was performed by HPLC (Waters, USA) using Pico-tag method following Cohen [29]. The amino acid determination was performed with acid hydrolysis using 6 N HCl at 110$^{o}$C for 24 h; and amino acid values were estimated in g 100 g$^{-1}$ sample.

### 2.4. Statistical analysis

Completely randomized design with four treatments, and each treatment with three replicates was applied for this experiment. One-way Analysis of Variance (ANOVA) was used for data analyses, and Duncan Multiple Range Test at a significant value of 95% ($P < 0.05$) was employed to indicate the difference among the treatment means. The data analyses were performed by SPSS software, version 17 [30].

## 3 Results and Discussion

Feed ingredients, formulation and proximate composition of the experimental diet were presented in Table 1. All diets were prepared to contain 45% protein and 18.5 KJ g$^{-1}$gross energy. Dietary crude protein, lipid and energy by analysis were similar among the experimental diets, but there was a small variance in ash and nitrogen free extract (NFE), where ash was lower and NFE was higher in diets F and S compared with diets C and W. Amino acid profiles (Table 2) varied slightly among the experimental diets. The highest total amino acid was shown by diet supplemented with fresh earthworm (W), and followed by diet C, S and F. The individual essential amino acids, specifically methionine; lysine and threonine were also parallel to the total amino acids profiles of the diets. Compared to the fish body, both total and individual essential amino acids were higher in all experimental diets than that of the fish body. These indicate that the quantity and quality of the essential amino acids of the experimental diets fulfill the optimum amino acids required by the fish, because the amino acid profile of the fish body is considered as an indicator of the amino acid balance in the fish diets [31, 32, 33, 34]. Temperature, dissolved oxygen (DO) and pH values of the waters during the experiment were 25.90-29.80$^{0}$C; 4.20-5.80 ppm and 6.4-7.36 respectively. Survival rates (SR) in all treatments ranged 88.3% to 95.83%, and the values were similar among all dietary treatments. The water quality values in this study were acceptable and supported a high survival rate and good growth of the fish.

Supplementation of the diet with fresh frog (F) and golden snail (S) did not influence growth performances compared with the control diet (C). However, feeding the fish with diet containing fresh earthworm (W) obtained significantly higher weight gain (WG) than the fish fed diet C. FER, PER and PR were not influenced by the experimental diets, but feed intake (FI) followed the same pattern as WG, indicating that the higher WG was contributed by high FI. Feed intake usually correlates to palatability of the diet [35], and high FI in earthworm supplemented diet in this study was probably due to better palatability. Fresh earthworm might act as natural feeding stimulant which gave strong odor and good flavor to the diet. It was reported that snakehead highly responded to feeds with strong smell [36]. The FI of fish has been known to correlate with the amount and the kind of dietary free amino acids [35, 37]. In this study, the amount of free amino acids such as aspartic acid, glutamic acid, serine, alanine, glycine, threonine which significantly contributed to the palatability of the diets [38], were higher in the earthworm supplemented diet than the other diets. These free amino acid may give palatability to the diet, in turn increase feed intake.

**Table 2.** Amino acid profiles of the experimental diets and snakehead (g 100 g−1sample**)**

| Amino acids | Snakehead | **Diets** | | | |
|---|---|---|---|---|---|
| | | C | F | S | W |

| | | | | | |
|---|---|---|---|---|---|
| Non-essential amino acid | | | | | |
| Aspartic acid | 2.89 | 3.78 | 3.22 | 3.56 | 3.87 |
| Glutamic acid | 4.22 | 5.57 | 5.17 | 5.34 | 5.85 |
| Serine | 1.20 | 2.01 | 1.55 | 1.88 | 1.94 |
| Glycine | 1.73 | 2.25 | 2.19 | 2.15 | 2.36 |
| Arginine | 1.28 | 1.77 | 1.37 | 1.56 | 1.86 |
| Proline | 1.52 | 2.18 | 1.82 | 1.93 | 2.31 |
| Tyrosine | 0.45 | 0.96 | 0.76 | 0.85 | 0.93 |
| Cysteine | 0.67 | 0.96 | 0.80 | 0.88 | 1.05 |
| Sub Total | 13.96 | 19.48 | 16.88 | 18.15 | 20.17 |
| Essential amino acid | | | | | |
| Histidine | 0.56 | 0.91 | 0.65 | 0.78 | 0.87 |
| Threonine | 0.87 | 1.13 | 1.08 | 1.09 | 1.22 |
| Alanine | 1.01 | 1.52 | 1.35 | 1.40 | 1.63 |
| Valine | 1.10 | 1.63 | 1.38 | 1.50 | 1.75 |
| Methionine | 0.60 | 0.84 | 0.65 | 0.72 | 0.87 |
| Isoleucine | 0.94 | 1.35 | 1.05 | 1.28 | 1.41 |
| Leucine | 1.34 | 3.04 | 2.60 | 2.87 | 3.14 |
| Phenylalanine | 0.86 | 1.16 | 1.04 | 1.12 | 1.20 |
| Lysine | 1.12 | 1.75 | 1.58 | 1.70 | 1.86 |
| Sub Total | 8.4 | 13.33 | 11.38 | 12.46 | 13.95 |
| Total | 22.36 | 32.81 | 28.26 | 30.61 | 34.12 |

The fact that the protein retention values were not significantly influenced by the experimental diets is an indication that all experimental diets had balanced profiles in their essential amino acids. Protein retention values usually depend on the quality of dietary essential amino acid profile [31, 32, 39, 40, 41], and the essential amino acid profile of the experimental diets in this study, especially lysine, methionine and threonine were balanced, as indicated by their profiles which were even higher than the essential amino acid profile of the snakehead. Therefore, the dietary nutrients were utilized efficiently, which in turn resulted in better protein retention.

**Table 3.** Growth performance of snakehead fed experimental diets for 60 days

| **Parameter** | **Diets** | | | |
|---|---|---|---|---|
| | **C** | **F** | **S** | **W** |
| Initial weight | 4.71±0.22 | 4.41±0.21 | 4.33±0.25 | 4.60±0.15 |
| Final weight | 13.98±1.38$^{a}$ | 14.67±0.57$^{a}$ | 13.66±0.25$^{a}$ | 15.36±0.15$^{b}$ |
| Survival rate | 88.33±3.12 | 89.17±8.25 | 88.38±5.89 | 95.83±2.36 |
| Weight gain | 8.93±1.22$^{a}$ | 9.19±0.37$^{a}$ | 9.33±0.19$^{ab}$ | 10.76±0.06$^{b}$ |
| Specific growth rate | 1.77±0.20 | 1.88±0.07 | 1.92±0.08 | 2.01±0.04 |
| Feed intake | 5.14±0.23$^{a}$ | 5.22±0.11$^{a}$ | 5.34±0.17$^{ab}$ | 5.62±0.23$^{b}$ |

| | | | | |
|---|---|---|---|---|
| Feed efficiency ratio | 4.05±0.53 | 3.83±0.23 | 3.84±0.19 | 3.85±0.16 |
| Protein efficiency ratio | 0.55±0.06 | 0.58±0.03 | 0.58±0.03 | 0.58±0.02 |
| Protein retention | 34.23±4.14 | 35.73±2.00 | 35.77±1.88 | 35.51±1.49 |

Values (triplicate±S.D) in the same row marked with the same superscript are not significantly different (P>0.05)

## 4 Conclusion

Supplementation of fresh earthworm in snakehead diet increased growth and feed intake, but inclusion of frog and golden snail in the diets did not affect any performance of the fish as compared with the control diet. Therefore, fresh earthworm may be supplemented in snakehead diets to improve growth and diet acceptability by the fish, which in turn reduces feed dependence on natural food.

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