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# Nile Tilapia (*Oreochromis niloticus*) Gill Powder as a Novel Feed Additive for Enhanced Histological Structure and Performance in Broilers

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## Abstract

The burgeoning population of Indonesia has led to a heightened demand for animal protein, particularly within the broiler industry. However, the commercial broiler feed tends to be costly due to its reliance on imported ingredients. Therefore, the potential utilization of Nile tilapia gills as an additional protein source for broiler feed has been explored in this study. The primary objective was to investigate how tilapia gill powder affects the histological structure of the small intestine, pectoralis muscle, and overall growth performance of broilers. For the study, a total of 200 day-old male Cobb-500 broiler chicks were utilized, employing a completely randomized design with five treatments and five replications. Each replication consisted of 8-day-old chicks. Tilapia gill powder was incorporated into the basal feed at varying concentrations: Control (basal feed), tilapia gill powder 1 (0.25% tilapia gill powder/kg basal feed), tilapia gill powder 2 (0.5% tilapia gill powder/kg basal feed), tilapia gill powder 3 (1% tilapia gill powder/kg basal feed), and tilapia gill powder 4 (2% tilapia gill powder/kg basal feed). Comprehensive histological analysis and growth performance assessments were carried out. The outcomes revealed that the tilapia gill powder 4 group, administered at an optimal concentration of 2% tilapia gill powder/kg basal feed, exhibited significant enhancements in small intestine morphology, pectoralis muscle structure, and overall chicken growth performance when compared to the control group. The supplementation of tilapia gill powder at this optimal concentration (2% tilapia gill powder/kg basal feed) showcased a favorable impact on broiler performance.

***Keywords:*** Feed additive, growth performance, pectoralis muscle, small intestine, tilapia gill powder

## Introduction

The growing population in Indonesia has led to an augmented demand for food, especially protein. Broiler chickens are widely favored and reasonably priced as a source of animal protein due to their cost-efficiency and proficient conversion of feed into meat (Choi et al., 2023). As per statistics provided by the Indonesian Ministry of Agriculture, the estimated consumption of broiler chicken meat per individual in Indonesia was 11.96 kg annually in 2019, 12.38 kg in 2020, and 12.78 kg in 2021. Moreover, in 2022, production of broiler chicken meat escalated to 4.34 million tons, with a nationwide consumption of 3.62 million tons. These numbers are projected to further rise in 2023, with an estimated production level of 4.23 million tons and a national consumption of 3.76 million tons (Ali et al., 2021).

To satisfy the growing demand for meat, the broiler chicken industry holds a critical role in meeting the nation's supply needs. A key aspect of successful broiler chicken farming is ensuring access to high-quality feed, with an emphasis on adequate protein and energy levels for optimal cell growth. In the formulation of poultry feed, protein supplements constitute a significant portion following energy-rich components (Skinner et al., 1992). The cost of poultry feed in Indonesia remains elevated due to heavy reliance on imported constituents such as corn and soybean meal (Alimon, 2009). The provision of feed stands as a pivotal determinant in the sustainability of broiler chicken farming, often comprising a substantial 60–70% of the total production costs (Sari et al., 2021). To alleviate these expenses, it is imperative to explore alternative raw materials for chicken feed production and lessen dependency on imports (Blatama et al., 2023). One viable ingredient for feed manufacturing is fish waste, presenting an innovative, cost-efficient, and unconventional source of animal protein. Furthermore, this resource is abundantly available for poultry farmers (Ingweye et al., 2008).

The Nile tilapia fish (*Oreochromis niloticus*) holds significant importance in Indonesian fisheries, contributing an annual production of 2–3 million tons (Firdaus et al., 2020). While tilapia meat is widely consumed, the unused parts are often discarded, potentially causing unpleasant odors and ecosystem harm if not managed appropriately. Specifically, the gills, considered waste, are crucial respiratory organs in fish, comprising cartilaginous arches and numerous red filaments rich in capillary blood vessels (Jumiati et al., 2021). Kandyliari et al., (2020) measured the nutritional content of 100 grams of gills from meagre fish (*Argyrosomus regius*) and gilthead sea bream (*Sparus aurata*), revealing their water (74.30% and 62.90%), ash (19.18% and 17.49%), fat (21.31% and 26.69%), carbohydrates (11.05% and 17.32%), and protein (48.46% and 38.50%) composition, respectively. Protein emerged as the primary nutritional component in both fish gills (Kandyliari et al., 2020).

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Prior studies have explored the utilization of fish gills as animal feed. Daud et al. (2020) studied the inclusion of waste from leubiem fish (*Chanthidermis maculatus*), encompassing skin, bones, heads, and gills, as a protein source in duck feed. The outcomes demonstrated significant impacts on feed consumption, body weight gain, final body weight, and egg production. Likewise, Leke et al. (2015) determined that gill flour from cakalang fish (*Katsuwonus pelamis*) could substitute fish meal in broiler chicken feed, enhancing performance indicators. These studies underscore the potential of marine or freshwater fish gills as an alternative feed source for broiler chickens. However, research addressing the effects of Nile tilapia gills on broiler chickens is limited. Therefore, this study aimed to investigate the potential of Nile tilapia gills as a feed additive by examining the morphology of the small intestine, pectoralis muscle, and growth performance of broiler chickens.

## Materials and Methods

### Ethical Approval

This study obtained ethical clearance from the Faculty of Veterinary Medicine at Universitas Gadjah Mada, Indonesia (Approval No: 093/EC-FKH/Eks./2022; Date: October 27, 2022).

### Proximate Analysis

The analysis of composition and nutritional content of tilapia gill powder (TGP) was carried out at the Centre for Feed and Nutrition Studies, Universitas Gadjah Mada, Indonesia. The analysis included assessing the composition and nutritional content of the TGP and measuring the calorie content.

### Feed Preparation

Tilapia gills for this study were sourced from Colombo and Demangan Markets in Yogyakarta, Indonesia. Subsequently, the gills were meticulously sorted, cleaned, and thoroughly washed. The cleaned gills were then placed on an aluminum foil-lined tray and dried in an oven at 65°C for 2–3 days. Following the drying process, the gills were ground into a powder using a blender, resulting in TGP. This TGP was mixed with basal feed (BF) obtained from Sari Rosa Asih Feedmill Inc., as outlined in Table 1. The TGP treatments were administered in specified ratios per kilogram of BF, namely, control (CO; BF), TGP1 (0.25% TGP/kg BF), TGP2 (0.5% TGP/kg BF), TGP3 (1.0% TGP/kg BF), and TGP4 (2% TGP/kg BF). These treatments were then provided ad libitum.

### Acclimation, Feeding, and Maintenance

Acclimation commenced from post-hatch until the chicks were 3 days old. During this period, the chickens were given basal feed, as outlined in Table 1, and provided with palm sugar water upon arrival. The chicks were carefully maintained in containers equipped with light bulbs, water, and feed supplemented with TGP. This arrangement was maintained until the chickens reached 14 days of age under constant 24-hour light conditions, with unrestricted access to feed and water. Body weight measurements were taken at 4, 6, and 14 days of age.

### Chicken Body Morphometric Measurements

At 4, 6, and 14 days, chicken body morphometric measurements were conducted using a midline measurement technique. The measured morphometric variables included body length, wing length, and body height. These measurements aimed to evaluate the influence of adding TGP on chicken growth. Morphometric traits were utilized as indicators to assess the growth performance of chickens (Blatama et al., 2023).

**Table 1.**
*Basal Feed Formulation and Nutrition Content*

| Composition | Percentage |
|---|---|
| Corn | 49.00 |
| Soybean meal | 29.00 |
| Rice bran | 9.80 |
| Full-fat soya | 5.40 |
| Crude palm oil | 3.00 |
| Dicalcium phosphate | 2.37 |
| Premix vitamin[a] | 0.03 |
| Premix mineral[b] | 0.06 |
| D, L-methionine | 0.22 |
| NaCl | 0.32 |
| Calcit | 0.50 |
| L-lysine HCl | 0.10 |
| L-threonine | 0.04 |
| Choline chloride 60% | 0.16 |
| Calculated composition[c] | |
| Metabolizable energy of poultry (kcal/kg) | 2904.02 |
| Crude protein (%) | 20.23 |
| Crude fat (%) | 8.30 |
| Fiber (%) | 3.37 |
| Lysine (%) | 1.22 |
| Methionine (%) | 0.53 |
| Methionine + cysteine (%) | 0.86 |
| Calcium (%) | 1.00 |
| Phosphorus, total (%) | 0.95 |
| Phosphorus, available (%) | 0.50 |
| Sodium (%) | 0.15 |
| Chloride (%) | 0.20 |

*Note*: [a]Premix vitamin provided the following per kilogram of diet (vitamin A: 15000 IU, vitamin $D_3$: 3000 IU, vitamin E: 22.5 mg, vitamin $K_3$: 3 mg, vitamin $B_1$: 3 mg, vitamin $B_2$: 9 mg, vitamin $B_6$: 4.5 mg, vitamin $B_{12}$: 30 µg, biotin: 30 µg, folic acid: 1.5 mg, niacin: 45 mg, pantothenic acid: 1.5 mg, vitamin C: 0 mg, choline: 2090 mg and 1242 mg).
[b]Premix mineral provided the following per kilogram of diet (Cu: 12 mg, Fe: 72 mg, Iodine: 0.9 mg, Mn: 84 mg, Se: 0.3 mg, Zn: 60 mg).
[c]Proximate, amino acids, minerals, and metabolizable energy was obtained from calculated values as in Haritadi et al. (2017).

### Measuring Feed Conversion Ratio

Daily calculations of feed given were made up to 14 days, and feed conversion ratio (FCR) was calculated by dividing feed intake by weight gain. The formula used to calculate the weight gain value was as follows:

$$Weight\ gain = \frac{Body\ weight\ at\ day\ 14 - Body\ weight\ at\ day\ 4}{10\ days}$$

According to Blatama et al. (2023), the FCR value was determined using the formula:

$$FCR = \frac{Total\ feed\ consumed}{Weight\ gain}$$

**Euthanasia, Organ Collection and Measurement**

For each treatment, five chickens aged 14 days were euthanized by neck decapitation. Visceral organs including the small intestine, heart, liver, spleen, bursa, and pectoralis muscle tissue were collected. Muscle tissue from the left side was collected to measure muscle area, while the right muscle tissue was prepared for histological analysis. The organ index was measured by dividing the organ weight by body weight and then multiplying the results by 100, following the formula by (Albab et al., 2022):

$$Organ\ Index(\%) = 100 \times \frac{Organ\ weight(g)}{Body\ weight(g)}$$

**Histological Preparations of the Duodenum, Jejunum, and Pectoralis Major Muscle**

Histological preparations of the duodenum, jejunum, and pectoralis major muscle were conducted using the paraffin method. The duodenum and jejunum were transversely cut to observe villi length, crypt depth, and goblet cells, and were stained with periodic acid Schiff–Alcian Blue. Villi, crypts, and goblet cells were observed and documented using a Leica microscope with 10 × 10 and 40 × 10 magnifications. The right pectoralis major muscle was cut to a 1 × 1 cm size and stained with hematoxylin–eosin (HE) to determine the area of the fasciculus and myofibers. Further observations of the fasciculus and myofibers were made under a Leica microscope at 10 × 10 and 40 × 10 magnifications. Myofiber area, fasciculus area, villi length, crypt depth, number, and goblet cells were measured using ImageJ software.

**Statistical Analysis**

A one-way analysis of variance (ANOVA) followed by Duncan's test was conducted for statistical analysis. The significance level was set at $p \leq .05$. All collected data were analyzed using The Statistical Package for Social Sciences version 25.0 software (IBM Corp.; Armonk, NY, USA) designed for Windows operating systems (Albab et al., 2022).

## Results

**Proximate Test**

The proximate analysis of TGP indicated water content of 3.02%, ash content of 51.52%, lipid content of 26.23%, protein content of 18.42%, carbohydrate content of 0.50%, and a total energy of 3220.51 cal/g (Table 2).

**Small Intestine Morphology**

Table 3 and Figure 1 present the morphology of the small intestine in broiler chickens supplemented with TGP. The TGP3 and TGP4 groups demonstrated significantly increased lengths of duodenal and jejunal villi compared to the CO and TGP1 groups. Furthermore,

**Table 2.**
*Proximate Test Results of Nile Tilapia (*Oreochromis niloticus*) Gill Powder*

| Parameter | Value (%) |
|---|---|
| Water | 3.02 |
| Ash | 51.52 |
| Lipid | 26.23 |
| Protein | 18.42 |
| Carbohydrate | 0.50 |
| Total energy (cal/g) | 3220.51 |

**Table 3.**
*Small Intestine Morphology of Broiler Chickens Supplemented with Nile Tilapia (*Oreochromis niloticus*) Gill Powder for 14 days*

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| **Variables** | **CO** | **TGP1** | **TGP2** | **TGP3** | **TGP4** | ***p*** |
| Duodenum | | | | | | |
| Villi length (μm) | 671.442 ± 74.986[a] | 770.535 ± 49.305[ab] | 781.743 ± 72.864[b] | 899.217 ± 53.020[c] | 1107.676 ± 78.470[d] | .000 |
| Crypt depth (μm) | 83.493 ± 8.796[a] | 94.240 ± 4.053[a] | 95.892 ± 3.248[a] | 111.403 ± 4.994[b] | 160.804 ± 18.133[c] | .000 |
| Villus–crypt ratio | 8.065 ± 0.727[a] | 8.191 ± 0.683[a] | 8.157 ± 0.776[a] | 8.098 ± 0.811[a] | 6.976 ± 1.092[a] | .238 |
| Goblet cells count | 119.806 ± 8.179[a] | 126.972 ± 12.495[a] | 142.361 ± 4.781[a] | 249.056 ± 29.940[b] | 387.111 ± 39.043[c] | .000 |
| Goblet cells area (μm$^2$) | 37.678 ± 6.175[a] | 39.956 ± 1.054[a] | 50.652 ± 4.851[b] | 67.532 ± 5.628[c] | 93.675 ± 9.801[d] | .000 |
| Jejunum | | | | | | |
| Villi length (μm) | 558.285 ± 27.115[a] | 570.198 ± 31.964[a] | 640.020 ± 15.835[b] | 697.660 ± 23.353[c] | 886.934 ± 25.883[d] | .000 |
| Crypt depth (μm) | 64.224 ± 7.532[a] | 66.722 ± 1.883[ab] | 73.401 ± 3.535[b] | 88.740 ± 3.003[c] | 114.342 ± 8.455[d] | .000 |
| Villus–crypt ratio | 8.766 ± 0.898[a] | 8.550 ± 0.502[a] | 8.728 ± 0.239[a] | 7.869 ± 0.378[a] | 7.785 ± 0.561[a] | .060 |
| Goblet cells count | 113.917 ± 10.291[a] | 136.334 ± 3.367[ab] | 164.334 ± 22.824[b] | 254.361 ± 30.417[c] | 400.583 ± 24.629[d] | .000 |
| Goblet cells area (μm$^2$) | 46.987 ± 5.432[a] | 50.562 ± 3.511[a] | 64.065 ± 2.395[b] | 76.979 ± 4.694[c] | 99.964 ± 3.747[d] | .000 |

*Note*: CO (0% TGP); TGP1 (0.25% TGP/kg BF); TGP2 (0.5% TGP/kg BF); TGP3 (1.0% TGP/kg BF); TGP4 (2.0% TGP/kg BF). Values with different superscripted letters in the same row indicate a significant difference ($p \leq .05$).
TGP, tilapia gills powder; BF, basal feed, mean ± SD.

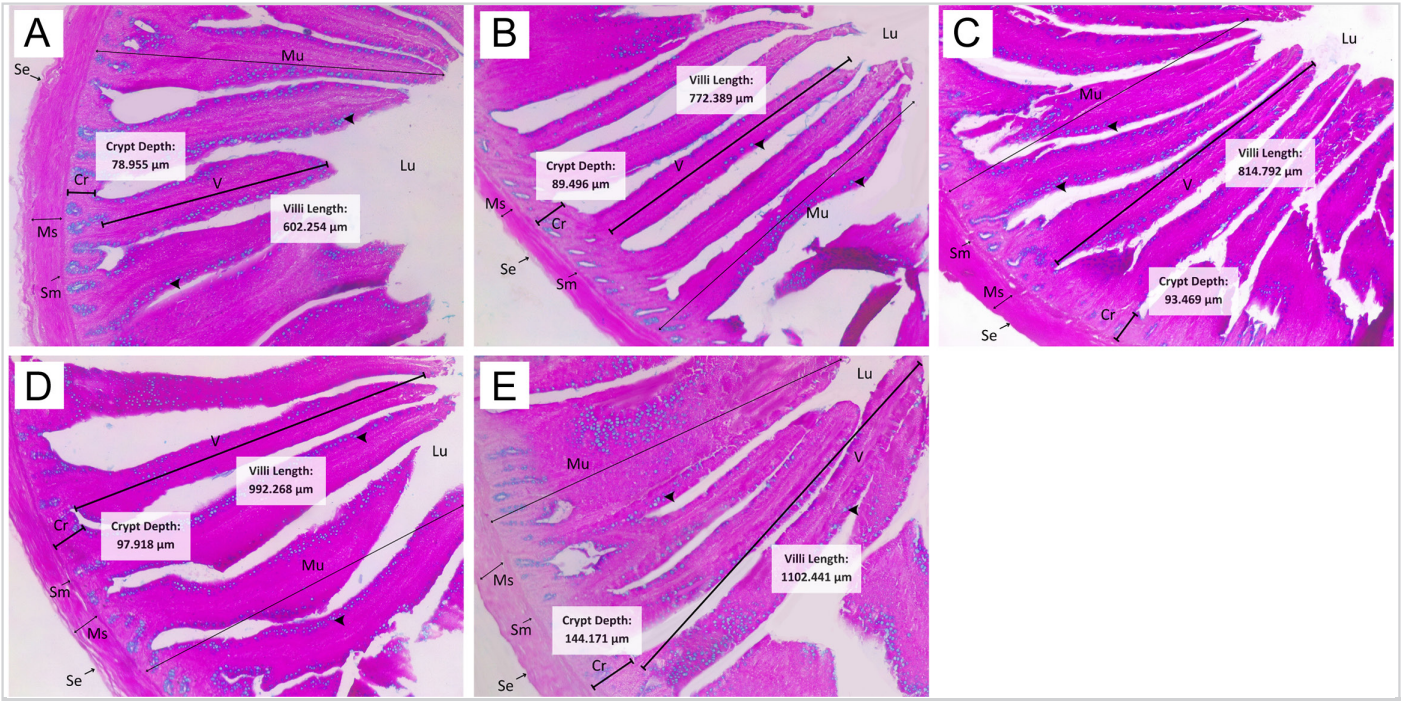


**Figure 1.**
*Transversal Section of Duodenum Morphology of Broiler Chickens Treated with Nile Tilapia (*Oreochromis niloticus*) Gill Powder (TGP) Treatments for 14 Days of Age, Showing Villi (V), Crypt (Cr), Tunica Mucosa (Mu), Tunica Submucosa (Sm), Tunica Muscularis (Ms), Tunica Serosa (Se), Lumen (Lu), and Goblet Cells (Arrowhead). Periodic acid Schiff–Alcian Blue staining, magnification 10×. (A) 0% TGP; (B) TGP1 (0.25% TGP/kg BF); (C) TGP2 (0.5% TGP/kg BF); (D) TGP3 (1.0% TGP/kg BF); (E) TGP4 (2.0% TGP/kg BF).*

a dose-dependent statistical difference was observed between the TGP3 and TGP4 groups as the TGP dosage increased. Moreover, the duodenal crypt depth also demonstrated significantly higher values, particularly in TGP3 and TGP4 in comparison to CO, TGP1, and TGP2. Conversely, the jejunum crypt depth displayed significant values, notably in TGP3 and TGP4 compared to CO and TGP1. Additionally, the TGP3 and TGP4 groups demonstrated significantly higher numbers of goblet cells in the duodenum compared to CO, TGP1, and TGP2 groups. The number of goblet cells in the jejunum for TGP3 and TGP4 also exhibited significant values compared to the CO and TGP1 groups. Furthermore, goblet cells in both the duodenum and jejunum were significantly larger in size for TGP2, TGP3, and TGP4 compared to CO and TGP1.

### Pectoralis Muscle Morphology

The morphology of the pectoralis muscle in broiler chickens treated with TGP is illustrated in Table 4 and Figure 2. The myofiber area in TGP2, TGP3, and TGP4 showed significantly different values compared to the CO and TGP1. Additionally, the total myofiber in TGP1, TGP2, TGP3, and TGP4 displayed significantly different values compared to the CO. The TGP4 group exhibited significant differences in muscle weight index compared to CO, TGP1, and TGP2.

### Viscera Organ Index

Table 5 revealed no differences in the heart and liver indices among all treatment groups and the CO. However, the spleen

**Table 4.**
*Pectoralis Major Muscle Morphology of Broiler Chickens Supplemented with Nile Tilapia (*Oreochromis niloticus*) Gill Powder for 14 days*

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| **Variables** | **CO** | **TGP1** | **TGP2** | **TGP3** | **TGP4** | ***p*** |
| Myofiber Area (µm²) | 307.30 ± 0.51[a] | 312.73 ± 0.36[a] | 337.30 ± 0.71[b] | 361.82 ± 1.42[c] | 365.82 ± 0.43[c] | 0.000 |
| Total myofiber | 135.50 ± 3.41[a] | 136.00 ± 0.82[b] | 153.50 ± 2.89[c] | 167.75 ± 6.60[c] | 168.00 ± 8.52[c] | 0.000 |
| Muscle weight index | 12.22 ± 1.07[a] | 12.50 ± 0.38[a] | 12.40 ± 0.31[a] | 13.87 ± 0.94[ab] | 15.44 ± 2.16[b] | 0.033 |

*Note*: CO (0% TGP); TGP1 (0.25% TGP/kg BF); TGP2 (0.5% TGP/kg BF); TGP3 (1.0% TGP/kg BF); TGP4 (2.0% TGP/kg BF). Values with different superscripted letters in the same row indicate a significant difference ($p \leq .05$).
TGP, tilapia gills powder; BF, basal feed, mean ± SD.

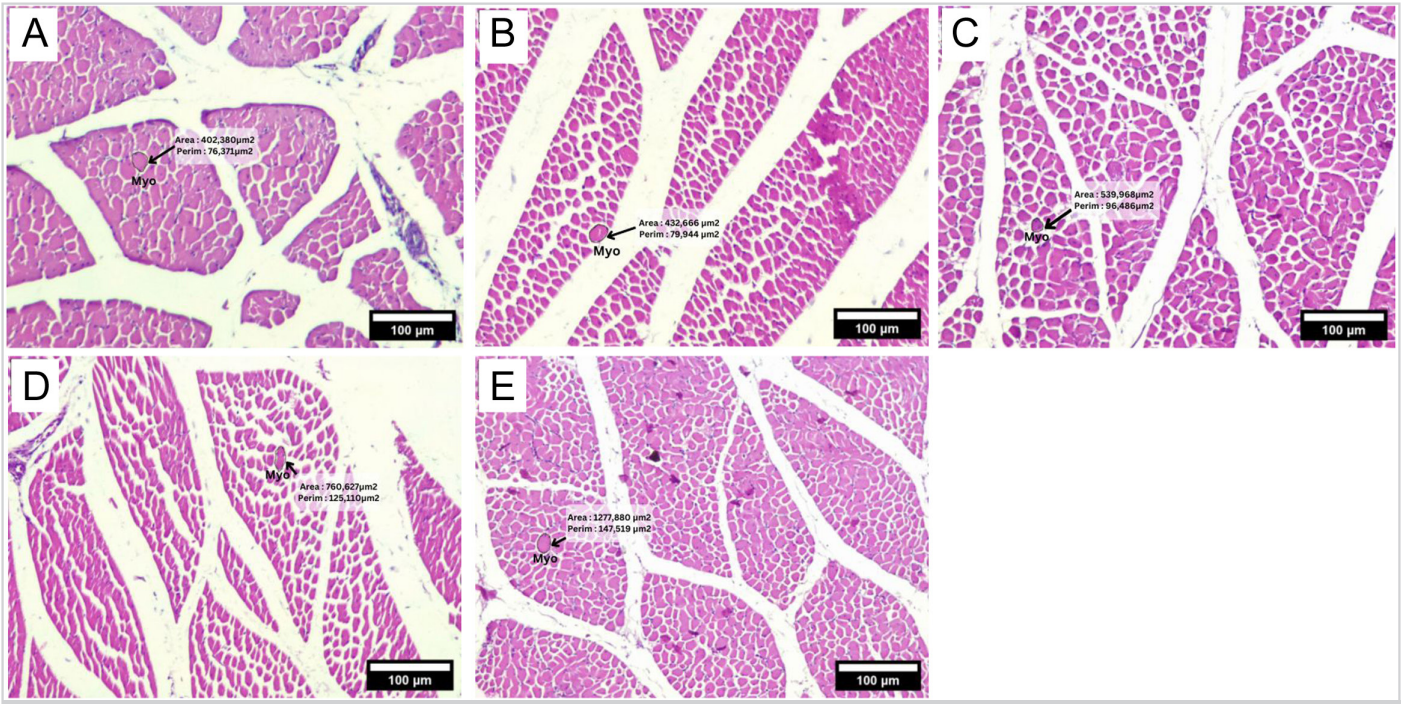


**Figure 2.**
*Transversal Section of Pectoralis Major Muscle Morphology of Broiler Chickens Treated with Nile Tilapia (*Oreochromis niloticus*) Gill Powder (TGP) Treatments for 14 Days of Age, Showing Myofiber (Myo) (Arrows). Hematoxylin–eosin, magnification 10×. (A) 0% TGP; (B) TGP1 (0.25% TGP/kg BF); (C) TGP2 (0.5% TGP/kg BF); (D) TGP3 (1.0% TGP/kg BF); (E) TGP4 (2.0% TGP/kg BF).*

index in TGP3 and TGP4 treatments showed a significant difference compared to CO, TGP1, and TGP2. Furthermore, the bursa index in all treatments exhibited a significant difference compared to the CO.

**Broiler Chicken Growth Performance**

Body weight at 4 days of age showed no differences among all treatment groups and the CO (Table 6). At 6 days of age, only the TGP3 group displayed a significant increase in body weight compared to CO, TGP1, and TGP2. For broiler chickens aged 14 days, all treatment groups demonstrated a significant increase in body weight compared to the CO. Values of FCR in all TGP treatments were lower than those in the CO group according to Table 6.

**Body Morphometric and Performance of Broilers**

Table 7 presents the morphometric results of broiler chickens aged 4, 6, and 14 days. At 14 days of age, the body length of broiler chickens in TGP4 significantly increased compared to CO, TGP1, TGP2, and TGP3, while the wing length of broiler chickens in TGP3 and TGP4 significantly increased compared to CO, TGP1, and TGP2. Conversely, chicken height in TGP3 and TGP4 significantly increased compared to CO, TGP1, and TGP2.

## Discussion

This research spanned a period of 14 days, encompassing a 3-day acclimation phase post-hatching. The initial 2 weeks following

**Table 5.**
*Viscera Organ Index of Broiler Chickens Supplemented with Nile Tilapia (*Oreochromis niloticus*) Gill Powder for 14 Days*

| | Treatments | | | | | |
|---|---|---|---|---|---|---|
| **Variables** | **CO** | **TGP1** | **TGP2** | **TGP3** | **TGP4** | ***p*** |
| Heart index | $0.582 \pm 0.495^{a}$ | $0.588 \pm 0.343^{a}$ | $0.671 \pm 0.146^{a}$ | $0.652 \pm 0.725^{a}$ | $0.755 \pm 0.056^{a}$ | .166 |
| Liver index | $2.836 \pm 0.071^{a}$ | $2.963 \pm 0.750^{a}$ | $2.928 \pm 0.155^{a}$ | $3.022 \pm 0.124^{a}$ | $3.202 \pm 0.040^{a}$ | .203 |
| Spleen index | $0.068 \pm 0.084^{a}$ | $0.081 \pm 0.013^{ab}$ | $0.103 \pm 0.007^{ab}$ | $0.129 \pm 0.008^{cd}$ | $0.156 \pm 0.030^{d}$ | .017 |
| Bursa index | $0.153 \pm 0.021^{a}$ | $0.241 \pm 0.036^{b}$ | $0.296 \pm 0.017^{bc}$ | $0.344 \pm 0.008^{cd}$ | $0.393 \pm 0.004^{d}$ | .000 |

*Note*: CO (0% TGP); TGP1 (0.25% TGP/kg BF); TGP2 (0.5% TGP/kg BF); TGP3 (1.0% TGP/kg BF); TGP4 (2.0% TGP/kg BF). Values with different superscripted letters in the same row indicate a significant difference ($p \leq .05$).
TGP, tilapia gills powder; BF, basal feed, mean ± SD.

**Table 6.**
*Growth Performance of Broiler Chickens Supplemented with Nile Tilapia (*Oreochromis niloticus*) Gill Powder for 14 Days*

| | | Treatments | | | | | |
|---|---|---|---|---|---|---|---|
| **Variables** | **Day** | **CO** | **TGP1** | **TGP2** | **TGP3** | **TGP4** | ***p*** |
| Body weight (g) | 4 | 49.75 ± 0.66[a] | 49.50 ± 1.15[a] | 49.91 ± 1.83[a] | 51.25 ± 1.56[a] | 49.50 ± 0.88[a] | .858 |
| | 6 | 59.67 ± 0.46[a] | 60.25 ± 0.43[a] | 60.17 ± 0.22[a] | 64.08 ± 1.09[b] | 62.00 ± 1.30[ab] | .018 |
| | 14 | 116.17 ± 0.87[a] | 123.00 ± 0.80[b] | 128.58 ± 1.79[c] | 137.00 ± 0.29[d] | 153.33 ± 1.45[e] | .000 |
| Feed intake (g/day) | | 26.05 ± 0.56[a] | 25.48 ± 0.75[a] | 24.93 ± 0.36[a] | 23.56 ± 1.26[a] | 23.97 ± 0.85[a] | .246 |
| Weight gain (g/day) | | 6.64 ± 0.15[a] | 7.35 ± 0.20[b] | 7.87 ± 0.22[b] | 8.58 ± 0.18[c] | 10.39 ± 0.21[d] | .000 |
| Feed conversion ratio | | 3.93 ± 1.13[d] | 3.47 ± 0.15[c] | 3.17 ± 0.04[bc] | 2.76 ± 0.20[b] | 2.31 ± 0.09[a] | .000 |

*Note*: CO (0% TGP); TGP1 (0.25% TGP/kg BF); TGP2 (0.5% TGP/kg BF); TGP3 (1.0% TGP/kg BF); TGP4 (2.0% TGP/kg BF). Weight gain indicates the average daily live weight gain over a period of 4 to 14 days. Values with different superscripted letters in the same row indicate a significant difference ($p \leq .05$).
TGP, tilapia gills powder; BF, basal feed, mean ± SD.

hatching, referred to as the brooding phase, hold significant importance in the growth of broiler chickens, constituting approximately 17% of the overall growth cycle. Therefore, effective management during this phase has the potential to amplify chicken body weight, establishing a fundamental basis for protein deposition during the productive phase (Payte et al., 2022).

A recent study by Blatama et al. (2023) demonstrated that incorporating 5% Goloba kusi (*Hornstedtia scottiana*) seeds with a crude protein content of 12.15% significantly enhanced the morphology of the small intestine, pectoralis muscle, and growth performance of broiler chickens compared to the control group. This suggests that feed rich in protein exerts a positive influence on the growth and development of chickens (Blatama et al., 2023). The proximate analysis of TGP revealed key nutritional components, including a low water content of 3.02% and a high ash content of 51.52%, indicative of its concentrated nature. The TGP demonstrated substantial lipid content at 26.23% and a noteworthy protein content of 18.42%, underscoring its potential as a protein-rich feed additive. Additionally, the carbohydrate content was minimal at 0.50%. The total energy content of 3220.51 cal/g suggests a calorific density suitable for supporting broiler growth. These findings emphasize the nutritional viability of TGP as a potential alternative protein source in broiler feed formulations, aligning with our study objective to explore cost-effective and locally available ingredients for sustainable poultry farming.

The small intestine, encompassing the duodenum, jejunum, and ileum, functions as the primary organ responsible for nutrient absorption and serves as an indicator of growth mechanisms (Awad et al., 2009; Setiawan et al., 2018). The efficiency of nutrient absorption within the small intestine is contingent upon its tissue structure, inclusive of villi length, crypt depth, and the optimal count of goblet cells that produce mucus (Saragih et al., 2019). In this study, the administration of TGP up to the 14th day of age resulted in an enhancement of the morphological structure of the duodenum and jejunum in broiler chickens, particularly in villi length, crypt depth, goblet cell count, and goblet cell area. Villi length contributes to improved nutrient absorption by expanding the surface area (Blatama et al., 2023). This closely correlates with an escalation in the production of brush border enzymes and the availability of nutrient

**Table 7.**
*Body Morphometric Measurements of Broiler Chickens Supplemented with Nile Tilapia (*Oreochromis niloticus*) Gill Powder for 14 Days*

| | | Treatments | | | | | |
|---|---|---|---|---|---|---|---|
| **Day** | **Variables (cm)** | **CO** | **TGP1** | **TGP2** | **TGP3** | **TGP4** | ***p*** |
| | Body length | 6.36 ± 0.20[a] | 6.59 ± 0.44[a] | 6.67 ± 0.36[a] | 6.70 ± 0.35[a] | 6.15 ± 0.41[a] | .367 |
| 4 | Wing length | 5.17 ± 0.15[a] | 5.09 ± 0.18[a] | 4.78 ± 0.29[a] | 5.04 ± 0.29[a] | 4.79 ± 0.15[a] | .646 |
| | Chicken height | 12.45 ± 0.30[a] | 12.48 ± 0.06[a] | 12.22 ± 0.21[a] | 12.53 ± 0.35[a] | 12.39 ± 0.12[a] | .898 |
| | Body length | 7.19 ± 0.08[a] | 6.96 ± 0.12[a] | 7.00 ± 0.07[a] | 7.48 ± 0.28[a] | 6.80 ± 0.51[a] | .080 |
| 6 | Wing length | 6.36 ± 0.13[a] | 5.97 ± 0.11[a] | 6.64 ± 0.07[a] | 6.95 ± 0.13[a] | 6.44 ± 0.43[a] | .085 |
| | Chicken height | 13.68 ± 0.38[a] | 13.62 ± 0.16[a] | 13.05 ± 0.25[a] | 13.46 ± 0.34[a] | 13.42 ± 0.48 [a] | .729 |
| | Body length | 9.44 ± 0.30[a] | 9.27 ± 0.38[a] | 9.70 ± 0.08[ab] | 10.51 ± 0.09[bc] | 11.32 ± 0.34[d] | .001 |
| 14 | Wing length | 7.29 ± 0.31[a] | 7.74 ± 0.20[a] | 7.55 ± 0.04[a] | 8.44 ± 0.12[b] | 9.14 ± 0.20[c] | .000 |
| | Chicken height | 14. 50 ± 0.09[a] | 14.86 ± 0.14[ab] | 15.20 ± 0.12[b] | 16.33 ± 0.15[c] | 16.78 ± 0.20[c] | .000 |

*Note*: CO (0% TGP); TGP1 (0.25% TGP/kg BF); TGP2 (0.5% TGP/kg BF); TGP3 (1.0% TGP/kg BF); TGP4 (2.0% TGP/kg BF). Values with different superscripted letters in the same row indicate a significant difference ($p \leq .05$).
TGP, tilapia gills powder; BF, basal feed, mean ± SD.

transporters, resulting in an expanded capacity for nutrient uptake. The observed increase in duodenal and jejunal villi lengths in the TGP3 and TGP4 groups, with a dose-dependent trend, suggests a positive correlation between TGP dosage and the enhancement of small intestine morphology. This result aligns with the known nutritional composition of fish gills, rich in proteins, which may contribute to improved intestinal structure. The progressive improvement in villi length may be attributed to the increased availability of essential nutrients, supporting the idea that TGP, particularly at higher concentrations, positively influences the histological structure of the small intestine.

Crypts play a vital role in villi proliferation and expedite the healing process of damaged villi tissues stemming from shedding, inflammation, or exposure to toxins caused by pathogens (Saragih et al., 2019). Deeper crypts facilitate swifter tissue renewal to support villi (Yang & Liao, 2019). Min et al. (2017) also noted that deeper crypts lead to heightened growth of enterocytes, a larger villus surface, and increased mucus secretion, thus augmenting nutrient absorption in the small intestine of broiler chickens. In this study, the elevated duodenal and jejunum crypt depth, particularly in TGP3 and TGP4, indicates that higher TGP concentrations may stimulate increased cell turnover in the duodenum and jejunum. This phenomenon could be linked to the nutritional content of TGP, potentially promoting better nutrient absorption and utilization, leading to enhanced crypt development. The significant differences in crypt depth suggest a potential mechanism through which TGP positively influences the cellular architecture of the duodenum and jejunum, contributing to improved performance in broilers. Further investigation into the specific nutritional components affecting crypt depth in different sections of the small intestine would provide valuable insights.

Goblet cells in the small intestine produce mucus to shield the epithelial cells from physical damage by luminal substances and prevent the invasion of pathogenic bacteria. A larger goblet cell area and higher goblet cell count translate to amplified mucus secretion, ultimately improving nutrient absorption and providing heightened protection to the villi surface against pathogenic attack (Blatama et al., 2023). The higher numbers and larger sizes of goblet cells in the duodenum and jejunum of TGP3 and TGP4 groups suggest that TGP, especially at higher concentrations, may stimulate mucin production and secretion. This enhancement in goblet cell characteristics may contribute to improved mucosal defense and nutrient absorption. The larger size of goblet cells further supports the idea that TGP could positively influence the mucosal integrity and protective functions of the small intestine in broilers. Overall, these findings underscore the potential of TGP as a novel feed additive, with TGP3 and TGP4 concentrations showing notable improvements in histological parameters related to the small intestine in broilers.

According to Lembayu et al. (2022), the inclusion of peanut hulls (*Arachis hypogaea*) in broiler feed, with a protein content of 12.97%, yielded noteworthy enhancements in the morphological characteristics of the duodenum and jejunum. Another study by Saragih et al. (2019) also documented that the addition of *Spirogyra jaoensis*, possessing a crude protein content of 18%, to starter feed led to improvements in small intestine morphology within the treatment groups compared to the control group. Meanwhile, Shazali et al. (2019) noted that the incorporation of dietary protein supplemented with amino acids resulted in enhanced growth performance, reduced pathogenic bacteria presence, increased beneficial bacterial counts, heightened small intestine villi height and crypt depth, as well as elevated ileal-digesta volatile fatty acid concentrations in broiler chickens.

In the analysis of visceral organ indices presented in Table 5, the absence of significant differences in the heart and liver indices among all treatment groups and the control suggests a consistent impact across these groups. This uniformity could be indicative of TGP's limited influence on these specific organs at the concentrations tested. It is also possible that these organs are less responsive to the varying concentrations of TGP in the diet during the study period. Conversely, the significant difference observed in the spleen index of TGP3 and TGP4 treatments compared to CO, TGP1, and TGP2 indicates a notable impact on spleen weight. This variation may be attributed to the immunomodulatory properties of TGP, as spleen size often reflects the immune response to dietary components. Furthermore, the significant difference in the bursa index across all TGP treatments compared to the CO suggests that the inclusion of TGP in broiler feed, regardless of concentration, had a discernible impact on the relative weight of the bursa. The bursa plays a crucial role in the development of the avian immune system, and alterations in its weight may reflect changes in immune function. The consistent impact across TGP treatments implies that TGP may have immunomodulatory effects even at lower concentrations, highlighting its potential role in influencing the immune system development in broilers.

Multiple studies have concurred on the significance of protein in facilitating muscle growth in chickens. Liu et al. (2015) indicated that breast muscle growth in Leuyang black bone chickens increased with the supplementation of crude protein for a 42-day period. Protein assumes a critical role in providing essential amino acids, with branched-chain amino acids (BCAA) particularly influential in driving muscle and organ development in chickens. Conversely, BCAA deficiency hinders the differentiation of myoblasts and primary satellite cells (Wang et al., 2022). The pectoralis muscle, situated in the thorax, boasts low levels of fat and an acidification rate, rendering it a suitable indicator for assessing the growth performance of broilers (Puspita et al., 2016). As illustrated in Table 4, protein in TGP can enhance myofiber area, total myofiber count, and the muscle weight index. The observed variations in myofiber area among the TGP2, TGP3, and TGP4 groups suggest that the inclusion of TGP in broiler feed at higher concentrations has a substantial impact on muscle fiber development. This outcome may be attributed to the rich protein content of tilapia gills, supporting the potential of fish gills as a protein source in animal feed and potentially enhancing the overall meat yield in broilers. Furthermore, the differences in total myofiber count in TGP1, TGP2, TGP3, and TGP4 compared to CO highlight the cumulative effect of TGP on muscle fiber proliferation. The significant variations imply that the inclusion of TGP in broiler feed positively influences the hyperplasia of myofibers, reinforcing the notion that TGP serves as an effective supplement for promoting muscle development. This finding is consistent with the broader objective of enhancing the overall growth performance of broilers, as outlined in the study. In relation to the muscle weight index, significant differences were observed in the TGP4 group, as compared to CO, TGP1, and TGP2. The findings underscore the importance of optimizing TGP concentration to achieve optimal outcomes in terms

of muscle structure and growth, providing valuable insights for practical applications in the broiler industry.

He et al. (2021) emphasized the fundamental role of protein in the growth, development, and overall health of poultry. It profoundly influences both feed consumption and the growth performance of broiler chickens. A prior investigation by Srilatha et al. (2017) substantiated that the inclusion of crude protein at concentrations of 21% and 19% engendered improved growth in broiler chickens. In this study, the absence of significant differences in body weight among treatment groups at 4 days of age suggests that the initial incorporation of TGP did not immediately impact early-stage growth. This may be attributed to the relatively short duration of exposure or the early developmental stage of the broiler chicks, where other factors might have a more pronounced influence on growth. The notable increase in body weight observed in the TGP3 group at 6 days of age implies that the concentration of 1% TGP/kg BF had a positive impact on growth during this intermediate stage. The enhanced growth performance could be linked to the nutritional composition of TGP, particularly its protein content, contributing to improved feed utilization and efficient weight gain in broilers. The consistent significant increase in body weight across all TGP-treated groups at 14 days compared to the control suggests that TGP supplementation positively influenced overall broiler growth during the later stages of the study. The lower FCR values in TGP treatments further indicate an improved efficiency of feed utilization, emphasizing the potential economic benefits of incorporating TGP into broiler diets. Regarding body morphometric measurementss at 14 days, the significant increase in body length in TGP4 and the heightened wing length in TGP3 and TGP4 compared to other groups underscore the positive influence of higher TGP concentrations on both skeletal and muscle development. Conversely, the increased chicken height in TGP3 and TGP4 may be attributed to the combined effects of TGP on bone and muscle growth. These morphometric enhancements align with the observed improvements in overall growth performance, emphasizing the potential of TGP as a valuable feed additive for broiler production.

## Conclusion and Recommendations

In conclusion, the Nile TGP shows promise as a feed additive for broiler chickens. Proximate analysis highlights its nutritional value, particularly as a concentrated protein source. Over 14 days, TGP positively influenced small intestine morphology, enhancing villi length, crypt depth, and goblet cell characteristics. Visceral organ indices revealed consistent effects on the heart and liver, while spleen and bursa indices showed significant differences, suggesting immunomodulatory potential. Importantly, TGP improved broiler growth performance, with increased body weight and morphometric enhancements. Future studies should optimize TGP concentration, assess long-term effects, and explore economic considerations for sustainable integration into broiler diets. Overall, TGP emerges as a promising, locally available feed additive with positive implications for broiler production.

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## References

Albab, L. U., Claudya, T. I., Oktafianti, R., Salsabila, N., Putri, R. D., & Saragih, H. T. S. S. G. (2022). Growth performance, morphometric of the small intestine, lymphoid organ, and ovary of laying hens supplemented with Dates (Phoenix dactylifera L.) extract in drinking water. *Veterinary World*, *15*(2), 350–359. [CrossRef]

Ali, P. R., Machfud, Sukardi, Noor, E., & Purnomo, D. (2021). The challenges in Indonesia poultry industry business. *Proceedings of the 11th annual international conference on industrial engineering and operations management Singapore*, *1*, 5433–5443. http://www.ieomsociety.org/singapore2021/papers/921.pdf

Alimon, A. R. (2009). Alternative raw materials for animal feed. *WARTAZOA. Indonesian Bulletin of Animal and Veterinary Sciences*, *19*(3), 117–124. [CrossRef]

Awad, W. A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, *88*(1), 49–56. [CrossRef]

Blatama, D., Salsabila, N., & Saragih, H. T. (2023). Goloba kusi (Hornstedtia scottiana [F. Muell.] K. Schum.) fruit as a feed additive to improve the histological structures and growth performance of broiler. *Veterinary World*, *16*(2), 329–340. [CrossRef]

Choi, J., Kong, B., Bowker, B. C., Zhuang, H., & Kim, W. K. (2023). Nutritional strategies to improve meat quality and composition in the challenging conditions of broiler production: A review. *Animals: An Open Access Journal from MDPI*, *13*(8), 1386. [CrossRef]

Daud, M., Yaman, M. A., & Zulfan, Z. (2020). Potensi penggunaan limbah ikan leubiem (Chanthidermis maculatus) sebagai sumber protein dalam ransum terhadap produktivitas itik petelur. *Livestock and Animal Research*, *18*(3), 217. [CrossRef]

Firdaus, R. M., Mulyono, M., & Farchan, M. (2020). Technical study and financial analysis of red tilapia cultivation (Oreochromis niloticus) in running water system using different feeds at PT ikan Bangun Indonesia (IWAKE) Bogor, West Java. *Journal of Aquaculture Science*, *5*(2), 88–98. [CrossRef]

Haritadi, H., Reksohadiprodjo, S., & Tilma, A. D. (2017). *Tabel komposisi pakan untuk Indonesia* (5th ed). Gadjah Mada University Press.

He, W., Li, P., & Wu, G. (2021). Amino acid nutrition and metabolism in chickens. In G. Wu (Ed.). *Amino Acids in Nutrition and Health* (Vol. *1285*, pp. 109–131). Springer International Publishing. [CrossRef]

Ingweye, J. N., Okon, B. I., Ubua, J. A., & Essien, A. I. (2008). Performance of broiler chickens fed fish and shrimp wastes. *Asian Journal of Animal Sciences*, *2*(2), 58–63. [CrossRef]

Jumiati, J., Rahmaningsih, S., & Sudianto, A. (2021). Mutu kerupuk limbah insang ikan kursi (Nemipterus japonicus) ditinjau dari analisis proksimat. *Jurnal Teknologi Pangan*, *15*(1), 1–11. [CrossRef]

Kandyliari, A., Mallouchos, A., Papandroulakis, N., Golla, J. P., Lam, T. T., Sakellari, A., Karavoltsos, S., Vasiliou, V., & Kapsokefalou, M. (2020). Nutrient composition and fatty acid and protein profiles of selected fish by-products. *Foods*, *9*(2), 190. [CrossRef]

Leke, J. R., Widyastuti, T., Mandey, J. S., Najoan, M., & Laihad, J. (2015). Penggunaan tepung insang cakalang (Katsuwonus pelamis) sebagai pengganti tepung ikan dalam beberapa level pemberian dan metode pengolahan terhadap performans ayam broiler. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia*, *1*, 771–775. [CrossRef]

Lembayu, R. P. L., Armandu, A. C., & Saragih, H. T. (2022). Histological structure of pectoralis thoracicus, small intestine, and growth performance of broiler chicken after supplementation of peanut hulls (Arachis hypogaea L.). *Jurnal Ilmu-Ilmu Peternakan*, *32*(1), 42–51. [CrossRef]

Liu, S., Niu, Z., Min, Y., Wang, Z., Zhang, J., He, Z., Li, H., Sun, T., & Liu, F. (2015). Effects of dietary crude protein on the growth performance, carcass characteristics and serum biochemical indexes of Lueyang black-boned chickens from seven to twelve weeks of age. *Revista Brasileira de Ciência Avícola*, *17*(1), 103–108. [CrossRef]

Min, Y. N., Liu, S. G., Qu, Z. X., Meng, G. H., & Gao, Y. P. (2017). Effects of dietary threonine levels on growth performance, serum biochemical indexes, antioxidant capacities, and gut morphology in broiler chickens. *Poultry Science*, *96*(5), 1290–1297. [CrossRef]

Payte, G. S., Purnamasari, L., Olarve, J. P., & Cruz, J. F. D. (2022). Correlation between body weight day old chick (DOC) and body weight each week from commercial farms in province of Rizal, Philippines. *Jurnal Ilmu Produksi Dan Teknologi Hasil Peternakan*, *10*(3), 126–131. [CrossRef]

Puspita, U. E., Utomo, R. T., Perdamaian, A. B. I., Lesmana, I., Arijuddin, H., Erwanto, Y., Daryono, B. S., & Saragih, H. T. S. G. (2016). Effect of varying levels of protein and energy in pre-stater feeds on pectoralis muscle development of Kampung super chicks (Gallus gallus gallus). *Asian Journal of Animal and Veterinary Advances*, *12*(1), 31–37. [CrossRef]

Saragih, H. T., Muhamad, A. A. K., Alfianto, A., Viniwidihastuti, F., Untari, L. F., Lesmana, I., Widyatmoko, H., & Rohmah, Z. (2019). Effects of Spirogyra jaoensis as a dietary supplement on growth, pectoralis muscle performance, and small intestine morphology of broiler chickens. *Veterinary World*, *12*(8), 1233–1239. [CrossRef]

Sari, Y. W., Widyarani, Sanders, J. P. M., & Heeres, H. J. (2021) The protein challenge: Matching future demand and supply in Indonesia. *Biofuels, Bioproducts and Biorefining*, *15*(2), 341–356. [CrossRef]

Setiawan, H., Jingga, M. E., & Saragih, H. T. (2018). The effect of cashew leaf extract on small intestine morphology and growth performance of Jawa Super chicken. *Veterinary World*, *11*(8), 1047–1054. [CrossRef]

Shazali, N., Loh, T. C., Foo, H. L., & Samsudin, A. A. (2019). Gut microflora and intestinal morphology changes of broiler chickens fed reducing dietary protein supplemented with lysine, methionine, and threonine in tropical environment. *Revista Brasileira de Zootecnia*, *48*, e20170265. [CrossRef]

Skinner, J. T., Waldroup, A. L., & Waldroup, P. W. (1992). Effects of dietary amino acid level and duration of finisher period on performance and carcass content of broilers forty-nine days of age. *Poultry Science*, *71*(7), 1207–1214. [CrossRef]

Srilatha, T., Reddy, V. R., Preetam, V. C., Rao, S. V. R., & Reddy, Y. R. (2017). Effect of different levels of dietary crude protein on the growth performance and carcass characteristics of commercial broilers at different phases of growth. *Indian Journal of Animal Research*, *14*(5), 285–292. [CrossRef]

Wang, M. M., Guo, H. X., Huang, Y. Y., Liu, W. B., Wang, X., Xiao, K., Xiong, W., Hua, H. K., Li, X. F., & Jiang, G. Z. (2022). Dietary leucine supplementation improves muscle fiber growth and development by activating AMPK/sirt1 pathway in blunt snout bream (Megalobrama amblycephala). *Aquaculture Nutrition*, *2022*, 7285851. [CrossRef]

Yang, Z., & Liao, S. F. (2019). Physiological effects of dietary amino acids on gut health and functions of swine. *Frontiers in Veterinary Science*, *6*, 169. [CrossRef]