

The Effect of Feeding Frequency with Different Levels of Protein and Carbohydrates on Growth, Survival Rate and Albumin Content of Snakehead Fish (*Channa striata*)

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Abstract:- The purpose of this study were to evaluate the frequency of feeding with various protein (P) - carbohydrate (C) content in feed which produces the best growth, survival and albumin content of snakehead fish (*Channa striata*). A factorial pattern with completely randomized basic design and two factors were used. The first was three different daily feeding frequencies, namely 2 times (F2), 4 times (F4) and 6 times (F6) while the second was the different protein-carbohydrate levels, namely P 35% - C 35% , P 40% - C 30%, and P 45% - C 25%. The obtained data was analyzed by analysis of variance. The results showed that the treatments produced the similar responses in survival rate of snakehead fish. The interaction between the differences in frequency of feeding and the different protein – carbohydrate levels had significant effect with $p < 0.05$ on individual growth and albumin content of snakehead fish. Average individual growth in the treatments P 35% - C 35% - F4, P 35% - C 35% - F6, P 40% - C 30% - F4, P 40% - C 30% - F6, P 45% - C 25% - F4 and P 45% - C 25% - F6 was not significantly different but it was higher and significantly different from other treatments. The highest albumin content was observed in the P 45% - C 25% - F6 treatment, but it was not significantly different compared to the P 35% - C 35% - F6, P 40% - C 30% - F6, P 45% - C 25% - F2 and P 45% - C 25% - F4. Based on the growth parameters, survival rate and albumin content, feed containing P 35% - C 35% with a feeding frequency of 4 times per day can be applied in rearing of snakehead.

Keywords: albumin, feeding frequency, growth, snakehead fish, protein- carbohydrate, survival rate.

1. Introduction

An Snakehead fish (*Channa striata*) is a freshwater fish that has high economic value (Mustafa *et al.*, 2012). This fish has very high albumin content, which is used as raw material for the pharmaceutical industry (Srivastava *et al.*, 2011). Albumin as a biomedical material can speed up the process of healing wounds after surgery. Application of snakehead extract to post-operative patients accelerates the process of tissue formation (Maryanto, 2004). The albumin content in snakehead fish is around 62.24 g per kg, while chicken eggs only contain 9.34 g per kg (Mustafa *et al.*, 2012). According to Mudjiharto (2007), snakehead albumin powder is more useful for healing white Wistar (*Rattus Copernicus*) wounds compared to albumin powder obtained from goldfish (*Cyprinus carpio*) and milkfish (*Chanos chanos*)

Common feed used by snakehead fish farmers, especially those whose seeds come from wild catches, is fresh feed, however fresh feed has several obstacles, including continuity of availability, unstable nutritional content and impractical provision, Based on this problems, therefore it is necessary replaced by artificial feed. Haryati *et al* (2021) has shown that snakehead fish can be reared with 100% artificial feed. The research also showed that

differences in the level of substitution of fresh feed, mackerel fish with artificial feed, gave the same response to growth, survival and albumin content of snakehead fish (*Channa striata*). The albumin content of snakehead fish at the beginning of the study was 16.36 mg per g of fish, while at the end of the study the albumin content of snakehead fish fed by 100% fresh mackerel fish, averaged 26.87 mg per g of fish, while those given 100% artificial feed averaged 20.20 mg per g. The albumin content produced in this study was relatively higher than the research found by Kusumaningrum *et al* (2014), where snakehead fish fed commercial feed with protein content of 32%, 29% and 26% produced average albumin content of 17.3 mg per g, 16.9 mg per g and 15.9 mg per g of fish.

Protein is the largest component in fish feed and is the most expensive among other feed ingredients. The results of research by Kpogue *et al* (2013) on fingerling snakehead (*Prachanna obscura*) showed that fish fed with a protein content of 50% produced the best growth and nutritional utilization compared to feed with a protein content of 30%, 40% and 60%. Study by Sagada *et al* (2017) on juvenile northern snakehead (*Channa argus*) showed that feed with a protein content of 45% and fat 9% produced the lowest growth response. Feed with 45% protein and 12% fat, 48% protein and 15% fat, 51% protein and 9% fat and 51% protein and 12% fat produces higher growth. However, using too much protein will actually cause high costs feed and resulting waste that can reduce the water quality. The results of research by Koshio *et al* (1993) on juvenile kuruma prawns showed that juvenile kuruma prawns fed by feed containing 21.0%, 31.4%, 41.6% and 50.3% protein, secreted ammonia-N of 31.2; 61.7; 102.3 and 114.8 ug/g/hour, respectively. Therefore, the protein content in feed has to be precisely calculate according to the needs of the species. Protein is optimized only for growth, while energy needs are met from other sources, including carbohydrates. (protein-sparing effect by carbohydrates) which is cheaper. However, ability of snakehead fish to utilize carbohydrates is limited.

One way to increase the ability of snakehead fish to utilize carbohydrates is to increase the frequency of feeding. Higher feeding frequency may increase the ability of the fish to digest and metabolize carbohydrates. Study by Zainuddin *et al* (2014) on vanamei shrimp showed that feeding frequency of 4 times per day can increase the ability to utilize carbohydrates by up to 40.1%. Apart from being environmentally friendly, carbohydrates are also cheaper

This study aims to evaluate the feeding frequency that can increase the ability of snakehead fish to utilize carbohydrates in terms of growth, survival rate and albumin content consequently environmentally friendly snakehead fish rearing can be achieved.

2. Materials And Methods

2.1. Time and Place of the Study.

This research was conducted from June to September 2023. The research was carried out at the Freshwater Fish Seed Center, Maros South Sulawesi. Feed production was carried out at the Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE) Maros. Proximate analysis of feed was carried out at the Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University. Analysis of feed amino acid composition was carried out at the Integrated Laboratory of the Agricultural Institute, Bogor, while analysis of albumin content was carried out at the Makassar Health Laboratory Center.

2.2. Tools and Materials

2.2.1. The Test Fish.

The test animals used in this study were snakehead fish with the fish length was 3 – 4 cm, the average weight was 0,25 g per head and the stocking density was 15 seeds per acuarium. Firstly, the fish seeds were acclimatized in the environment and the feed adaptation was carried out during 3 days before the treatnents

2.2.2. Research container.

The container used in the research were aquarium with 27 taps 70 x 50 x 50 cm.

2.2.3. The experimental diets.

The feed used was crumble – shaped and the ingredient composition is presented in Table 1, while the proximate analyses results of each treatment is presented in Table 2.

Table 1. Ingredient composition of experimental diets

Ingredient composition	P35%-C 35%	Treatments	
		P 40%-C 30%	P45%-C 25%
Fish flour	25	35	45
Shrimp head flour	6	6	6
Soybean meal	20	20	20
Corn starch	10	10	10
Azole flour	10	10	10
Bran flour	25	15	5
Fish oil	1	1	1
Vitamin – mineral mix	3	3	3

Table 2. Proximate composition of experimental diets

Nutritional composition (%)	P 35% - C 35%	Treatments	
		P 40% - C 30%	P 45% - C 25%
Crude protein	36.17	39.92	44.83
Lipid	11.13	10.92	10.05
Nitrogen Free Extract (NFE)	32.84	27.52	21.71
Crude fiber	3.41	3.44	3.18
Ash	16.44	18.20	20.24
Gross Energy (Kkal/kg)	4,4125	4,2656	4.0344

Remarks 1. Except for water, all fractions are expressed in dry mater

2.3. Experimental design.

This study was conducted using a factorial completely randomized design with two factors and three replications.

The first factor was the feeding frequency, respectively:

(F2) feeding frequency of twice per day

(F4) feeding frequency of four times per day and

(F6) feeding frequency of six times per day.

The second factor was the level of protein (P) - carbohydrates (C) in the feed were:

Feed with the level 35% protein and 35% carbohydrate (P 35% -C 35%)

Feed with the level 40% protein and 30% carbohydrate (P40% - C 30%) and

Feed with the level 45% protein and 25% carbohydrate (P 45% - C 25%)

Fish were fed 10% biomass per day. Frequency of feeding 2 times per day at 07.00 and 17.00, 4 times per day at 07.00, 10.30, 14.00 and 17.00, 6 times per day at 07.00, 09.00, 11.00, 13.00, 15.00 and 17.00

2.4. The Observed Variables

2.4.1. Feed Quality. Feed quality was evaluated based on the amino acid composition.

2.4.2. Average individual weight growth (g) = $W_t - W_0$

Where: W_t = average individual weight at the end of the study (g)

W_0 = average individual weight at the start of the study (g)

2.4.3. Survival Rate (%)

The survival rate (SR) = $N_t / N_0 \times 100$

Where N_0 = number of fish at the start of the study

N_t = number of fish at the end of the study

2.4.4. Albumin content

Albumin content analysis of snakehead fish albumin levels was carried out at the beginning and end of the study.

2.5. Data Analysis.

To evaluate the effect of treatment on growth, survival rate and albumin content in fish was used analysis of variance. If the analysis proved that significant treatment followed by W -Tukey test to determine which treatment produced the best response The amino acid content of the feed was analyzed descriptively based on the needs of snakehead fish

3. Results And Discussion

3.1. Results

3.1.1. Amino Acid Composition of Feed and Snakehead Fish Requirement

The amino acids composition of the feed and amino acid requirement of snakehead fish is presented in Table 3.

Table 3. Amino acid composition of feed (% in feed) and in snakehead fish

Amino Acid	P 35%– C 35%	P 40% - C 30%	P 45% - C 25%	*Snakehead fish requirement (% in feed)
Non essential Amino Acid				
Aspartic	3,02	3,23	3,48	1,79
Glutamic acid	5,14	5,55	5,83	2,85
Serine	1,22	1,28	1,36	0,58
Glisine	1,76	1,92	2,12	0,71
Alanine	1,80	1,99	2,20	1,14
Tirosine	0,83	0,88	0,97	0,67
Essential amino Acid				
Histidine	0,75	0,79	0,85	0,40
Threonine	1,21	1,31	1,43	0,83
Arginine	1,55	1,61	1,67	1,26
Methionine	0,55	0,61	0,73	0,67
Valine	1,53	1,70	1,79	0,97
Fenilalanine	1,47	1,60	1,69	0,81
Isoleusine	1,43	1,59	1,67	0,95

Amino Acid	P 35% - C 35%	P 40% - C 30%	P 45% - C 25%	*Snakehead fish requirement (% in feed)
Leusine	2,43	2,61	2,89	1,58
Lysine	2,13	2,36	2,58	0,93
Tryptophan	0,47	0,47	0,66	0.13
Total amino acid	27,3	29,5	31,92	

Note: * : Evi Fitriyani, Nani Naraenah, Ika Meidy Deviarni (2020)

3.1.2. Average Individual Weight Growth and Survival Rate of Snakehead Fish.

Average individual weight growth and survival rate of snakehead fish was presented in Table 4

Table 4. Average individual weight growth and survival rate of snakehead fish

Treatments	Average individual weight growth (g)	Survival rate (%)
P35% - C35% - F2	0,19±0,0019 ^a	59,99±11,547 ^a
P35% - C35% - F4	0,38±0,0019 ^c	57,77±3,851 ^a
P35% - C35% - F6	0,44±0,0241 ^c	60,00±3,851 ^a
P40% - C30% - F2	0,28±0,0224 ^b	62,22±3,851 ^a
P40% - C30% - F4	0,39±0,0006 ^c	57,77±3,851 ^a
P 40% - C30% -F6	0,40±0,0014 ^c	57,78±7,702 ^a
P45% - C25% - F2	0,31±0,0024 ^b	62,22±3,851 ^a
P 45% - C25% - F4	0,37±0,0086 ^c	57,78±10,18 ^a
P45% - C25% - F6	0,42±0,0073 ^c	62,22±16,775 ^a

Note: the same letter in the same column indicates that they are not different ($P > 0.05$)

The results of the analysis of variance showed that the differences in protein-carbohydrate levels of feed and the frequency of feeding as well as the interaction between the two had a significant effect on the average individual growth of snakehead fish ($P < 0.05$) but did not have a significant effect on the survival rate of snakehead fish ($P > 0.05$).

The results of the W-Tukey test showed that the average growth of individual snakehead fish in the treatment with 35% protein content, 35% carbohydrates, feeding frequency 2 times per day (P35% – C 35% - F2) was the lowest and was significantly different from the other treatments. . Individual growth in the P40% - C 30% - F2 and P 45% – C25% - F2 treatments was not significantly different but was significantly different from the other treatments. Average individual growth in treatments 35% P – 35% C - F4, P 35% - C 35% - F6, P40% – C 30% - F4, P40% – C 30% -F6, P45% - C25% - F4 and P 45% – C 25% - F6 was not significantly different but was higher and significantly different from other treatments. The interaction between protein-carbohydrate levels and feeding frequency on growth can be seen in Figure1

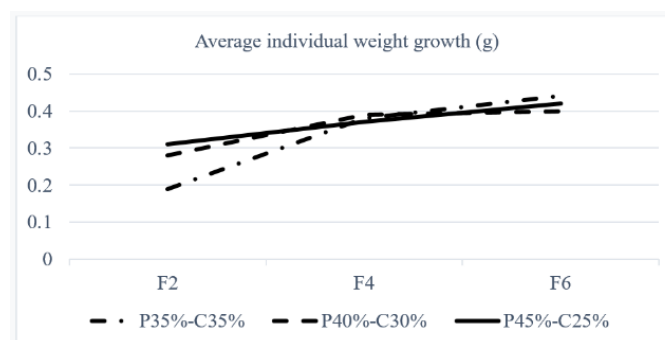


Figure 1. The interaction between protein-carbohydrate levels and feeding frequency on growth

Differences in protein - carbohydrate content of feed and frequency of feeding did not have a significant effect ($P>0.05$) on the survival rate of snakehead fish. The results of this research indicate that the feed consumed was sufficient for the maintenance process

The survival rate in this study was higher than the results of the study by Haryati et al (2019), which ranged from 40.83 - 57.5%

3.1.3. Albumin content of snakehead fish.

The average albumin content of snakehead fish at the beginning and end of the study was presented in Table 5

Table 5. Average albumin content of snakehead fish at the beginning and end of the study

Treatments	Albumin content (mg per g of fish)
Beginning of the research	8,95
End of the research	
P35% - C35% - F2	9,85±0,577 ^a
P35% - C35% - F4	11,33±0,839 ^b
P35% - C35% - F6	11,71±1,007 ^{bc}
P40% - C30% - F2	11,01±0,990 ^b
P40% - C30% - F4	11,44±1,155 ^{bc}
P 40% - C30% - F6	11,40±1,400 ^{bc}
P 45% - C25% - F2	12,19±0,595 ^c
P 45% - C25% - F4	12,22±0,430 ^c
P45% - C25% - F6	12,38±0,457 ^c

Note: the same letter in the same column indicates that they are not different ($P>0.05$)

The results of data analysis showed that the protein-carbohydrate content of the feed, the frequency of feeding and the interaction between the protein-carbohydrate content of the feed and the frequency of feeding had an effect on the albumin content. The albumin content was lowest in the P35%- C 35%-F2 treatment and significantly different from other treatments . The highest albumin content was in the P45%-C25%-F6 treatment but was not significantly different from the P35%-C35%-F6, P40%-C30% F4, P40% -C30%-F6, P45%-C25%-F2 and P45%-C25%-F4 treatments.. The interaction between protein-carbohydrate levels and feeding frequency on the albumin content of snakehead fish can be seen in Figure 2.

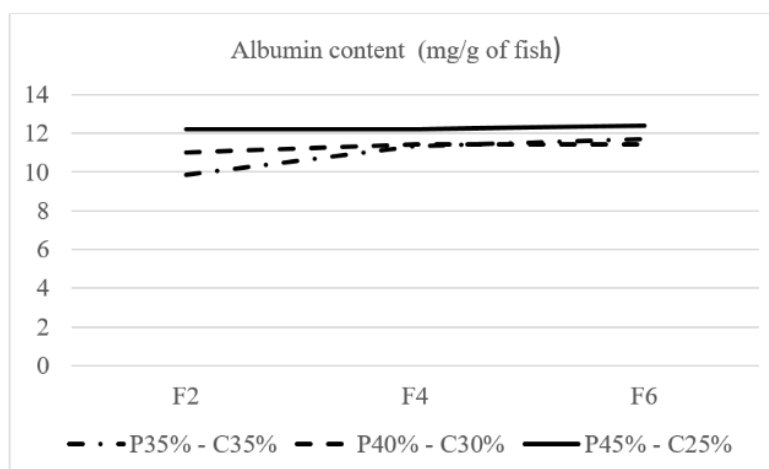


Figure 2. The interaction between protein-carbohydrate levels and feeding frequency on albumin content of snakehead fish

4. Discussion

Protein quality correlates with essential amino acids. Amino acid imbalance will result in low availability of one or more essential amino acids in the feed, along with low protein retention and high ammonia excretion. The ten essential amino acids in feed P45% - C 25% was higher than snakehead fish needs. Only the amino acid methionine in feed P 35% - C 35% (0.55%) and feed P 40% - C 30% (0.61%) was lower than snakehead fish needs (0.67%). According to Borlongan (1992) methionine deficiency can be replaced by the amino acid cystine. The replacement value of cystine for methionine is around 50%. Based on the opinion of Borlongan (1992), methionine deficiency in treatments P 35% - C 35% and P 40% - C 30% can be replaced by the amino acid cystine.

The average individual growth of snakehead fish in the P35% - C35% - F2 treatment was the lowest and different from other treatments. Average individual growth in treatments P35% - C35% - F4, P35% - C35% - F6, P40% - C30% - F4, P40% - C30% - F6, P45% - C25% - F4 and P45% - C25% - F6 was not different but was higher and different from other treatments. This is caused by the digestibility of the feed, the research results of Haryati *et al* (2023) showed that the digestibility of snakehead fish fed with a protein content of 35%, carbohydrates 35%, frequency of feeding 2 times per day was the lowest and different from other treatments. The lowest carbohydrate digestibility was in the P45- C25 - F2 treatment but was not different from the P35% -C35% - F2 and P40% - C30%-F2 treatments but was different from the other treatments. The highest carbohydrate digestibility was in the P35% - C35%-F6 treatment but was not different from the P45 - C25 -F6, P45% -C25%-F4, P40% -C30%-F6 and P35%-C35%-F4 treatments but different from other treatments. The higher the digestibility of feed, the nutrients and energy that can be used for growth also increase.

Research on the effect of feeding frequency on carbohydrate utilization was carried out by Tung and Shiau (1991) on tilapia fish. The results of this research showed that fish fed with a carbohydrate content of 44% with a feeding frequency of six times per day produced better weight gain, feeding efficiency, protein deposition and energy than those fed twice per day. Research on rainbow trout, which was a carnivorous fish, also produces the same pattern. The specific growth rate, total amount of feed consumed, feed efficiency and protein and energy retention in fish fed continuously were better than those fed twice per day (Hung and Storebakken, 1994). The limited ability of fish to utilize carbohydrates was caused by low digestibility and low plasma glucose regulation (Hung and Storebakken, 1994). The low regulation of plasma glucose was thought to be caused by insulin deficiency (Hung and Storebakken, 1994). Enzymes that play a role in glucose metabolism may also be influenced by the frequency of feeding which in turn will affect the fish's ability to utilize carbohydrates. By being able to use carbohydrates as an energy source, the protein contained in the feed can be used for growth

The albumin content increased at the end of the study. The albumin content in this study was highest in the P 45%- C 25% - F6 treatment but was not different compared to P35% - C35% - F6, P40% - C30% - F6, P45% - C25% - F2 and P45% - C25% - F4. The lowest albumin content was in the P35% - C35% - F2 treatment. The average individual weight growth in this treatment was also the lowest. The high albumin content in this treatment is because the digestibility of protein and carbohydrates in this treatment is also high, the energy needed for metabolic processes can be supplied from carbohydrates, so that more protein is used for growth. High growth will also produce a high albumin content . The results of research by Haryati *et al* (2021), show that the albumin content of snakehead fish has an average individual weight of 0.8 g, which was 20.20 mg per g of fish, while the average individual weight of 1.78 g was 29.66 mg per g of fish. The results of Jamal's research (2022) show that the albumin content of snakehead fish measuring 10 - 20 cm, an average of 8.5 cm and 21 - 30 cm, an average of 23 cm respectively was 38.87 mg/g fish and 50.32 mg/g fish.

5. Conclusion

Based on the growth parameters, survival rate and albumin content in rearing snakehead fish can be fed with a protein content of 35%, carbohydrates 35% with a feeding frequency of 4 times per day.

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