Optimization of Feeding With Organic Chromium Supplement in Different Concentrations on the Ammonia Excretion and the Growth of Snakehead Fish Seeds (*Channa Striata*)

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Abstract. The purposes of this study were 1) to evaluate the optimal carbohydrate-protein levels to suppress ammonia excretion in the media of snakehead fish farming and 2) to evaluate the optimal organic chromium concentration as a feed supplement that can increase the snakehead growth and survival rate. Based on the analysis of variance result, it showed that the treatment of chromium concentration and the carbohydrate-protein levels as well as the interaction between the two of them brought the effect ($P < 0,05$) on the ammonia excretion and the growth of snakehead fish seeds (Channa striata). Duncan's multiple range test results showed that the treatments produced the ammonia excretion and obtained the highest growth from the feeding with the levels of 35% carbohydrate, 40% protein, and 5ppm of chromium. Meanwhile, the treatment resulting the lowest growth obtained from the feeding with the levels of 40% carbohydrate, 35% protein, and 3ppm of chromium, and this was not significantly different from the obtained result with 25% carbohydrate, 40% protein, and 3ppm of chromium levels.

Keywords: carbohydrate-protein, organic chromium supplement, snakehead fish, the ammonia excretion

I. INTRODUCTION

Snakehead fish is a type of freshwater fish which is easy to be cultivated because it has a good tolerance ability in water quality parameters. However, for the farmers the obstacle is the high level of protein needed in its feed. This is because the nature of the fish as meat-eating organism (carnivores) that requires protein ranging from 45%- 60% [1] and feed is one of the prominent components towards the success of farming business, and at the same time can lead to the failure due to the high ammonia excretion in the media of farming.

Protein is an expensive source of feed energy especially that comes from fish meal. It is the most important substance of all nutrients required by fish because it is a constituent substance and the main energy source [2]. In fish, proteins are more effectively used as an energy source than carbohydrates [3]. This is due to the low ability of fish in regulating plasma glucose concentration which is apparently caused by the insulin hormone deficiency [4].

The low ability of fish in utilizing blood glucose for energy metabolism associated with the bioactivity and the insulin performance capacity, where at the cellular level glucose requires transport facilities, namely glucose transporter (GLUT) in order to pass through the cell membrane and go inside the cytosol before being metabolized into energy. It is therefore necessary to make various efforts to increase the activities of glucose transporter so that the use of proteins as an energy source can be reduced and the utilization of carbohydrates as an energy source can be increased. Proteins are expected to be used for the growth and the replacement of damaged tissue, but not as an energy source. Meanwhile, the use of carbohydrates by fish is expected to increase the carbohydrate levels and to reduce the protein levels in artifical feed composition which can lower the feed prices.

One alternative that can be developed to overcome the above problem is to provide an organic chromium supplement into the feed as a micronutrient that has a major role to boost insulin activities to bring the glucose into the cell and further to convert the glucose into energy. A number of research on chromium supplementation into feed has been done mostly on herbivorous fish such as on the addition of Cr that may increase blood glucose transport in gold fish [5], on carp fish [6];[7], on the role of yeast chromium supplement in the carbohydrate utilization in carp fish [8], and on the effect of chromium on the growth performance of catfish [9]. However, research related to the chromium supplement incorporated through *Rhyzopus oryzae* fungus which is then applied to snakehead fish has not been found. Therefore, research on the role of organic chromium as a feed supplement to improve the effectiveness of carbohydrate utilization in snakehead fish needs to be conducted. Thus, the purposes of this study were to evaluate the combination of the carbohydrate-protein levels and an organic chromium concentration that resulted the ammonia excretion and the best growth of snakehead fish seeds.

II. RECEARCH METHODS

A. Time and Place of the Study. The study was conducted from February 2017 to September 2017. The incorporation of the organic chromium in *Rhyzopus oryzae* fungus through fermentation process was conducted in the laboratory of Biotechnology PKP Hasanuddin University of Makassar. Meanwhile, the atomic absorption analysis to determine the amount of the organic chromium produced through fungus was carried out in the laboratory of Agricultural Research Institute of Maros Regency. Besides, the feed preparation and the nutrition content analysis were done in BALITKANTA Nutrition Laboratory of Maros Regency. Last, testing the feed towards the snakehead fish seeds was implemented in Balai Benih Ikan (Fish Seed Hatchery) of Bantimurung, Maros Regency.

B. Tools and Materials

1. The Test Fish. The snakehead fish seeds imported from Special Region of Jogjakarta province were used in this study. The fish' length was 3-4 cm, the weight was 0.8-0.85g, and the stocking density was 20 seeds/container. Firstly, the fish seeds were acclimatized in the environment for 1 hour and the feed adaptation was carried out during 1 week before the treatments.

2. **The Test Feed** The feed used was pellet-shaped with the chemical compositions of the ingredients as shown in Table 1. The seeds were fed at satiation 3 times a day at 7:00, at 12:00, and 17:00 (Western Indonesian Time)

Table 1. The chemical composition of the raw ingredients feed provided for each treatment

No	TREATMENTS	COMPOSITION (%)				
		ASH (%)	PROTEIN (%)	FAT (%)	FIBER (%)	BETN (%)
1	(C.40%, P.35%, Cr. 3ppm)	18,19	35,33	6,91	5,79	33,78
2	(C.40%, P.35%, Cr. 5ppm)	18,14	35,35	6,93	5,74	33,84
3	(C.40%, P.35%, Cr. 7ppm)	18,16	35,33	6,92	5,75	33,84
4	(C.35%, P.40%, Cr. 3ppm)	18,20	39,90	6,67	5,46	29,77
5	(K, 35%, P.40%, Cr. 5ppm)	18,17	39,97	6,62	5,45	29,79
6	(C. 35%, P.40%, Cr. 7ppm)	18,22	39,80	6,73	5,52	29,73
7	(C.30%, P.45%, Cr. 3ppm)	17,85	45,06	6,81	3,05	27,23
8	(C.30%, P.45%, Cr. 5ppm)	17,87	45,02	6,81	3,05	27,25
9	(C.30%, P.45%, Cr. 7ppm)	17,85	45,06	6,84	3,01	27,24
10	(C.25%, P.50%, Cr. 3ppm)	18,51	49,95	6,30	4,02	21,22
11	(C.25%, P.50%, Cr. 5ppm)	19,10	49,75	6,03	4,01	21,11
12	(C.25%, P.50%, Cr. 7ppm)	18,55	49,90	6,32	4,01	21,22

3. The Farming Container . The container used for observing the ammonia excretion was a 1m x 1m x 1m aquarium. Meanwhile, for the growth and the survival rate evaluation, the size of a net container used was 0.5m x 0.5m x 1m. There were 20 fish seeds for each container.

4. Research design. This research used factorial completely randomized design. The first factor was the different carbohydrate and protein levels in the feed, and the second factor was the chromium supplementation with different concentrations in the feed. The treatments are as follows:

The carbohydrate and protein levels for each treatment was determined based on the optimal protein and carbohydrate requirements of snakehead fish from the results of previous research

Feed with the levels of 40% carbohydrate and 35% protein
Feed with the levels of 35% carbohydrate and 40% protein
Feed with the levels of 30% carbohydrate and 45% protein
Feed with the levels of 25% carbohydrate and 50% protein
Each carbohydrate-protein level was triplicate.

The second factor was the addition of chromium into the feed with different concentrations. The treatments are as follows:

3ppm of chromium concentration
5ppm of chromium concentration
7ppm of chromium concentration

Each treatment of chromium concentration in the feed was also triplicate to obtain 12 treatment combinations and 36 experimental units.

The Observed Variables

1. The ammonia excretion measured in 1 to 5 hours periods of the treatment
2. Absolute Growth of Biomass

$PB = Wt - Wo$

Description:
PB = Growth of biomass
Wt = Fish biomass at the end of treatment (g)
Wo = Fish biomass at the beginning of treatment (g)

Data analysis. The data obtained in this study were analyzed using analysis of variance. If the treatment had significant effect, then Duncan's multiple range test analysis would be conducted

III. RESULTS AND DISCUSSION

Ammonia Excretion in One to Five Hours Periods of Treatment

The mean value of carbohydrate-protein levels in the feed supplemented with different organic chromium towards the ammonia levels in 1-5 hours periods of treatment is presented in Table 2.

Table 2. The concentration of ammonia (ppm) in 1-5 hours periods of treatment with different carbohydrate-protein levels and organic chromium concentrations in the feed

treatment	Mean Result of Ammonia Expression Contents / Clock				
	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
(C.40%,P.35%,Cr.3 ppm)	$0{,}007\pm0{,}001^{a}$	$0{,}014\pm0{,}001^{b}$	$0{,}030\pm0{,}001^{a}$	$0{,}033\pm0{,}002^{d}$	$0{,}033\pm0{,}002^{ed}$
(C.40%,P.35%,Cr.5 ppm)	$0{,}005\pm0{,}001^{b}$	$0{,}007\pm0{,}000^{d}$	$0{,}020\pm0{,}0006^{c}$	$0{,}031\pm0{,}002^{ed}$	$0{,}031\pm0{,}004^{g}$
(C.40%,P.35%,Cr.7 ppm)	$0{,}006\pm0{,}001^{a}$	$0{,}013\pm0{,}001^{b}$	$0{,}031\pm0{,}0006^{a}$	$0{,}045\pm0.002^{c}$	$0{,}045\pm0{,}009^{gf}$
(C.35%,P.40%,Cr.3 ppm)	$0{,}006\pm0{,}001^{b}$	$0{,}012\pm0{,}002^{cb}$	$0{,}025\pm0{,}005^{b}$	$0{,}028\pm0.002^{fe}$	$0{,}028\pm0{,}002^{d}$
(C.35%,P.40%,Cr.5 ppm)	$0{,}005\pm0001^{dc}$	$0{,}010\pm0{,}002^{c}$	$0{,}009\pm0{,}0000^{e}$	$0{,}018\pm0{,}008$	$0{,}018\pm0{,}002^{g}$
(C.35%,P.40%,Cr.7 ppm)	$0{,}004\pm0{,}000^{cb}$	$0{,}012\pm0{,}002^{cb}$	$0{,}019\pm0{,}0006^{c}$	$0{,}025\pm0{,}007^{fe}$	$0{,}025\pm0{,}003^{ed}$
(C.30%,P.45%,Cr.3 ppm)	$0{,}006\pm0{,}001^{a}$	$0{,}007\pm0{,}006^{d}$	$0{,}010\pm0.0006^{ed}$	$0{,}029\pm0.001^{fe}$	$0{,}029\pm0{,}004^{c}$
(C.30%,P.45%,Cr.5 ppm)	$0{,}005\pm0{,}001^{cb}$	$0{,}006\pm0{,}006^{d}$	$0{,}009\pm0{,}0006^{e}$	$0{,}006\pm0{,}0030$	$0{,}006\pm0{,}002^{g}$
(C.30%,P.45%,Cr.7 ppm)	$0{,}006\pm0{,}001^{ba}$	$0{,}008\pm0.0006^{d}$	$0{,}014\pm0{,}0006^{d}$	$0{,}029\pm0{,}005^{fe}$	$0{,}029\pm0.012^{fe}$
(C.25%,P.50%,Cr.3 ppm)	$0{,}006\pm0{,}001^{b}$	$0{,}013\pm0{,}004^{a}$	$0{,}029\pm0{,}0006^{a}$	$0{,}092\pm0{,}003^{b}$	$0{,}092\pm0{,}002^{a}$
(C.25%,P.50%,Cr.5 ppm)	$0{,}004\pm0{,}001^{f}$	$0{,}009\pm0.0034^{b}$	$0{,}024\pm0{,}001^{b}$	$0{,}051\pm0{,}0006^{c}$	$0{,}051\pm0{,}002^{c}$
(C.25%,P.50%,Cr.7 ppm)	$0{,}003\pm0{,}001^{fe}$	$0{,}011\pm0{,}0023^{cb}$	$0{,}033\pm0{,}006^{a}$	$0{,}114\pm0{,}0044^{a}$	$0{,}114\pm0{,}006^{b}$

The of analysis of variance result showed that feed with different carbohydrate-protein levels and chromium concentrations as well as the interactions of both had a significant effect ($P < 0.05$) on the ammonia concentration in the snakehead fish seeds farming in one to five hours periods of treatment.

In addition, the result of Duncan's multiple range test (1-hour measurement) showed that feed with 40% carbohydrate, 35% protein, and 3 ppm of chromium produced the highest concentration of ammonia excretion (0.007 ppm) and was not significantly different ($P < 0.05$) from the feed with 40% carbohydrate, 35% protein, and 7 ppm of chromium as well as with 30% carbohydrate, 45% protein, and 3 ppm of chromium. However, it was significantly different from the other treatments. The feed with 25% carbohydrate, 50% protein, and 5 ppm of chromium produced the lowest ammonia excretion concentration (0.0053 ppm) and it was not different with the result from the feed with 25% carbohydrate, 50% protein, and 7 ppm of chromium. However, this result had significant difference from the other treatments.

Duncan's test result in 2-hour measurement showed that feed with 25% carbohydrate, 50% protein, and 3 ppm of chromium had the highest concentration of ammonia excretion (0.0177 ppm) and was significantly different from the other treatments. On the other hand, the lowest (0.0063 ppm) concentration was obtained from the feed with 30% carbohydrate, 45% protein, and 5 ppm of chromium. This was not significantly different from the feed with 40% carbohydrate, 35% protein, and 5 ppm of chromium, with 40% carbohydrate, 35% protein, 5ppm of chromium, and with 45%

carbohydrate, 30% protein as well as with 45% carbohydrate, 30% protein, and 5 ppm of chromium. This result, however, had significant difference from the rest treatments. In 3-hour measurement, Duncan's test result also recorded that feed with 25% carbohydrate, 50% protein, and 7 ppm of chromium gained the highest ammonia excretion concentration (0.033 ppm), while the feed with 30% carbohydrate, 45% protein, and 5 ppm of chromium gained the lowest ammonia excretion concentration.

Further, this Duncan's test result in 4-hour measurement recorded that feed with 25% carbohydrate, 50% protein and 7 ppm of chromium got the highest concentration of ammonia excretion (0.114 ppm) and it was significantly different from the other treatments. However, the lowest concentration of ammonia excretion (0.018 ppm) was recorded from the feed with 35% carbohydrate, 40% protein, and 5 ppm of chromium but it was significantly different from the other treatments.

Lastly, in 5-hour treatment Duncan's test result showed that the treatment of feeding with 25% carbohydrate, 50% protein and 3 ppm of chromium resulted ammonia excretion with the highest concentration

(0.179 ppm) and had significant difference from the other treatments. However, the feed with 30% carbohydrate, 45% protein, 5 ppm of chromium showed the lowest concentration of ammonia excretion (0.043 ppm). It was not different from the result of the feed with 35% carbohydrate, 40% protein, and 5 ppm of chromium, as well as with 40% carbohydrate, 35% protein, and 5ppm chromium, but it had different result from the other treatments.

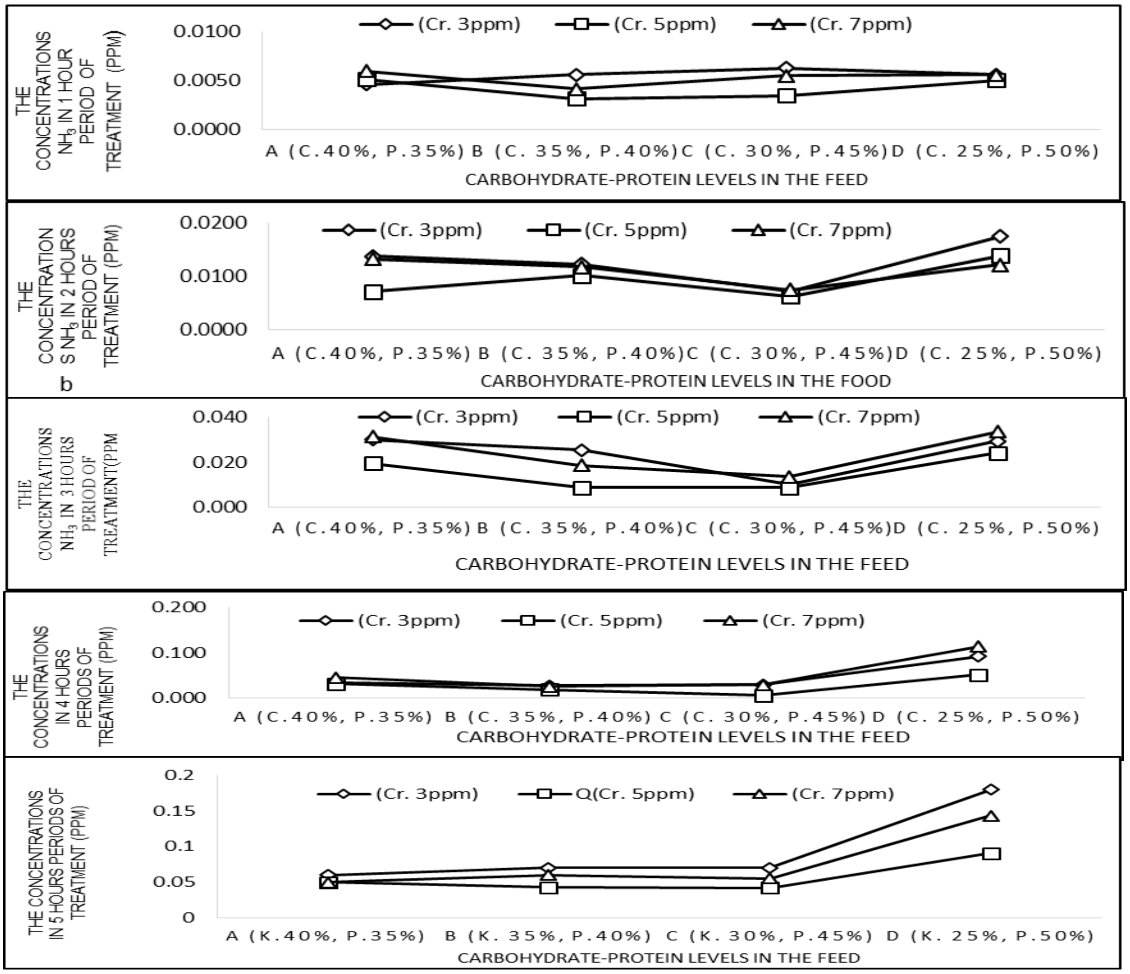

Figure 1. The interaction of carbohydrate-protein levels and organic chromium supplement in different concentratios in 1-5 hour periods of treatment

Based on the analysis result as shown in Figure 1, the treatment combination of 35% carbohydrate-40% protein, and 30% carbohydrate-45% protein with 5 ppm of organic chromium showed that in 1 to 5 hour periods of treatment, the ammonia excretion was significantly the lowest among other treatment combinations. This means that a 5 ppm of chromium supplement can increase blood glucose influx into cells and cause glucose able to immediately used as a source of energy to meet energy metabolism needs. This is in line with [10] and [11] that chromium at optimum concentration may increase the glucose influx in blood, so carbohydrates can act as *proteins sparing effect* in tissue formation. In these treatments, many retentions were done in the protein. Subsequently,the protein could be utilized for the growth more efficiently so that the ammonia production could be reduced in the media of farming.

In the treatment combination of 25% carbohydrate, 50% protein, and 3 ppm of organic chromium showed that the ammonia excretion significantly had the highest concentration. This high concentration showed that the protein level in the feed exceeded the required protein level for the growth of snakehead fish. It caused the fish had an *exessive protein syndrome.* Therefore, the protein cannot be used optimally for its growth, but it will be thrown into the environment in the form of ammonia[11], [12], [13]

The analysis result on the carbohydrate-protein levels and the organic chromium supplementation concentrations on the ammonia concentration in 1 to 5 hour periods of treatment as presented in Figure 1a, 1b, 1c, 1d, and 1e are in line with [14]. They state all nitrogens in the feed given to fish, 25% are used by fish to grow, 60% is expended in the form of NH 3 and 15% is excreted with feces. Thus, the potential supply of ammonia into fish farming is about 75% nitrogen level in the feed. Furthermore[15] state that 70-

80% nitrogen in the feed is converted to ammonia through direct excretion or through mineralization by bacteria. Last, 33% nitrogen contained in the fish feed will be excreted by fish and can be recycled [16].

The Absolute Growth. The analysis of variance result in Table 3 showed the different levels of carbohydrate-protein and the chromium concentration as well as the interaction between two of them had a significant effect on the absolute growth of the snakehead fish seeds ($P < 0.05$).

Table 3. Biomass growth of the snakehead fish seeds *(Channa striata)* fed with the organic chromium supplement with different doses

Treatments	Mean (g)
K..40%, P.35%, Cr. 3ppm	3,13±0,003[f]
K .40%, P.35%, Cr. 5ppm	3,68±0,101[c]
K. 40%, P.35%, Cr. 7ppm	3,44±0,141[d]
K. 35%, P.40%, Cr. 3ppm	3,25±0,047[e]
K. 35%, P.40%, Cr. 5ppm	4,82±0,015[a]
K. 35%, P.40%, Cr. 7ppm	3,82±0,017[b]
K. 30%, P.45%, Cr. 3ppm	3,16±0,021[fe]
K .30%, P.45%, Cr. 5ppm	4,82±0,017[a]
K. 30%, P.45%, Cr. 7ppm	3,52±0,067[d]
K. 25%, P.50%, Cr. 3ppm	3,09±0,057[f]
K. 25%, P.50%, Cr. 5ppm	4,77±0,035[a]
K. 25%, P.50%, Cr. 7ppm	3,50±0,656[d]

As presented in Table 3, the results of the study found that the treatment combination of 35% carbohydrate-40% protein supplemented with 5ppm of organic chromium showed the highest absolute growth (159.0%) among the other treatment combinations. While the combination of treatment of 40% carbohydrate-35% protein supplemented with 3ppm of organic chromium showed the lowest absolute growth (104.4%) of all the treatments.

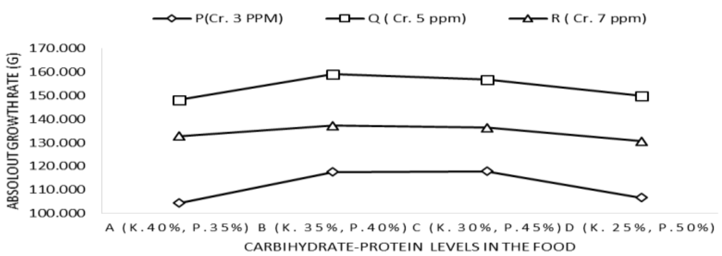

Figure 2. The interaction of carbohydrate-protein levels and organic chromium supplement concentrations towards the absolute growth rate(g) of the snakehead fish seeds.

The treatment with 35% carbohydrate and 40% protein supplemented with 5 ppm of organic chromium could obtain high absolute growth rate because 5 ppm of chromium is the optimal concentration related to the role of chromium in increasing the potential performance of insulin in mobilizing blood glucose into cells. According to [17],when chromium is able to increase insulin receptor sensitivity, insulin will be able to mobilize glucose into cells faster then to be converted into energy. Then, the amount of proteins for the growth gets increasing. Conversely, if carbohydrates are not able to utilize effectively through the addition of chromium, then proteins will be catabolized into energy that will affect the growth.

Survival Rate. Survival rate is a percentage value of fish that lives during the farming period (Effendie, 1997 *in* [13] this study, the survival of the snakehead fish reached 100% for all the treatments. In addition, there was no difference among those survival rates of the treatments. This fact is due to the feeding at satiation that does not make the snakehead fish lack of feed to avoid the cannibalism that can decrease the fish' survival.

The high percentage of survival rate is associated with the adequate feed provided. This 100% survival rate showed that the snakehead fish seeds were in a decent living condition.

IV. CONCLUSIONS.

of the results showed that overall ammonia excressions showed the lowest values in the carbohydrate treatment of 35% -protein 40%, crom 5ppm and the highest absolute growth was obtained in the treatment of 35% carbohydrate, 40% protein, 5ppm chromium. While survival gets 100% for all treatments.

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