Jurnal Natur Indonesia
23(1), April 2025: 1-9

p-ISSN 1410-9379
e-ISSN 2503-0345

Effect of Chitosan Extracted from Vannamei Shrimp (*Litopenaeus vannamei*) Shells in Feed on the Growth Performance and Digestibility of Nile Tilapia (*Oreochromis niloticus*)

Pengaruh Penambahan Kitosan yang Diekstrak dari Cangkang Udang Vannamei (*Litopenaeus vannamei*) dalam Pakan terhadap Pertumbuhan dan Daya Cerna Ikan Nila (*Oreochromis niloticus*)

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ABSTRACT

Chitosan extracted from vannamei shrimp shells has the potential to act as an attractant for Nile tilapia (*Oreochromis niloticus*) while also enhancing growth performance and digestibility in various fish species. This study evaluated the impact of chitosan supplementation in feed on Tilapia's growth rate and digestibility. The experiment was designed using a Completely Randomized Design (CRD) with five treatment groups and three replications. Fish were fed diets containing chitosan at 0%, 1%, 2%, 3%, and 4% for 56 days. 525 tilapia fingerlings (2.50±0.41 g) were stocked in 15 floating net cages (1x1x1 m^3). Feed was provided at 10% of the fish biomass three times daily at 08:00 AM, 01:00 PM, and 05:00 PM. The results indicated that chitosan supplementation significantly ($P<0.05$) influenced Tilapia's specific growth rate and digestibility. Among the tested levels, the 4% chitosan treatment yielded the highest values, including overall digestibility (52.38%), protein digestibility (74.72%), protein retention (27.27%), feed efficiency (34.77%), specific growth rate (3.33%), and 100% survival rate. These findings suggest that incorporating chitosan into tilapia feed can enhance growth performance and nutrient utilization.

Keywords: Attractant, Chitosan, Fish Digestibility, Growth, Vannamei,

ABSTRAK

Kitosan yang diekstrak dari cangkang udang vannamei berpotensi berperan sebagai atraktan bagi ikan nila (*Oreochromis niloticus*) serta meningkatkan kinerja pertumbuhan dan daya cerna pada berbagai spesies ikan. Penelitian ini bertujuan untuk mengevaluasi pengaruh suplementasi kitosan dalam pakan terhadap laju pertumbuhan dan daya cerna ikan nila. Percobaan ini dirancang menggunakan Rancangan Acak Lengkap (RAL) dengan lima kelompok perlakuan dan tiga ulangan. Ikan diberi pakan dengan kandungan kitosan sebesar 0%, 1%, 2%, 3%, dan 4% selama 56 hari. Sebanyak 525 ekor benih ikan nila (2,50±0,41 g) dipelihara dalam 15 unit keramba jaring apung (1x1x1 m^3). Pakan diberikan sebanyak 10% dari bobot biomassa ikan sebanyak tiga kali sehari, yaitu pada pukul 08.00, 13.00, dan 17.00 WIB. Hasil penelitian menunjukkan bahwa suplementasi kitosan secara signifikan ($P<0,05$) memengaruhi laju pertumbuhan spesifik dan daya cerna ikan nila. Dari berbagai level perlakuan yang diuji, penambahan kitosan sebanyak 4% menghasilkan nilai tertinggi, termasuk daya cerna total (52,38%), daya cerna protein (74,72%), retensi protein (27,27%), efisiensi pakan (34,77%), laju pertumbuhan spesifik (3,33%), dan tingkat kelangsungan hidup sebesar 100%. Penelitian ini menunjukkan bahwa penambahan kitosan dalam pakan ikan nila dapat meningkatkan kinerja pertumbuhan serta pemanfaatan nutrisi secara optimal.

Kata Kunci: Atraktan, Daya Cerna Ikan, Kitosan, Pertumbuhan, Vanamei.

Received: 15 January 2025
Accepted: 15 March 2025

INTRODUCTION

Tilapia is a widely cultivated freshwater fish favoured by Southeast Asian aquaculture farmers (Ashuri, 2016). Its popularity stems from its remarkable adaptability to diverse aquatic environments, making it a species with significant development potential (Muahiddah & Diamahesa, 2023). Additionally, Tilapia is highly valued for its thick flesh, rich nutritional content, and affordable price, contributing to its strong consumer demand (Putra et al., 2017). However, despite its advantages, tilapia farmers continue to face challenges that hinder optimal profitability. One of the primary concerns is the high feed cost, while independent feed formulations remain less effective due to their low palatability. To address this issue, incorporating attractants in artificial feed is essential to enhance its appeal to fish. One promising attractant that can be utilized is chitosan (Bakshi et al., 2020).

In aquaculture, chitosan is an attractant and a feed supplement for fish (Aathi et al., 2013). Diets supplemented with chitosan have been shown to stimulate appetite, enhance digestion and nutrient absorption, and increase protein levels, ultimately improving fish growth performance. Several studies have explored the application of chitosan in aquafeeds. For example, Zaki et al. (2015) reported that supplementing the diet of European seabass (*D. labrax*) with 2% chitosan resulted in the highest feed efficiency of 25%. Similarly, research by Khayrurraja et al. (2023) demonstrated that incorporating chitosan and liquid probiotics in the diet of giant gourami (*O. gouramy)* yielded the highest specific growth rate of 3.07%. Furthermore, Syaputra et al. (2023) highlighted that adding chitosan to fish feed significantly improved protein digestibility. These findings suggest that chitosan has the potential to enhance both growth and digestibility in fish.

Chitosan is a biopolymer derived from chitin-containing glucosamine and is typically sourced from shrimp shells and other crustacean byproducts (Rochima, 2014). According to Mahatmanti et al. (2022), the production of chitosan involves three key stages: demineralization, deproteinization, and deacetylation. Recently, chitosan has gained recognition as a potential growth-promoting agent in aquaculture. However, research on its effects remains limited. Therefore, further studies are necessary to evaluate the impact of chitosan supplementation on the growth and digestibility of Tilapia in feed.

MATERIALS AND METHOD

Time and place of research

This research was conducted from May to July 2024. The preparation of feed materials, formulation of experimental feed, and fish maintenance were carried out at the Fish Nutrition Laboratory, Faculty of Fisheries and Marine Science, Universitas Riau. Chemical analysis of feed and fish samples was performed at the Agricultural Product Analysis Laboratory, Universitas Riau. The Cr_2O_3 analysis was conducted at the Fish Nutrition Laboratory, IPB University.

Methods

The test fish used in this study were Tilapia fingerlings, totalling 625 fish with an average size of 2.50±0.41 g. The fish is obtained from Oikos hatcheries in Pekanbaru. The fish were reared in 15 net cages made of mesh netting (1 mm mesh size) with dimensions of 1x1x1 m^3, stocked at a density of 25 fish per cage. The test fish were placed in cages and acclimated for one week to minimize stress. During this adaptation period, they were initially fed commercial feed, which was gradually replaced with the test feed. After seven days of acclimation, the fish underwent a 24-hour fasting period to empty their digestive tracts and gain full weight. Following this, they were weighed to determine their initial rearing weight. The fish were then fed the test diet at a rate of 10% of their biomass, with a feeding frequency of three times a day at 08.00 AM, 01.00 PM, and 05.00 PM for 56 days. Fish weight was sampled every 14 days to adjust the feed quantity accordingly. Any dead fish were recorded and weighed to assess survival rates.

For the digestibility trials, the fish were placed in 10 aquariums with dimensions of 60x40x40 cm^3, each stocked at a density of 25 fish per aquarium (Mulantika et al., 2020). The experimental feed was formulated and prepared in pellet form. The feed ingredients included fish meal, soybean meal, wheat flour, and rice bran. Additional ingredients included vitamin mix, mineral mix, fish oil, Cr_2O_3 as an indicator, and chitosan.

The research method employed was an experimental method using a Completely Randomized Design (CRD) with one factor, consisting of five treatments and three replications. The treatments in this study were as follows:

P0 = Feed without chitosan

P1 = Addition of 1% chitosan per kg of feed
P2 = Addition of 2% chitosan per kg of feed
P3 = Addition of 3% chitosan per kg of feed
P4 = Addition of 4% chitosan per kg of feed

Preparation of chitosan

The shrimp shell was obtained from a shrimp seller in Pasir Lima Kapas Panipahan District, Rokan Hilir Regency. The type of shrimp used was vannamei shrimp. The shrimp shells were thoroughly washed and then sun-dried until dry and brittle enough to be crushed. After drying, the shells were finely ground using a blender and sieved with a strainer. The shrimp shells then underwent the chitosan synthesis process, which consists of three stages: demineralization, deproteinization, and deacetylation (Mahatmanti et al., 2022). The results of the proximate analysis of the chitosan are presented in Table 1.

Table 1. Protein and moisture composition in chitosan

No.	Parameters	Values (%)
1	Protein	32.50
2	Moisture	8.75

Source: Results of Analysis of Agricultural Product Technology Laboratory, Universitas Riau

Preparation of experimental feed

The preparation of experimental feed was carried out by formulating and adjusting the composition of each ingredient to meet the desired protein requirement for Tilapia, which is 30% (Hamed et al., 2024). The proportion of chitosan was determined according to the needs of each treatment, while the quantities of other ingredients were adjusted based on calculations. The composition of the experimental feed is presented in Table 2:

Table 2. Composition of artificial feed and results of proximate analysis of feed.

Ingredients	Proteins ingredients	Treatments (Chitosan %)				
		P0(0)	P1(1)	P2(2)	P3(3)	P4(4)
Fish Meal	49.35	36.00	36.00	36.00	36.00	36.00
Soybean Meal	31.70	31.00	31.00	31.00	31.00	31.00
Wheat Flour	10.00	13.00	13.00	13.00	13.00	13.00
Rice Bran	7.90	14.00	14.00	14.00	14.00	14.00
Vit. Mix	0.00	2.00	2.00	2.00	2.00	2.00
Min. Mix	0.00	2.00	2.00	2.00	2.00	2.00
Fish Oil	0.00	2.00	2.00	2.00	2.00	2.00
Total		100.00	100,00	100.00	100.00	100.00
Chitosan		0.00	1,00	2.00	3.00	4.00
Proximate Analysis (%)						
Proteins		29.34	29.73	29.87	29.86	29.66
Lipid		8.30	8.20	8.20	8.15	8.25
Crude Fiber		6.80	6.65	6.70	6.60	6.50
Moisture		8.10	8.00	7.95	8.15	8.10
Ash		5.20	5.15	5.00	5.10	4.95
BETN		49.91	50.10	50.25	50.67	50.90

Source: Results of Analysis of Agricultural Product Technology Laboratory, Universitas Riau

Pisciculture

The test fish were put into cages and then adapted for a week to prevent the fish from experiencing stress during the adaptation period. The test fish were given commercial feed and then slowly changed to the test feed. After 7 days of adaptation, the fish are fasted for 24 hours, and the digestive tract is emptied to obtain full weight. Then, test fish were weighed to determine initial rearing weight. Fish are given food tests as much as 10% of the biomass with a feeding frequency of 3 times a day, namely at 08.00 AM, 01.00 PM and 05.00 PM, for 56 days of maintenance. Fish weight sampling is done every 14 days to adjust the number of feeds given. Dead fish were recorded and weighed to find out the survival of fish.

Observation parameters

Feed digestibility is calculated using the Watanabe (1988):

$$FD = (1 - \frac{a}{a'}) \times 100$$

Description:

FD : The fish digestibility,
a' : Cr_2O_3 level in feces
a : Cr_2O_3 level in the feed

Protein digestibility was calculated using the Watanabe (1988):

$$PD = 1 - \frac{a}{a'} - \frac{b}{b'} \times 100$$

Description:

PD : Protein digestibility
b : Protein in feed
a : Cr_2O_3 levels in feed
b' : Protein in feces
a' : Cr_2O_3 levels in feces

Protein retention was calculated using the Watanabe (1988):

$$PR = \frac{\text{increase in body protein (g)}}{\text{total protein consumed (g)}} \times 100\%$$

Description:

PR : Protein retention

Feed efficiency is calculated using the equation Zonneveld et al. (1991):

$$FE = \frac{(Wt + Wd) - Bo}{F}$$

Description:

FE : Feed efficiency
Wd : Dead fish biomass weight
Wt : Final biomass weight
F : Amount of feed consumed by fish
Wo : Initial biomass weight

The specific growth rate is calculated using the equation Afzriansyah et al. (2014):

$$SGR = \frac{(Wt + Wd) - Bo}{F} \times 100\%$$

Description:

SGR : Specific growth rate
Wt : Average weight at the end of the study
Wo : Average weight at the beginning of the study
t : Length of the study.

Fish survival is calculated using the equation Handayani et al. (2014):

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

Description:

SR : Survival rate
Nt : Final number of live fish
No : The number of live fish at the start of the study.

Water quality observations supported survival data by analyzing key environmental parameters. The measured parameters included temperature, pH, and dissolved oxygen. These measurements were taken at the study's beginning, middle, and end to monitor changes over time.

Data Analysis

The data collected during the study was presented in tabular form. To assess the effect of treatments on the tested parameters, statistical analysis was conducted using the Completely Randomized Design (CRD) model (Hanafiah, 2005). Data analysis was performed using the SPSS Version 23 program to conduct ANOVA, and if the probability value was (P<0.05), it indicated a significant effect of chitosan administration on the measured parameters. Further analysis was carried out using the Newman-Keuls test to determine treatment differences. Meanwhile, water quality data was presented in tabular form and analyzed descriptively.

RESULT AND DISCUSSION

Feed digestibility

Fish feed digestibility is breaking down and absorbing nutrients from the feed consumed. Feed digestibility data can be seen in Table 3.

Table 3. Digestibility of Tilapia in each treatment

Replications	Treatments (Chitosan%)				
	P0(0)	P1(1)	P2(2)	P3(3)	P4(4)
1	43.82	44.44	45.65	48.45	51.92
2	45.05	45.65	46.24	48.98	52.83
Total	88.87	90.09	91.89	97.43	104.75
Mean	$44.44±0.86^{a}$	$45.05±0.85^{a}$	$45.95±0.41^{a}$	$48.72±0.37^{b}$	$52.38±0.64^{c}$

Note: Different superscript letters within the same line indicate differences among treatments (P<0.05).

Table 3 shows that Tilapia's highest feed digestibility value is 52.38%. The high feed digestibility in the P4 treatment (4% chitosan) is likely due to the glucosamine content in chitosan, which enhances the feed digestibility value in fish (Aathi et al., 2013). The presence of microorganisms supporting digestion can influence feed digestibility. This aligns with Kusharto (2006), who stated that microbial growth provides benefits such as accelerating nutrient absorption and boosting immunity. Glucosamine has been shown to increase the population of microorganisms. This is consistent with the research by Mukti et al. (2018), which stated that the addition of chitosan in feed improves the digestibility of protein and lipids.

The differences in feed digestibility values across treatments are thought to be due to variations in the amount of chitosan used in each treatment. The higher the chitosan content in the feed, the greater the feed digestibility value that fish can utilize for growth. This supports the statement by Mukti et al. (2018), which emphasized that the addition of chitosan in feed enhances feed digestibility.

Protein digestibility

Protein digestibility refers to the ability of the fish's digestive system to break down and absorb protein from feed. The digested and absorbed protein is utilized for various bodily functions, including growth, tissue repair, and energy production (Jobling, 2016). Protein digestibility data can be found in Table 4.

Table 4. Protein digestibility of Tilapia in each treatment

Replications	Treatments (Chitosan%)				
	P0(0)	P1(1)	P2(2)	P3(3)	P4(4)
1	71.37	71.58	71.74	73.04	74.49
2	71.79	72.01	72.25	73.23	74.95
Total	142.16	143.59	143.99	146.27	149.44
Mean	$71.58±0.30^{a}$	$71.80±0.30^{a}$	$72.00±0.36^{a}$	$73.14±0.13^{b}$	$74.72±0.32^{c}$

Note: Different superscript letters within the same line indicate differences among treatments (P<0.05).

The highest protein digestibility value was observed in the P4 treatment (4% chitosan), reaching 74.72%. This is attributed to the high feed digestibility value of 52.38% in the P4 treatment. The increase in protein digestibility aligns with the improvement in feed digestibility. The primary mechanism underlying the effect of chitosan on protein digestibility is its ability to enhance the activity of proteolytic enzymes such as protease. According to Kusharto (2006), fish fed with chitosan-supplemented feed exhibit increased digestive enzyme activity, ultimately improving protein breakdown and absorption efficiency.

Research by Syahputra et al. (2023) on catfish (*Clarias gariepinus*) demonstrated that the addition of chitosan to feed significantly enhances protein digestibility. This improvement is due to the interaction of chitosan with gut microflora, which supports protease activity, and the reduction of antinutritional compounds in the feed that could otherwise inhibit protein digestion.

Protein retention

Protein retention represents the amount of protein stored and utilized by fish to form new tissues during the rearing period (Samsudin et al., 2010). Protein retention data can be found in Table 5.

Table 5 shows that Tilapia's highest protein retention value was 27.27%. The protein retention value obtained in the P4 treatment (4% chitosan) was higher than in other treatments, indicating that the test fish in the P4 treatment were better able to convert feed protein into body protein than the other treatments. Chitosan helps enhance protein digestibility by improving digestion and increasing protease enzyme activity, which breaks down protein into amino acids that are more easily absorbed. With more protein being digested and absorbed, the fish's body can utilize protein more effectively, ultimately increasing protein retention (Heptarina et al., 2010).

Table 5. Protein retention of Tilapia in each treatment

Replications	Treatments (Chitosan%)				
	P0(0)	P1(1)	P2(2)	P3(3)	P4(4)
1	18.78	19.19	22.78	24.68	26.11
2	19.07	21.79	21.69	24.22	26.40
3	17.74	18.96	22.78	23.87	29.29
Total	55.59	59.94	67.25	72.77	81.80
Mean	18.53±0.69[a]	19.98±1.57[a]	22.42±0.62[b]	24.26±0.40[b]	27.27±1.75[c]

Note: Different superscript letters within the same line indicate differences among treatments (P<0.05).

The protein retention value in the P4 treatment aligns with the high feed and protein digestibility values shown in Tables 4 and 5. The low crude fiber content also contributes to the high protein retention value. The crude fiber content in the P4 treatment was 6.50%, facilitating the absorption of feed protein. The fish more easily digests feed containing 4% chitosan (Islami et al., 2013). The protein retention value obtained in this study is higher than that of Udo et al. (2018), who reported the highest % protein retention value of 15% in African catfish (*C. gariepinus*) fed with a diet supplemented with 1% nano-chitosan. In contrast, this study's highest protein retention value for Tilapia was 27.27%, indicating that adding 4% chitosan to the feed can significantly improve protein retention in Tilapia.

Feed efficiency

Feed efficiency is one of the parameters used to measure how efficiently fish can utilize the provided feed. The higher the feed efficiency value, the more efficiently the fish utilize the feed for consumption and growth. Feed efficiency data is presented in Table 6.

Table 6. Feed efficiency of Tilapia in each treatment.

Replications	Treatments (Chitosan%)				
	P0(0)	P1(1)	P2(2)	P3(3)	P4(4)
1	25.32	26.81	30.89	32.37	32.96
2	26.34	28.94	30.08	30.72	34.68
3	24.75	26.64	30.90	30.46	36.67
Jumlah	76.42	82.39	91.87	93.54	104.31
Average	25.47±0.80[a]	27.46±1.28[a]	30.62±0.47[b]	31.18±1.03[b]	34.77±1.85[c]

Note: Different superscript letters within the same line indicate differences among treatments (P<0.05).

The feed efficiency of Nile tilapia reared during the study ranged from 25.47% to 34.77%. This value is considered optimal, as according to the study by Islami et al. (2013), the feed efficiency of Tilapia typically ranges from 18.18% to 48.68%. Therefore, the P4 treatment (4% chitosan) indicates that the fish could digest the feed well and utilize it optimally for growth. This is likely due to the glucosamine derived from chitosan, which plays a role in feed digestibility. According to Haryanto et al. (2014), feed digestibility is directly proportional to feed efficiency, meaning that feed efficiency will also be high if feed digestibility is high. Furthermore, the high feed efficiency value in the P4 treatment (4% chitosan) is likely due to the protein content of the feed, which meets the nutritional needs of Nile tilapia. This aligns with Hamed et al. (2024), who stated that feeding protein matching fish's nutritional requirements results in more efficient feeding.

The feed efficiency value obtained in this study is higher than the study by Zaki et al. (2015), which added 2% chitosan to the white seabass (*D. labrax*) feed, resulting in the highest feed efficiency value of 25%. This indicates that adding 4% chitosan can improve feed efficiency in Tilapia.

Specific growth rate

The specific growth rate of Tilapia in the treatment where chitosan was added to the feed showed changes according to the increase in the percentage of chitosan added to the feed (Ekaputri et al., 2018). The specific growth rate can be seen in Figure 1.

Figure 1 shows that the addition of different chitosan to feed produces different specific growth rates. During the 14 days of maintenance, fish growth for each treatment was relatively the same because the fish were still adapting to the environment and the test feed given. Then, fish growth experienced a fairly significant increase from the 28th to the end of rearing. The highest specific growth rate value for Tilapia was found in the P4 treatment, namely 3.33%. Each treatment experienced a different specific growth rate; the feed containing the most chitosan experienced higher growth.

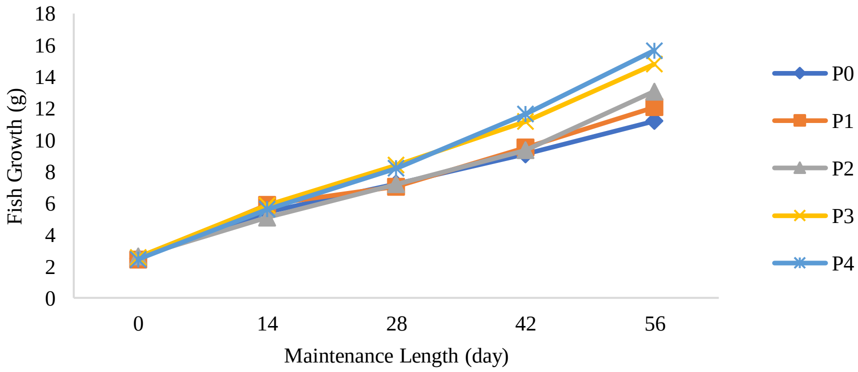

Figure 1. Growth of Tilapia during research

The highest specific growth rate was in treatment P4, namely 3.33%. This is because the P4 treatment (4% chitosan) has a fairly high feed efficiency value of 34.77%. High growth is directly proportional to high feed efficiency values; optimal feed utilization will provide good growth in fish. Apart from that, the feed contains complete nutrients such as protein, carbohydrates, and lipids (Table 2), which are converted into energy the fish needs for growth (Sukarman & Ramadhan, 2015). However, the balance between protein, lipids, and carbohydrates must be right so the fish gets energy and sufficient nutrients for growth and other bodily functions.

The addition of chitosan to feed can play an important role in the nutrient absorption process (Ekaputri et al., 2018). Kurniasih & Kartika (2011) found that chitosan from vannamei shrimp shells contains glucosamine. The glucosamine content in chitosan can support the high specific growth rate values in the P4 treatment. Besides glucosamine, chitosan is a natural ingredient that can stimulate the immune system, accelerate wound healing, and have antibacterial properties, so chitosan can be an alternative in preventing disease in fish.

The lowest value of specific growth rate in this study was P0 (0% chitosan) at 2.68%. This happens because the feed does not contain chitosan, so the feed given is difficult for the fish's body to digest and reduces the absorption of nutrients by the intestines in the feed digestion process so that much of the feed is wasted through feces as a result of which energy requirements for metabolism and growth become slower (Zaki et al., 2015).

The specific growth rate value in this study was higher than research by Khayrurraja et al. (2023) regarding the addition of chitosan and liquid probiotics to gourami fish feed, which resulted in the highest specific growth rate of 3.07%, in the study This produces the highest specific growth rate value, namely 3.33%. This shows that the addition of 4% chitosan can increase the specific growth rate of Tilapia.

Survival rate

Table 7 shows that the survival value of Tilapia ranges from 97–100%. The survival value of Tilapia with 4% chitosan in the feed provides the highest feed digestibility level of 100%. The high survival rate in rearing tilapia fry shows that the fish can adapt to the environment and can make good use of the feed provided. This follows the statement by Zaki et al. (2015) that the addition of chitosan to fish feed can improve the non-specific immune system and minimize mortality rates.

Table 7. The survival rate of Tilapia in each treatment

Replications	Treatments (Chitosan%)				
	P0(0)	P1(1)	P2(2)	P3(3)	P4(4)
1	100	92	100	100	100
2	96	100	100	100	100
3	96	100	96	96	100
Total	292	292	296	296	300
Mean	97±2.30	97±4.61	99±2.30	99±2.30	100±0.00

Water Quality

Measuring water quality in fish farming is very important for the growth and survival of the raised fish. Data from water quality measurements in maintenance containers can be seen in Table 8.

Table 8. Data from water quality measurements

Parameters	Values			Water quality standards
	Beginning	Middle	Finish	
Temperature (°C)	28	28.5	27.5	27.32
pH	7.5	7.4	7.2	6-8
Dissolved oxygen (mg/L)	3.9	4.2	4.1	3-4

In Table 8, it can be concluded that the range of water quality in each treatment is still within the tolerance standards for Tilapia, where the water quality is still in good condition when rearing Tilapia. Based on water quality standards in PP No. 22 (2021), the water temperature in fish farming ranges from 27-32°C, pH ranges from 6-8, and dissolved oxygen ranges from 3-4 mg/L.

CONCLUSION

Based on the results of the research, it can be concluded that adding chitosan from vannamei shrimp shells to tilapia feed for 56 days significantly affects feed digestibility, protein digestibility, protein retention, feed efficiency, and specific growth rate. The addition of chitosan, as much as (4%), is the best result, namely increasing feed digestibility by 52.38%, protein digestibility by 74.72%, protein retention by 27.27%, feed efficiency by 34.77%, specific growth rate by 3 .33% and survival of 100%.

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