Original article DOI: 10.19027/jai.24.1.21-31

Effectiveness of NSP enzyme and organic chromium supplementation in low-protein feed on carbohydrate utilization in Pomfret *Colossoma macropomum*

Efektivitas suplementasi enzim NSP dan kromium organik pada pakan rendah protein terhadap pemanfaatan karbohidrat pada ikan bawal *Colossoma macropomum*

Maihardiyanti Faiqotul Himmah[1], Ichsan Achmad Fauzi[1], Julie Ekasari[1], Muhammad Raditya Gumelar[2], Muhammad Agus Suprayudi[1]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java 16680, Indonesia.
[2]P.T. Aquacell Indo Pacific, Jln Pedurenan No 5, Gunung Sindur, Bogor, West Java 16340, Indonesia
Corresponding author: muhammadsu@apps.ipb.ac.id

(Received January 17, 2024; Revised March 6, 2024; Accepted September 18, 2024)

ABSTRACT

This study aims to evaluate the effectiveness of NSP enzyme and organic chromium supplementation in low-protein *Colosoma macropomum* diet to increase carbohydrate utilization. The diets contains isoprotein (20% Protien) and isoenergy (3,500 kcal GE/kg). Factorial design with two factors, namely NSP enzyme (0 and 1 g/kg) and organic chromium (0, 1, and 2 mg/kg). Fish were reared in an aquarium measuring 80×50×35 cm^3 and containing 75 L of water and stock at the density of 15 fish and reared for 60 days. The test parameters used were postprandial blood glucose levels, antioxidant activity, glycogen in muscle and liver, and growth performance. The results showed that the highest postprandial blood glucose levels when the NSP enzyme was administered. Furthermore, the lowest malondialdehyde (MDA) values and the highest superoxide dismutase (SOD) and glutathione peroxide (GPx) values were obtained in fish fed feed containing 1 g/kg of NSP enzyme and 1 mg/kg organic chromium. Fish fed feed containing 1 g/kg NSP enzyme and 1 mg/kg organic chromium showed significant values in liver and muscle glycogen as well as growth performance compared to other treatments. From this research, it can be concluded that supplementation of NSP enzyme and organic chromium at doses of 1 g and 1 mg/kg of feed produces the best growth performance and anti-oxidant capacity in pomfret fish.

Keywords: antioxidant, glycogen, growth performance, postprandial blood glucose

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi efektivitas suplementasi enzim NSP dan kromium organik pada pakan ikan bawal *Colosoma macropomum* rendah protein untuk meningkatkan pemanfaatan karbohidrat. Pakan yang digunakan mengandung iso protein (20%) dan isoenergic 3500 kkal. Rancangan faktorial dengan dua faktor yakni enzim NSP (0 dan 1 g/kg) dan kromium organik (0, 1, dan 2 mg/kg). Ikan bawal dipelihara dalam akuarium dengan ukuran 80×50×35 cm^3 dan berisi air 75 L diisi 15 ekor ikan dan dipelihara selama 60 hari. Adapun parameter uji yang digunakan adalah pengukuran kadar glukosa darah *postprandial*, aktivitas antioksidan, glikogen, dan kinerja pertumbuhan. Hasil penelitian menunjukkan kadar glukosa darah *postprandial* tertinggi pada pemberian enzim NSP. Selanjutnya nilai malondialdehyde (MDA) terendah dan *superoxide dismutase* (SOD) dan *glutathione peroxide* (GPx) tertinggi didapat pada ikan yang diberi pakan mengandung pada 1 g/kg enzim NSP dan 1 mg/kg kromium organik. Ikan yang diberi pakan yang mengandung 1 g/kg enzim NSP dan 1 mg/kg kromium organik menunjukkan nilai signifikan pada glikogen hati dan otot serta kinerja pertumbuhan dibanding perlakuan lainnya. Penelitian ini dapat menyimpulkan bahwa suplementasi enzyme NSP dan Kromium organik pada dosis 1 g dan 1 mg/kg pakan menghasilkan kinerja pertumbuhan dan kapasitas antioksidan terbaik pada ikan bawal.

Kata kunci: antioksidan, glikogen, glukosa darah *postprandial*, kinerja pertumbuhan

INTRODUCTION

Pomfret is a fish from the Amazon River that began to enter and be cultivated in Indonesia around 1980 (Woynarovich & Anrooy, 2019). Pomfret production in Indonesia in 2014 was 62,633.4 tons and increased in 2019 to 66,569.55 tons (KKP, 2022). The advantages of pomfret are not only fast growth, tolerance of high water quality, disease resistance, and easy cultivation (Taufiq *et al*., 2016; Santos *et al*., 2021). Feed is an important factor in pomfret cultivation because feed costs reach 85-98% of production costs (Suprayudi *et al*., 2023).

Protein supplements are one of the most expensive and limiting feed ingredients (Kim *et al*., 2019; Parrini *et al*., 2023). The higher protein content of feed makes it more expensive. Therefore, reducing protein levels can be a solution to lower production costs (Welengane *et al*., 2019). A decrease in feed protein content is usually offset by increased carbohydrate content (Suprayudi *et al*., 2023).

Carbohydrates are the cheapest source of dietary energy. Proper utilization of carbohydrates in fish feed contributes to directing the use of protein for fish growth, and reducing the residual nitrogen that will be produced. In addition, carbohydrate utilization, when balanced with protein and lipids in the diet, can reduce the amount of protein in the diet (Silva *et al*., 2020). However, increasing carbohydrates in feed will increase non-starch polysaccharides which are antinutrients that can reduce digestibility (Budiansyah *et al*., 2023).

The digestibility of carbohydrate source materials in feed can be increased by adding non-starch polysaccharidase (NSP) enzymes (Ambarwati & Iriyanti, 2017). The contents in NSP enzymes include cellulase, xylanase, glucanase, and pentosanase (Rastiti, 2020). The application of enzymes in feed aims to help degrade complex compounds into simple ones and continue in the digestive tract (Ramadhan *et al*., 2020). The results of research from Rastiti (2020) state that the provision of 0.4 g/kg NSP enzyme can increase the digestibility of water hyacinth flour in tilapia feed.

Pomfret fish are included in the omnivorous fish group. Omnivorous fish are generally less able to utilize carbohydrates in feed (Puteri *et al*., 2020). Yanto *et al*. (2019) stated that omnivorous and herbivorous fish can utilize 30-40% carbohydrates while carnivorous fish are only able to utilize 10-20% carbohydrates. The difference in the ability to utilize carbohydrates is due to the limited ability of fish digestive organs to digest feed carbohydrates and the availability of the hormone insulin in transferring glucose into cells as an energy source. The higher the carbohydrate digested by the fish, the higher the blood glucose. If glucose cannot be utilized, the fish will be exposed to hyperglycemia which causes oxidative stress (Wang *et al*., 2014).

Insulin is a polypeptide hormone that regulates carbohydrate metabolism and is a major effector in carbohydrate homeostasis (Nalle & Juanda, 2020). Lobo *et al*. (2022) stated that carbohydrate utilization can be improved through the provision of organic chromium in feed. Organic chromium is an essential micromineral that plays a role in increasing insulin activity (Trivedi *et al*., 2019). The main role of organic chromium in its involvement in the interaction between insulin and receptor cells is as a complex compound called glucose tolerance factor (GTF) or chromodulin that functions to trigger insulin activity to carry a lot of glucose into the cell.

The cell will convert glucose into energy. This additional energy is a source for protein synthesis, tissue growth, cell maintenance, and increased fertility (Sari *et al*., 2009; Asad *et al*., 2019). Organic chromium can also improve the performance of several digestive organs, increase carbohydrate metabolism, and increase protein retention. Organic chromium as an essential micromineral, of course, has a certain range value to function optimally.

Some research results that have been conducted related to the addition of organic chromium in feed in various types of fish show different optimal doses. Nalle and Juanda (2020) stated that the optimal level of organic chromium that can be given in dumbo catfish feed is as much as 3 mg/kg. Lobo *et al.* (2022) research states that the addition of organic chromium at a dose of 2,5 mg/kg gets optimal results in cantang grouper. Based on these studies, it is necessary to determine the optimal dose of the addition of NSP enzymes and organic chromium to the fish feed.

MATERIALS AND METHODS

Experimental design

This study used an experimental method. The experimental design used was a two-factor factorial design consisting of NSP enzyme dosage

(0 g/kg and 1 g/kg) and organic chromium dosage (0 mg/kg, 1 mg/kg, and 2 mg/kg) with three replications each. The experimental design is listed in Table 1.

Experimental diets

The experimental diet in this study was a 20% protein commercial feed from PT. Wonokoyo Jaya Corporindo to which NSP enzyme and organic chromium were added. The mixing of NSP enzyme and organic chromium was done by repelleting. The results of proximate analysis on the experimental diets are listed in Table 2.

Fish Maintenance

The fish used in this study was pomfret fish (*Colossoma macropomum*) measuring 5-6.5 cm in length with an average initial weight of 9 g obtained from farmers in Bogor. Each aquarium measuring 80×50×35 cm^3 and containing 75 L of water was filled with 15 fish. Before the fish were stocked, an acclimatization process was carried out first to familiarize the fish to the rearing container. The fish were satisfied for one day before being given the treatment feed which aims to remove metabolic waste from the feed that has been given before. The frequency of feeding was three times per day done at satiation. Feed was given directly into the aquarium at 08.00, 12.00, and 16.00 WIB for 60 days. The amount of feed given was recorded to calculate the amount of feed consumption given each day. Sampling of total fish body weight was done every 15 days. Water quality in the rearing medium was kept good by gradually changing the water every day with a volume of 30-70%. The results of water quality measurements including pH, temperature, dissolved oxygen, and total ammonia nitrogen, carried out at the beginning, middle, and end of the study are presented in Table 3.

Postprandial blood glucose

Measurement of postprandial blood glucose levels followed the method of Watanabe (1988) which was measured at 0 hours fasting and after being fed the treatment diet at 1, 3, 5, and 8 hours. Blood was taken intravenously using a syringe that had been rinsed with ethylene diamine tetra-acetic acid (EDTA) solution to prevent coagulation. Blood glucose levels were analyzed based on Wedemeyer and Yasutake (1977) by reacting blood plasma samples with ortho-

Table 1. Experimental design.

Treatments	Ingredients	
	NSP enzyme[1] (g/kg) [N]	Organic chromium[2] (mg/kg) [K]
N0K0	0	0
N0K1	0	1
N0K2	0	2
N1K0	1	0
N1K1	1	1
N1K2	1	2

Note: [1]NSP enzyme with Rovizyme Aqua WB brand from PT Aquacell Indo Pasifik; [2]organic chromium with ChromIGFin 1% brand from PT Aquacell Indo Pasifik.

Table 2. Proximate analysis of the experimental diets.

Ingredients	Treatments					
	N0K0	N0K1	N0K2	N1K0	N1K1	N1K2
Protein (%)	20.93	20.75	20.82	20.95	20.78	20.98
Lipid (%)	6.47	6.98	6.33	6.80	6.27	6.33
Moisture (%)	8.82	8.29	8.25	8.87	8.13	8.78
Ash (%)	7.84	7.96	7.77	7.88	7.66	7.80
Crude fiber (%)	11.13	11.48	11.10	11.25	11.40	11.40
NFE[1]	44.81	44.54	45.73	44.25	45.76	44.71
GE[2](Kcal/kg diet)	3575.61	3584.75	3602.76	3587.66	3594.23	3561.05

Note: [1]NFE: nitrogen-free extract; [2]GE: Gross energy = (%protein × 5.6 kcal) + (%NFE ×4.1 kcal) + (%lipid × 9.4 kcal) (Watanabe, 1988).

toluidine solution and glacial acetic acid, and then measuring the optical density at a wavelength of 635 nm.

Antioxidant activity

Antioxidant activities observed included malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx). MDA analysis was analyzed on 0.5 g liver samples using the MDA lipid peroxidation colorimetric test kit from Abcam, UK. MDA analysis was following the method of Ohkawa *et al*. (1979). SOD enzyme activity was analyzed on 0.5 g of liver samples using the SOD colorimetric test kit from Abcam, UK. The standard used in this analysis was SOD enzyme (EC 1.15.1.1). SOD analysis was following McCord and Fridovich (1969). GPx enzyme activity was analyzed on 0.5 g liver samples using glutathione peroxidase activity colorimetric assay kit from Abcam, UK.

Liver and muscle glycogen

Glycogen analysis was performed on the liver and muscle. A total of 0.5 g of liver or fish muscle was taken and extracted with 2 mL of 30% KOH solution, incubated in a boiling water bath for 30 minutes, then placed at room temperature until cool. 96% alcohol as much as 5 mL was added to the sample. Centrifugation was carried out at 3,000 rpm for 10 minutes to separate the glycogen precipitate in the sample (Suarsana *et al*., 2010). The supernatant was discarded and 2 mL of distilled water was added. 96% alcohol as much as 5 mL was added to the sample and heated for five seconds. Centrifugation for five minute and discard the supernatant. The sample was then added with 1N H_2SO_4 as much as 2 mL, heated for two hours, and continued with the glucose test. The absorbance was then measured using a spectrophotometer with a wavelength of 635 nm.

Growth parameters

The growth parameters observed in this study were survival rate (SR), daily growth rate (DGR), feed conversion ratio (FCR), protein retention (PR), and fat retention (FR). The fish survival rate during the study was calculated using the following formula:

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

Note:
SR = Survival rate (%)
Nt = Number of test fish at the end of rearing (fish)
No = Number of test fish at the beginning of rearing (fish)

The calculation of DGR using the formula:

$$DGR = \left[\sqrt[t]{\frac{Wt}{W0}} - 1\right] \times 100$$

Note:
DGR = Daily growth rate (%)
T = Observation period (days)
Wt = Average weight of live and dead fish at the end of the study (g)
W_0 = Average weight of fish at the beginning of the study (g)

The FCR calculation uses a formula that refers to Takeuchi (1988), which is as follows:

$$FCR = \frac{F}{Wt - W0 + Wd}$$

Note:
FCR = Feed conversion ratio
W_t = Total fish weight at the end of rearing (g)
W_0 = Total fish weight at the beginning of rearing (g)

Table 3. Results of water quality.

Treatments	Water quality			
	temperature (°C)	pH	DO (mg/L)	TAN (mg/L)
N0K0	7.21 – 7.33	29.1 – 31.4	4.9 – 6.3	0.15 – 0.27
N0K1	7.26 – 7.35	28.3 – 31.2	5.1 – 6.1	0.15 – 0.26
N0K2	7.15 – 7.35	29.6 – 31.2	5.2 – 6.3	0.14 – 0.27
N1K0	7.21 – 7.33	29.1 – 30.9	4.9 – 6.3	0.16 – 0.27
N1K1	7.21 – 7.36	29.1 – 31.2	4.9 – 6.3	0.15 – 0.27
N1K2	7.21 – 7.35	30.2 – 31.4	5.1 – 5.9	0.14 – 0.27

Note: DO = dissolved oxygen; TAN = total ammonia nitrogen.

Wd = Total weight of dead fish during rearing (g)
F = Total of feed consumed (g)

Protein retention is calculated with the following formula (Takeuchi 1988):

$$PR\ (\%) = \frac{F - 1}{P} \times 100$$

Note:
PR = Protein retention (%)
F = Total fish protein at the end of rearing (g)
I = Total fish protein at the beginning of rearing (g)
P = Total of protein consumed by fish (g)

Fat retention was calculated through proximate analysis of test fish body fat at the beginning and end of the study with the following formula (Takeuchi 1988):

$$FR\ (\%) = \frac{F - 1}{P} \times 100$$

Note:
RL = Fat retention (%)
F = Total fish fat at the end of rearing (g)
I = Total fish fat at the beginning of rearing (g)
P = Total of fat consumed by fish (g)

Data Analysis

The data obtained were tabulated using Microsoft Excel software. Before data analysis, normality tests and homogeneity tests were conducted. Data analysis used is two-way ANOVA (Analysis of Variance) to determine the effect of the treatment given, if there are significant results then the calculation is continued with DMRT (Duncan's Multiple Range Test) (Al-Arif, 2016).

RESULTS AND DISCUSSIONS

Results

Postprandial blood glucose

Measurement of blood glucose levels at each observation time fluctuated with significant differences between NSP enzyme and organic chromium supplementation diets. Two-way ANOVA analysis on postprandial blood glucose level measurements (Table 4) showed that NSP enzyme supplementation had a significant effect on this parameter, except at hour 0 and hour 8 where blood glucose levels had returned to basal values. Meanwhile, organic chromium supplementation had a significant effect on this parameter, except at hour 0. A significant interaction was also shown in the postprandial blood glucose parameter, especially at the 0th, 5th, and 8th observation hours, indicating that the effect of NSP enzyme supplementation and organic chromium both affected blood glucose levels.

Antioxidant activity

The effect of NSP enzyme and organic chromium supplementation on antioxidant activity is presented in Table 5. The results of two-way ANOVA analysis on MDA, SOD, and GPx parameters showed that NSP enzyme supplementation had a significant effect on MDA and GPx parameters, while not significantly different on SOD parameters. Organic chromium supplementation showed significant results on MDA, SOD, and GPx parameters. A significant interaction was also shown on MDA, SOD, and GPx parameters.

Liver and muscle glycogen

The effect of NSP enzyme and organic chromium supplementation on liver and muscle glycogen is presented in Table 6. The results show significant differences between treatments.

The results of two-way ANOVA analysis on the measurement of liver and muscle glycogen levels showed that NSP enzyme supplementation had a significant effect on the measurement of liver and muscle glycogen levels. Similarly, organic chromium supplementation had a significant effect on the measurement of liver and muscle glycogen levels. A significant interaction was also shown in the measurement of liver and muscle glycogen levels.

Growth parameters

The effect of NSP enzyme and organic chromium supplementation on growth parameters is presented in Table 7. Two-way ANOVA analysis on growth parameters showed that NSP enzyme supplementation had a significant effect on the parameters of fat retention, total feed consumption, daily growth rate, and feed conversion ratio, while not significantly different on the parameters of survival rate and protein retention. Likewise, organic chromium supplementation showed the same results, which had a significant effect on the parameters of protein retention, fat retention, total feed consumption, daily growth rate, and feed

Table 4. Postprandial blood glucose levels (mg/dL) of *Colossoma macropomum* fed with NSP enzyme and organic chromium supplementation.

Treatments	Hour				
	0	1	3	5	8
N0K0	81.50 ± 0.19[a]	98.94±0.48d	81.50 ± 0.19[a]	129.48±0.97ab	81.50 ± 0.19[a]
N0K1	81.60 ± 0.10[a]	94.80±0.19e	81.60 ± 0.10[a]	126.46±2.27ab	81.60 ± 0.10[a]
N0K2	81.99 ± 1.64[a]	98.36±1.64d	81.99 ± 1.64[a]	132.72±2.27a	81.99 ± 1.64[a]
N1K0	81.60 ± 0.29[a]	107.03±0.67c	81.60 ± 0.29[a]	125.23±0.54ab	81.60 ± 0.29[a]
N1K1	81.79 ± 1.45[a]	111.47±1.45b	81.79 ± 1.45[a]	102.16±3.24d	81.79 ± 1.45[a]
N1K2	81.80 ± 0.10[a]	121.10±0.10a	81.80 ± 0.10[a]	121.06±4.64bc	81.80 ± 0.10[a]
NSP Enzyme Level (g/kg)					
0	81.70 ± 0.25[a]	97.37 ± 2.08[b]	110.36 ± 8.59[b]	129.55 ± 3.21[a]	84.67 ± 3.19[a]
1	81.73 ± 0.26[a]	113.20 ± 5.94[a]	146.85 ± 5.85[a]	112.81 ± 8.86[b]	84.63 ± 3.16[a]
Organic Chromium Level (mg/kg)					
0	81.55 ± 0.25[a]	102.99 ± 4.09[b]	123.31 ± 16.42[b]	122.35 ± 7.17[a]	89.04 ± 0.71[a]
1	81.70 ± 0.24[a]	103.13 ± 8.40[b]	127.41 ± 25.30[b]	114.31 ± 12.47[b]	82.40 ± 0.76[b]
2	81.89 ± 0.14[a]	109.73 ± 11.43[a]	135.10 ± 13.38[a]	126.89 ± 6.88[a]	82.51 ± 0.51[b]
Two Way Anova					
NSP Enzyme Level	NS	P<0.01	P<0.01	P<0.01	NS
Organic Chromium Level	NS	P<0.01	P<0.01	P<0.01	P<0.01
NSP Enzyme x Organic Chromium	NS	P<0.01	P<0.01	NS	NS

Note: mean ± standard deviation (n=3) followed by different letters in the same column indicate significant differences (P<0.05) based on DMRT α=5%. NS: not significant.

Table 5. Antioxidant activity of *Colossoma macropomum* fed with NSP enzyme and organic chromium supplementation.

Treatments	Parameters		
	MDA (mMol/g)	SOD (% *inhibition ratio*)	GPx (U/mg protein)
N0K0	2.51 ± 0.01[ab]	53.03 ± 2.14[d]	55.77 ± 0.17[d]
N0K1	2.40 ± 0.03[c]	56.61 ± 0.75[cd]	57.08 ± 0.10[c]
N0K2	2.28 ± 0.04[d]	62.2 ± 3.14[ab]	62.52 ± 0.24[b]
N1K0	2.52 ± 0.02[a]	53.14 ± 2.32[d]	55.99 ± 0.05[d]
N1K1	1.96 ± 0.04[e]	63.9 ± 1.96[a]	64.51 ± 0.97[a]
N1K2	2.45 ± 0.03[bc]	57.93 ± 0.56[bc]	61.76 ± 0.56[b]
NSP Enzyme Level (g/kg)			
0	2.40 ± 0.10[a]	57.29 ± 4.40[a]	58.46 ± 2.93[b]
1	2.31 ± 0.25[b]	58.32 ± 4.75[a]	60.75 ± 3.61[a]
Organic Chromium Level (mg/kg)			
0	2.51 ± 0.01[a]	53.08 ± 2.24[b]	55.88 ± 0.16[c]
1	2.18 ± 0.23[c]	60.25 ± 3.93[a]	60.79 ± 3.78[b]
2	2.37 ± 0.09[b]	60.08 ± 3.11[a]	62.14 ± 0.57[a]
Two Way Anova			
NSP Enzyme Level	P<0.01	NS	P<0.01
Organic Chromium Level	P<0.01	P<0.01	P<0.01
NSP Enzyme x Organic Chromium	P<0.01	P<0.01	P<0.01

Note: mean ± standard deviation (n=3) followed by different letters in the same column indicate significant differences (P<0.05) based on DMRT α=5%. MDA: malondialdehyde; SOD: superoxide dismutase; GPx: glutathione peroxide; NS: not significant.

Tabel 6. Glycogen levels of *Colossoma macropomum* fed with NSP enzyme and organic chromium supplementation.

Treatments	Parameters	
	Liver Glycogen (mg/100 mL)	Muscle Glycogen (mg/100 mL)
N0K0	0.54 ± 0.01^{d}	0.84 ± 0.01^{c}
N0K1	0.57 ± 0.01^{bc}	0.85 ± 0.01^{c}
N0K2	0.62 ± 0.01^{a}	0.87 ± 0.01^{b}
N1K0	0.56 ± 0.01^{cd}	0.85 ± 0.01^{c}
N1K1	0.63 ± 0.01^{a}	0.93 ± 0.01^{a}
N1K2	0.59 ± 0.01^{b}	0.86 ± 0.01^{bc}
NSP Enzyme Level (g/kg)		
0	0.58 ± 0.03^{b}	0.85 ± 0.02^{b}
1	0.59 ± 0.03^{a}	0.87 ± 0.04^{a}
Organic Chromium Level (mg/kg)		
0	0.55 ± 0.01^{b}	0.84 ± 0.01^{c}
1	0.60 ± 0.03^{a}	0.89 ± 0.04^{a}
2	0.60 ± 0.01^{a}	0.87 ± 0.02^{b}
Two Way Anova		
NSP Enzyme Level	P<0.01	P<0.01
Organic Chromium Level	P<0.01	P<0.01
NSP Enzyme x Organic Chromium	P<0.01	P<0.01

Note: mean ± standard deviation (n=3) followed by different letters in the same column indicate significant differences (P<0.05) based on DMRT α=5%. NS: not significant.

Table 7. Growth performance of *Colossoma macropomum* fed with NSP enzyme and organic chromium supplementation.

Treatments	Parameters					
	SR (%)	PR (%)	FR (%)	F (g)	DGR (%)	FCR (%)
N0K0	100 ± 0.00^{a}	25.40 ± 0.23^{c}	44.84 ± 1.87^{c}	402 ± 3^{c}	1.84 ± 0.01^{d}	1.44 ± 0.01^{a}
N0K1	100 ± 0.00^{a}	27.10 ± 0.92^{b}	52.31 ± 1.44^{b}	425 ± 7^{b}	1.95 ± 0.01^{c}	1.39 ± 0.01^{b}
N0K2	100 ± 0.00^{a}	29.59 ± 0.26^{a}	52.82 ± 1.01^{b}	421 ± 2^{b}	2.01 ± 0.01^{b}	1.32 ± 0.02^{c}
N1K0	100 ± 0.00^{a}	25.51 ± 0.48^{c}	46.64 ± 1.14^{c}	397 ± 2^{c}	1.85 ± 0.01^{d}	1.42 ± 0.02^{a}
N1K1	100 ± 0.00^{a}	30.35 ± 0.30^{a}	59.07 ± 1.77^{a}	449 ± 3^{a}	2.11 ± 0.01^{a}	1.29 ± 0.01^{d}
N1K2	100 ± 0.00^{a}	26.36 ± 0.04^{bc}	53.40 ± 0.28^{b}	428 ± 5^{b}	1.98 ± 0.02^{bc}	1.38 ± 0.01^{b}
NSP Enzyme Level (g/kg)						
0	100 ± 0.00^{a}	27.37 ± 1.81^{a}	49.99 ± 3.93^{b}	416 ± 11^{b}	1.93 ± 0.07^{b}	1.38 ± 0.05^{a}
1	100 ± 0.00^{a}	27.41 ± 2.13^{a}	53.04 ± 5.22^{a}	425 ± 21^{a}	1.98 ± 0.11^{a}	1.36 ± 0.06^{b}
Organic Chromium Level (mg/kg)						
0	100 ± 0.00^{a}	25.46 ± 0.38^{c}	45.75 ± 1.79^{c}	400 ± 3^{c}	1.84 ± 0.01^{c}	1.44 ± 0.02^{a}
1	100 ± 0.00^{a}	28.72 ± 1.76^{a}	55.69 ± 3.74^{a}	437 ± 13^{a}	2.03 ± 0.08^{a}	1.34 ± 0.05^{b}
2	100 ± 0.00^{a}	27.98 ± 1.63^{b}	53.11 ± 0.80^{b}	424 ± 5^{b}	1.99 ± 0.02^{b}	1.35 ± 0.03^{b}
Two Way Anova						
NSP Enzyme Level	NS	NS	P<0.01	P<0.01	P<0.01	P<0.01
Organic Chromium Level	NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
NSP Enzyme x Organic Chromium	NS	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01

Note: mean ± standard deviation (n=3) followed by different letters in the same column indicate significant differences (P<0.05) based on DMRT α=5%. SR: survival rate; PR: protein retention; FR: fat retention; F: total feed consumption; DGR: daily growth rate; FCR: feed conversion ratio; NS: not significant.

conversion ratio. A significant interaction was also shown on the parameters of protein retention, fat retention, total feed consumption, daily growth rate, and feed conversion ratio.

Discussions

The treatment of NSP enzyme and organic chromium supplementation showed a significant increase in blood glucose levels. Digestibility of feed carbohydrates occurs when there is absorption of nutrients into the cell wall and the level of carbohydrate utilization can be seen from blood glucose levels. Increased intake of digestible carbohydrates results in increased blood glucose levels (Scharma *et al*., 2018). Glucose in the blood can be immediately utilized as an energy source to meet metabolic energy needs with the help of the hormone insulin (Sari *et al*., 2009). Factors that cause the slow process of glucose regulation in fish are due to the lack of insulin production, the small number of insulin receptors, and the absence of insulin-sensitive glucose transporter 4 (GLUT4) in peripheral tissues (Forbes *et al*., 2019).

The results of this study showed that the treatment added with NSP enzyme showed a high increase in blood glucose levels compared to the treatment without the addition of NSP enzyme. This is because NSP enzymes help simplify complex compounds in low-protein feed so as to increase carbohydrate digestibility. NSP enzymes function to break down complex cell wall structures in plant-based raw materials. NSP enzymes are a mixture of various enzymes, namely cellulase, xylanase, glucanase and pentosanase. Meanwhile, the treatment added with organic chromium showed a faster decrease in blood glucose levels than the treatment without the addition of organic chromium. This is because organic chromium functions to accelerate the utilization of blood glucose by cells in the metabolic process which can be indicated by a decrease in blood glucose levels (Subandiyono & Hastuti, 2008).

Based on the results of this study, the best results occurred in the N1K1 treatment which contained 1 g/kg NSP enzyme and 1 mg/kg organic chromium. This is to the research of Rastiti (2020) who obtained the best digestibility of feed with water hyacinth flour with the addition of 0.4 g/kg NSP enzyme in tilapia feed. According to Lobo *et al.* (2022) the optimum dose of organic chromium addition to cantang grouper feed is 2,5 mg/kg. This is different from the best results of organic chromium doses obtained in this study, due to differences in fish species and the addition of NSP enzymes to the feed.

The higher the carbohydrate ingested by the fish, the higher the blood glucose. If glucose cannot be utilized, the fish will be exposed to hyperglycemia which causes oxidative stress (Wang *et al*., 2014). Oxidative stress is a condition where the imbalance of antioxidants and free radicals can cause cell damage. Oxidative stress causes an increase in melondialdehyde (MDA) levels and a decrease in the activity of the body's antioxidant enzymes such as superoxide dismutase (SOD) and glutathion peroxidase (GPx).

The results obtained in this study showed that MDA obtained the lowest results in the N1K1 treatment. While the SOD and GPx tests obtained the highest results in the N1K1 treatment. These results indicate that the addition of 1 g/kg NSP enzyme and 1 mg/kg organic chromium can reduce the risk of exposure to hyperglycemia because blood glucose can be utilized faster than without the addition of NSP enzyme or organic chromium. Organic chromium is also an antioxidant additive that can be used as a dietary supplement to enhance antioxidant capacity and ultimately strengthen health (Han *et al*., 2021).

SOD and GPx are antioxidant enzymes that have important roles. SOD functions in protection from oxidant damage generated by reactive oxygen species (ROS) in terms of dismutation of the toxic superoxide anion radical into less toxic hydrogen peroxide, which is then neutralized into oxygen and water by catalase. Furthermore, GPx catalyzes the reduction of lipid peroxide and hydrogen peroxide using glutathione to protect against the accumulation of lipid peroxide and other oxidants, thus preventing oxidant damage (Ernawati *et al*., 2020). SOD is an antioxidant enzyme that is mostly located in the liver. The higher the SOD enzyme activity, the lower the lipid oxidation product. High levels of MDA in the body indicate high levels of free radicals.

The condition of excess free radicals in the body causes an imbalance between the endogenous antioxidant capacity in counteracting free radicals, causing cell damage (Sofian *et al*., 2016). The difference in blood glucose levels between treatments indicates that supplementation of NSP enzymes and organic chromium in feed triggers an increase in blood glucose accompanied by insulin secretion, causing the process of storing energy reserves in the form of body glycogen or increased glycogenesis. Feed carbohydrates that are digested in limited amounts are converted

in the form of glycogen in the muscles, then in smaller fractions will be converted into fat in the liver through the process of lipogenesis (Bou *et al*., 2015). Insulin plays a role in carbohydrate metabolism, namely spurring the process of glycogenesis and lipogenesis. Glycogenesis is a process of glycogen formation as reserve energy derived from excess glucose as a source of metabolic energy both in the liver and muscle (Sari *et al*., 2009).

The results obtained from this study showed that the N1K1 and N0K2 treatments had the highest liver glycogen yield (0.63 ± 0.01^{a}; 0.62 ± 0.01^{a}). While the results of muscle glycogen N1K1 treatment got the highest results (0.93 ± 0.01^{a}). The provision of organic chromium affects the amount of glycogen stored. High levels of glycogen in the liver is an energy reserve that can be quickly used by the body when energy deficiency (Sari *et al*., 2009). NSP enzyme and organic chromium supplementation showed growth parameters, namely survival rate, which were not significantly different between treatments.

While in the parameters of protein retention, fat retention, total feed consumption, daily growth rate, and feed conversion ratio, the best results were obtained in the N1K1 treatment. This indicates that the addition of 1 g/kg NSP enzyme and 1 mg/kg organic chromium can increase the utilization of energy contained in the feed, especially carbohydrates efficiently for various activities without disturbing the amount of protein used for growth. Magalhaes *et al*. (2016) stated that NSP enzymes can improve growth performance and feed conversion. These results are in accordance with the research of Sari *et al*. (2009) which showed that the addition of organic chromium at a dose of 3.2 mg/kg can increase protein retention and fat retention in bream.

Organic chromium can increase the efficiency of feed protein utilization or increase body protein deposition for growth. Organic chromium is known to increase protein synthesis and will further increase fish growth. This is related to the role of organic chromium on insulin and amino acid increase. The ability to protect RNA from heat denaturation, increase RNA synthesis and decrease cortisol can increase amino acid synthesis and protein. The presence of carbohydrates optimizes the utilization of non-protein energy sources, energy production from non-protein sources will increase the capacity of the protein sparing effect which maximizes the role of protein for the improvement of biological structure and biological activity (Rifai *et al*., 2022), reduces protein catabolism as energy thereby increasing protein retention and increasing fish growth (Van Doan *et al*., 2021).

CONCLUSION

Supplementation of 1 g/kg NSP enzyme and 1 mg/kg organic chromium in pomfret fish feed can improve energy utilization from carbohydrate source materials based on postprandial blood glucose test results, antioxidant activity, liver and muscle glycogen, and growth parameters.

ACKNOWLEGMENT

The authors would like to thank to PT. Aquacell Indo Pasifik for their support funding in this study.

REFERENCES

Al-Arif MA. 2016. Experimental Design Textbook. Surabaya: Lentera Jaya Madina.

Ambarwati L, Iriyanti N. 2017. Blood profiles from native chicken use of enzyme in non strack polysacharida (NSP). Jurnal SAINTEK Peternakan dan Perikanan 1: 16–22.

Asad F, Mubarik MS, Ali T, Zahoor MK, Ashrad R, Qamer S. 2019. Effect of organic and in-organic chromium supplementation on growth performance and genotoxicity of *Labeo rohita*. Saudi Journal Biological Sciences 26: 1140–1145.

Bou M, Todorcevic M, Torgersen J, Skugor S, Navarro I, Ruyter B. 2015. De novo lipogenesis in Atlantic salmon adipocytes. Biovhimica et Biophysica Acta 1860: 86–96.

Budiansyah A, Haroen U, Resmi, Syafwan, Ramlah. 2023. Performance of broiler chicken with treatment of buffalo rumen liquid as a source of enzymes in corn and soybean meal-based rations. Buletin Peternakan Tropis 4: 69–87.

Ernawati, I'tishom R, Sudjarwo SA. 2020. Role of MDA, SOD and GPx expression on protective mechanism of xanthone against 2-methoxyethanol-decreased number of sertoli cell in mice. Interciencia Journal 45: 104–114.

Forbes JLI, Kostyniuk DJ, Mennigen JA, Weber JM. 2019. Unexpected effect of insulin on glucose intolerance in trout. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 316: 387–394.

Han M, Chen Y, Li J, Dong Y, Miao Z, Li J, Zhang L. 2021. Effects of organic chromium sources on growth performance, lipid metabolism, antioxidant status, breast amino acid and fatty acid profiles in broilers. Journal of the Science of Food and Agriculture 101: 3917–3926.

Kim SW, Less JF, Wang L, Yan T, Kiron V, Kaushik SJ, Lei XG. 2019. Meeting global feed protein demand: challenge, opportunity, and strategy. Annual Review of Animal Biociences 7: 21–43.

Lobo C, Tjendanawangi A, Rebhung F. 2022. Effect of addition of chromium-yeast (Cr organic) in feed on growth of cantang grouper (*Epinephelus fuscogutattus lanceolatus*). Jurnal Aquatik 5: 16–23.

Magalhaes R, Lopes T, Martins N, Diaz-Rosales P, Couto A, Pousao-Ferreira P, Olivia-Teles A, Peres H. 2016. Carbohydrases suplementation increased nutrient utilization in white seabream (*Diplodus sargus*) juveniles fed high soybean meal diets. Aquaculture 463: 43–50.

McCord JM, Fridovich I. 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). The Journal of Biological Chemistry 244: 6049–6055.

Nalle MMD, Juanda SJ. 2020. Efisiensi pemanfaatan pakan melalui penambahan kromium (Cr^{+3}) terhadap pertumbuhan ikan lele dumbo *Clarias* sp. Jurnal Vokasi Ilmu-ilmu Perikanan 1: 1–7. (In Indonesian).

Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95: 351–358.

Parrini S, Aquilani C, Pugliese C, Bozzi R, Sirtori F. 2023. Soybean replacement by alternative protein sources in pig nutrition and its effect on meat quality. Animals 13: 1–30.

Puteri BJ, Subandiyono, Hastuti S. 2020. The role of chromium (Cr^{+3}) in artificial feed on the level of efficiency of feed utilization and growth of catfish (*Clarias* sp.). Jurnal Sains Akuakultur Tropis 4: 161–170.

Ramadhan RF, Montesqrit, Marlida Y. 2020. Production of thermostable cellulase enzymes from Ng2 bacteria using various sources of cellulose from agricultural and plantation waste. Jurnal Ilmu dan Teknologi Peternakan 8: 64–72.

Rastiti AMS. 2020. Digestibility of Water Hyacinth Flour (*Eichhornia crassipes*) treated with HCl and NSP Enzyme in Tilapia (*Oreochromis niloticus*) [Thesis]. Bogor (ID): Institut Pertanian Bogor.

Rifai R, Jusadi D, Suprayudi MA, Alimuddin, Nuryati S. 2022. Evaluation of dietary α-lipoic acid supplementation on the growth performance and physiological status of striped catfish Pangasianodon hypophthalmus. Jurnal Akuakultur Indonesia 21: 198–206.

Santos RB, Izel-Silva J, Fugimura MMS, Suita SM, Ono EA, Affonso EG. 2021. Growth performance and health of juvenile tambaqui, *Colossoma macropomum*, in a biofloc system at different stocking densities. Aquaculture Reserarch.

Sari EP, Mokoginta I, Jusadi D. 2009. The effect of providing chromium-yeast in feed on the growth performance of baung fish (*Hemibagrus nemurus* Blkr). Jurnal Ilmu-ilmu Perairan dan Perikanan Indonesia 16: 17–23.

Scharma JW, Haidar MN, Ceurden I, Kaushik SJ. 2018. Ebergy efficiency of digestible protein, fat a for growth in rainbow trout and *Nile tilapia*. British Journal of Nutrition 119: 782–791.

Silva AF, Copatti CE, de Oliveira EP, Bonfa HC, de Melo FVST, Camargo ACS, Melo JFB. 2020. Effect of whole banana meal inclusion as replacement for corn meal on digestibility, growth performance, haematological and biochemical variables in practical diets for tambaqui juveniles (*Colossoma macropomum*). Aquaculture Reports 17: 1–8.

Sofian S, Jusadi D, Nuryati S. 2016. Growth and antioxidant status of gourami fish given different levels of astaxanthin supplementation. Jurnal Akuakultur Indonesia 15: 24–31.

[KKP] Kementerian Kelautan dan Perikanan. 2022. Fisheries Production. https://statistik.kkp.go.id/home.php?m=prod_ikan_prov&i=2#panel-footer. [22 March 2022].

Suarsana IN, Priosoeryanto BP, Wresdiyati T. 2010. Sintesis glikogen hati dan otot pada tikus diabetes yang diberi ekstrak tempe. Jurnal Veteriner 11: 190–195.

Subandiyono dan Hastuti S. 2008. Post prandial blood glucose pattern and growth of "sangkuriang dumbo catfish (*Clarias gariepinus*) maintained with organic chromium feed. Aquacultura Indonesia 9: 31–38.

Suprayudi MA, Jusadi D, Setiawati M, Ekasari

J, Fauzi IA. 2023. Increased Protein and Feed Efficiency of Aquatic Organisms. Bogor (ID): IPB Press.

Taufiq T, Firdus F, Imelda AI. 2016. Growth of freshwater pemfret (*Colossoma macropomum*) in the growth performance of pomfret (*Colossoma macropomum*) fed several life feeds. Jurnal Ilmiah Mahasiswa Kelautan dan Perikan Unsyiah 1: 355–365.

Takeuchi T. 1988. *Laboratory Work Chemical Evaluation of Dietary Nutrition*. p. 179–229, In Watanabe T. Fish Nutrition and Mariculture. Tokyo (JP): Kanagawa International Fisheries Training Center.

Trivedi SP, Patil SS, Garg DD, Savsani HH, Pawar MM, Gohel BC. 2019. Effect of dietary supplementation of organic chromium on feed intake, growth performance and economics in commercial broiler chickens. Journal of Entomology and Zoology Studies 7: 374–378.

Van Doan H, Lumsangkul C, Hoseinifar SH, Harikrishnan R, Balasundaram C, Jaturasitha S. 2021. Effects of coffee silverskin on growth performance, immune response, and disease resistance of Nile tilapia culture under biofloc system. Aquaculture 543: 736995.

Wang J, Ai Q, Mai K, Xu H, Zuo R. 2014. Dietary chromium polynicotinate enhanced growth performance , feed utilization , and resistance to *Cryptocaryon irritans* in juvenile large yellow croaker (*Larmichthys crocea*). Aquaculture 432: 321–326.

Welengane E, Sado RY, Bicudo AJdA. 2019. Protein-sparing effect by dietary lipid increase in juveniles of the hybrid fish tambatinga (♀*Colossoma macropomum* × ♂*Piaractus brachypomus*). Aquaculture Nutrition 25: 1–9.

Woynarovich A, Van Anrooy R. 2019. Field guide to the culture of Tambaqui (*Colossoma macropomum*, Cuvier, 1816). Rome: Food and Agriculture Organization of the United Nations.

Yanto H, Setiadi AE, Kurniasih D. 2019. Influence of the different levels of carbohydratein diets on the growth performance of tengadak (*Barbonymus schawenfeldii*). Jurnal Ruaya 7: 39–46.