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Evaluation of food digestibility in Nile tilapia fish *Oreochromis niloticus* given NSP enzymes and organic chromiium

Evaluasi kecernaan pakan pada ikan nila *Oreochromis niloticus* yang diberi enzim NSP dan kromium organik

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ABSTRACT

Feed is a crucial factor in tilapia farming because it contributes 92% of the total production cost. This study aims to evaluate the digestibility and utilization of carbohydrates in low-protein feed supplemented with NSP enzymes and organic chromium. The research method employed a factorial completely randomized design with treatments of organic chromium (0, 1, 2) and NSP enzymes (0, 1). The study used 18 aquariums, each measuring 50×40×35 cm^3. The fish used were tilapia with an average weight of 33.09 ± 0.06 g per fish. The feed used was commercial feed supplemented with organic chromium and NSP enzymes. The study focused on digestibility performance, digestive enzyme activity, growth performance, and antioxidant status. The results showed that the treatments E1C1 and E0C2 resulted in better total digestibility, energy digestibility, and protein digestibility compared to the control and other treatments. Lipase, protease, and amylase enzymes in tilapia fed with the E1C1 treatment showed significantly higher results ($P<0.05$) compared to the control. Growth performance in tilapia fed with NSP enzymes and organic chromium did not show significant differences from the control ($P>0.05$). However, antioxidant status, specifically SOD and GPx, was significantly higher in the E0C1 treatment compared to the control ($P<0.05$). The conclusion of this study is that the addition of NSP enzymes and organic chromium to low-protein feed can improve digestibility and optimize carbohydrate utilization, with the best results achieved with the E1C1 treatment.

Keywords: antioxidant, digestive enzymes, Nile tilapia, nutrient digestibility

ABSTRAK

Pakan menjadi factor krusial dalam usaha budidaya ikan nila karena memiliki kontribusi sebesar 92% dari total biaya produksi. Penelitian ini bertujuan untuk mengevaluasi kecernaan dan kemampuanpemanfaatan karbohidrat pada pakan protein rendah yang diberienzim NSP dan kromium organik. Metode penelitian ini menggunakan rancangan acak lengkap faktorial dengan perlakuan kromium organic (0,1,2) dan enzim NSP (0,1). Wadah yang digunakan merupakan aquarium yang berukuran 50×40×35 cm^3 sebanyak 18 unit. Ikan yang digunakan dalam penelitian ini adalah ikan nila dengan bobot rata-rata 33,09 ± 0,06 g/ekor. Pakan yang digunakan merupakan pakan komersial yang diberi penambahan kromium organik dan enzim NSP. Penelitian ini difokuskan pada kinerja kecernaan, aktivitas enzim pencernaan, kinerja Pertumbuhan serta status Antioksidan. Hasil penelitian menunjukkan bahwa perlakuan E1C1 dan E0C2 menghasilkan nilai kecernaan total, kecernaan energi dan kecernaan protein yang lebih baik dibanding perlakuan kontrol dan perlakuan lainnya. Enzim lipase, protease dan amilase pada ikan nila yang diberipakan perlakuan E1C1 menunjukkan hasil yang lebih tinggi secarasignifikan ($P<0,05$) terhadap kontrol. Kinerja pertumbuhan pada ikan nila yang diberienzim NSP dan kromium organik menghasilkan nilai yang tidak signifikan dengan kontrol ($P>0,05$). Walaupun demikian, status antioksidan yang dihasilkan yaitu SOD dan GPx pada perlakuan E0C1 menghasilkan nilai yang lebih tinggi secara signifikan terhadap kontrol ($P<0,05$). Kesimpulan dari penelitian ini adalah penambahan enzim NSP dan kromium organik pada pakan rendah protein mampu meningkatkan kinerja kecernaan dan memanfaatkan karbohidrat dengan optimal. Hasil terbaik pada perlakuan E1C1

Kata kunci: antioksidan, enzim pencernaan, kecernaan nutrient, ikan nila

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the dominant freshwater aquaculture species in Indonesia. According to the Central Bureau of Statistics (BPS, 2023), Nile tilapia production in 2018 reached 1.171.699 tons, increasing to 1.300.518 tons in 2021. To maintain this production level, intensive aquaculture practices are required. Feed plays a crucial role in Nile tilapia farming, contributing approximately 92% of the total production cost (Suprayudi *et al*., 2023). Among feed components, protein is one of the most expensive nutrients, significantly impacting feed costs. The high cost of feed is not proportional to the market price of Nile tilapia, necessitating cost-effective feeding strategies. The use of low-protein feed (18–22%) has been proposed as a potential solution to mitigate this issue (Zaidy *et al*., 2021).

Protein reduction in aquafeed is typically compensated by an increased carbohydrate content. However, the utilization of carbohydrates in fish diets presents several challenges, primarily due to the limited ability of fish to metabolize carbohydrates and the presence of non-starch polysaccharides (NSPs). NSPs can be classified into soluble NSPs (pectin, mannan, arabinoxylan, and β-glucan) and insoluble NSPs (cellulose and hemicellulose) (Kumar *et al*., 2012). In general, soluble NSPs are more digestible than their insoluble counterparts (Sing & Kim, 2021). NSPs affect gastric motility by slowing gastric emptying, allowing sufficient time for fiber digestion.

According to Manik and Arleston (2021), Nile tilapia has a limited capacity to digest NSPs. Consequently, higher carbohydrate inclusion in feed leads to increased NSP content, reducing digestibility due to the limited enzymatic capacity of the fish. The digestibility of carbohydrate sources can be enhanced through the supplementation of non-starch polysaccharide (NSP) enzymes. Non-starch polysaccharide (NSP) enzymes comprise glucanase, mannanase, cellulase, xylanase, pectinase, and amylase (Tsion & Kumar, 2018; Angriani *et al*., 2021). Research by Dwinanti *et al.* (2023) reported that the supplementation of 5% *Lemna sp.* flour combined with 10 g/kg NSP enzyme resulted in optimal growth rates and survival rates in kissing gourami (*Helostoma temminckii*).

Suharyanti *et al*. (2021) further explained that NSP enzymes effectively hydrolyze complex-structured carbohydrates into simpler compounds, facilitating their absorption. However, fish generally exhibit limited capability in utilizing dietary carbohydrates (Rostika *et al*., 2020). As an omnivorous species, Nile tilapia can efficiently utilize dietary carbohydrate levels of up to 50% or even higher (Nanariain *et al*., 2017). The efficiency of carbohydrate utilization in fish is closely associated with insulin activity, which regulates glucose metabolism to fulfill energy requirements for growth (Puteri *et al*., 2020). Insulin sensitivity can be enhanced by chromium supplementation (Nur *et al*., 2020).

Chromium (Cr) is an essential trace mineral required for carbohydrate, protein, and lipid metabolism in animals (Suryadi *et al*., 2018). Chromium is available in both organic and inorganic forms; however, organic chromium (Cr-yeast) exhibits superior bioavailability compared to its inorganic counterparts (Costa & Murphy, 2019). Khaeriyah (2018) demonstrated that dietary organic chromium supplementation stimulates insulin activity via glucose tolerance factor (GTF), facilitating glucose uptake into cells and its subsequent conversion into energy. Elevated insulin levels in the bloodstream accelerate glucose transfer to target cells, ensuring its immediate utilization as an energy source while reducing protein catabolism for energy production (Rostika *et al*., 2020). However, insulin deficiency can disrupt glucose homeostasis, leading to oxidative stress due to increased free radical production or decreased antioxidant system activity (Prawitasari, 2019).

Several studies have investigated the effects of dietary organic chromium supplementation on fish species. For instance, Rakhmawati *et al.* (2018) reported that supplementation with chromium picolinate (CrPic) at 1 mg/kg and chromium yeast (CrYst) at 2 mg/kg improved growth performance and blood biochemical parameters in red tilapia. Similarly, Yanto *et al.* (2017) demonstrated that dietary supplementation with Cr^{3+} at 1.55 mg/kg enhanced the growth performance of *Leptobarbus hoevenii* (Jelawat fish). These studies suggest that organic chromium supplementation positively influences fish growth performance. While extensive research has been conducted on the use of NSP enzymes and organic chromium individually in Nile tilapia nutrition, studies investigating their combined effects on carbohydrate utilization remain limited. Therefore, this study aims to evaluate the digestibility and carbohydrate utilization efficiency in low-protein diets supplemented with NSP enzymes and organic chromium.

MATERIALS AND METHOD

Experimental design

This study was conducted from June 2022 to January 2023 at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University (Institut Pertanian Bogor). The experimental design employed a completely randomized factorial design (CRD) with two factors and three replicates. The treatments consisted of non-starch polysaccharide (NSP) enzyme supplementation (0 and 1 g/kg) and organic chromium supplementation (0, 1, and 2 mg/kg).

The preparation of fish tank and fish rearing

The experimental diet used in this study was a commercial feed containing 19.53% protein, 6.68% lipid, and 59.38% carbohydrate. The feed was supplemented with organic chromium and NSP enzymes according to the treatment groups. Total of 18 aquarium (each measuring of 50×40×35 cm^3) were used, with each tank filled with 60 liters of water. The test fish used in this study were Nile tilapia (*Oreochromis niloticus*) with an initial body weight of 33.09 ± 0.06 g per fish, stocked at a density of 10 fish per aquarium. The fish were reared for 50 days and fed ad libitum three times daily at 07:00, 12:00, and 17:00 WIB. During the rearing period, water quality parameters were maintained within optimal ranges temperature is between 29–30.5°C, pH is between 6.57–7.12, dissolved oxygen (DO) is between 5.0–5.6 mg/L, and ammonia (NH_3) is between 0.0004–0.0156 mg/L.

Digestibility performance (fecal collection)

Digestibility assessment was conducted using the fecal collection method. Fecal collection commenced on the fourth day after the fish were fed the experimental diet and continued until a sufficient amount of feces was obtained for analysis. Feces were collected by siphoning using a siphon tube and a fine-mesh filter to separate the fecal matter.

Fecal collection was performed twice daily, approximately 30 minutes to 1 hour after feeding. The collected feces were stored in sample bottles and preserved in a freezer to prevent decomposition. Subsequently, the fecal samples were dried using an oven and analyzed for protein, energy, and Cr_2O_3 content. The absorbance values were measured using a spectrophotometer at a wavelength of 350 nm (Rahmatia, 2016).

Observation parameters

Total digestibility (TD)

The total digestibility (TD) of Nile tilapia fingerlings can be calculated using the equation proposed by Takeuchi (1988):

$$TD = 100 - (100 \times \frac{a}{a'})$$

Note:
TD = Total digestibility (%)
a = Cr_2O_3 in feed (%)
a' = Cr_2O_3 in feces (%)

Nutrient digestibility (protein, total dietary carbohydrates, energy)

Nutrient digestibility, including protein, total dietary carbohydrates, and energy, can be calculated using the following formula (Takeuchi, 1988):

$$\int KN = [1 - (\frac{\{\%Cr_2O_3 \text{ in feed}}{\{\%\text{nutrient in feed}} \times \frac{\%\text{nutrient in feces}\}}{\%\ Cr_2O_3 \text{ in feed}\}})] \times 100$$

Energy digestibility

Energy digestibility can be calculated using the following formula (Takeuchi, 1988):

$$\int KN = [1 - (\frac{\{\%Cr_2O_3 \text{ in feed}}{\{\%\text{energy in feed}} \times \frac{\%\text{energy in feces}\}}{\%Cr_2O_3 \text{ in feed}\}})] \times 100$$

Measurement of digestive enzyme activity

The measurement of digestive enzyme activity (protease, amylase, and lipase) was conducted at the end of the study. For each measurement, three fish were randomly sampled from each treatment group, and their body weight was recorded. Fish dissection was performed by making an incision from the anal opening along the medio-ventral line toward the anterior, near the pectoral fins, using surgical scissors. The incision was made carefully to avoid damaging internal organs.

The upper muscle tissue was then separated using forceps to expose the digestive tract. The intestine, from the anterior to the posterior section, was carefully removed. The collected digestive organs were placed into labeled sample bags and stored in a freezer at -80°C (Cahyadi *et al.*, 2020). Digestive enzyme activity was measured by homogenizing the fish intestines with distilled water at a ratio of ten times the tissue weight to obtain a crude enzyme extract.

The extract was then stored in a refrigerator at -20°C until enzyme activity analysis. Protease activity was determined following the method of Bergmeyer and Grassi (1983), using casein as the substrate and tyrosine as the standard. One unit of protease enzyme activity was defined as the amount of enzyme that releases 1 mg of tyrosine in 10 minutes at 37°C. Amylase activity was assessed based on the Worthington method (1993), using starch as the substrate and maltose as the standard.

One unit of amylase enzyme activity was defined as the amount of enzyme that releases 1 mg of maltose from starch within three minutes at 95°C and pH 6.9. Lipase activity was determined using the Borlongan method (1990), with olive oil as the substrate. The free fatty acids released from the enzymatic hydrolysis of triglycerides in a stable olive oil emulsion were titrated with NaOH. One unit of lipase enzyme activity was defined as the volume of 0.05 N NaOH required for neutralization.

Specific growth rate (SGR)

The specific growth rate (SGR) of fish is calculated using the formula proposed by Huisman (1987):

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

Note:
SGR = specific growth rate (%)
T = observation period (days)
Wo = initial body weight (g)
Wt = final body weight (g)

Feed conversion ratio (FCR)

The feed conversion ratio (FCR) is calculated using the following formula (Takeuchi, 1988):

$$RKP = \frac{F}{[(Wt+Wd) - W0]}$$

Note:
FCR = Feed conversion ratio (%)
W_t = Final fish biomass (g)
W_0 = Initial fish biomass (g)
W_d = Fish biomass mortality during rearing (g)
F = Total feed during rearing (g)

Antioxidant activity analysis

Antioxidant activity was measured at the end of the rearing period. One or two fish from each replicate, after fasting for 24 hours, were selected and dissected. The dissection process began by making an incision from the anal opening along the medio-ventral line toward the anterior near the pectoral fins using scissors. The incision was made carefully to avoid damaging internal organs, and the upper muscle tissue was then opened using forceps. The liver was carefully removed according to the testing requirements and placed into a labeled sample plastic bag. The liver sample was then stored in a freezer at -40°C (Ali *et al*., 2015).

SOD activity was measured in the liver by taking a 0.3 g sample. The sample was analyzed using the SOD colorimetric test kit (Sigma-Aldrich, Merck KGaA, US). After the analysis, the results were read using a spectrophotometer at a wavelength of 450 nm. SOD levels were measured 24 hours after the last feeding. GPx activity was measured using the glutathione peroxidase activity colorimetric assay kit (Abcam, UK). The analysis was conducted by measuring absorbance at 460 nm.

MDA analysis was performed to evaluate oxidative stress conditions. A 0.5 g sample of liver tissue was taken for the analysis, using the MDA lipid peroxidation colorimetric test kit (Sigma-Aldrich, Merck KGaA, US). The liver sample was placed into a labeled plastic/bottle sample and stored in a freezer at -40°C. The analysis was then conducted, and the results were read using a spectrophotometer at a wavelength of 530-540 nm. MDA levels were measured 24 hours after the last feeding.

Data analysis

The collected data were tabulated using Microsoft Excel software. Data analysis was performed using Analysis of Variance (ANOVA) to determine the effect of treatments. If significant results were found, Duncan's Multiple Range Test (DMRT) was used for further calculations (Al-Arif, 2016).

RESULTS AND DISCUSSION

Results

Digestibility performance

Chromium oxide (Cr_2O_3) was added to the experimental feed at a concentration of 0.6% as a digestibility indicator. The digestibility

performance in Nile tilapia fed with the experimental diets is presented in Table 1. Based on the results of the Two-Way ANOVA, the addition of NSP enzymes and organic chromium to the low-protein feed significantly influenced digestibility compared to the control (P<0.05). The Nile tilapia fed with the treatment of 1 g/kg NSP enzyme and 1 mg/kg chromium (E1C1) and the treatment of 0 g/kg NSP enzyme and 2 mg/kg chromium (E0C2) showed significantly higher digestibility compared to the control treatment (P<0.05). The treatment with 1 g/kg NSP enzyme and 2 mg/kg chromium (E1C2) showed no significant difference compared to the treatment with 0 g/kg NSP enzyme and 1 mg/kg chromium (E0C1) (P>0.05), but the result was significantly lower than the control treatment (P<0.05). Meanwhile, the treatment with 1 g/kg NSP enzyme and 0 mg/kg chromium (E1C0) showed no significant difference compared to the control (P>0.05).

The Nile tilapia fed with the addition of 1 g/kg NSP enzyme and 1 mg/kg chromium (E1C1) and the treatment with 0 g/kg NSP enzyme and 2 mg/kg chromium (E0C2) showed significantly higher energy digestibility compared to the control treatment (P<0.05). The Nile tilapia fed with the addition of 1 g/kg NSP enzyme and 1 mg/kg chromium (E1C1) and the treatment with 0 g/kg NSP enzyme and 2 mg/kg chromium (E0C2) showed significantly higher protein digestibility compared to the control treatment (P<0.05). However, the treatment with 1 g/kg NSP enzyme and 2 mg/kg chromium (E1C2) resulted in a lower value that did not differ significantly from the control (P>0.05). The Nile tilapia fed with the experimental diets containing NSP enzymes and organic chromium showed no significant difference compared to the control treatment (P>0.05). The interaction between the treatments of NSP enzyme and organic chromium was significant (P<0.05). However, the overall effect of adding NSP enzymes did not produce a significant difference (P>0.05).

Digestive enzyme activity

The results of the digestive enzyme activity testing on Nile tilapia fed with the experimental diet are presented in Table 2. Based on the Two-Way ANOVA results, in general, the feed supplemented with NSP enzyme and organic chromium showed significant results compared to the control group (P<0.05). The fish fed with 1 g/kg NSP enzyme and 1 mg/kg chromium (E1C1) showed significantly higher lipase activity compared to the control group (P<0.05). The interaction between NSP enzyme and organic chromium showed a very significant result (P<0.05).

The fish fed with 1 g/kg NSP enzyme and 1 mg/kg chromium (E1C1) showed significantly higher protease activity compared to the control group (P<0.05). However, the treatment with 1 g/kg NSP enzyme and 0 mg/kg chromium (E1C0) showed no significant difference compared to the control group (P>0.05). The interaction between

Table 1. Digestibility performance of Nile tilapia after supplementation with NSP enzyme and organic chromium in low-protein feed.

Treatments	Observation parameters (%)			
	KT	KE	KP	KK
E0C0	47.86 ± 1.48^{bc}	59.49 ± 1.48^{a}	74.79 ± 1.12^{ab}	36.18 ± 2.33^{b}
E0C1	44.64 ± 2.54^{ab}	58.96 ± 2.59^{a}	83.35 ± 2.92^{c}	24.76 ± 5.14^{a}
E0C2	49.26 ± 2.40^{c}	79.94 ± 1.85^{c}	85.25 ± 1.16^{c}	34.49 ± 0.70^{b}
E1C0	48.52 ± 1.58^{c}	61.15 ± 1.55^{a}	76.11 ± 1.97^{b}	36.59 ± 2.29^{b}
E1C1	48.65 ± 1.64^{c}	81.91 ± 0.25^{c}	86.42 ± 1.50^{c}	32.11 ± 1.36^{b}
E1C2	41.66 ± 1.10^{a}	72.36 ± 0.63^{b}	71.64 ± 2.33^{a}	20.53 ± 2.27^{a}
Two Way Anova				
Level of enzymes (g/kg)	NS	P<0.05	NS	NS
Level of chromium (mg/kg)	NS	P<0.05	P<0.05	P<0.05
Enzymes X chromium	P<0.05	P<0.05	P<0.05	P<0.05

Note: total digestibility (TD), energy digestibility (ED), protein digestibility (PD), and carbohydrate digestibility (CD): not significant (ns). Enzyme (E); chromium (C); doses of 0, 1, 2. Different letters on the same row indicate a significant difference in treatment (P<0.05). Values presented are the mean and standard deviation.

NSP enzyme and organic chromium also showed no significant result (P>0.05). However, the addition of NSP enzyme and organic chromium had a significant effect (P<0.05).

The fish fed with 1 g/kg NSP enzyme and 1 mg/kg chromium (E1C1) showed significantly higher amylase activity compared to the control group (P<0.05). However, the treatment with 1 g/kg NSP enzyme and 0mg/kg chromium (E1C0) showed no significant difference compared to the control group (P>0.05). The interaction between NSP enzyme and organic chromium showed no significant result (P>0.05). But the addition of organic chromium showed a significant result (P<0.05).

Growth performance

The results of the growth performance testing on Nile tilapia fed with the experimental diets are presented in Table 3. Based on the Two-Way ANOVA results, overall, the feed supplemented with NSP enzyme and organic chromium did not show any significant difference compared to the control group (P>0.05). The interaction between NSP enzyme and organic chromium also showed no significant result (P>0.05).

Table 2. Digestive enzyme activity in Nile tilapia fed with diets supplemented with nsp enzyme and organic chromium in low-protein feed.

Treatments	Observation parameters (IU/mL)		
	Lipase	Protease	Amylase
E0C0	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.50 ± 0.05^{a}
E0C1	0.11 ± 0.00^{d}	0.02 ± 0.00^{c}	0.68 ± 0.00^{cd}
E0C2	0.08 ± 0.00^{c}	0.01 ± 0.00^{b}	0.54 ± 0.02^{ab}
E1C0	0.04 ± 0.00^{b}	0.01 ± 0.00^{a}	0.55 ± 0.07^{ab}
E1C1	0.13 ± 0.00^{e}	0.02 ± 0.00^{d}	0.74 ± 0.04^{d}
E1C2	0.08 ± 0.00^{c}	0.02 ± 0.00^{bc}	0.61 ± 0.04^{bc}
Two Way Anova			
Level of enzymes (g/kg)	P<0.05	P<0.05	NS
Level of chromium (mg/kg)	P<0.05	P<0.05	P<0.05
Enzymes X chromium	P<0.05	NS	NS

Note: Enzyme (E); chromium (C); doses of 0, 1, 2; not significant (NS). Different letters on the same row indicate a significantly different treatment effect (P<0.05). The values presented are the averages and standard deviations.

Table 3. Growth performance of Nile tilapia after supplementation of NSP enzyme and organic chromium in low-protein feed.

Treatments	Observation parameters			
	Bt (gr)	FC (gr)	RKP (%)	SGR (%)
E0C0	588.34 ± 128.88^{a}	481.83 ± 158.22^{a}	1.96 ± 0.29^{a}	1.18 ± 0.37^{a}
E0C1	624.16 ± 95.58^{a}	496.20 ± 58.21^{a}	1.76 ± 0.35^{a}	1.26 ± 0.30^{a}
E0C2	530.07 ± 79.55^{a}	371.90 ± 106.62^{a}	1.93 ± 0.24^{a}	0.92 ± 0.30^{a}
E1C0	568.95 ± 83.28^{a}	432.30 ± 116.36^{a}	1.85 ± 0.14^{a}	1.07 ± 0.29^{a}
E1C1	627.86 ± 131.80^{a}	518.73 ± 197.09^{a}	1.78 ± 0.16^{a}	1.25 ± 0.41^{a}
E1C2	535.15 ± 113.03^{a}	382.40 ± 101.11^{a}	2.13 ± 0.72^{a}	0.93 ± 0.42^{a}
Two Way Anova				
Level of enzymes (g/kg)	NS	NS	NS	NS
Level of chromium (mg/kg)	NS	NS	NS	NS
Enzymes X chromium	NS	NS	NS	NS

Note: Initial biomass (Bo), final biomass (Bt), feed consumption (FC), eed conversion ratio (FCR), specific growth rate (SGR), not significant (NS), enzyme (E), chromium (C), doses of 0, 1, 2. Letters that differ on the same row indicate significantly different effects (P<0.05). The values presented are averages and standard deviations.

Antioxidant activity

The antioxidant testing on tilapia fed with experimental diets is presented in Table 4. Based on the results of Two-Way ANOVA, overall, Superoxide Dismutase (SOD) in tilapia fed with diets supplemented with NSP enzyme and organic chromium showed significantly higher results compared to the control (P<0.05). The addition of NSP enzyme did not give significant results (P>0.05). However, the interaction between NSP enzyme and organic chromium showed very significant results (P<0.05). Glutathione Peroxidase (GPx) in tilapia fed diets with added NSP enzyme and organic chromium, overall, showed significantly higher results compared to the control (P<0.05). However, the treatment with 0 g/kg NSP enzyme and 1 mg/kg chromium (E0C1) showed significantly higher results compared to the other treatments (P<0.05).

The interaction between NSP enzyme and organic chromium showed very significant results (P<0.05). As for Malondialdehyde (MDA), tilapia fed the treatment with 0 g/kg NSP enzyme and 1 mg/kg chromium (E0C1) showed significantly lower results compared to the control (P<0.05). Meanwhile, other treatments showed no significant difference from the control (P>0.05). The interaction between NSP enzyme and organic chromium showed no significant results (P>0.05).

Water quality

The temperature in the maintenance media ranged from 29–30.5°C, while the pH ranged from 6.57–7.12. The dissolved oxygen (DO) level was between 5–5.6 mg/L, and ammonia levels ranged from 0.0004–0.0156 mg/L. All water quality parameters observed were within the optimum standards for tilapia cultivation, in accordance with BSN (2009).

Discussion

The results of this study indicate an interaction between the addition of NSP enzymes and organic chromium (Table 2). Based on the results of the Two-Way ANOVA, the treatment with the addition of 1 g/kg NSP enzyme and 1 mg/kg organic chromium (E1C1), as well as the addition of 0 g/kg NSP enzyme and 2 mg/kg organic chromium (E0C2), produced better total digestibility (KT), energy digestibility (KE), and protein digestibility (KP) compared to the control treatment. However, the addition of the NSP enzyme did not have a significant effect on KT, KP, and KE. This indicates that fish have very limited ability to digest carbohydrates, and the level of NSP enzyme used was not sufficient to optimally break down complex carbohydrate chains into simpler forms.

According to the study by Maas *et al*. (2018), the addition of xylanase and phytase enzymes in high-NSP feed did not have a significant effect on the digestibility of crude protein, crude fat, and total carbohydrate fractions in Nile tilapia. Similarly, Dwinanti *et al.* (2023) found that in tambakan fish, the addition of NSP enzymes was unable to break down anti-nutritional substances in *Lemna* sp. flour, resulting in suboptimal utilization of the protein and carbohydrates

Table 4. Antioxidant performance in Nile tilapia after supplementation with NSP enzyme and organic chromium in low-protein diet

Treatments	Observation parameters		
	SOD (% inhibition)	GPx (nmol)	MDA (μM)
E0C0	49.96 ± 0.78^{a}	91.17 ± 1.15^{a}	5.11 ± 0.36^{b}
E0C1	83.89 ± 2.46^{d}	99.04 ± 0.20^{d}	4.52 ± 0.02^{a}
E0C2	73.91 ± 4.97^{c}	98.88 ± 1.38^{d}	4.58 ± 0.18^{ab}
E1C0	75.11 ± 1.41^{c}	92.86 ± 1.60^{ab}	4.73 ± 0.45^{ab}
E1C1	64.62 ± 3.79^{b}	94.58 ± 1.31^{cd}	4.96 ± 0.51^{ab}
E1C2	75.87 ± 4.01^{c}	96.52 ± 2.58^{d}	4.64 ± 0.16^{ab}
Two Way Anova			
Level of Enzymes (g/kg)	NS	P<0.05	TS
Level of Chromium (mg/kg)	P<0.05	P<0.05	TS
Enzymes X chromium	P<0.05	P<0.05	TS

Note: Superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA), not significant (NS). enzyme (E), chromium (C), doses of 0, 1, 2. Different letters on the same line indicate significantly different effects of the treatments (P<0.05). The values presented represent the mean and standard deviation.

contained in *Lemna* sp. flour. However, the findings of Maas *et al.* (2020) showed different results in Nile tilapia, where common quality (CQ) and low quality (LQ) feeds resulted in higher digestibility values for crude protein, crude fat, carbohydrates, and energy compared to CQ and LQ feeds without enzyme supplementation. Likewise, Anugrah (2018) reported that in Nile tilapia, the digestibility values of protein, energy, and total nutrients in rubber seed hydrolyzed with an enzyme cocktail (88.18%, 82.61%, 84.65%, and 70.70%) were higher than those in rubber seed flour (TBK) and defatted rubber seed flour (TBKD).

The level of nutrient digestibility can be influenced by several factors, including feed composition, fish size, and the amount of feed consumed. According to Nurfitasari *et al.* (2020), factors affecting feed digestibility include fish age, feed type, chemical and physical properties of the feed, nutritional content, as well as the type and amounts of enzymes present in the digestive tract. In addition to improving digestibility, one of the other effects of adding exogenous enzymes to feed, such as NSP enzymes, is enhancing the sensitivity of endogenous enzyme production. Susanto *et al.* (2017) stated that the addition of exogenous enzymes in feed can increase the production of endogenous enzymes such as amylase for breaking down carbohydrates, protease for breaking down proteins, and lipase for breaking down fats, supporting the endogenous production of digestive enzymes in fish.

Furthermore, Liang *et al.* (2022) explained that specifically, the addition of exogenous enzymes, such as protease, can break down dietary proteins into peptides, improving their digestibility. Thus, exogenous protease can supplement the low secretion levels in the fish digestive tract, assisting endogenous enzymes in completely digesting nutrients and enhancing their utilization. The digestive enzyme activity in Nile tilapia fed a low-protein diet supplemented with NSP enzymes and organic chromium showed significantly higher amylase, protease, and lipase enzyme values compared to the control ($P<0.05$). The results indicate that amylase activity was the highest, followed by lipase and protease activity (Table 3). The high activity of amylase and lipase suggests that Nile tilapia can optimally utilize carbohydrates in feed as an energy source. However, the interaction between NSP enzymes and organic chromium did not yield significant results for protease and amylase enzymes ($P>0.05$). Additionally, the addition of NSP enzymes did not have a significant effect on amylase enzyme activity ($P>0.05$).

The obtained values suggest that NSP enzymes can secrete endogenous enzymes according to the nutrient composition of the feed and the amount of enzyme used. Meanwhile, chromium plays a role in glucose utilization derived from carbohydrates as an energy source, allowing proteins to be used for growth and body structure formation. Sagita *et al.* (2017) also explained that the composition of feed ingredients determines enzyme utilization the more nutrients digested, the better the energy absorption and distribution throughout the body. This is supported by Bilal *et al.* (2016), who reported that NSP-ase supplementation at a 25% inclusion level did not provide beneficial effects, possibly due to excessive crude fiber in the feed.

Similarly, Liang *et al.* (2022) stated that the addition of a combination of exogenous protease and endogenous digestive enzymes can benefit fish growth. Fish produce endogenous enzymes from their gastrointestinal system or gut microbiota. The addition of exogenous amylase can enhance metabolic activity and regulate blood glucose levels, while cellulase-enriched feed increases digestive enzyme activity (e.g., protease and amylase) in grass carp compared to the control group (Liang *et al*., 2022). Findings that were reported by Hua *et al*. (2012) and Emami *et al.* (2015), showing that the addition of organic chromium in low-protein diets plays a crucial role in carbohydrate and lipid metabolism by effectively improving glucose tolerance and reducing insulin resistance.

Hou *et al.* (2018) also stated that the addition of chromium yeast (CY) significantly enhances intestinal lipase activity, possibly related to glucose metabolism, thereby influencing lipid metabolism in vivo. In general, digestive enzyme activity correlates with fish growth (Slamet & Aslianti, 2016). The higher the digestive enzyme activity, the better the growth rate. The study results in Table 4 show that Nile tilapia fed a low-protein diet supplemented with NSP enzymes and organic chromium exhibited no significant differences ($P>0.05$) in growth parameters, including initial biomass (Bo), final biomass (Bt), total feed consumption (JKP), feed conversion ratio (RKP), and specific growth rate (SGR), compared to the control. This suggests that digestion and energy production were already optimal, but the low protein content in the feed limited fish growth. Moreover, the interaction

between NSP enzyme and organic chromium supplementation in this study did not significantly affect the growth of Nile tilapia. The levels of NSP enzymes and organic chromium used were not sufficient to improve feed digestibility.

Feed digestibility is influenced by crude fiber content, and the low-protein feed used in this study also led to increased blood glucose levels. Consequently, chromium utilization in this study was not optimal in enhancing insulin activity for glucose transfer into body cells as energy. According to Liu *et al.* (2022), NSP negatively impacts digestive enzyme activity and intestinal morphology, ultimately reducing nutrient digestibility and fish growth. These findings align with Mokoginta *et al.* (2005), who reported that administering chromium in the form of organic Cr to common carp resulted in the best growth and protein retention at 1.6–2.2 ppm Cr+3. However, in Nile tilapia (O. niloticus), growth was relatively unchanged, but the highest protein retention was observed at 3.9 ppm Cr+3.

Growth occurs during metabolic processes, during which an imbalance between free radicals (reactive oxygen and nitrogen species) and the body's antioxidant defense system can lead to oxidative stress. This aligns with Prawitasari (2019), who defines oxidative stress as a condition caused by increased free radical (ROS) production or decreased antioxidant defense activity. Menon *et al.* (2023) further explain that oxidative stress occurs when there is a discrepancy between the amount of ROS produced and accumulated and the fish's ability to eliminate them through antioxidants or repair the resulting damage. Oxidative stress can alter glucose metabolism genes and disrupt energy production and storage. It also affects gene expression related to energy metabolism, maturation, reproduction, immunological and antioxidant enzymes, and other cellular defense proteins in fish (Jomova *et al*., 2023).

Following ROS production in the body, the antioxidant defense system is activated, leading to increased activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutamate transaminase, and glutamate oxaloacetate transaminase. SOD is a crucial antioxidant enzyme that catalyzes the conversion of the superoxide anion (O2*) into hydrogen peroxide (H2O2) and molecular oxygen (O2) (Pratiwi *et al*., 2022). GPx is a peroxidase enzyme that removes free radicals in vivo and is used to assess the body's antioxidant function. Meanwhile, malondialdehyde (MDA) is commonly used to determine lipid peroxidation levels (Hou *et al*., 2018).

In Nile tilapia fed a low-protein diet supplemented with NSP enzymes and organic chromium, SOD and GPx levels were significantly higher than in the control group ($P<0.05$). The interaction between NSP enzymes and organic chromium also had a significant effect on SOD and GPx ($P<0.05$) but not on MDA levels ($P>0.05$). The supplementation of NSP enzymes and organic chromium in this study effectively reduced lipid peroxidation (MDA), as indicated by the increased SOD and GPx levels. This finding aligns with Hou *et al.* (2018), who reported that chromium supplementation significantly enhances GSH-Px levels and reduces MDA content. Similarly, Elbaz *et al.* (2023) found that broiler chickens fed fermented canola meal with exogenous enzyme supplementation exhibited improved antioxidant status, with increased SOD and significantly reduced MDA levels.

However, in this study, NSP enzyme supplementation did not significantly affect SOD levels ($P>0.05$). It is suspected that the NSP enzyme levels used in the low-protein diet were insufficient to maximally enhance the antioxidant defense system, particularly SOD, in Nile tilapia. This is consistent with the findings of Zhou *et al.* (2015), who compared two fish species, Wuchang fish and common carp, fed high-carbohydrate and normal-carbohydrate diets. Their results showed that the high-carbohydrate diet group exhibited lower liver SOD and total antioxidant capacity (T-AOC), indicating that high-carbohydrate diets reduce the liver's antioxidant capability in both species. These findings suggest that organic chromium plays a more significant role in enhancing antioxidant enzyme activity, supported by the observation that NSP enzyme supplementation did not significantly affect SOD levels ($P>0.05$).

The higher the levels of antioxidant enzymes, the better the protection against oxidative damage, helping to prevent or mitigate oxidative stress caused by pollutants, radiation, or extreme environmental conditions (Halliwell *et al*., 2015; Pham-Huy *et al*., 2008). Therefore, antioxidant enzymes play a crucial role in fish survival. When fish survival rates are high, overall fish production improves.

CONCLUSION

The addition of Non-Starch Polysaccharide (NSP) enzymes and organic chromium (Cr) in a low-protein diet can enhance digestion performance by up to 80% and optimize carbohydrate utilization. The best results were observed in the treatment with the addition of NSP enzymes at 1 g/kg and organic chromium at 1 mg/kg (E1C1).

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