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INCLUSION EFFECT OF NUCLEOTIDE ON GROWTH AND PROTEIN RETENTION IN PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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ABSTRACT

The growth of aquaculture in the world is expected to continue to increase. Intensification of vanamei shrimp farming is one of the best possibilities to increase aquaculture production while taking into account the limiting factors. Limited supply, price, and environmental factors are issues in meeting the needs of fish meals in feed. This study aimed to evaluate the effectiveness of nucleotides (NT, Nucleoforce Aqua ™, Bioiberica, SAU, Spain) as feed additives produced commercially in optimizing feed formulations from plant-based ingredients in the aquaculture of white shrimp (*Litopenaeus vannamei*). A 60-day growth trial was conducted to evaluate five treatments: Basal-1 with 10% fish meal; Basalt-2 with 6% fish meal; Diet-1: 10% fish meal + 0.1% NT ; Diet-2: 8% fish meal + 0.1% NT and Diet-3: 6% fishmeal + 0.1% NT. As a feed additive produced commercially in optimizing feed formulations from vegetable ingredients in the aquaculture of vannamei shrimp *Litopenaeus vannamei*, the concentration of 0.1% nucleotide formulation has been able to provide good results. The growth performance of the tested shrimp was significantly related to the parameters of increasing biomass, final weight (FBW), feed conversion (FCR), feed protein retention, and average daily growth (ADG) ($p < 0.05$) and TGC. The best results is Diet-1 (10% FMNT treatment) with the best protein content of 37.06% ± 0.15, showed the best performance of growth parameters (ADG, FBW, SR, TGC, PER, and RP) and lowest FCR. The addition of 0.1% nucleotide proves that the functional and nutritional properties of NT have many advantages in increasing the growth rate and more efficient protein absorption. Therefore, 0.1% NT can be recommended as a supplement in shrimp feed. So this study explains that the nucleotide addition of 0.1% in the feed provides an affirmative effect on growth performance and protein content parameters of the whole body of white vanamei shrimp.

KEYWORDS: Nucleotide; growth performance; feed analysis; *Litopenaeus vannamei*

INTRODUCTION

Aquaculture growth is expected to continue to increase until it reaches 109 million tons by 2030 (Hancz, 2020). The intensification of vanamei shrimp culture is one of the best possibilities for increasing aquaculture production while still paying attention to limiting factors such as soil and water and disease outbreaks (Junda, 2018). Meanwhile, the use of high commercial feeds with high protein levels also seems to have become a marker of the current status of the intensification of aquaculture. Along with the provision of these feeds, fish meal has generally been considered a preferred protein source due to its nutritional properties, the content of essential amino acids, fatty acids, vitamins, minerals and growth factors, high palatability, and absence of anti-nutritional factors (Novriadi *et al.,* 2021). Limited supply, price, and environmental factors are issues in meeting the needs of fish meals in feed. So that efforts to intensify aquaculture, which will affect the demand for fish meal, it is necessary to find alternative protein sources to ensure the sustainability of aquaculture (Shao *et al.,* 2019; Tevez & Regaza, 2016). Soybean meal (SBM) has been applied as a raw material for fish and shrimp feed formulations, accompanied by efforts to improve nutrition.

Adding additives to the feed can improve the nutritional content, including nucleotides (Andrino *et al.*, 2012). Previously the role of nucleotides and their metabolites in aquatic organisms was rarely studied (Ringø *et al.,* 2011). However, recently the positive

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effects of various nucleotides related to growth yields and performance of fish and crustacean feed utilization have shown consistent results (Burrells *et al.,* 2001; Ringo *et al.,* 2011). The crustaceans and shrimp use antenna chemoreceptors where amino acids and nucleotides are the two main molecules in identifying food (Hancz, 2020). Likewise, Métailler *et al.* (2009) and Hossain *et al.* (2020) suspected that the feed attracting properties of the nucleotides promote rapid feed intake so that nutrient *leaching* can be avoided and shrimp can benefit, especially to support the growth performance of the complete nutritional properties contained in the feed. An exogenous supply of nucleotides can promote the growth of crustaceans at an early stage by meeting all the specific dietary requirements required for high cell replication rates (Hossain *et al.,* 2020).

Continuing the research by Novriadi *et al.* (2021) in a controlled laboratory that recommended adding 0.1% nucleotide to feed, it was able to overcome the effect of substitution of fish meal on soybean meal. The optimum dose was found at an added concentration of 0.1% nucleotide with 8% and 10% fish meal. So, further discussion is needed about the effect of nucleotide additions in feed formulations with different fish meal inclusions in commercial culture conditions and their economic level for shrimp culture efficiency in the future.

MATERIALS AND METHODS

Research Design

This study used an experimental method by placing the influence of feed with nucleotide added (NT) as much as 0.1% in the feed formulation at different levels of inclusion of fish meal (Table 1). Two basal feeds were prepared with 10% and 6% fishmeal inclusion rates. The addition of fish meal was combined with soybean meal flour (SBM), corn gluten meal (CGM), and wheat products (WP) as the main ingredients. Basal-1 is designed based on a similar approach to feed in the Indonesian market with the respective ratio of SBM, FM, CGM, and WP of 43%, 10%, 10%, and 17%. Basal-2 was designed by lowering the fish meal (FM) level from 10% to 6% and increasing the addition of SBM from 43 to 49.5%. Experimental feeds on Diets 1, 2, and 3 were formulated with 0.1% nucleotide supplementation to complete the reduction of FM concentration from 10% to 8 and 6%. As shown in Table 1, the treatment on the nucleotide supplemented feed was labeled as Diet-1 (10 % FMNT), Diet-2 (8% FMNT), and Diet-3 (6% FMNT).

The experimental feed was produced at the Feed Production Unit of the Center for Aquaculture Production Business Services (BLUPPB) Karawang Dusun Sukajadi, North Pusakajaya Village, RT01 RW04, Cilebar District, Karawang Regency, West Java Province. Feed production used a commercial method with a double extruder (CXE 65 E, Jinan Shengrun, China). Before the feed molding process, each raw material was ground using a flour machine to obtain a particle size of <200 ìm using a disk mill machine. Then all the ingredients are mixed in a mixer with a capacity of 50 kg. The heater temperature on the machine is set at 110°C for 14 seconds on the five pieces of *bearing*. In the end, the temperature is set at 62°C, and all adjustments are made electronically on the visual operation display. The *die head* pressure is set to approximately 50 bar, and the cutting screw speed is maintained at 423 rpm. Feed sizes were extruded using 1, and 2 mm dies, producing 1.5 and 2.5 mm feed granules. Furthermore, the feed is dried in an electric conveyor oven until the humidity reading is below 6%. The pellets were dried at 107°C with a maximum feed temperature output of about 88°C. The feed is then cooled to ambient air temperature to a final moisture content of less than 12%. All finished feed is cooled in light and fan temperature. During approximately 3-5 hours, the feed is aerated and then put into a 25 kg sack/sack and labeled according to the feed treatment. Then the feed is stored at room temperature until further use.

Growth Test

The growth test was carried out on a commercial pond Module 4 Marine and Fisheries Field Practice Installation (IPLKP) Fisheries Business Expert Polytechnic (AUP) Serang Campus Jalan STP Raya Karangantu, Kasemen District, Serang City, Banten Province. A total of five treatment formulations of feed with ten replications were placed on 50 units of cages mesh size 1 mm in a pond area of 1,200 m^2. Waring containers with an area of 4m^2 measuring (L×W×H) 2 2 ×1 meters were placed using a completely randomized design method with bamboo as the net construction. Each cage was randomly filled with vanamei shrimp seeds from CV. Salira Benur Technique, Serang Regency, Banten Province has as many as 400 fish (100 fish/m^2) with the average initial weight of 1.06 ±0.01 g. Following the vanamei shrimp culture system, the maintenance process was carried out for 60 days. The experimental pond was equipped with aeration using a 2 HP blower for each pond with 1 point of 5 cm aeration bubble stone, equipped with a 0.5 HP wheel as the main supplier of the pond aeration system.

Ten replicate groups of 5 experimental feed treatments were labeled after going through a complete randomization process with feeding times four times a day at 07:00, 11:00, 15:00, and 20:00 WIB. Based on the history of previous studies and the assumption of normal growth, the feed was calculated in a feeding program with a feed conversion rate (FCR) of 1.5.

Table 1. Composition (% as is) of diets containing inclusion levels of nucleotide and fed to *L. vannamei* for 60 days

Ingredients (% *as is*)	Diet Code				
	Basal-1	Basal-2	Diet-1	Diet-2	Diet-3
Nucleotide	0.00	0.00	0.1	0.1	0.1
Soybean meal	43.00	49.50	43.00	44.80	49.50
Menhaden Fishmeal	10.00	6.00	10.00	8.00	6.00
Corn Gluten Meal	10	10	10	10	10
Menhaden fish oil	5.64	5.64	5.64	5.64	5.64
Soy-Lecithin	1.00	1.00	1.00	1.00	1.00
Corn starch	8.06	5.56	7.96	8.16	5.46
Wheat products	17.00	17.00	17.00	17.00	17.00
Mineral premix [a)]	0.70	0.70	0.70	0.70	0.70
Vitamin premix [b)]	1.90	1.90	1.90	1.90	1.90
KP-dibasic	2.5	2.50	2.5	2.5	2.5
Choline chloride	0.2	0.2	0.2	0.2	0.2
Protein content	36.48	36.17	37.06	36.57	36.00
Total fat	6.64	6.05	6.14	6.32	7.39
Carbohydrate	42.06	37.81	31.93	34.69	35.52
Ash content	9.25	9.38	9.55	9.56	9.52
Moisture content	7.58	10.61	10.61	10.10	10.24
Crude fiber	2.65	3.44	1.77	1.89	1.62

*) According to the formulation recommended by Novriadi *et al.* (2021)

[a)] Trace mineral premix (g/100 g premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.550; ferrous sulfate, 2,000; magnesium sulfate anhydrous, 13,862; manganese sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13,193; alpha-cellulose, 69,664. f

[b)] Vitamin premix (g/kg premix): thiamin HCL , 4.95; riboflavin, 3.83; pyridoxine HCL , 4.00; Ca-pantothenate, 10.00; nicotinic acid, 10.00; biotin, 0.50; folic acid, 4.00; cyanocobalamin, 0.05; inositol, 25.00; vitamin A acetate (500,000 IU/g), 0.32; vitamin D3 (1000,000 IU/g), 80.00; menadione, 0.50; alpha-cellulose, 856.81.

During the experiment, the daily feed was calculated with a feeding rate of 3-5% of the total biomass per day and adjusted based on mortality and average body weight obtained after sampling every two weeks.

Growth sampling and water quality observation

The first sampling was carried out in the third week of maintenance using a hand net (drain) with a diameter of 0.5 m and a mesh size of 1 cm. Each cage was randomly taken with 10-20 shrimp to be weighed and then averaged. Shrimp sampling was carried out every two weeks throughout the rearing cycle. Water quality observations (DO, pH, temperature, salinity) were monitored twice/day at 06.00 – 07:00 and 17.00 – 18.00 using a hand refractometer (ATAGO, Japan) for salinity. Electrometry for dissolved oxygen and temperature (Lutron, 2007). DO 5510), and pH (ATC pH meter). The use of equipment is carried out according to the instructions for use and calibrated as needed. The water chemistry test will be carried out at the Fish and Environmental Disease Examination Center (LP2IL) Serang, Banten, and the Central Laboratory of AHS Jakarta. Total ammonia-nitrogen (TAN), nitrate, common bacteria and total Vibrio sp were measured weekly using a national standard protocol. The test carried out at BBPBAP Jepara (Jepara, Central Java, Indonesia) and AHS Central Laboratory (Central Proteina Prima, Tangerang, Banten, Indonesia). At the end of the reared period, all shrimp were grouped and weighed to calculate final biomass, av-

$$ADG = \frac{AFBW}{DOC} x\ 100$$

$$FCR\ (\%) = \frac{Feed\ Intake}{(Wt + D) - Wo} x\ 100$$

$$FBW = \frac{AIFW - AIIW}{AIIW} x\ 100$$

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

$$TGC = \frac{FBW^{1/3} - IBW^{1/3}}{\Sigma TD} x\ 1000$$

$$PER = \frac{Wt - Wo}{Pt} x\ 100$$

$$RP(\%) = \frac{P_t - P_0}{P_p} x\ 100$$

erage daily growth (ADG), final body weight (FBW), feed conversion ratio (FCR), survival percentage (SR), thermal growth coefficient (TGC) Powell *et al.* (2020), protein efficiency ratio (PER), and protein retention (RP) based on Hu *et al.* (2008) as follows:

Statistical Analysis

The data were analyzed using regression and one-way analysis of variance (ANOVA) to determine significant differences between treatments, followed by Duncan's Multiple Range Test (DMRT) to determine differences between treatments test feed. All statistical analyzes were performed using the IBM SPSS Statistics 26 system.Analyzes were performed using the IBM SPSS Statistics 26 system.

RESULTS AND DISCUSSION

Test Feed Analysis

The feed proximate profile was analyzed at the Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia. The test feed Basal-1 (10% FM), which was the control close to commercial feeds, generally showed a protein proximate of 36.48% ± 0.1 better than Basal-2 (6% FM) with a protein content of 36.17% ± 0.13. The use of nucleotide additives of 0.1% in the inclusions of fish meal 10%FMNT, 8%FMNT, and 6%FMNT to soybean meal flour in the test feed significantly impacted the increasing the protein value of the feed. It was stated that 10% FMNT gave the best effect from each treatment of feed formulation, namely 37.06% ± 0.15. The positive trend of the impact of 8%FMNT supplementation was still able to provide a better protein value than the Basal-1 feed treatment, which was 36.57% ± 0.01. At the same time, the inclusion of 6% TIN did not have a positive effect on the protein content of the feed, which was 36% ± 0.01 lower than that of the Basal-2 diet.

Growth Performance

The results of the use of test feed proved to have a significant effect on aspects of growth and survival of shrimp during 60 days of rearing. The growth performance of the test shrimp had a significant effect on growth (Figure 1 and 2). As for survival, there were no significant results between feed treatments (Figure 3 and 4; $p > 0.05$). The growth parameters presented a significant performance in increasing biomass, final body weight (FBW), feed conversion ratio (FCR), and average daily growth (ADG) ($p < 0.05$).

The increase in biomass, final average weight, decrease FCR , and average daily growth of the Basal-1 test feed was better than that of the Basal-2 test feed (6%TI). The treatment of feed formulation with nucleotide addition of 0.1% in the test feed of 10% fish meal, 8% fish meal, and 6% fish meal, in general, was not significantly different from the results of growth trials on Basal-1 feed. Other data showed that fish meal substituted with increased soybean meal on Diet-2 and Diet-3 gave significantly better results than 6% fish meal without nucleotides (Basal-2). The presentation of the data obtained from the growth test results, statistically, the use of 0.1% nucleotide supplementary feed has been confirmed to give good results in general cultivation conditions in commercial ponds.

This study evaluates the effectiveness of commercial nucleotides (Nucleoforce ™) in field conditions as a confirmation of previous research by Novriadi *et al.* (2021). Using 0.1% nucleotide additives in 10, 8 and 6% fish meal with reduced inclusions of fish meal was substituted for soybean meal. Furthermore, the results of the intensive culture simulation in commercial ponds were analyzed on shrimp growth performance, water quality, feed nutrient profile, shrimp body composition, and the effect of nucleotide use on the production cost of aquaculture during the 60-day rearing period. Based on the test results, nucleotide supplementation was proven to increase growth in FBW, ADG, and this was seen in the ADG value in shrimp with 10% FMNT feed treatment better than 10% FM treatment without nucleotides. These results follow Tahmasebi-Kohyani *et al.* (2012) that 0.15-0.2% NT in the feed can increase the percentage gain in

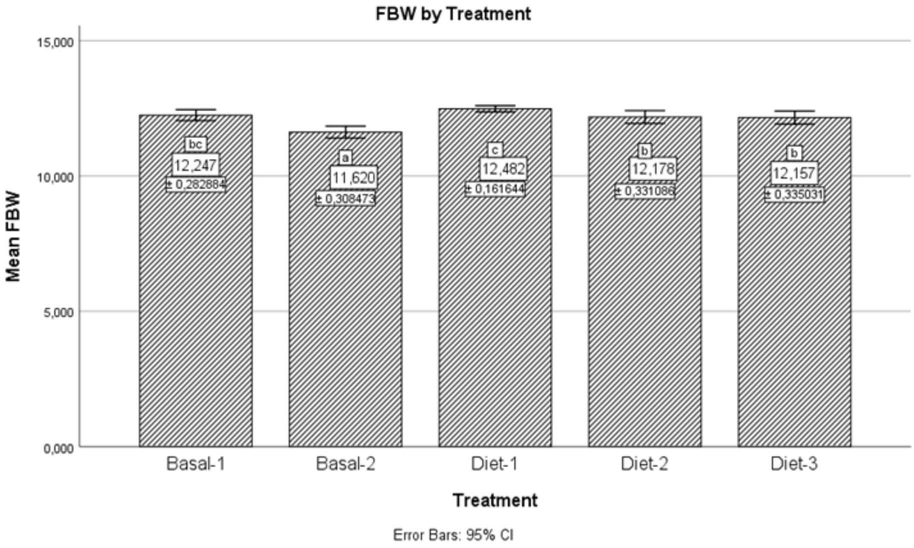
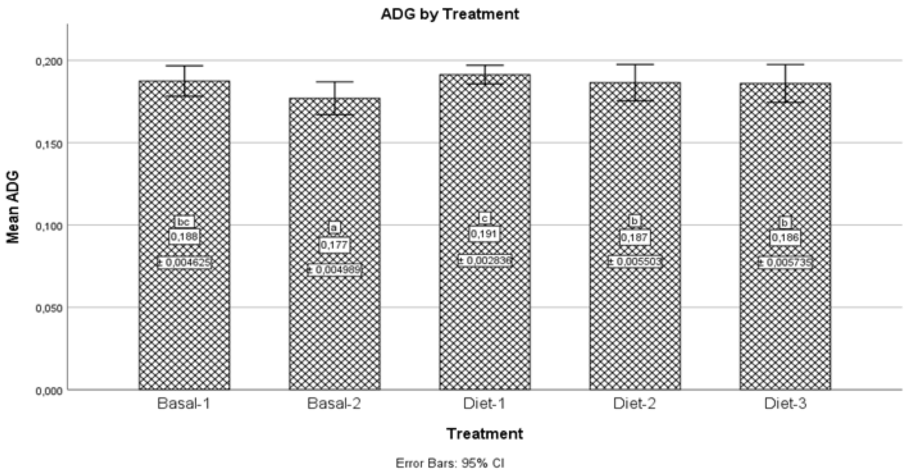
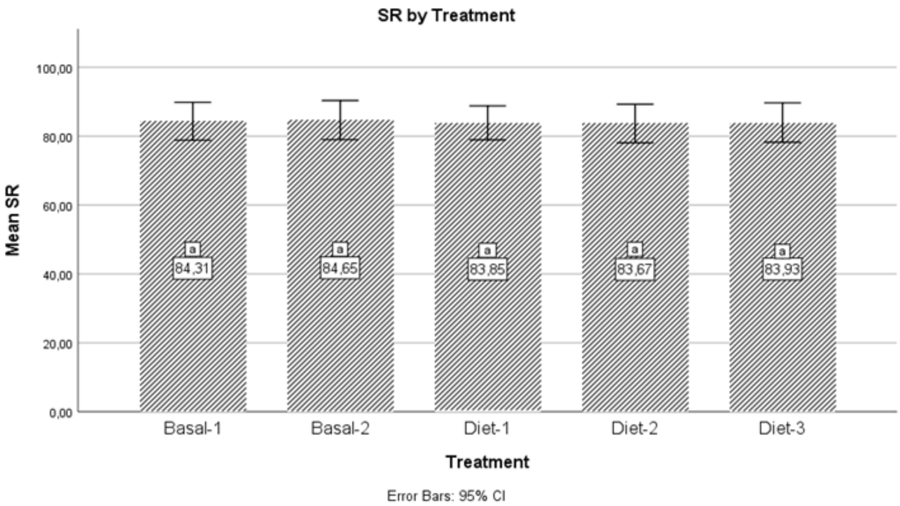


Figure 1.Weight of individual shrimp (g).



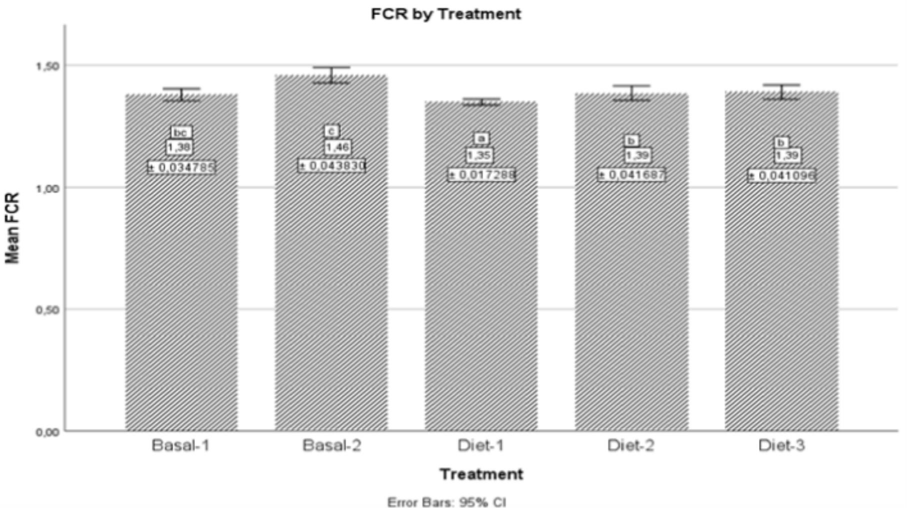
Figures 2. Average daily growth.



Figures 3. Shrimp survival rate.

body weight (WG) and feed efficiency (FE) of fish better.

Meanwhile, the ADG of shrimp treated with 10%TI (Basal-1) feed was not significantly different from that of 6% TIN (Diet-3). This means that a decrease in the amount of fish meal content by 4% in the feed, accompanied by the addition of 0.1% nucleotides to the reduction of fish meal protein substituted by soybean meal flour has the same impact on the growth rate of shrimp. These results are in line with the research of Andrino *et al.* (2012) where the addition of 0.2% nucleotide can significantly produce specific growth rates and better feed conversion efficiency than shrimp growth without nucleotide addition. This study also confirms the research of Novriadi *et al.* (2021) on a field test scale in commercial ponds influenced by fluctuations in physical and chemical conditions of water in general cultivation conditions.



Figures 4. Feed conversion ratio.

The physiological function of nucleotides in providing biochemical genetic information, mediating energy metabolism and cell signaling, and as a component of coenzymes is one of the important aspects in the development of functional feeds (Ringø *et al.,* 2011; Carver, 1993; Cosgrove, 1998). Nucleotides as low molecular weight intracellular compounds and building blocks of DNA and RNA play important roles in physiological, and almost all biochemical processes (Hossain *et al.,* 2020; Ringo *et al.* 2011; Segara, 2021) and are also considered has an immunostimulating effect on shrimp (Guo *et al.,* 2016; Novriadi *et al.,* 2021; Xiong *et al.,* 2018). As an aim to develop an economical feed by reducing the level of inclusion of fish meal, the use of nucleotides has been found to reduce the negative impact of using alternative proteins to replace fish meal and not reduce the growth performance and health of aquaculture organisms (Hossain *et al.,* 2020).

The physiological function of nucleotides in providing biochemical genetic information, mediating energy metabolism and cell signaling, and as a component of coenzymes is one of the important aspects in the development of functional feeds (Ringø *et al.,* 2011; Carver, 1993; Cosgrove, 1998). Nucleotides, as low molecular weight intracellular compounds and building blocks of DNA and RNA, play important roles in physiological and almost all biochemical processes (Hossain *et al.,* 2020; Ringo *et al*, 2011; Segara, 2021) and are also considered as an immunostimulating effect on shrimp (Guo *et al.,* 2016; Novriadi *et al.,* 2021; Xiong *et al.,* 2018). As an aim to develop an economical feed by reducing the inclusion rate of FM, the use of NT has been found to reduce the negative impact of using alternative protein instead of using FM and has no negative impact on the growth and health performance of cultured organisms (Hossain *et al.,* 2020).

Thermal Growth Coefficient

Based on the study's results in Figure 5, the TGC Diet-1 value (0.7621) was significantly different from all treatments, with the addition of 0.1% nucleotide in the feed could affect better energy absorption. Nucleotide addition of 0.1% in the use of 8% and 6% fish meal (Diet-2 & Diet-3) did not give a significantly different effect ($p>0.05$) on the growth of Basal-1 shrimp with the use of 10% fish meal without nucleotide affixes. Optimal temperature is a condition for shrimp to grow quickly and efficiently. Small shrimp can grow rapidly at high temperatures (up to 30 °C), while large shrimp (>15 g) grow fast at 27 °C). Shrimp reared at a temperature of 27°C have a lower FCR value (growing efficiently) compared to shrimp reared at temperatures above or below it (Wyban et al., 1995).

The thermal growth coefficient (TGC) was used as a model to predict fish growth. TGC relates to feed, rearing, fish size, and temperature factors. This model is quite popular because it is easy and flexible to use. Growth data at certain temperatures can be used to predict the growth of shrimp/fish reared at different temperatures (Hussain *et al*., 2021; Powell *et al*., 2019; Al-Dubakel *et al*., 2011; Rheido *et al*., 2022; Thorarensen & Farrell, 2011). The average growth rate of fish depends on the size and is strongly influenced by feed, temperature, photoperiod, and other environmental conditions. The average daily growth (ADG) decreased along with the increase in shrimp weight, so temperature significantly affected the ADG value. The rise in temperature (up to the optimal maintenance temperature) is proportional to the increase in ADG. Based on this, assessing growth rates based on ADG under conditions of fish reared in very different temperature ranges is difficult.

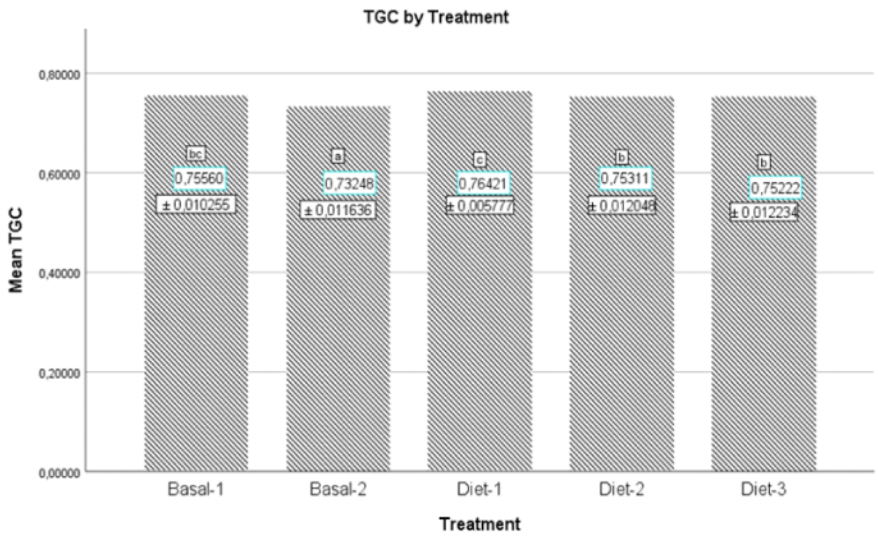


Figure 5. TGC of shrimp in each feed treatment.

Body Composition

The results of the analysis of the whole body composition of shrimp are presented in Table 2. Juvenile proximate at the beginning of the test (1 g) was placed as control, and after 60 days of rearing, proximate was carried out on the shrimp samples from the test results for each feed treatment. Diet-1 and Diet-2 feed treatments had the best effect on increasing the protein content of shrimp meat (24.72% and 24.88%) compared to other feed treatments. Basal-2 feed (6%FM) produces the highest moisture and carbohydrate content of shrimp. In general, the nutritional content of all test feeds was able to provide an increase in the nutritional profile from the juvenile phase to the end of the test shrimp rearing process.

The calculations and statistics on the protein efficiency ratio in all test feed showed that the level of protein efficiency was not significantly different ($p > 0.05$). The protein values in the Basal-2 feed gave the lowest efficiency results, and the Basal-1, Diet-1, and Diet-3 test feed gave relatively the same values (Figure 6). In contrast to the results of calculations and statistics, the retention of feed protein in the shrimp body (Figure 7) showed that all treatments were significantly different ($p < 0.05$).

This study showed that 0.1% nucleotide additions in the Diet-1 and Diet-2 flour inclusions resulted in the absorption of protein in the shrimp body which was much better than the Diet-3 treatment. Nucleotides were not previously considered a source of essential nutrients in feed development programs. Many assume that all organisms can supply sufficient amounts of nucleotides for their physiological needs through a de novo synthesis process or a “salvage pathway*”* which means recycling nucleotides from dead cells (Huu, 2016; Andrino *et al.,* 2012). Previously it was thought that crustaceans had a limited capacity to produce nucleotides *de novo*. So that this study is at least able to explain the de novo synthesis of nucleotides or the “*Salvage Pathway”* that consumes a lot of metabolic energy (Andrino *et al.,* 2012) in shrimp so that the addition of nucleotides through the intake of feed supplements (Krüger & Werf, 2018) gives better results. It has been reported that nucleotides’ ability to induce specific activation and nonspecific immune systems can increase aquatic organisms' resistance to various pathogens, including viral, bacterial, and parasitic (Hossain *et al.,* 2020). Andrino *et al.* (2012) revealed that the survival of vanamei shrimp was higher in shrimp fed nucleotide diets compared to shrimp not fed nucleotide diets. However, the results of this study showed no significant difference.

The proximate composition of the shrimp body showed a higher amount of protein absorption (protein retention) in the group that received 0.1% nucleotide treatment feed. Protein retention is a comparison value of the amount of protein stored in the form of tissue in the shrimp body with the amount of protein consumed by shrimp from feed (Barrows & Hardy (2001); Khalida *et al.,* 2017). The calculation of the best protein retention results explained that adding 0.1% nucleotides in the feed obtained better protein retention in the shrimp body compared to the feed without the addition of nucleotides. Diet-2 feed treatment occupied the highest yield with 27.32% protein retention. This result means that for every consumption of 36.57 grams of protein feed consumed by shrimp, it produces benefits for metabolism and growth of (0.2732 $\times$ 36.57 grams) or 10 grams. Protein retention was better in Diet-1 and Diet-3 diets compared to Basal-1 and Basal-2 feeds without nucleotide additions showing the ability of nucleotides to increase protein digestibility in better feeds. In addition, comparable values for other nutrients include total fat, water content, carbohydrates and crude fiber content. With respect to higher protein levels in the nucleotide group, it is associated with the ability of nucleotides to influence protein biosynthesis by regulating the intracellular nucleotide pool (Hossain *et al.,* 2020). This is closely related to the role of nucleotides as the building blocks of DNA and RNA which are then transcribed and translated to form

Table 2. Shrimp body composition on juvenile size (control) and after 60 days of rearing

Ingredients (% *as is*)	Diet Code					
	Control	Basal-1	Basal-2	Diet-1	Diet-2	Diet-3
Protein content	14.89	22.42	22.29	24.72	24.88	23.65
Total fat	1.01	0.28	0.35	0.31	0.33	0.29
Carbohydrate	0.65	1.56	2.45	1.88	1.7	2.14
Ash content	3.76	1.59	1.78	1.71	2.27	1.82
Moisture content	79.69	68.72	72.77	69.43	69.96	69.72
Crude fiber	0.44	0	0	0	0	0

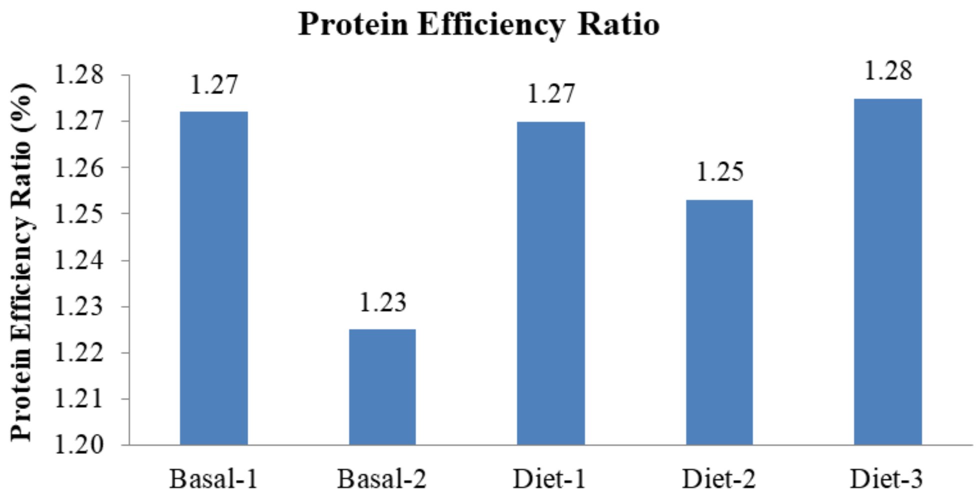


Figure 6. Protein efficiency ratio (%).

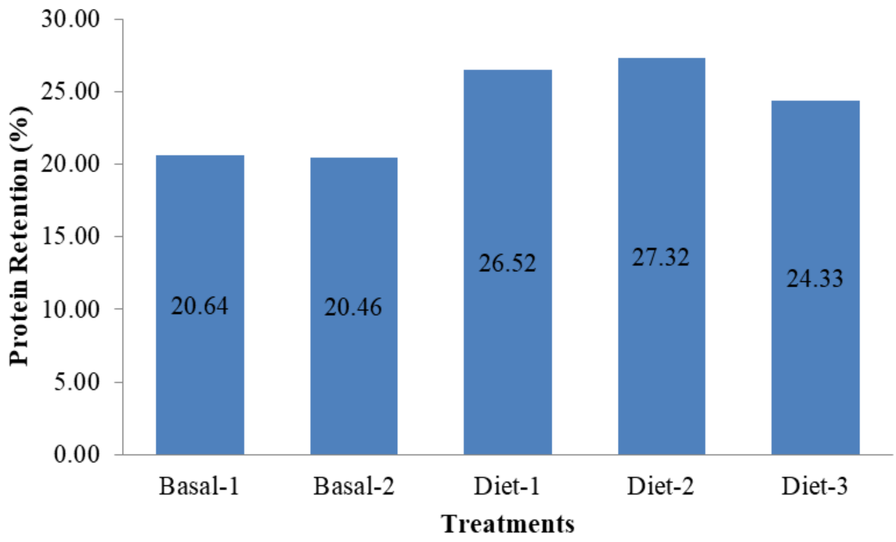


Figure 7. Protein retention on five treatments.

proteins in the shrimp body (Kurland, 1978; Hossain *et al.,* 2020) where the activity of RNA in the ribosome affects the increase in protein synthesis (Astuti *et al.,* 2015). So it can be assumed that adding nucleotides in the feed activates the "salvage pathway" de novo synthesis, so naturally more nucleotides are produced by reusing nucleic acids from dead cells without losing their function (Mamidala *et al.,* 2020).

Water Quality

The results of the measurement of water quality conditions (mean ± standard deviation) during the testing process in aquaculture ponds observed were temperature, DO, salinity, pH, ammonia, nitrite, nitrate, and phosphate are shown in Table 3. The pH value of the water ranged from 7.79 ± 0.12, dissolved oxygen 6.09 ± 0.41 mg/L, salinity ranged from 33.17 ± 0.75 ppt, and temperature 28.74 ± 0.39 °C. Meanwhile, ammonia (NH_3-N) was 0.014 ± 0.006 mg/L, nitrite (NO_2-N) 0.022 ± 0.007 mg/L, nitrate (NO_3-N) 4.659 ± 1,248 and phosphate (PO_4 N) 0.023 ± 0.006 mg/L.

Table 3. Water quality measurement during shrimp maintenance

Parameter	Unit	Average Value
Dissolved Oxygen	mg/L	6.09±0.41
Temperature	°C	28.74 ± 0.39
Salinity	mg/L	33.17±0.75
pH	units	7.79 ± 0.12
Ammonia (NH_3-N)	mg/L	0.014±0.006
Nitrite (NO_2-N)	mg/L	0.022 ± 0.007
Nitrates (NO_3-N)	mg/L	4.659±1.248
Phosphate (PO_4-N)	mg/L	0.023±0.006

Water quality is crucial in the enlargement of vanamei shrimp because it is one of the determinants of the success of rearing vanamei shrimp. Shrimp survival is determined by the degree of acidity (pH), salt content (salinity), dissolved oxygen content (DO), ammonia content, H_2S, water brightness, plankton content, and others (Pratama *et al.,* 2017). The physical condition of the water in the experiment showed a relatively stable value except for the salt content (33.17 ppt ± 0.75), which was above the normal range according (Badan Standardisasi Nasional, 2006) but still within the range of cultivation conditions in general (Konkeo, 1994). This condition can be seen at a high enough salinity that does not impact growth performance. The chemical conditions of the water were still in the relatively normal range, where the nitrate level was 4.659 ppm ± 1.248, but nitrate was not toxic to shrimp at concentrations below 50 ppm (Syah *et al.,* 2017; Boyd & Clay, 2002).

CONCLUSION

As a feed additive produced commercially in optimizing feed formulations from vegetable ingredients in the aquaculture of vannamei shrimp *Litopenaeus vannamei*, the concentration of 0.1% nucleotide formulation has been able to provide good results. The growth performance of the tested shrimp was significantly related to the parameters of increasing biomass, final weight (FBW), feed protein retention, average daily growth (ADG), reducing feed conversion (FCR) ($p<0.05$) and TGC. The best results is 10% FMNT treatment (Diet-1) with the best protein content of

37.06% ± 0.15, showed the best performance of growth parameters (ADG, FBW, SR, TGC, PER, and RP) and lowest FCR. The addition of 0.1% nucleotide proves that the functional and nutritional properties of NT have many advantages in increasing the growth rate and more efficient protein absorption. Therefore, 0.1% NT can be recommended as a supplement in shrimp feed. So this study explains that the nucleotide addition of 0.1% in the feed provides an affirmative effect on growth performance and protein content parameters of the whole body of white vanamei shrimp.

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