

Evaluation of feeds with different NSP levels on digestive enzyme activity, intestinal histology, and digestibility of Nile tilapia *Oreochromis niloticus*

Evaluasi pakan NSP kadar berbeda terhadap aktivitas enzim pencernaan, histologi usus dan pencernaan ikan Nila *Oreochromis niloticus*

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ABSTRACT

The increasing use of plant-based feed ingredients may lead to an increase in the level of non-starch polysaccharides (NSP) in fish feed. This study was conducted to assess the effects of varying dietary NSP levels on digestive enzyme activity, intestinal histology, postprandial blood glucose levels, and feed digestibility in Nile tilapia (*Oreochromis niloticus*). The present study consisted of three treatments, namely control-diet (Low-NSP, 11.59%), Medium-NSP (22.51%), and High-NSP (31.76%) with five replications for each treatment. Nile tilapia with an initial weight of 17.04 ± 0.11 g were kept in aquariums at a density of 15 fish for 30 days. The results showed that the low NSP feed resulted in higher protease, lipase, and amylase enzyme activity compared to the other treatments. The low NSP treatment had greater villi height and wider surface area in the midgut area of the intestine, whereas the high NSP treatment showed higher villi height and surface area in the hindgut area. Protein, fat, dry matter, energy, and phosphorus digestibility were significantly higher in the low NSP ($P < 0.05$). Fish in the low NSP treatment showed higher postprandial blood glucose levels compared to the other treatments. Furthermore, growth performance and retention of protein, lipid, and phosphorus were higher in fish maintained in the low NSP treatment ($P < 0.05$) compared to other treatments. In conclusion, the present study demonstrated that different dietary levels of NSP resulted in different levels of digestive enzyme activities, changes in intestinal structure, feed digestibility as well as growth and nutrient retention in Nile tilapia.

Keywords: enzyme activity, feed digestibility, Nile tilapia, non-starch polysaccharides (NSP)

ABSTRAK

Meningkatnya penggunaan bahan pakan nabati dapat menyebabkan peningkatan *non-starch polysaccharide* (NSP) dalam pakan ikan. Penelitian ini bertujuan untuk mengevaluasi pengaruh kadar NSP yang berbeda pada pakan ikan terhadap aktivitas enzim pencernaan, histologi usus, kadar glukosa darah *postprandial* dan pencernaan pakan ikan nila (*Oreochromis niloticus*). Penelitian ini terdiri dari tiga perlakuan, yaitu low NSP (11,59%), medium NSP (22,51%), dan high NSP (31,76%) dengan lima ulangan untuk setiap perlakuan. Ikan nila yang digunakan dengan berat awal $17,04 \pm 0,11$ g, dipelihara di akuarium dengan kepadatan 15 ikan selama 30 hari. Hasil penelitian menunjukkan bahwa pakan low NSP menghasilkan aktivitas enzim protease, lipase, dan amilase lebih tinggi dibandingkan dengan perlakuan lainnya. Perlakuan low NSP memiliki tinggi villi yang lebih panjang dan luas permukaan yang lebih lebar di area midgut usus, sedangkan perlakuan high NSP menunjukkan tinggi villi dan luas permukaan yang lebih tinggi di area hindgut. Kecernaan protein, lemak, total, energi, dan fosfor secara signifikan lebih tinggi pada low NSP ($P < 0,05$). Ikan dengan perlakuan low NSP menunjukkan kadar glukosa darah *postprandial* yang lebih tinggi dibandingkan dengan perlakuan lainnya. Selanjutnya, kinerja pertumbuhan dan retensi protein, lemak, dan fosfor lebih tinggi pada ikan yang dipelihara pada perlakuan low NSP ($P < 0,05$) dibandingkan dengan perlakuan lainnya. Kesimpulan pada penelitian ini menunjukkan bahwa kadar NSP dalam pakan yang berbeda menghasilkan kadar aktivitas enzim pencernaan yang berbeda, perubahan struktur usus, pencernaan pakan serta pertumbuhan dan retensi nutrisi pada ikan nila.

Kata kunci: aktivitas enzim, ikan nila, pencernaan pakan, *non-starch polysaccharide* (NSP)



INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed fishery commodities worldwide because it has a fast growth rate, tolerance to various environmental conditions, resistance to stress and diseases, and easy acceptance of formulated feed (Islam *et al.*, 2025; El-Sayed *et al.*, 2025; Vega *et al.*, 2025). Global Nile tilapia production has increased annually, from 1.7 million tons in 2005 to 4.4 million tons in 2020, making it important for aquaculture production (FAO, 2022). The high cost of feed remains one of the main challenges in Nile tilapia culture. This dependency on feed poses a significant risk to the sustainability of the aquaculture industry, as feed costs account for approximately 75.5–91.2% of total fish production costs (Suprayudi, 2018). To mitigate the high cost of feed, the aquaculture feed industry has increasingly turned to plant-based feed ingredients.

The increasing use of plant-based feed ingredients has the potential to increase the content of *non-starch polysaccharides* (NSP) in fish feed (Deng *et al.*, 2021). The NSP content in plant raw materials commonly used in feed production can range from 22.7% to 50.5% (Ren *et al.*, 2020). NSP are complex carbohydrates other than starch and free sugars with long polymer structures consisting of many monosaccharide units and are a major component of plant cell walls (Marcotuli *et al.*, 2020). NSP components such as lignin, cellulose, hemicellulose, and pectin are generally indigestible by fish due to the limited ability of fish to produce degrading enzymes such as cellulase, glucanase, and xylanase (Maas *et al.*, 2020; Dey *et al.*, 2024). NSP is classified as fiber and is considered a component that is difficult for fish to digest so it is often regarded as having limited nutritional value or even as an anti-nutrient (Sinha *et al.*, 2011; Zhang *et al.*, 2025).

Numerous studies in fish have shown that elevated NSP levels can adversely affect feed digestibility and growth performance. The study by Liu *et al.* (2022b) showed that diets containing NSP above 9% resulted in a decrease in specific growth rate, protein efficiency, and nutrient digestibility. In rainbow trout, NSP inclusion up to 24.8% altered the composition of intestinal microbiota and serum metabolites and reduced growth performance (Zhou *et al.*, 2022). In Nile tilapia, high NSP ingredients such as soybean meal (217 g NSP/kg) or additional NSP sources

such as guar gum have been shown to damage intestinal villus morphology and decrease nutrient utilization (Wang *et al.*, 2023). However, specific types and amounts of NSP have been reported to positively influence the digestive tract structure, intestinal microflora, and fish growth (Liu *et al.*, 2022a).

Certain types of NSP act as prebiotics that can be fermented by beneficial bacteria, including *Lactobacillus* and *Bifidobacterium*, resulting in the production of short-chain fatty acids (SCFAs) that reduce intestinal pH and inhibit pathogenic bacteria. This process enhances the integrity of the intestinal mucosa, improves villus morphology, and increases the efficiency of nutrient absorption. The combined effects ultimately contribute to improved feed utilization and support better growth performance (Singh *et al.*, 2021; Jha *et al.*, 2021; Morgan *et al.*, 2023). Additionally, the presence of NSP in feed at certain levels is reported to be a source of organic carbon, facilitating the growth of biofloc forming bacteria (Kabir *et al.*, 2020). Research conducted by Kabir *et al.* (2020) showed that feed containing NSP increased the abundance of natural food in aquaculture ponds and increased the comparable C:N ratio.

This feed is expected to be able to meet the nutritional requirements of Nile tilapia while simultaneously serving as a source of organic carbon for heterotrophic bacteria in biofloc systems, which can be further utilized by Nile tilapia. However, studies evaluating the direct effect of different NSP inclusion levels in formulated diets for Nile tilapia remain limited. Therefore, further research is necessary to evaluate digestibility, digestive enzyme activity and intestinal histology in Nile tilapia.

MATERIALS AND METHODS

Preparation of maintenance media and test animals

This study was conducted from September 2023 to November 2023 at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Indonesia. Nile tilapia with an initial weight of 17.04 ± 0.11 g and an initial length of 10.91 ± 0.12 cm were obtained from the experimental pond hatchery unit at the Department of Aquaculture, IPB University, and randomly distributed into aquariums measuring $60 \times 50 \times 50$ cm³. The experiment consisted of 15 treatment units

and 5 additional units for growth testing using commercial feed as the control, with a stocking density of 15 fish. Each aquarium was equipped with an aeration system to maintain dissolved oxygen levels. Maintenance was carried out for 30 days by giving test feed three times daily at 08:00, 12:00, and 16:00 at satiation. Water quality measurements conducted during the study indicated that the temperature of the rearing water ranged 28.2–29.4°C, dissolved oxygen (DO) 4.14–4.94 mg/L, pH 7.11–7.48 and nitrate 5–20 mg/L, nitrite 0.00–0.25 mg/L, ammonia 0.00–0.50 mg/L.

Preparation of the test feed

The experimental diets were prepared by weighing the feed ingredients according to the formulated composition to achieve a protein level of 28% (Table 1). The feed ingredients were then mixed using a mixer machine until homogeneous. The addition of chromium oxide (Cr₂O₃) which was used as a marker for determining digestibility tests was added at 0.5% and then water was added until homogeneous. The feed mixture was molded into pellets. The feed pellets were oven-dried at 50°C for 6–12 hours. The treatments consisted of

Table 1. Formulation and proximate of Nile tilapia feed with different levels of non-starch polysaccharides.

Raw Material (%)	Feed Treatment (%)		
	Low NSP	Medium NSP	High NSP
Soybean meal	31	30	28
Rice bran	-	-	7.45
Fishmeal	4	4	4
Corn gluten meal	5	6.29	-
Meat bone meal	8	7	6
Cassava starch	13.9	-	-
Corn gluten feed	-	6	9.5
Wheat flour	28.55	21	13.5
Palm kernel meal	-	16.16	22
Crude palm oil	3	3	3
Fish oil	1.5	1.5	1.5
PMC binder	0.3	0.3	0.3
Vitamin mix ¹	1	1	1
Mineral mix ¹	0.5	0.5	0.5
Monocalcium phosphate	1	1	1
Lysine	0.75	0.75	0.75
Methionine	0.75	0.75	0.75
Choline chloride	0.15	0.15	0.15
NaCl	0.1	0.1	0.1
Cr ₂ O ₃ ²	0.5	0.5	0.5
Total	100	100	100
Proximate Content in Dry Matter (%)			
Protein (%)	28.42	28.87	28.91
Fat (%)	5.19	7.01	7.16
Ash (%)	8.82	9.36	9.70
Crude fiber (%)	4.79	8.74	13.65
NFE (%) ³	53.19	46.01	40.58
Gross energy (kcal/kg) ⁴	4278.36	4181.32	3974.32

Note: ¹Commercial product, ²Cr₂O₃= chromium oxide, ³NFE = nitrogen-free extract, ⁴Gross energy (GE) of dry weight feed was calculated based on protein = 5.64 kcal/g, fat = 9.44 kcal/g, and carbohydrate/NFE = 4.11 kcal/g (Watanabe, 1998).

three types of feed, namely control–diet (Low–NSP, 11.59%), Medium–NSP (22.51%), and High–NSP (31.76%). Additionally, the growth performance of Nile tilapia fed the commercial diet was evaluated.

Chemical analysis

The feed underwent proximate analysis to determine its protein, fat, ash, moisture, crude fiber, and nitrogen-free extract (NFE) contents (AOAC, 2012). Non-starch polysaccharides (NSP) content in the feed was calculated based on Kabir *et al.* (2020) using the formula: total carbohydrate – (starch + free sugars). Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were analyzed by Soest (1963). Phosphorus content in the feed, fish body, and feces was analyzed by Reitz *et al.* (1960). Digestibility parameters measured in this study included total digestibility (dry matter) and the digestibility of protein, fat, and energy in the test feed. Fecal collection began four days after chromium administration and was performed twice daily, approximately 30–60 minutes after feeding (Oktaviani *et al.*, 2025).

Digestive enzyme activity was evaluated on day 30 of the experiment. At the end of the rearing period, the fish were fasted for 24 hours. The collected digestive organs were placed in labeled sample bags and stored in a freezer at –80°C (Cahyadi *et al.*, 2020), after which analyses were conducted to determine protease activity (Cupp-Enyard & Aldrich, 2008), amylase activity (Worthington, 1993), and lipase activity (Borlongan, 1990). Blood glucose measurements were performed postprandially. Fish were fasted for 24 hours prior to initial blood sampling, followed by feeding. Subsequent blood samples were taken at 1, 2, 4, 6, and 8 hours after feeding (Rakhmawati, 2018).

The histological measurement of Nile tilapia intestines was divided into two sections, namely the midgut and the hindgut. Hindgut samples were taken 3–5 cm before the anus, and each section of the intestine was cut into 1 cm segments (Tran-Ngoc *et al.*, 2019). Intestinal morphometric observations included the intestinal length ratio (IRL), villi height (VH), crypt depth (CD), villi surface area (VSA) based on Iji *et al.* (2001) and the ratio of villus height to crypt depth (RVHCD) (Fard *et al.*, 2014) using the following formula:

$$\text{Ratio of villus height to crypt depth} = \frac{\text{Average villus height } (\mu\text{m})}{\text{Average crypt depth } (\mu\text{m})}$$

Growth performance

Growth performance parameters included individual final weight, specific growth rate (SGR), absolute growth rate (AGR), total feed consumption (TFC), feed conversion ratio (FCR), protein retention (PR), fat retention (FR), phosphorus retention (PHR) and survival rate (SR).

Data analysis

The data obtained were tabulated using Microsoft Excel 2019 software. Digestibility parameters, postprandial blood glucose, digestive enzyme activity, phosphorus content analysis, fish intestinal morphometrics, and growth performance were analyzed using SPSS version 22, followed by normality and homogeneity tests. Statistical analysis was performed using one-way analysis of variance (ANOVA) with a 95% confidence level. If significantly different results were found, a DMRT (Duncan's Multiple Range Test) was applied. Water quality and fish intestinal histology were analyzed using descriptive and image analysis.

RESULTS AND DISCUSSION

Results

Analysis of NSP, ADF, NDF, and phosphorus content

The results of the analysis of NSP, ADF, NDF, and phosphorus content in feed with different levels of NSP are presented in Table 2. The analysis revealed that the highest values for NSP, ADF, NDF, and phosphorus were observed in the high NSP treatment compared to the medium and low NSP treatments.

Enzyme activity analysis

The enzyme activity tests in the digestive tract of Nile tilapia fed with diets containing different levels of NSP are presented in Table 3. The activities of protease, lipase, and amylase in the low NSP treatment were significantly higher than those in the other NSP treatments ($P < 0.05$).

Histology and morphometrics of fish intestine

The fish intestinal histology analysis included both the midgut and hindgut. The results of the histological observations for each treatment group are presented in Figure 1 and Table 4. Based on observations, fish in the low NSP treatment had higher villi height and surface area in the midgut

compared to the medium NSP and high NSP treatments. In contrast, the fish in the high NSP treatment showed higher villi height and surface area in the hindgut.

Based on morphometric analysis, Nile tilapia fed with the high NSP diet exhibited an increased intestinal length ratio compared to the other treatments ($P < 0.05$). In the midgut, the low NSP

Table 2. Content of NSP, ADF, NDF, and Phosphorus in treated diets with different levels of NSP.

Analysis	Feed Treatment		
	Low NSP	Medium NSP	High NSP
NSP (%)	11.59	22.51	31.76
ADF (%)	4.78	10.57	18.01
NDF (%)	18.23	26.76	33.54
P (%)	0.344	0.378	0.380

Note: Non-starch polysaccharides (NSP), acid detergent fiber (ADF), neutral detergent fiber (NDF), phosphorus (P).

Table 3. Enzyme activities in the digestive tract of Nile tilapia fed with different levels of NSP.

Enzyme activity analysis (IU/mL)	Feed Treatment			
	Low NSP	Medium NSP	High NSP	¹ Initial fish
Protease	0.02 ± 0.00 ^b	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00
Lipase	0.07 ± 0.01 ^b	0.06 ± 0.02 ^{ab}	0.05 ± 0.00 ^a	0.03 ± 0.00
Amylase	0.59 ± 0.03 ^b	0.57 ± 0.06 ^{ab}	0.52 ± 0.06 ^a	0.45 ± 0.00

Note: ¹The initial fish values are provided as baseline information prior to treatment and were excluded from the statistical evaluation. Values are presented as mean ± standard deviation. Different uppercase letters in different rows indicate significantly different treatment effects ($P < 0.05$).

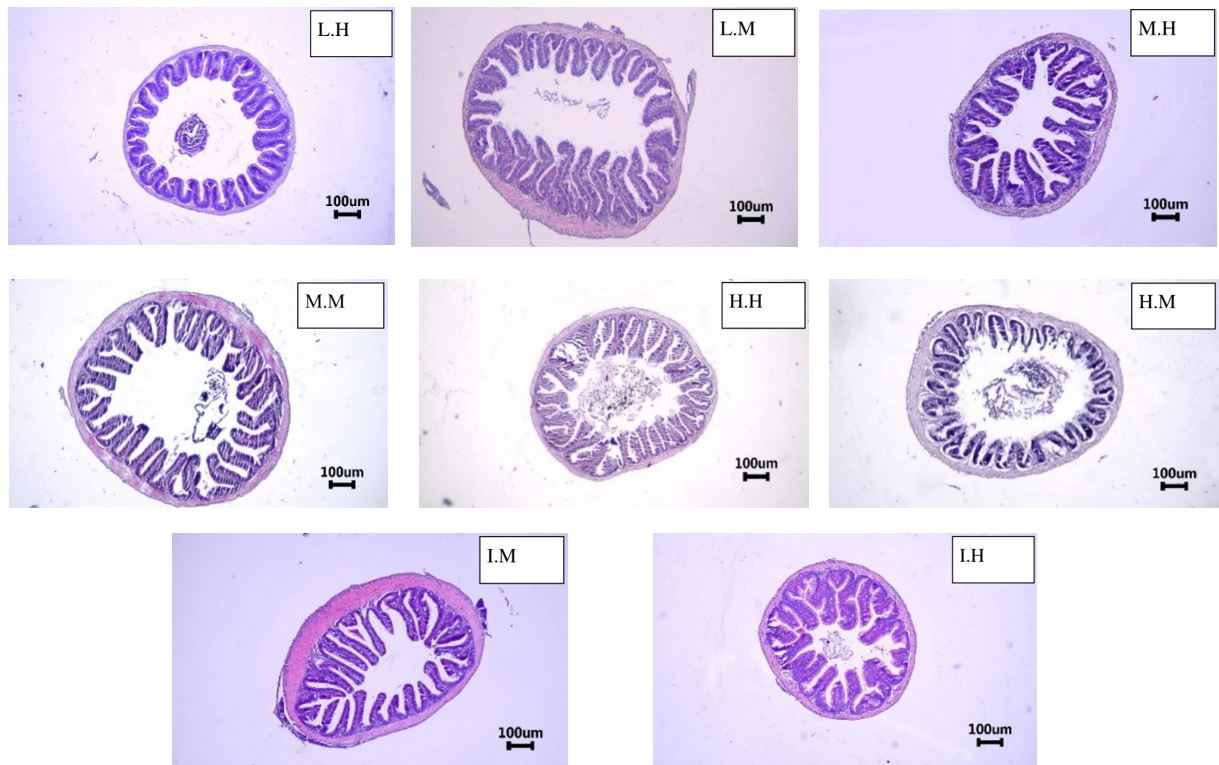


Figure 1. Intestinal histology of Nile tilapia fed with different NSP levels in the midgut and hindgut intestine. Low NSP midgut intestine (L.M), low NSP hindgut intestine (L.H), medium NSP midgut intestine (M.M), medium NSP hindgut intestine (M.H), high NSP midgut intestine (H.M), high NSP hindgut intestine (H.H), Initial fish midgut intestine (I.M), initial fish hindgut intestine (I.H).

treatment exhibited a greater villi length and surface area compared to the other treatments. In contrast, the high NSP treatment showed greater villi length and surface area in the hindgut. Furthermore, the ratio of villi length to crypt depth in both the midgut and hindgut was highest in the low NSP treatment ($P < 0.05$), but had a lower value than the initial fish.

Digestibility performance

Digestibility of diets with different NSP content showed significant differences between treatments (Table 5), with the low NSP treatment consistently showing higher nutrient and energy digestibility than the other treatments ($P < 0.05$).

Postprandial blood glucose

Measurement of postprandial blood glucose levels of Nile tilapia was carried out at hours 0, 1,

2, 4, 6, and 8. The results indicated that fish in the low-NSP treatment exhibited a greater increase in blood glucose levels than those in the other NSP treatments, with peak glucose absorption observed 2nd hour. In contrast, the medium and high NSP treatments exhibited peak blood glucose absorption at the 4th hour post-feeding. Blood glucose levels in all treatments declined and returned to near-basal conditions by the 8th hour.

Growth Performance

The growth performance of Nile tilapia fed diets containing different NSP levels after 30 days of rearing is presented in Table 6. Fish fed the low-NSP diet exhibited significantly higher final individual weight, absolute growth rate, and specific growth rate than those in the other treatments ($P < 0.05$). In contrast, the high-NSP

Table 4. Intestinal morphometrics of Nile tilapia fed with different levels of NSP in the midgut and hindgut intestine.

Parameters	Intestinal Morphometrics			
	Low NSP	Medium NSP	High NSP	Initial Fish
ILR	4.11 ± 0.03 ^b	4.34 ± 0.06 ^c	4.75 ± 0.15 ^d	3.44 ± 0.17 ^a
MIVH (µm)	223 ± 14.20 ^b	213 ± 7.59 ^b	137 ± 5.38 ^a	230 ± 7.31 ^b
HIVH (µm)	184 ± 11.40 ^a	207 ± 15.30 ^{ab}	220 ± 14.20 ^b	212 ± 6.81 ^b
MICD (µm)	42.07 ± 1.23 ^{ab}	44.64 ± 3.67 ^b	36.25 ± 0.95 ^a	37.72 ± 4.56 ^a
HICD (µm)	39.60 ± 1.26 ^a	45.93 ± 1.25 ^b	52.11 ± 1.94 ^c	37.05 ± 1.33 ^a
MIVSA (µm)	367 ± 16.25 ^b	338 ± 13.16 ^b	215 ± 15.43 ^a	365 ± 16.67 ^b
HIVSA (µm)	302 ± 2.09 ^a	361 ± 14.98 ^{bc}	379 ± 14.91 ^c	348 ± 15.21 ^b
RVHMICD	5.30 ± 0.29 ^{bc}	4.77 ± 0.25 ^b	3.79 ± 0.25 ^a	6.15 ± 0.86 ^c
RVHHICD	4.64 ± 0.27 ^a	4.51 ± 0.43 ^a	4.23 ± 0.20 ^a	5.73 ± 0.25 ^b

Note: ¹Intestinal length ratio (ILR), midgut intestinal villi height (MIVH), hindgut intestinal villi height (HIVH), midgut intestinal crypt depth (MICD), hindgut intestinal crypt depth (HICD), midgut intestinal villi surface area (MIVSA), hindgut intestinal villi surface area (HIVSA), Ratio of villi height to midgut intestinal crypt depth (RVHMICD), Ratio of villi height to hindgut intestinal crypt depth (RVHHICD). Values are presented as mean ± standard deviation. Different superscript letters in different rows indicate significantly different treatment effects ($P < 0.05$).

Table 5. Digestibility performance of Nile tilapia treated with different levels of NSP in the diet

Digestibility Analysis (%)	Digestibility Value		
	Low NSP	Medium NSP	High NSP
Protein Digestibility	88.66 ± 0.80 ^c	79.94 ± 0.59 ^b	77.20 ± 0.68 ^a
Fat Digestibility	84.21 ± 0.89 ^c	79.55 ± 0.19 ^b	78.10 ± 0.86 ^a
Carbohydrate Digestibility	73.14 ± 0.83 ^b	72.22 ± 0.60 ^{ab}	71.89 ± 0.79 ^a
Total Digestibility	60.15 ± 0.79 ^c	51.86 ± 0.47 ^b	48.46 ± 0.64 ^a
Energy Digestibility	79.17 ± 0.49 ^c	76.49 ± 0.21 ^b	75.24 ± 0.50 ^a
Phosphorus Digestibility	79.99 ± 0.08 ^c	54.19 ± 0.12 ^b	35.04 ± 0.04 ^a

Note: Values are presented as mean ± standard deviation. Different superscript letters in different rows indicate significantly different treatment effects ($P < 0.05$).

treatment resulted in the highest feed consumption and feed conversion ratio ($P < 0.05$). Protein, lipid, and phosphorus retention were also significantly higher in fish fed the low-NSP diet compared with the other NSP treatments ($P < 0.05$). Furthermore, Nile tilapia fed the commercial diet demonstrated better growth performance, including higher final individual weight, absolute growth rate, and specific growth rate, as well as lower feed consumption and feed conversion ratio than fish fed the NSP experimental diets. Survival rates did not differ significantly among treatments, and no mortality was observed during the rearing period ($P > 0.05$).

Discussion

Non-starch polysaccharides (NSP) are the main components of plant cell walls, commonly referred to as dietary fiber, and are poorly digested by fish due to the limited endogenous enzymes capable of degrading complex polysaccharides (Liu *et al.*, 2022a; Liang *et al.*, 2022). The presence of anti-nutritional factors, including NSP, phytate, acid detergent fiber (ADF), and neutral detergent fiber (NDF) in the diet may reduce nutrient digestibility in fish (Fauzan *et al.*, 2022; Verdegem *et al.*, 2023; Mishra *et al.*, 2024). The analyses of NDF, ADF, and phosphorus (P) indicated that their concentrations increased with increasing levels of

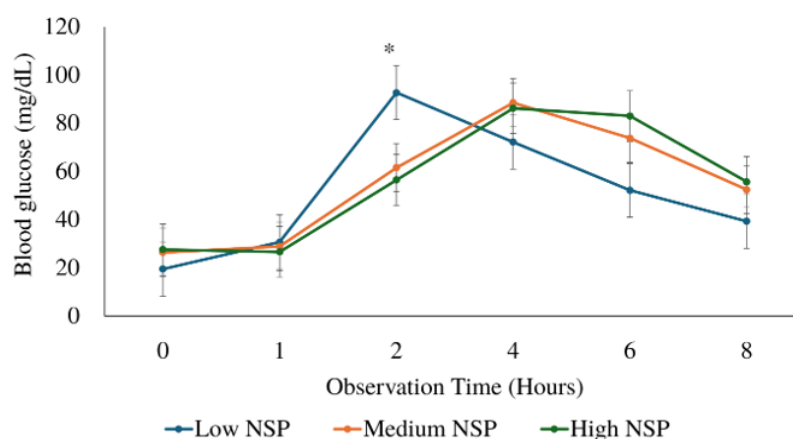


Figure 2. Postprandial blood glucose levels of Nile tilapia at 0, 1, 2, 4, 6 and 8 hours after feeding with different levels of NSP. (*) Indicates significant difference ($P < 0.05$).

Table 6. Growth performance of Nile tilapia fed with different levels of NSP.

Parameters	Feed Treatment			
	Low NSP	Medium NSP	High NSP	Commercial Feed
W0 (g)	17.02 ± 0.07 ^a	17.10 ± 0.12 ^a	17.02 ± 0.07 ^a	17.02 ± 0.16
Wt (g)	37.10 ± 0.07 ^c	36.77 ± 0.07 ^b	36.51 ± 0.20 ^a	38.71 ± 0.19
AGR (g/day)	0.67 ± 0.00 ^b	0.66 ± 0.00 ^a	0.65 ± 0.01 ^a	0.72 ± 0.01
SGR (%/day)	2.60 ± 0.01 ^b	2.55 ± 0.02 ^a	2.54 ± 0.02 ^a	2.74 ± 0.03
SR (%)	100 ± 0.00 ^a	100 ± 0.00 ^a	100 ± 0.00 ^a	100 ± 0.00
TFC (g)	429 ± 1.92 ^a	447 ± 1.30 ^b	480 ± 1.92 ^c	428 ± 1.67
FCR	1.42 ± 0.00 ^a	1.51 ± 0.01 ^b	1.64 ± 0.01 ^c	1.31 ± 0.01
PR (%)	39.71 ± 1.77 ^c	27.17 ± 1.73 ^b	21.74 ± 0.56 ^a	–
FR (%)	79.39 ± 20.98 ^c	48.97 ± 10.58 ^a	42.20 ± 8.20 ^a	–
PHR (%)	1.99 ± 0.62 ^b	1.42 ± 0.19 ^{ab}	1.03 ± 0.56 ^a	–

Note: ¹Individual initial weight (W0), individual final weight (Wt), absolute growth rate (AGR), specific growth rate (SGR), survival rate (SR), total feed consumption (TFC), feed conversion ratio (FCR), protein retention (PR), fat retention (FR), and phosphorus retention (PHR). Different *superscript* lowercase letters in the same row indicate significantly different treatment effects ($P < 0.05$). ²Commercial feed: values were not measured (–) and were not included in the statistical analysis. Values are presented as mean ± standard deviation. Different superscript letters in different rows indicate significantly different treatment effects ($P < 0.05$).

NSP in the feed (Table 2). Dietary NSP has been reported to influence digestive enzyme activity. In this study, the activities of protease, amylase, and lipase increased at the initial stage but decreased with increasing levels of dietary NSP (Table 3). This reduction in enzyme activity is presumably attributed to the interaction between NSP and increased intestinal viscosity, which is associated with the type, concentration, and solubility of NSP (Deng *et al.*, 2021).

Elevated intestinal digesta viscosity caused by NSP can reduce the diffusion of substrates and digestive enzymes, thereby limiting their effective interaction at the intestinal mucosal surface and ultimately decreasing digestive efficiency (Zheng *et al.*, 2021). Diets containing NSP resulted in variations in fish health status. Histological examination of the intestine revealed alterations in intestinal structure and internal morphology in fish fed NSP-containing diets compared with pre-treatment conditions, which may affect digestion and nutrient absorption (Figure 1 and Table 4). These changes are presumed to be associated with increased viscosity within the gastrointestinal lumen (Sinha *et al.*, 2011; Liu *et al.*, 2022c). Elevated luminal viscosity can disrupt intestinal morphology and function by accelerating villus cell loss, inducing villus atrophy, and increasing crypt cell proliferation and crypt depth (Jha *et al.*, 2019). In the midgut, shorter villi with narrower surface areas were observed in fish fed high NSP diets, whereas in the hindgut, increased villus height and crypt depth appeared as adaptive responses to enhance nutrient absorption in the distal intestine.

A similar result was reported by Wang *et al.* (2023) & Schneider *et al.* (2025) that dietary soybean meal and plant-based NSP affected intestinal morphology including villus height and goblet cell populations in Nile tilapia. The reduction in villus height or surface area may also be related to increased peristaltic activity and accelerated digesta transit time in the gastrointestinal tract, thereby reducing the contact time between nutrients and the intestinal surface and resulting in suboptimal absorption. Shortened villi accompanied by increased crypt depth can impair nutrient absorption and increase secretory activity in the gastrointestinal tract, negatively affecting intestinal function (Maryam *et al.*, 2024). Consistent with this, Liu *et al.* (2022b) reported that dietary NSP levels above 3% significantly increased the intestinal

length index, while levels above 9% affected nutrient digestibility and intestinal morphology in largemouth bass (*Micropterus salmoides*). The present study demonstrated that increasing dietary NSP levels reduced the digestibility of protein, lipid, carbohydrate, total nutrients, energy, and phosphorus (Table 5).

High dietary NSP content increases digesta viscosity, which inhibits digestive enzyme activity and reduces the utilization of digestible carbohydrates, proteins, and lipids (Tran-Tu *et al.*, 2019; Oliveira *et al.*, 2021). This effect is presumed to result from the water holding capacity of NSP, which forms a viscous gel in the intestinal lumen, creating a physical barrier on the surface of feed particles that limits enzyme access to substrates. Consequently, the diffusion and mixing rates between digestive enzymes and their substrates are reduced, making nutrient hydrolysis less effective. In addition, NSP can entrap bile salts and increase luminal viscosity, thereby limiting micelle formation, reducing lipase access to triglyceride surfaces, and ultimately decreasing lipid digestion and absorption (Grundy *et al.*, 2016; Pabois *et al.*, 2020). The combined effects of these mechanisms decrease the time and surface area of nutrient contact with the intestinal mucosa, ultimately lowering the digestibility and retention of carbohydrates, proteins, and lipids.

Increased viscosity arising from the water-binding and gel-forming properties of NSP represents the primary mechanism by which digestive enzyme activity and nutrient utilization are inhibited (Wang *et al.*, 2024; Da Cruz *et al.*, 2024). Overall, fish digestibility is closely correlated with enzymatic activity and intestinal morphology (Sinha *et al.*, 2011; Deng *et al.*, 2021). High dietary inclusion of NSP is known to affect the metabolism and utilization of nutrients such as glucose, lipids, amino acids, and minerals (Sinha *et al.*, 2011; Wang *et al.*, 2024). Based on the results obtained, the blood glucose levels of tilapia in the low NSP treatment had the highest value which increased at the 2nd hour after feeding and began to decrease at the 4th hour. The increase in glucose levels indicates that in the low NSP treatment, fish can quickly utilize carbohydrate levels in the feed. At the 4th hour, blood glucose levels in the medium and high NSP treatments reached their peak point and lasted until the 6th hour then decreased in the following hour until approaching basal conditions at the 8th hour in all treatments (Figure 2).

The prolonged glucose absorption time in the medium and high NSP treatments is consistent with the study by Faiqotul Himmah *et al.* (2025), which reported that feeding without the addition of NSP enzymes can reduce and delay blood glucose absorption. This mechanism is attributed to the ability of NSP to disperse in aqueous environments and form gel-like matrices, thereby increasing the viscosity of gastrointestinal contents. This increase in viscosity is associated with delayed gastric emptying and feed transit time, which can reduce the rate of glucose absorption (Giuntini *et al.*, 2022; Lu *et al.*, 2023). This effect is presumably due to the ability of NSP to decrease nutrient diffusion rates and limit enzyme substrate interactions, resulting in reduced carbohydrate hydrolysis into glucose. Consequently, slower gastric emptying, prolonged feed transit time, and impaired hydrolysis ultimately decrease the availability of glucose and energy for utilization by fish.

The low protein and lipid retention observed in this study were presumably attributable to reduced enzymatic activity and nutrient digestibility in fish, resulting from increased viscosity due to the consumption of high NSP diets (Maas *et al.*, 2019; Kabir *et al.*, 2020). The increase in digestive viscosity induced by dietary NSP has been shown to impair lipid emulsification and reduce lipolysis, thereby diminishing intestinal lipid digestion and absorption, as higher polysaccharide-induced viscosity can inhibit enzyme diffusion and limit access of lipase to lipid substrates (Karim *et al.*, 2024). In addition, Nile tilapia exhibited decreased phosphorus retention values, which were consistent with the reduced phosphorus digestibility observed, indicating that higher NSP levels in the diet impair phosphorus absorption. This reduction in phosphorus retention and digestibility is likely due to the abundance of anti-nutritional factors in plant-based feed ingredients that interfere with mineral metabolism, such as phytate. Phytate cannot be efficiently hydrolyzed by nonruminant animals including fish, due to their lack of endogenous phytase and its presence in plant-based diets reduces phosphorus and other mineral availability, negatively affecting nutrient utilization (Naz *et al.*, 2023; El-Dein *et al.*, 2025).

High dietary levels of phytate and NSP can reduce phosphorus digestibility in fish by interacting and forming complexes that increase digesta viscosity and limit enzyme access. Supplementation with exogenous phytase can

mitigate these effects (Rodrigues *et al.*, 2023). This elevated intestinal viscosity may exacerbate the effect of phytate in binding phosphorus, thereby reducing its availability for further absorption. Moreover, NSP and phytate are also known to bind minerals, disrupting homeostasis, particularly phosphorus (Lall & Kaushik, 2021; Liu *et al.*, 2022b). Feeding with different NSP levels affected growth performance parameters, including final individual weight, absolute growth rate, and specific growth rate. The highest values were observed in the low NSP treatment compared with the other NSP treatments, although they remained lower than those of the commercial diet (Table 6).

The presence of NSP in the diet can adversely affect intestinal physiology and function, thereby reducing nutrient digestibility and growth performance (Jiang *et al.*, 2022; Vinasyiam *et al.*, 2023; Tarigan *et al.*, 2025). Reduced fish growth rates and nutrient digestibility in high NSP diets lead to an increased feed conversion ratio (Dey *et al.*, 2025). In the present study, both the feed conversion ratio and feed intake increased with higher dietary NSP levels, indicating that fish were less able to utilize diets with high NSP content. This finding is consistent with Deng *et al.* (2021), who reported that NSP significantly decreased the daily growth coefficient and protein efficiency ratio while increasing the feed conversion ratio. High levels of dietary NSP can disrupt nutrient digestibility and utilization in fish, requiring NSP degrading enzymes to enhance nutrient absorption (Oktaviani *et al.*, 2025; Faiqotul Himmah *et al.*, 2025).

CONCLUSION

Feeding Nile tilapia with NSP-containing diets revealed that the low NSP treatment (11.59%) yielded the best overall performance compared with the other treatments, as indicated by enzyme activity, intestinal histology, postprandial blood glucose levels and nutrient digestibility.

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REFERENCES

- [AOAC] Association of Official Analytical Chemists. 2012. Official Methods of Analysis of The Association of Official Analytical Chemists. AOAC International. Arlington, Virginia.
- Borlongan LG. 1990. Studies on the lipases of Milkfish *Chanos chanos*. *Aquaculture*. 89: 315-325.
- Cahyadi U, Jusadi D, Fauzi IA, Sunarma A. 2020. Supplemental enzyme in artificial diets for north african catfish larvae *Clarias gariepinus* burchell, 1822. *Jurnal Ikhtologi Indonesia* 20: 155–169.
- Cupp-Enyard C, Aldrich S. 2008. Sigma's non-specific protease activity assay - Casein as a substrate. *Journal of Visualized Experiments*. 19:899.
- Da Cruz TP, Wernick B, Kozu AY K, Gonçalves GS, Furuya VRB, Adeshina I, Gatlin DM, Furuya WM. 2024. Dietary β -mannanase supplementation decreases digesta viscosity, improves growth and modulates gut microbiota in juvenile Nile tilapia, *Oreochromis niloticus* fed a soybean meal-based diet. *Aquaculture* 583: 740606.
- Deng J, Zhang X, Sun Y, Mi H, Zhang L. 2021. Effects of different types of non-starch polysaccharides on growth, digestive enzyme activity, intestinal barrier function and antioxidant activity of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Reports* 21: 100864.
- Dey BK, Verdegem MCJ, Nederlof MAJ, Beddow JM, Masagounder K, Mas-Munoz J, Schrama JW. 2024. Effect of temperature on the digestibility of non-starch polysaccharide-rich ingredients in Nile tilapia (*Oreochromis niloticus*). *Aquaculture Reports* 39: 102426.
- Dey BK, Verdegem, MCJ, Nederlof MAJ, Mas-Munoz J, Schrama JW. 2025. Energy utilisation efficiencies of digestible starch and non-starch polysaccharide in Nile tilapia. *Animal* 19: 12–101696.
- El-Dein AN, Ezzat A, Aboseif, AM, Flefil NS. 2025. Enhancing micronutrients bioavailability for tilapia reared in green water system using rice bran fermented with newly isolated phytase-producer, *Pediococcus pentosaceous*, maintained both economic and environmental sustainability. *Discover Food* 5: 155.
- El-Sayed AFM, Fagnon MS, Hamdan AM, Chabrillat T, Kerros S, Zeid SMS. 2025. Dietary plant-based mixture improves feed efficiency, gross profit, physiological performance, gene expression and gut health of Nile tilapia (*Oreochromis niloticus*). *Biology* 14: 1–22.
- Faiqotul Himmah, M Achmad Fauzi I, Ekasari J, Raditya Gumelar M, Muhammad Agus Suprayudi. 2025. Effectiveness of NSP enzyme and organic chromium supplementation in low-protein feed on carbohydrate utilization in Pomfret *Colossoma macropomum*. *Jurnal Akuakultur Indonesia* 24: 21–31.
- [FAO] Food and Agriculture Organization of the United Nations. 2022. Towards Blue Transformation. 2000-2020. Rome, Italy: FAO.
- Fard SH, Toghiani M, Tabeidian SA. 2014. Effect of oyster mushroom wastes on performance, immune responses and intestinal morphology of broiler chickens. *International Journal of Recycling of Organic Waste in Agriculture* 3: 141–146.
- Fauzan, Suprayudi MA, Fauzi IA, Ekasari J. 2022. Utilization of hydrolyzed corncob as a carbohydrate source in diets for red Nile tilapia *Oreochromis niloticus*. *Jurnal Akuakultur Indonesia* 21: 32–40.
- Giuntini EB, Chi SP, Zylstra RT, Racanicci AMC. 2022. The effects of soluble dietary fibers on glycemic response. *Foods* 11: 3934.
- Grundy MML, Edwards CH, Mackie AR, Gidley MJ, Butterworth PJ, Ellis PR. 2016. Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. *British Journal of Nutrition* 116: 816–833.
- Iji, PA, Hughes RJ, Choct M, Tivey DR. 2001. Intestinal structure and function of broiler chickens on wheat-based diets supplemented with a microbial enzyme. *Asian-Australasian Journal of Animal Sciences* 14: 54–60.
- Islam SF, Islam MN, Islam F, Hosne AM. 2025. Adaptability of Nile tilapia (*Oreochromis niloticus*) in biofloc systems: Effects on growth, feed efficiency, water quality, and economic viability. *Journal of Aquaculture and Fish Health* 14: 271–283.
- Jha R, Fohse JM, Tiwari UP, Li L, Willing BP. 2019. Dietary Fiber and Intestinal Health of Monogastric Animals. *Frontiers in veterinary science* 6: 48.
- Jha R, Fohse JM, Tiwari UP, Li L, Willing BP.

2021. Dietary fiber in poultry nutrition and their effects on nutrient utilization. *Journal of Animal Science and Biotechnology* 82: 1–16.
- Jiang Z, Zhang X, Niu J, Wang P, Zhou X. 2022. Effects of dietary non-starch polysaccharides on growth performance, digestive enzyme activities, and intestinal health of Nile tilapia (*Oreochromis niloticus*). *Aquaculture Reports* 26: 101312.
- Kabir KA, Verdegem MCJ, Verreth JAJ, Phillips MJ, Schrama JW. 2020. Dietary non-starch polysaccharides influenced natural food web and fish production in semi-intensive pond culture of Nile tilapia. *Aquaculture* 528: 735506.
- Karim A, Mohammadi L, Osse EF, Aider M, Saqui-Salces M, Khalloufi S. 2024. Effect of polysaccharide-induced viscosity on the digestion of proteins, fats, and carbohydrates in food: A comprehensive review and future perspectives. *Trends in Food Science and Technology* 153: 104757.
- Lall SP, Kaushik SJ. 2021. Nutrition and Metabolism of Minerals in Fish. *Animals* 11: 2711.
- Liang Q, Zhang X, Li Y, Chen H, Wang J. 2022. Application of exogenous enzymes to improve digestibility of non-starch polysaccharides in aquaculture feed. *Applied Microbiology and Biotechnology* 106: 3513–3528.
- Liu Y, Deng J, Tan B, Xie S, Zhang W. 2022a. Effects of soluble and insoluble non-starch polysaccharides on growth performance, digestive enzyme activity, antioxidant capacity, and intestinal flora of juvenile genetic of improvement of farmed tilapia (*Oreochromis niloticus*). *Frontiers in Marine Science* 9: 872577.
- Liu Y, Fan J, Huang H, Zhou H, Cao Y, Zhang Y, Jiang W, Zhang W, Deng J and Tan B. 2022b. High dietary non-starch polysaccharides detrimental to nutrient digestibility, digestive enzyme activity, growth performance, and intestinal morphology in Largemouth bass, *Micropterus salmoides*. *Frontiers in Nutrition* 9: 1015371.
- Liu Y, Fan J, Zhou H, Zhang Y, Huang H, Cao Y, Zhang W, Deng J, Tan B. 2022c. Feeding juvenile largemouth bass (*Micropterus salmoides*) with carboxymethyl cellulose with different viscous: Impacts on nutrient digestibility, growth, and hepatic and gut morphology. *Frontiers in Marine Science* 9: 1023872.
- Lu K, Xu Y, Zhang Y, Wang Y, Song X, Sun J. 2023. Effect of viscous soluble dietary fiber on glucose and lipid metabolism. *Frontiers in Nutrition* 10: 1253312.
- Maas RM, Verdegem MCJ, Schrama JW. 2019. Effect of non-starch polysaccharide composition and enzyme supplementation on growth performance and nutrient digestibility in Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition* 25: 622–632.
- Maas RM, Verdegem MCJ, Wiegertjes GF, Schrama JW. 2020. Carbohydrate utilization by tilapia: A meta-analytical approach. *Reviews in Aquaculture* 12: 1851–1866.
- Marcotuli I, Colasuonno P, Hsieh YSY, Fincher GB, Gadaleta A. 2020. Non-starch polysaccharides in durum wheat: A review. *International Journal of Molecular Sciences* 21: 2933.
- Maryam, Shah SZH, Fatima M, Nadeem H, Ashraf S, Hussain M. 2024. Roles of dietary supplementation of exogenous protease in low fishmeal aquafeed – A mini review. *Annals of Animal Science* 24: 27–39.
- Mishra S, Khumujam SD, Nottanalan H, Kantal D. 2024. Unraveling the complexities of antinutritional factors: Implications for fish nutrition and feeding practices. *Handbook of Sustainable Aquaculture and Fisheries*. Elite Publishing House. 115 pp.
- Morgan NK, Walk CL, Bedford MR. 2023. Advances in prebiotics for poultry: role of the caeca and oligosaccharides derived from NSP. *Animal Production Science* 63: 1911–1925.
- Naz S, Abbas G, Fatima A, Chatha AMM, Nawaz A, Alrefaei AF, Almutairi MH, Rehman M. U, Gul S, Naz S. 2023. Effects of supplemental phytase on growth, nutrient digestibility and anti-oxidant enzyme activity in the gills of juvenile mrigal, *Cirrhinus mrigala* (Hamilton, 1882) fed distillers dried grains with soluble based diets. *Journal of Applied Animal Research* 51: 789–796.
- Oktaviani A, Suprayudi MA, Setiawati, M., Fauzi, IA. 2025. Evaluation of food digestibility in Nile tilapia fish *Oreochromis niloticus* given NSP enzymes and organic chromium. *Jurnal Akuakultur Indonesia* 24: 136–147.
- Oliveira KRB, Peres H, Oliva-Teles A, Marconi JN, Paulino RR, Diogenes AF, Viegas

- EMM. 2021. Maize distillers dried grains with solubles alter dietary digestibility and improve intestine health of pacu, *Piaractus mesopotamicus* juveniles. *British Journal of Nutrition* 125:1 331–1343.
- Pabois O, Antoine-Michard A, Zhao X, Omar J, Ahmed F, Alexis F, Harvey RD, Grillo I, Gerelli, Y, Grundy, MML, Bajka B, Wilde PJ, Dreiss CA. 2020. Interactions of bile salts with a dietary fibre, methylcellulose, and impact on lipolysis. *Carbohydrate Polymers* 231: 115741.
- Rakhmawati. 2018. Evaluasi pemanfaatan energi non protein dan status kesehatan ikan nila merah (*Oreochromis niloticus*) yang diberi pakan berkromium. [Dissertation]. Bogor (ID): Sekolah Pascasarjana. Institut Pertanian Bogor.
- Reitz LL, Smith WH, Plumlee MP. 1960. Analytical Chemistry. Animal Science Department. West Lafayette. Ind. 1730 pp.
- Ren S, Cai C, Cui G, Ni Q, Jiang R, Su X, Wang Q, Chen W, Zhang Z, Wu P, Lu J, Ye Y. 2020. High dosages of pectin and cellulose cause different degrees of damage to the livers and intestines of *Pelteobagrus fulvidraco*. *Aquaculture* 514: 734445.
- Rodrigues EJD, Ito PI, Ribeiro LFM, de Carvalho PLPF, Xavier WDS, Guimaraes MG, Junior ACF, Pezzato LE, Barros MM. 2023. Phytase Supplementation under Commercially Intensive Rearing Conditions: Impacts on Nile tilapia Growth Performance and Nutrient Digestibility. *Animals* 13: 136.
- Schneider TLS, Scheid RC, Peixoto NC, Lazzari R. 2025. Metabolic and intestinal morphometric responses of Nile tilapia fed diets containing soybean and protease. *Animals*. 15:349.
- Singh AK, Cowieson AJ, Ravindran V. 2021. Effects of Dietary Fiber on Nutrients Utilization and Gut Health in Poultry. *Animals*. 11: 181.
- Sinha AK, Kumar V, Makkar HPS, De Boeck G, Becker K. 2011. Non-starch polysaccharides and their role in fish nutrition. *Food Chemistry* 127: 1409–1426.
- Soest V. 1963. Use of Detergents in the Analysis of Fibrous Feeds. II. A Rapid Method for the Determination of Fiber and Lignin. *Journal of AOAC International* 46: 829–835.
- Suprayudi MA. 2018. Peran ilmu nutrisi ikan dan kemandirian bahan pakan lokal dalam mendukung produksi akuakultur berkelanjutan. Orasi Ilmiah Guru Besar IPB, Auditorium Rektorat Gedung Andi Hakim Nasution, Institut Pertanian Bogor <https://www.scribd.com/document/871085610/OrationIPBUniv-AgusSuprayudi-21stJul18>. PT. IPB Press. [21 Juli 2018].
- Tarigan NB, Amal Jr M, Ekasari J, Keesman KJ, Verdegem M. 2025. Nitrogen, phosphorus, and carbon dynamics in biofloc system of Nile tilapia fed with high non-starch polysaccharides diet. *Aquaculture* 596: 741714.
- Tran-Ngoc KT, Haidar MN, Roem AJ, Sendao J, Verreth JAJ, Schrama JW. 2019. Effects of feed ingredients on nutrient digestibility, nitrogen/energy balance and morphology changes in the intestine of Nile tilapia (*Oreochromis niloticus*). *Aquaculture Research* 50: 2577–2590.
- Tran-Tu LC, Bosma RH, Verstegen MWA, Schrama JW. 2019. Effect of dietary viscosity on digesta characteristics and progression of digestion in different segments of the gastrointestinal tract of striped catfish (*Pangasianodon hypophthalmus*). *Aquaculture* 504: 114–120.
- Vega FM, Romero MC, Jiménez OV, Barrantes MG. 2025. Enhanced growth and reduced fat accumulation in Nile tilapia (*Oreochromis niloticus*) through spirulina supplementation: a costbenefit analysis. *Egyptian Journal of Aquatic Research* 51: 415–422.
- Verdegem MCJ, Kabir KA, Phillips M. 2023. Nutritious pond: Enhancing protein use efficiency in aquaculture systems. In: *Our future proteins*. VU University Press, Amsterdam, pp. 163–173.
- Vinasyiam A, Kokou F, Ekasari J, Schrama JW, Verdegem MCJ, 2023. Effects of high wheat bran input on the performance of a biofloc system for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture Reports* 33: 101853.
- Wang S, Xu G, Zou J. 2024. Soluble non-starch polysaccharides in fish feed: implications for fish metabolism. *Fish Physiology and Biochemistry* 50: 1–22.
- Wang T, Zhou R, He Y, Guo C, Zhang J, Wang, Y. 2023. Xylanase improves the intestinal barrier function of Nile tilapia (*Oreochromis niloticus*) fed with soybean meal. *Journal of Animal Science and Biotechnology* 14: 53.
- Watanabe T. 1998. *Fish Nutrition and Mariculture*. Tokyo: Kanagawa fisheries training center. Japan International Cooperation Agency. 233 pp.

- Worthington V. 1993. Worthington Enzyme Manual. Enzymes and Related Biochemicals Worthington Chemical. New Jersey. USA: 399 pp.
- Zhang Y, Maas RM, Hilgers P, Horstmann ZP, Kokou F, Staessen TWO, Schrama JW. 2025. Effect of nonstarch polysaccharides and bile acid supplementation on nutrient digestibility, bile acid balance, faecal waste production and characteristics of yellowtail kingfish (*Seriola lalandi*). *Aquaculture Reports* 41: 102708.
- Zheng X, Chen Y, Hu B, Huang Z, Qin Y. 2021. Effects of different types of non-starch polysaccharides on growth, digestive enzyme activity, intestinal barrier function, and antioxidant activity of rainbow trout. *Aquaculture Report* 20: 100684.
- Zhou H, Liu Y, Fan J, Huang H, Deng J, Tan B. 2022. Feeding rainbow trout with different types of non-starch polysaccharides: impacts on serum metabolome and gut microbiota. *Metabolites* 12: 1167.