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Growth, Feed Efficiency, and Health Status of *Tilapia* sp. Fed with New Technology Promoter Binder Fortified Diet

Ating Yuniarti [1,*], I Made D. Mahariawan [1], Wahyu E. Kusuma [1], Bagus R. Hidayat [2] and Anik M. Hariati [1]

[1] Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Malang 65145, Indonesia; imd_mahariawan@ub.ac.id (I.M.D.M.); wahyuendrak@ub.ac.id (W.E.K.); a_hariati@ub.ac.id (A.M.H.)

[2] Numega Nutrition Pte., Ltd., 111 North Bridge Road Peninsula Plaza, Singapore 179098, Singapore; bagusdrh@gmail.com

* Correspondence: ating_y@ub.ac.id

Abstract: Developing a sustainable feed with minimal or no fishmeal in tilapia production is a challenge to this industry. New Technology Promoter Binder (NTPB), a guanidinoacetic acid, is a nutritional supplement to enhance the energy metabolism of the muscle and brain tissue of vertebrates. This study aimed to evaluate several plant-based diet formulations with zero and minimal use of fishmeal enriched with NTPB on the growth, feed efficiency, and health status of *Tilapia* sp. The experimental diets in this study were conducted based on four formulations (minimum fishmeal/FM and zero fishmeal/NFM-based diets) with 0, 0.6, and 1.2 g·kg^{-1} feed of NTPB supplementation. The research indicated that the various diets given to tilapia affected the growth performance of fish in terms of growth parameters and feed efficiency. Tilapias fed with NTPB grew better than those without NTPB in both FM- and NFM-based diets. The addition of NTPB was safe for tilapia as demonstrated in the blood glucose, urea, and creatinine levels, which were normal for healthy fish. The viscerosomatic and hepatosomatic indexes of tilapia fed with the experimental diets showed no distinct differences. Adding NTPB to tilapia diets increased the hardness and amino acid contents of the tilapia's muscle, which would benefit consumers.

Keywords: feed efficiency; feed supplement; fortification; guanidinoacetic acid

Key Contribution: This study provided an evaluation of whether supplementation of the New Technology Promoter Binder (NTPB), a creatine precursor in plant-based fish feed formulation (with minimum or zero fishmeal), affects the growth performance, feed efficiency and health status of *Tilapia* sp. Positive results were reported and the optimal NTPB supplementation seemed to be 0.6 g·kg^{-1} of feed. This feed additive was safe for tilapia as observed in their blood, viscerosomatic and hepatosomatic index. Furthermore, the enhanced muscle hardness and amino acid content would provide other benefits for consumers.

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1. Introduction

Tilapia is the most promising aquaculture species in Indonesia, with a production of 364,747.10 metric tons in 2020 [1]. In the global market, tilapia has become an important export fish from many developing countries including Indonesia. Over the past 20 years, the supply of Indonesian tilapia export items to the US market has increased steadily [2]. The high tilapia production in Indonesia requires increasingly expensive inputs and relies heavily on formulated diets.

In the feed industry, fishmeal in formulated diets is often regarded as the gold standard of dietary protein sources. However, there is cultural and economic pressure to discover alternative proteins. Plant proteins such as soybean meal and corn gluten meal are often used as fishmeal substitutes in aquafeeds. Yet, even when high plant protein (>50%) diets were

used to ensure the necessary nutrient balance, the growth performance attained was inferior to that of fish given fishmeal-based diets [3,4]. Another promising high protein source to replace fishmeal is poultry by-product meal (PBM) which is available in high quantities, cheap, and, highly sustainable for the feed industry [5]. Many studies recorded the success of fish meal replacement by PBM in both freshwater and marine water species [6–12]. High variation in the nutritional composition of PBM resulted in certain essential amino acid deficiencies, high ash contents and varied digestibility value [13,14]. Therefore, a lot of studies have been carried out on re-formulating aquafeeds with innovative components and nutritional supplements that complement fishmeal alternatives and assist aquaculture species to satisfy their needs.

NTPB, a guanidinoacetic acid (GAA), is a naturally occurring amino acid derivative that works as a direct precursor to creatine [15]. Creatine is a key component in the energy metabolism of the muscle and brain tissue of vertebrates [16]. Many studies have shown positive results of GAA as a performance-enhancing agent in several animals. In pigs, supplementation of 1.0 $g \cdot kg^{-1}$ GAA increased the carcass quality and energy metabolism [17]. Dietary fortification of 0.6–1.2 $g \cdot kg^{-1}$ GAA improved the growth performance of broilers [18,19] and reduced heat stress [20]. The addition of 0.6 or 0.9 $g \cdot kg^{-1}$ DM GAA improved growth performance, nutrient digestion, and ruminal fermentation in bulls [21]. In lambs, positive results were also discovered with dietary supplementation of 500–1000 $mg \cdot kg^{-1}$ DM GAA. For fish, 0.3–1.2 $g \cdot kg^{-1}$ GAA increased the growth and flesh quality of carp based on a vegetable meal diet with 28% crude protein (CP) [22] and 30% CP of the diet [23]. The growth performance of tilapia also increased with fortification of 0.6–1.2 $g \cdot kg^{-1}$ GAA in their diet with 35% CP and 3500 $kcal \cdot kg^{-1}$ [24].

To date, developing sustainable feeds to support maximal growth while still being cost effective has become an essential topic to be studied. Some interesting research suggested that diets with minimal or no fishmeal inclusion will be viable in the future with careful formulations [25,26]. Egerton et al. [27] suggested the use of a minimum 5% fishmeal to provide unidentified growth factors which are assumed to be naturally occurring trace and ultra-trace chemicals such as amines and steroids. The objective of this study was to evaluate several plant-based formulations with zero or minimal use of fishmeal enriched with NTPB on the growth, feed efficiency, and health status of *Tilapia* sp.

2. Materials and Methods

2.1. Materials

The experimental diets were produced based on four formulations, with 0.6 and 1.2 $g \cdot kg^{-1}$ and without NTPB supplementation in minimum fish meal inclusion (FM) and zero fish meal (NFM). All diets were formulated with 35% of crude protein and the metabolizable energy of 3100 $cal \cdot kg^{-1}$ (Table 1). The diets were prepared in the form of a sinking pellet by an electric fish pellet machine with a diameter of 2 mm. Those were then stored in plastic bags at −20 °C during the time of use. After pelleting, the diets were subjected to proximate analysis [28]. The NTPB (Numega Nutrition, Singapore) used in this study was GAA (85%) and some assistant factors. The GAA in NTPB products originated from the synthesis of glycin and arginine.

Table 1. The experimental diets and their nutritional composition.

Ingredient (gr)	A (NFM_0)	B (NFM_0.6)	C (NFM_1.2)	D (FM_0)
Fish meal	0.0	0.0	0.0	50.0
Poultry By-Product meal	100.0	100.0	100.0	50.0
Soybean meal	325.1	325.4	325.5	311.5
Corn Gluten meal	200.0	200.0	200.0	200.0
Corn Yellow	168.8	168.5	167.5	168.0
Rice Bran	18.5	18.9	19.2	22.1
Pollard	1.1	0.1	0.1	12.0

Table 1. *Cont.*

Ingredient (gr)	A (NFM_0)	B (NFM_0.6)	C (NFM_1.2)	D (FM_0)
Cassava	95.0	95.0	95.0	95.0
Distiller's dried grains with solubles	50.0	50.0	50.0	50.0
Crude Palm Oil	35.0	35.0	35.0	35.0
CMC	5.0	5.0	5.0	5.0
Vitamin HC	0.5	0.5	0.5	0.5
Mineral	1.0	1.0	1.0	1.0
NTPB	0.0	0.6	1.2	0.0
Total	1000	1000	1000	1000
Dry matter (%)	89.02	92.51	91.73	91.42
Crude Protein (%)	34.05	34.09	33.69	33.31
Fat (%)	6.20	7.87	6.98	6.31
Ash (%)	6.67	6.65	6.59	6.57
Fiber (%)	6.53	3.82	6.01	5.73
NFE (%)	46.55	47.57	46.73	48.08
ME (kcal·kg^{-1})	3.55	3.74	3.61	3.58

NFM_0 = non fish meal, with no NTPB, NFM_0.6 = non fish meal, with 0.6 g·kg^{-1} NTPB, NFM_1.2 = non fishmeal, with 1.2 g·kg^{-1}, FM_0 = 5% fishmeal with no NTPB, NFE = nitrogen free extract, ME = metabolizable energy.

2.2. Methodology

The experiment was conducted in the fish-rearing unit of the Department of Aquaculture Faculty of Fisheries and Marine Science University of Brawijaya. Five treatment diets were administered in quadruplicate using a completely randomized method with a trial unit of a 20-L volume aquarium (size 50 × 30 × 30 cm). Fifteen fish (±15 g) of sex-reversed tilapia were stocked in each of the 20 aquaria according to treatment. The animal management protocol for this experiment was approved by the bioethics and animal welfare committee University of Brawijaya with an ethical clearance number of 154-KEP-UB-2024.

Before placing the fish in a randomly selected recirculated aquarium among the four treatments, the average fish biomass of each stocked aquarium was recorded. The feed was given three times daily, with as much as 3% biomass, and adjusted for every sampling period. The aquaria were cleaned daily after the last feeding through siphoning. Water exchange was conducted at a rate of a maximum 10% of the reservoir tank set with drilled well water every day.

Water quality parameters such as temperature, dissolved oxygen, and pH were monitored daily, while ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) were measured every 1 month using a spectrophotometer method. The temperature, pH, and dissolved oxygen of all treatments were maintained in optimal conditions for fish culture, which ranged from 24.67 to 24.82 °C, 7.7 to 7.71, and 5.73 to 5.75 ppm, respectively. The chemical parameters of water quality such as ammonia, nitrite, and nitrate in this study were considered similar and still in acceptable levels for tilapia which leveled at 0.023 ± 0.0004 ppm, 0.063 ± 0.001 ppm, and 0.16 ± 0.0001 ppm, respectively.

The number of fish was monitored and counted daily. Sampling of fish weight was conducted every 10 days by taking all fish from each aquarium and adjusting the amount of feed according to the growth. All fish were harvested after 60 days. Samples from each replication were packed in polythene and stored in a freezer (−20 °C) for proximate analysis. The proximate composition of fish (initial and final sampling) was conducted based on the AOAC method [28]. Dry matter was calculated by drying in an oven at 105 °C for 24 h, crude protein (NX 6.25) by the Kjeldahl method and crude fat with ether extraction. Nitrogen free extract was estimated by the reduction method. The profile of essential amino acids (EAAs) of the fish carcass fed with experimental diets was determined by using HPLC [28].

2.3. The Measurement of Parameters and Data Analysis

2.3.1. Growth Performances

The following formulas were used to evaluate the survival rate and growth performance among treatments.

$$Survival\ rate\ (SR.\ \%) = \frac{total\ fish\ harvested}{total\ fish\ stocked} \times 100$$

$$Feed\ intake\ \left(FI\ \%.day^{-1}\right) = \frac{(100 \times dry\ feed\ intake)}{\left(\frac{final\ body\ weight + initial\ body\ weight}{2}\right)/days}$$

$$Specific\ Growth\ Rate\ \left(SGR.\%\ BWd^{-1}\right) = \frac{Ln\ (final\ body\ weight) - Ln(initial\ body\ weight)}{number\ of\ feeding\ day} \times 100$$

$$Feed\ Conversion\ Ratio\ \left(FCR.\ gr.gr^{-1}\right) = \frac{feed\ consumption}{body\ weight\ gain}$$

$$Protein\ Retention\ (PRE,\ \%) = \frac{(final\ weight \times protein\ content\ in\ final\ weight) - (Initial\ weight \times protein\ content\ in\ initial\ weight)}{(dry\ feed\ intake \times fat\ content\ in\ diet)} \times 100$$

$$Fat\ Retention\ (FRE,\ \%) = \frac{(final\ weight \times fat\ content\ in\ final\ weight) - (Initial\ weight \times fat\ content\ in\ initial\ weight)}{(dry\ feed\ intake \times fat\ content\ in\ diet)} \times 100$$

$$Energy\ Retention\ (ERE,\ \%) = \frac{(final\ weight \times energy\ content\ in\ final\ weight) - (Initial\ weight \times energy\ content\ in\ initial\ weight)}{(dry\ feed\ intake \times energy\ content\ in\ diet)} \times 100$$

2.3.2. Body Indices, Fish Health Status and Filet Texture

After the feeding trial ended, four fish from each replication were collected for evaluating the body indices:

$$Hepatosomatic\ index\ (HSI) = 100 \times \frac{liver\ weight}{body\ weight}$$

$$Viscerosomatic\ index\ (VSI) = 100 \times \frac{viscera\ weight}{body\ weight}$$

The health status of tilapia was assessed through the biochemical content of fish blood. At the end of the experimental period, blood samples were collected from the caudal vein of 3 fish/aquariums, kept at room temperature for 20 min to allow for clotting, and stored in the refrigerator for 4 h. After centrifugation at 3000 rpm for 10 min, the clear serum was carefully isolated and refrigerated at −20 °C for further analysis of biochemical parameters. The blood chemical parameters were urea, creatinine, and glucose. Urea, creatinine, and glucose were assayed using ready-made kits manufactured by Human Company Germany.

The filet texture was analyzed based on Wiriyapattanasub et al. [24]. The filets were removed from the dorsal body portion, approximately 1.5 cm above the lateral line. The filets were cut into cubes around 2-2-1 cm in size. The cubes were placed so that the muscle fibers were horizontally orientated and squeezed using a 35 mm cylindrical aluminum probe at a speed of 2 mm s^{-1} and a trigger force of 5 g. The maximum force obtained during compression (gF) was recorded.

2.4. Statistical Analysis

Data were expressed as mean ± standard deviation. Analysis of variance (one-way ANOVA) was used to determine the level of significance between different growth and other parameters with SPSS version 20.0. Significant levels were considered at $p < 0.05$ and means were compared using Duncan's multiple range test.

3. Results

3.1. The Growth Performance of Tilapia sp.

All experimental diets were safe for the tilapia as no deaths were encountered during the study. The various diets given to tilapia affected the growth performances of fish ($p < 0.05$) (Table 2). Generally, the growth performances of tilapia in this experiment were higher when fed with NTPB fortification.

Table 2. The growth performance of experimental fish during the study (mean ± standard deviation, n = 16).

Diet	Initial Weight (g)	Final Weight (g)	SR (%)	FI (%·day^{-1})	SGR (% BW·day^{-1})	FCR
A(NFM_0)	16.20 ± 0.25	45.33 ± 0.51 ^{a}	100 ± 0.00 ^{a}	62.37 ± 0.59 ^{a}	1.47 ± 0.02 ^{a}	2.14 ± 0.04 ^{a}
B (NFM_0.6)	16.77 ± 0.58	49.82 ± 0.31 ^{b}	100 ± 0.00 ^{a}	68.77 ± 0.37 ^{b}	1.55 ± 0.02 ^{b}	2.08 ± 0.03 ^{b}
C (NFM_1.2)	16.40 ± 0.30	48.46 ± 0.62 ^{b}	100 ± 0.00 ^{a}	67.32 ± 0.69 ^{b}	1.54 ± 0.02 ^{b}	2.10 ± 0.03 ^{b}
D (FM_0)	15.68 ± 0.79	45.58 ± 0.43 ^{a}	100 ± 0.00 ^{a}	62.97 ± 0.89 ^{a}	1.53 ± 0.06 ^{b}	2.10 ± 0.04 ^{b}

SR = survival rate, FI = feed intake, SGR = specific growth rate, FCR = feed conversion ratio. The different superscript letters are significantly different as determined by Duncan's post hoc test ($p < 0.05$).

3.2. Nutrient Retention of Tilapia sp.

This study showed that the experimental diets significantly influenced the nutrient retention in *tilapia* ($p < 0.05$) (Table 3).

Table 3. Nutrient retention of tilapia fed with the experimental diets (n = 16).

Diet	PRE (%)	FRE (%)	ERE (%)
A (NFM_0)	24.66 ± 0.12 ^{a}	27.40 ± 0.13 ^{a}	13.77 ± 0.06 ^{a}
B (NFM_0.6)	27.12 ± 0.20 ^{b}	29.93 ± 0.22 ^{b}	15.83 ± 0.11 ^{c}
C (NFM_1.2)	27.06 ± 0.33 ^{b}	29.78 ± 0.3 ^{b}	15.67 ± 0.19 ^{c}
D (FM_0)	25.43 ± 0.45 ^{a}	28.38 ± 0.57 ^{a}	14.67 ± 0.27 ^{b}

PRE = protein retention, FRE = fat retention, ERE = energy retention. The different superscript letters are significantly different as determined by Duncan's post hoc test ($p < 0.05$).

3.3. Glucose, Urea, and Creatinine Concentration

Glucose, urea, and creatinine concentrations were measured to evaluate the physiological status of fish after NTPB supplementation. As presented in Table 4, the glucose, urea, and creatinine levels in tilapia were affected by NTPB supplementation ($p < 0.05$).

Table 4. Glucose, urea, and creatinine concentration of tilapia fed with the experimental diets (n = 16).

Diet	Glucose (mg·dL^{-1})	Urea (mg·dL^{-1})	Creatinine (mg·dL^{-1})
A (NFM_0)	52.00 ± 0.82 ^{a}	1.90 ± 0.11 ^{a}	0.24 ± 0.01 ^{a}
B (NFM_0.6)	56.50 ± 0.96 ^{c}	3.25 ± 0.13 ^{b}	0.31 ± 0.01 ^{c}
C (NFM_1.2)	55.25 ± 0.48 ^{bc}	3.19 ± 0.12 ^{b}	0.29 ± 0.03 ^{b}
D (FM_0)	53.50 ± 1.91 ^{ab}	3.00 ± 0.11 ^{b}	0.28 ± 0.01 ^{b}

The different superscript letters are significantly different as determined by Duncan's post hoc test ($p < 0.05$).

3.4. Viscerosomatic Index (VSI), Hepatosomatic Index (HSI), and Muscle Texture of Tilapia sp.

As shown in Table 5, the VSI and HSI of tilapia were not significantly different among treatments ($p > 0.05$). On the other hand, the supplementation of NTPB in the diet influenced the tilapia meat's texture ($p < 0.05$)

Table 5. HSI, VSI, and muscle texture of tilapia fed with the experimental diets.

Diet	HSI (%)	VSI (%)	Meat Texture (gF)
A(NFM_0)	0.68 ± 0.01 [a]	5.66 ± 0.08 [a]	1465.84 ± 20.6 [a]
B (NFM_0.6)	0.67 ± 0.01 [a]	5.65 ± 0.07 [a]	1830.38 ± 8.8 [d]
C (NFM_1.2)	0.67 ± 0.04 [a]	5.65 ± 0.10 [a]	1779.40 ± 19.8 [c]
D (FM_0)	0.69 ± 0.01 [a]	5.67 ± 0.02 [a]	1555.07 ± 6.6 [b]

The different superscript letters are significantly different as determined by Duncan's post hoc test ($p < 0.05$).

3.5. Free Amino Acid in Tilapia sp. Muscle

The nutrient composition of the muscle is an important factor in determining the quality of fish meat (Table 6). The protein and amino acid concentrations can affect the value of nutrients, flavor, and function of the fish muscle. In general, feed with fish meal inclusion had higher TAAs compared to that without fish meal.

Table 6. Free amino acid in *Tilapia* sp. muscle under NTPB supplementation (% wet weight).

Amino Acid	A (NFM_0)	B (NFM_0.6)	C (NFM_1.2)	D (FM_0)
L-Serine	0.69 ± 0.13	0.69 ± 0.04	0.71 ± 0.04	0.74 ± 0.08
L-Glutamin Acid	2.43 ± 0.03	2.23 ± 0.07	2.25 ± 0.10	2.25 ± 0.05
L-Phenylalanine	0.73 ± 0.07	0.74 ± 0.04	0.74 ± 0.05	0.82 ± 0.08
L-Isoleucine	0.76 ± 0.05	0.72 ± 0.05	0.71 ± 0.08	0.78 ± 0.01
L-Valine	0.85 ± 0.07	0.80 ± 0.10	0.81 ± 0.08	0.87 ± 0.06
L-Alanine	1.08 ± 0.08	1.17 ± 0.16	1.20 ± 0.08	1.13 ± 0.03
L-Arginine	1.21 ± 0.06	1.28 ± 0.05	1.27 ± 0.05	1.36 ± 0.04
L-Glycine	1.32 ± 0.06	1.66 ± 0.04	1.73 ± 0.08	1.55 ± 0.04
L-Lysine	1.39 ± 0.15	1.25 ± 0.06	1.28 ± 0.08	1.27 ± 0.17
L-Aspartic Acid	1.39 ± 0.06	1.28 ± 0.06	1.33 ± 0.16	1.31 ± 0.06
L-Leucine	1.33 ± 0.08	1.28 ± 0.01	1.26 ± 0.11	1.38 ± 0.03
L-Tyrosine	0.58 ± 0.04	0.50 ± 0.16	0.49 ± 0.03	0.56 ± 0.17
L-Proline	0.80 ± 0.00	0.99 ± 0.05	1.00 ± 0.07	0.88 ± 0.04
L-Threonine	0.79 ± 0.06	0.78 ± 0.06	0.78 ± 0.07	0.87 ± 0.04
L-Histidine	0.36 ± 0.11	0.39 ± 0.04	0.37 ± 0.11	0.41 ± 0.06
∑ EAA	7.99 ± 0.35	7.71 ± 0.33	7.68 ± 0.34	8.31 ± 0.35
∑ NEAA	7.71 ± 0.63	8.01 ± 0.54	8.21 ± 0.55	7.85 ± 0.54
TAA	15.69 ± 0.50	15.72 ± 0.48	15.89 ± 0.49	16.16 ± 0.46

EAA = essential amino acid, NEAA = non-essential amino acid, TAA = total amino acid.

4. Discussion

All the experimental diets were safe for the tilapia as no deaths were reported in this study. This finding is in line with research by Wiriyapattanasub et al. [24] that the addition of NTPB at levels of 0.6 g·kg^{-1}, 1.2 g·kg^{-1}, and 1.8 g·kg^{-1} resulted in a 100% survival rate of Nile tilapia. Several factors influenced fish survival rates including the physical condition of fish, declining water quality, and environmental parameters [29]. Based on this study, the survival rates could also be maintained by monitoring the fish condition and water quality.

It was hypothesized that the addition of NTPB provided a high protein synthesis in tilapia, thus potentially improving SGR values. The presence of NTPB in the fish feed also could increase creatine deposition in the muscles. Creatine is considered an essential molecule in energy homeostasis through the creatine and phosphocreatine (PCr) system. A high supply of creatine in the body of tilapia could enhance energy utilization and protein synthesis and then increase fish growth. The higher growth performances were possibly due to the improved protein synthesis as there was an increase in creatine availability. This was in line with several research studies that showed that the given NTPB accelerated the absolute weight gain and feed intake of some organisms [16,17,30]. The NTPB is a more stable molecule and is cheaper. The supplementation of NTPB to feed formulations

exhibited the potential to accelerate fish growth. The NTPB consumed by organisms would convert into creatine via guanidinoacetic methyltransferase (GAMT) and then be phosphorylated to phosphocreatine [31]. Compared to SGR, the lower the feed conversion ratio (FCR) value, the more optimal the utilization of feed to produce fish meat. Some researchers reported that adding an appropriate level of NTPB to feed could decrease the FCR value [32,33]. This condition influenced the fish to utilize the protein provided and the energy obtained, which could be processed optimally to produce an increase in fish weight, and also affected the FCR value [34,35].

Protein retention is the ratio between the amount of protein stored in the form of tissues in the fish body and the amount of protein consumption obtained in the fish feed. In this study, the addition of NTPB increased protein retention by more than 27%. Following the protein retention, higher fat retention was also found in this study by the addition of NTPB. The high fat content in the feed showed that the fat content in the fish's body also tends to increase. Fat retention describes the ability of fish to store fat and utilize it. The high fat retention in fish can be used to produce energy for increasing its growth. The fat retention value also determined the calorie intake of the fish; hence, fish with a high fat retention level would have a high calorie intake [36]. The addition of NTPB to fish feed also influenced the energy retention of tilapia. Energy was obtained from the breakdown of chemical ligaments through the process of oxidation reactions to specific components such as proteins, fats, and carbohydrates to produce simpler compounds such as amino acids, fatty acids, and glucose. Therefore, it could be absorbed by the body to be used or stored by the fish. The higher value of energy retention in the tilapia body indicated that the energy content in the feed derived from protein was good. Thus, it influenced fish to use the energy to synthesize and absorb protein in the body instead of energy storage [37,38].

This study revealed that the diet affected the glucose blood level of *tilapia*. The higher blood glucose level in this study was found in tilapia fed with NTPB fortification. Glucose is a carbohydrate that plays an important part in animal bioenergetics by being converted to chemical energy (ATP), which can then be represented as mechanical energy [39]. As a creatine precursor, GAA can directly enhance ATP and phosphocreatine levels, as well as glucose breakdown, to generate energy [40]. High glucose levels were also detected in fish serum treated with GAA supplementation in tilapia [41] and bullfrog [40]. Inconsistent with the result of this study, GAA supplementation reduced the blood glucose level in ducks [42]. Furthermore, the decrease in glucose could be attributed to the inclusion of GAA in diets and improved creatine and ATP levels, resulting in lower glucose needed for energy supply. However, the blood glucose levels were still in the normal range for fish (40–90 $mg{\cdot}dL^{-1}$) [43].

The addition of NTPB resulted in a significantly higher serum creatinine concentration than without NTPB. Creatinine is a creatine metabolite and a by-product of energy production in muscle tissue [44–46]. Because fish excrete creatinine via the kidney, creatinine blood levels can be used to assess the efficiency of renal filtration [47]. This study showed a general increase in creatinine with NTPB supplementation. The elevated creatinine levels are caused by the breakdown of creatine in the fish's muscles [46]. As a precursor of creatine, NTPB supplementation increased ATP and phosphocreatine contents. This condition moved the muscle cells into the energy filling state, which could result in increased creatine metabolism to creatinine, the only product of creatine decomposition. This finding was in line with a study in the bullfrog [40]. The creatinine produced by creatine was influenced by muscle function, meat consumption, and creatine de novo production [45]. Furthermore, the blood creatinine levels showed the balance between creatinine production and clearance. GAA added to diets is lost mostly through creatine and creatinine, with minimal excretion in the form of GAA through the kidney [47]. Concerning health, rapid and prolonged decreases in creatinine production have been seen during diseases. Normal creatinine values range from 0.8 to 1.4 $mg{\cdot}dL^{-1}$ [48] Furthermore, higher urea levels may arise from poor excretion, higher synthesis, and lower urine clearance by the kidney, or by decreased degradation of nitrogenous compounds [49]. In some fish species, urea levels of

up to 20 mg·dL^{-1} are considered normal [48]. Taken together, this finding confirmed that the use of NTPB is safe for fish with no response to stress.

Viscerosomatic and hepatosomatic Index (VSI and HSI) parameters showed the indices of fish metabolism, particularly digestion and absorption, the production and release of digestive enzymes, and glucose metabolism [50]. The accumulation of excess fat in fish will affect the fish's health. Adipose tissues are the main lipid storage in vertebrates. The major sites of deposition are visceral, liver, and muscle. The lower HSI and VSI indicate less fat deposited in the liver and abdominal capacity. Similar findings were revealed in the study of tilapia fed with a higher maltose diet [50] and grouper fed with higher amounts of corn starch [51]. Based on the value of VSI and HSI, all tilapias given the experimental diets were normal, with no distinct abnormalities.

The acceptance by the consumer is closely related to the intrinsic structure and the textural features of the fish muscle, especially the hardness. Increased muscular hardness is used to denote an improvement in fish muscle quality [52]. Furthermore, this vital index depends largely on the structure of connective tissue. The hardness of tilapia fed with NTPB-containing diets is considerably higher compared to that in fish fed without NTPB. Several studies showed positive results of GAA supplementation on the muscle quality of fish [24,53,54] and flesh of broilers [55], pigs [17], and ducks [42]. Dong et al. [53] discovered that enhanced muscle hardness was likely connected to an increase in new muscle fiber controlled by Myogenic Regulatory Factors (MRFs) and Myostatin (MSTN). Furthermore, enhanced muscle hardness was likely linked to increased collagen synthesis and decreased collagen degradation. The supplementation of NTPB also increased total amino acid (TAA) levels in tilapia. These free amino acids and reducing sugars in the muscles can generate better flavors [56]. Similar findings found an improvement in amino acids as GAA was added to the diet of tilapia [24] and pigs [56,57]. The umami amino acid glutamate was increased in lean pigs with GAA addition to the pig's diet [56].

5. Conclusions

The addition of NTPB in tilapia diets, which were formulated with zero or minimal use of fishmeal, resulted in higher growth and feed efficiency for *Tilapia* sp. Several physiological parameters, such as glucose, urea, and creatinine levels, of tilapia were affected by NTPB fortification, yet this feed additive was considered safe for the fish. This condition was also supported by the viscerosomatic and hepatosomatic index. NTPB fortification increased the hardness and essential amino acid content of tilapia muscle, perhaps increasing customer preference.

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only, and did not involve in the process and result of the analysis, 2. Bagus R. Hidayat carried out supervision during the research.

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