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Katsuwonus pelamis Liver Powder Supplementation in Broilers: Small Intestinal and Skeletal Muscle Histology in Relation to Growth Performance

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ABSTRACT

Significant skipjack tuna (*Katsuwonus pelamis*) by-products are generated from Indonesia's abundant marine resources, and utilizing nutrient-rich materials such as discarded liver for animal feed offers a sustainable alternative to environmentally harmful waste disposal. This study aimed to evaluate the effects of dietary supplementation with *K. pelamis* liver powder (KPP) on the histological structure of the small intestine and skeletal muscles, and growth performance in broilers. A total of 300 mixed-sex *Cobb* 500 day-old chicks were raised for 17 days and randomly assigned to four dietary treatments containing 0%, 1%, 3%, and 5% KPP. Growth performance was evaluated, and samples of the small intestine, skeletal muscles, and bone were collected on day 18 after an 8-hour fasting period and processed using standard histological procedures. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test. The results demonstrated that KPP supplementation enhanced multiple physiological variables in a concentration-dependent manner. Broilers receiving 3%–5% KPP showed significantly increased villus length, crypt depth, and goblet cell number and area across all small intestinal segments, along with higher skeletal muscle weight, fasciculus area, myofiber size, and myofiber number. Thoracic bone measurements were higher in KPP-fed groups. These improvements were accompanied by greater body weight gain and reduced feed conversion ratio (FCR), indicating enhanced growth performance, while hepatic histology showed no evidence of fatty liver. In conclusion, dietary inclusion of 3%–5% KPP supported broiler intestinal and skeletal muscle histology, improved growth performance, and promoted sustainable utilization of fishery by-products.

Keywords: broiler; growth performance; Katsuwonus pelamis liver powder; skeletal muscle; the small intestine

INTRODUCTION

Indonesia is an archipelago country consisting of more than 17,000 islands and has a very high level of biological and economic potential. This country holds the largest marine diversity in the world and accommodates a broad spectrum of fishery resources (Hutama *et al*., 2016). The most economically important and ecologically unsustainable fish in Indonesia includes skipjack tuna (*Katsuwonus pelamis*), which has been consumed domestically and exported (Sala *et al*., 2023). Increasing production of *K. pelamis* generates substantial by-products, such as visceral organs, particularly liver, which are often discarded without further utilization, and improper disposal of these wastes tends to cause environmental problems. *K. pelamis* liver contains high protein levels, reaching 47.95% as reported by Jeerakul *et al*. (2024). Liver by-product has a significant nutritional value that can be further investigated and possibly used in the development of various value-added products, including animal feed and other protein-based formulations. Efficient use of liver provides a potential economical return from production of value-added products and contributes to reducing environmental impacts associated with fish processing waste.

The demand for protein in poultry feed is still largely dependent on imported ingredients, mainly soybean meal, making the feed industry vulnerable to international price fluctuations and limited availability (Maulidiah, 2020). This reliance increases production costs and constrains the sustainability of the national poultry industry, even as public demand for chicken meat continues to rise in correspondence with the increasing consumption of animal protein (Santoso, 2023). Therefore, broiler farming plays a crucial role in ensuring the adequacy of chicken meat consumption in Indonesia, with per capita broiler meat consumption growing at an annual rate of 7.39% over the period 2012-

2022 (Kementerian Pertanian, 2022). Protein in poultry feed is a critical factor in supporting growth, metabolism, and performance of broilers. Chicken meat and eggs are significant sources of animal protein, providing essential nutrition for humans (Santoso, 2023). A high-protein feed can enhance the microscopic morphology of the small intestine, increase nutrient absorption, pectoralis muscle development for better metabolism, and promote bone growth to improve broiler growth performance (Saragih *et al*., 2024). Preparation of fish meal from local sources, using discarded parts such as liver, might represent a good alternative to reduce imports and ensure sufficient feed quality (Adugna *et al*., 2020). The use of *K. pelamis* liver powder (KPP) in broiler diet is a cost-effective and environment-friendly intervention in raising poultry with local feed resources.

Previous studies examined the use of *K. pelamis* by-products, such as offal or liver in animal feed (Adugna *et al*., 2020; Jeerakul *et al*., 2024), with a primary focus on proximate composition or growth performance and failure to assess tissue-level responses. *K. pelamis* liver contains very high protein content and a favorable amino acid profile (Jeerakul *et al*., 2024), suggesting that the physiological effects may differ from those of other tuna species. All previous studies have not evaluated the effects of KPP on gut and skeletal muscle histomorphology or general growth performance in broilers. Therefore, this study was designed to provide the first histological assessment of KPP as a protein source in broiler feed.

MATERIALS AND METHODS

Ethical Approval and Study Location

All procedures related to animal handling, sampling, husbandry, and experimentation were approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Gadjah Mada (No. 87/EC-FKH/int./2024). The experiment was carried out between October 2024 and April 2025 at the Sawitsari Research Station and the Laboratory of Developmental Structures and Animal Histology, Faculty of Biology, Universitas Gadjah Mada.

Proximate and Calorie Test of KPP

The analysis of KPP for moisture, ash, fat, protein, carbohydrate, and caloric content was conducted at Pusat Studi Pangan dan Gizi, Pusat Antar Universitas (PAU), Universitas Gadjah Mada. Moisture and ash content were measured through the thermogravimetric method, while protein and fat content were analyzed using the Kjeldhal and Soxhlet extraction methods, respectively. Carbohydrate was calculated by difference (AOAC, 2006) and caloric value was determined using a Bomb Calorimeter (AOAC, 2006).

Diet Formulation

The basal feed used was a composition developed by Haritadi *et al*. (2017), as reported in the study of Blatama *et al*. (2023), shown in Table 1. The nutritional composition of the feed for all broiler treatments is presented in Table 2. To ensure the taxonomic identity of the sample, identification of the species K. *pelamis* was carried out at the Animal Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada. Liver obtained from Tamperan fishing port (Pacitan, East Java, Indonesia) was sun-dried, incubated for three 24-hour cycles, and subsequently ground into a fine powder.

Feed Formulation, Husbandry Practices, and Experimental Setup

A total of 300 Cobb 500 day-old-chick (DOC) broilers with an initial body weight of 45.78 ± 2.653 g (mean ± SD) were obtained from PT Japfa Comfeed, Indonesia, and randomly allocated into four dietary treatments. These included a basal diet as control (CON), and basal diets supplemented with 1% (KPP1), 3% (KPP2), or 5% KPP (KPP3). Broilers were housed

Table 1. Basal feed formulation and nutrient content of broiler for 17 days (Haritadi *et al*., 2017)

Composition of feed (%)	Single feed
Corn	49
Soybean meal	29
Rice bran	9.8
Full-fat soya	5.4
Crude palm oil	3
Dicalcium phosphate	2.37
Premix vitamin [a]	0.03
Premix mineral [b]	0.06
D. L-methionine	0.22
NaCl	0.32
Calcite (calcium carbonate)	0.5
L-lysine HCl	0.1
L-threonine	0.04
Choline chloride 60%	0.16
Total	100
Calculated composition [c]	
Metabolizable energy of poultry (kcal/kg)	2.904.02
Crude protein (%)	20.23
Crude fat (%)	8.3
Fiber (%)	3.37
Lysine (%)	1.22
Methionine (%)	0.53
Methionine + cystine (%)	0.86
Calcium (%)	1
Phosphorus. total (%)	0.95
Phosphorus. available (%)	0.5
Sodium (%)	0.15
Chloride (%)	0.23

Notes: Basal feed formulation & nutrient content of broiler for 17 days. [a] Premix vitamin provided the following per kilogram of diet (Vitamin A: 15000 IU, Vitamin D3: 3000 IU, Vitamin E: 22.5 mg, Vitamin K3: 3 mg, Vitamin B1: 3 mg, Vitamin B2: 9 mg, Vitamin B6: 4.5 mg, Vitamin B12: 30 mcg, biotin: 30 mcg, 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 were obtained from calculated values.

Table 2. Calculation of *Katsuwonus pelamis* liver powder (KPP)

Treatments	Basal feed (%)	Liver powder (%)	Filler (%)	Crude protein (%)	Crude protein from liver powder (%)	Total crude protein (%)
CON	95	0	5	20.23	0	19.22
KPP1	95	1	4	51.87	0.52	19.74
KPP2	95	3	2	51.87	1.56	20.78
KPP3	95	5	0	51.87	2.59	21.81

Note: CON = control (basal feed (BF)), KPP1 = 1% KPP supplemented/kg BF, KPP2 = 3% KPP supplemented/kg BF, KPP3 = 5% KPP supplemented/kg BF, KPP = *Katsuwonus pelamis* liver powder. The basal feed was maintained at an identical proportion (95%) across all groups. Fillers function as supplementary ingredients used to bring the feed formulation to 100% without providing additional energy for broilers.

in pens measuring 82.5 × 59 × 45.5 cm under 24-h light conditions, at 35–36 °C (Oke *et al*., 2020), which was maintained in the 17-day experimental period, with ad libitum access to feed and water, in five replications of 15 broilers each. Feeding commenced on day 4 after hatching, and one broiler per replication was euthanized on day 18 through neck decapitation.

Body Weight, Feed Intake, and Feed Conversion Ratio (FCR)

Body weight (BW) was measured on days 0, 4, 7, 10, 14, and 17. Daily feed intake (FI) was recorded, and FCR was determined as total kg feed intake per kg of body weight gain.

Sample Collection and Histological Preparation

Broilers were fasted for 8 hours before being slaughtered. Five broilers per treatment group were randomly selected at the completion of the experimental period, leading to a total of 20. Small intestine (duodenum, jejunum, ileum), skeletal muscle (pectoralis major, iliotibialis, gastrocnemius), and liver were dissected, rinsed with 0.9% NaCl, and processed for histology, and then the thigh weight was measured post-mortem. Intestinal sections were analyzed for villus length, crypt depth, and goblet cell number/area using PAS-AB staining, while skeletal muscle and liver sections were stained with hematoxylin-eosin. Microscopy observations were carried out under 10×10 and 40×10 magnifications, using a *Leica DM750* microscope and capturing nine fields per sample. Histomorphometric measurements were conducted with *ImageJ* software.

Thoracic Bone Length Measurement

Thoracic bones (clavicle, coracoid, sternum) were dissected, boiled in 100 ^{o}C for 10-15 minutes to remove attached tissue, and measured with calipers. Clavicle length was measured from the coracoid junction to the wing tip, sternum from keel to opposite end, and coracoid from clavicle junction to the distal articular surface (Tabassum *et al*., 2015).

Statistical Analysis

Data were assessed for normality and homogeneity of variance. Variables meeting these assumptions were subjected to *one-way* analysis of variance (ANOVA), based on the following statistical model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where Y_{ij} is the observation for treatment i in replicate j; μ is the overall mean; T_i is the fixed effect of dietary treatment (control, 1%, 3%, 5% KPP); and ε_{ij} is the residual error. Significant differences were set at $p \leq 0.05$, and all analyses were performed using SPSS version 26.0 (IBM, USA).

RESULTS

Proximate and Calorie Test

The results of proximate analysis and caloric assessment for *K. pelamis* liver are shown in Table 3. The content of water, ash, fat, protein, carbohydrate, and calories was 21.54%, 3.63%, 11.74%, 51.87%, 11.22%, and 4,939,181 Cal/g, respectively.

Broiler Small Intestine Histology

Dietary supplementation with KPP (1%–5%) significantly enhanced intestinal morphology compared to the control (CON). In KPP1–KPP3, both villus and crypt depth in the duodenum, jejunum, and ileum were higher, with the highest values observed in KPP3 ($p<0.05$). Additionally, goblet cell number and area were significantly greater in KPP-supplemented groups, with KPP3 showing the largest and most numerous goblet cells across all intestinal segments ($p<0.05$) (Table 4, Figure 1).

Broiler Skeletal Muscle Histology

KPP supplementation significantly improved skeletal muscle development compared to the control

Table 3. Proximate and calorie content of *Katsuwonus pelamis* liver powder (KPP)

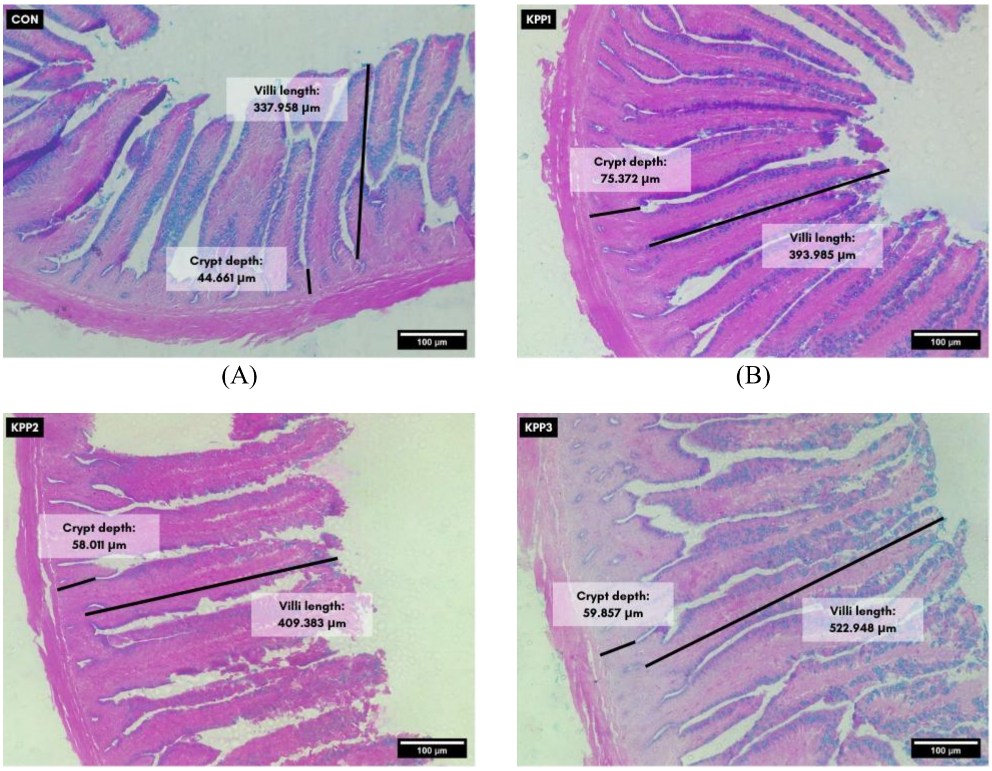
Nutrient contents	Value	Unit
Water	21.54	%
Ash	3.63	%
Fat	11.74	%
Protein	51.87	%
Carbohydrate	11.22	%
Calories	4,939.181	Kal/g

Note: Proximate composition (g/100 g).

Table 4. Intestinal histomorphology of broilers after administering *Katsuwonus pelamis* liver powder at 18 days old

Variables	Treatments				p-value
	CON	KPP1	KPP2	KPP3	
Duodenum					
Villi length (μm)	600.27 ± 6.665^a	631.55 ± 6.443^b	664.83 ± 6.032^c	879.09 ± 8.305^d	≤ 0.001
Crypt depth (μm)	89.33 ± 2.012^a	100.59 ± 2.277^b	114.31 ± 2.478^c	151.12 ± 2.542^d	≤ 0.001
Goblet cell area (μm^2)	22.33 ± 0.507^a	24.66 ± 0.422^b	27.73 ± 0.585^c	36.39 ± 0.639^d	≤ 0.001
Number of goblet cells	103.67 ± 2.095^a	114.47 ± 2.496^b	126.18 ± 2.632^c	140.91 ± 3.302^d	≤ 0.001
Jejunum					
Villi length (μm)	511.95 ± 5.099^a	579.51 ± 6.531^b	598.99 ± 5.783^c	655.25 ± 4.381^d	≤ 0.001
Crypt depth (μm)	86.83 ± 2.389^a	97.89 ± 2.046^b	109.86 ± 2.249^c	123.95 ± 2.192^d	≤ 0.001
Goblet cell area (μm^2)	20.42 ± 0.466^a	23.43 ± 0.503^b	27.96 ± 1.516^c	32.94 ± 0.624^d	≤ 0.001
Number of goblet cells	100.78 ± 4.257^a	110.31 ± 3.906^a	129.67 ± 4.812^b	137.64 ± 5.305^b	≤ 0.001
Ileum					
Villi length (μm)	449.23 ± 7.125^a	470.40 ± 6.660^b	530.26 ± 5.536^c	569.54 ± 5.725^d	≤ 0.001
Crypt depth (μm)	68.50 ± 2.053^a	75.06 ± 1.999^b	88.88 ± 1.702^c	96.46 ± 2.326^d	≤ 0.001
Goblet cell area (μm^2)	19.98 ± 0.407^a	22.38 ± 0.548^b	25.54 ± 0.491^c	29.58 ± 0.524^d	≤ 0.001
Number of goblet cells	96.44 ± 2.054^a	102.09 ± 2.484^a	110.09 ± 2.248^b	114.67 ± 1.942^b	≤ 0.001

Note: CON=Control, BF=Basal feed, KPP: *Katsuwonus pelamis* liver powder, KPP1: 1% KPP supplemented/kg BF, KPP2: 3% KPP supplemented/kg BF, and KPP3: 5% KPP supplemented/kg BF. Data are shown as mean ± standard error of the mean (SEM). Different superscript letters (a–d) within the same row denote statistically significant differences among values (p<0.05).



(A) (B)

Figure 1. Transverse sections of jejunal villi and crypts in 18-day-old broilers fed diets supplemented with *Katsuwonus pelamis* liver powder (KPP), observed under 10 × 10 magnification. A: Control (CON), basal feed (BF) without KPP; B: KPP1, 1% KPP supplemented/kg BF; C: KPP2, 3% KPP supplemented/kg BF; D: KPP3, 5% KPP supplemented/kg BF. Broilers supplemented with KPP showed higher villus length and deeper crypts than those in the CON group.

(CON). Pectoralis weight, muscle area, fasciculus area, myofiber area, and myofiber number were higher in KPP-treated groups, with the greatest values observed in KPP3 (p<0.05) (Tables 5–8, Figure 2). All treatment groups showed significant increases in pectoralis weight, fasciculus area, and myofiber metrics relative to CON (p<0.05), suggesting enhanced muscle growth and structural development.

Liver Fat Content Measurement

Histological evaluation of 18-day-old broilers showed no significant differences in hepatic fat deposition between the control (CON) and KPP-supplemented groups (KPP1–KPP3) (p<0.05) (Table 9, Figure 3). This observation suggested that dietary KPP supplementation did not induce fatty liver.

Broiler Thoracic Bones Length

Dietary supplementation with KPP (1%–5%) significantly increased the clavicle, sternum, and coracoid length compared to the control (CON) ($p<0.05$) (Table 10).

Broiler Growth Performance

KPP significantly enhanced body weight gain between days 7 and 17 ($p<0.05$) and increased thigh weight compared to the control (CON) ($p<0.05$) (Table 11). Consequently, FCR was significantly lower in KPP-treated groups ($p<0.05$), reflecting more efficient growth.

Table 5. Histological structure of pectoralis major muscle in broilers after administration of *Katsuwonus pelamis* liver powder at 18 days old

Variables	Treatments				p-value
	CON	KPP1	KPP2	KPP3	
Muscle weight (g)	20.00 ± 0.707^{a}	22.80 ± 0.374^{b}	27.20 ± 0.374^{c}	32.80 ± 0.374^{d}	≤ 0.001
Muscle area (cm)	20.84 ± 0.120^{a}	24.10 ± 0.070^{b}	27.76 ± 0.092^{c}	33.62 ± 0.346^{d}	≤ 0.001
Fascicle area (μm^2)	80,925.26 ± 5,237.594^{a}	85,387.47 ± 7,861.205^{a}	112,956.3 ± 11,697.848^{b}	114,843.5 ± 8,710.003^{b}	0.009
Myofiber area (μm^2)	300.10 ± 48.834^{a}	391.20 ± 20.432^{ab}	469.60 ± 40.762^{bc}	642.70 ± 68.521^{c}	0.001
Total myofiber per fascicle	174.60 ± 8.628^{a}	210.30 ± 12.044^{bc}	230.60 ± 9.427^{cd}	251.40 ± 11.169^{d}	≤ 0.001

Note: CON=Control, BF=Basal feed, KPP: *Katsuwonus pelamis* liver powder, KPP1: 1% KPP supplemented/kg BF, KPP2: 3% KPP supplemented/kg BF, KPP3: 5% KPP supplemented/kg BF. Data are shown as mean ± standard error of the mean (SEM). Different superscript letters ($^{a–d}$) within the same row denote statistically significant differences among values ($p<0.05$).

Table 6. Histological structure of pectoralis minor muscle in broilers after administration of *Katsuwonus pelamis* liver powder at 18 days old

Variables	Treatments				p-value
	CON	KPP1	KPP2	KPP3	
Fascicle area (μm^2)	41,979.304 ± 1,167.495^{a}	75,927.635 ± 3,400.711^{b}	109,484.186 ± 3,531.501^{c}	236,108.23 ± 1,156.079^{d}	≤ 0.001
Myofiber area (μm^2)	151.87 ± 7.119^{a}	194.47 ± 16.291^{b}	214.40 ± 9.802^{b}	254.80 ± 14.609^{c}	≤ 0.001
Total myofiber per fascicle	136.01 ± 38.333^{a}	196.54 ± 40.667^{b}	355.26 ± 28.494^{c}	507.34 ± 59.781^{d}	≤ 0.001

Note: CON=Control, BF=Basal feed, KPP: *Katsuwonus pelamis* liver powder, KPP1: 1% KPP supplemented/kg BF, KPP2: 3% KPP supplemented/kg BF, KPP3: 5% KPP supplemented/kg BF. Data are shown as mean ± standard error of the mean (SEM). Different superscript letters ($^{a–d}$) within the same row denote statistically significant differences among values ($p<0.05$).

Table 7. Iliotibialis muscle histomorphology in broilers after administration of *Katsuwonus pelamis* liver powder at 18 days old

Variables	Treatments				p-value
	CON	KPP1	KPP2	KPP3	
Fascicle area (μm^2)	15,139.863 ± 568.657^{a}	17,791.265 ± 390.203^{b}	26,587.647 ± 498.203^{c}	46,060.185 ± 1,624.819^{d}	≤ 0.001
Myofiber area (μm^2)	144.87 ± 6.995^{a}	145.20 ± 0.580^{a}	208.13 ± 7.311^{b}	276.27 ± 15.979^{c}	≤ 0.001
Total myofiber per fascicle	70.48 ± 1.315^{a}	116.69 ± 7.157^{b}	146.10 ± 5.231^{c}	177.42 ± 9.802^{d}	≤ 0.001

Note: CON=Control, BF=Basal feed, KPP: *Katsuwonus pelamis* liver powder, KPP1: 1% KPP supplemented/kg BF, KPP2: 3% KPP supplemented/kg BF, KPP3: 5% KPP supplemented/kg BF. Data are shown as mean ± standard error of the mean (SEM). Different superscript letters ($^{a–d}$) within the same row denote statistically significant differences among values ($p<0.05$).

Table 8. Histological structure of the gastrocnemius muscle in broilers after administration of *Katsuwonus pelamis* liver powder at 18 days old

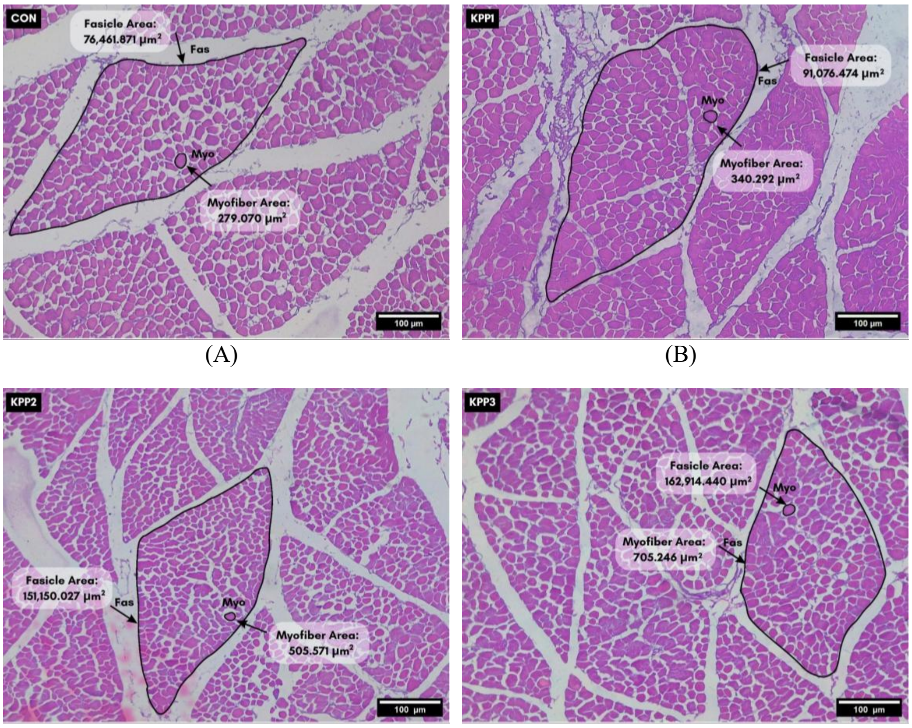
Variables	Treatments				p-value
	CON	KPP1	KPP2	KPP3	
Fascicle area (μm^2)	16,728.596 ± 637.216^{a}	36,638.610 ± 1,003.201^{b}	49,574.300 ± 1,410.014^{c}	117,234.88 ± 6,551.474^{d}	≤ 0.001
Myofiber area (μm^2)	79.29 ± 3.127^{a}	120.92 ± 4.193^{b}	169.10 ± 8.939^{c}	319.45 ± 14.918^{d}	≤ 0.001
Total myofiber per fascicle	79.60 ± 5.817^{a}	95.67 ± 5.155^{a}	203.47 ± 15.865^{b}	230.47 ± 7.534^{b}	≤ 0.001

Note: CON=Control, BF=Basal feed, KPP: *Katsuwonus pelamis* liver powder, KPP1: 1% KPP supplemented/kg BF, KPP2: 3% KPP supplemented/kg BF, KPP3: 5% KPP supplemented/kg BF. Data are shown as mean ± standard error of the mean (SEM). Different superscript letters ($^{a–d}$) within the same row denote statistically significant differences among values ($p<0.05$).

Table 9. Percentage of fat content in broiler liver on day 18 after administration of *Katsuwonus pelamis* liver powder

Variables	Treatments				p-value
	CON	KPP1	KPP2	KPP3	
Percentage of fat content in the liver (%)	26.22 ± 0.439^{a}	26.47 ± 0.620^{b}	25.82 ± 0.714^{ab}	24.61 ± 0.564^{a}	0.215

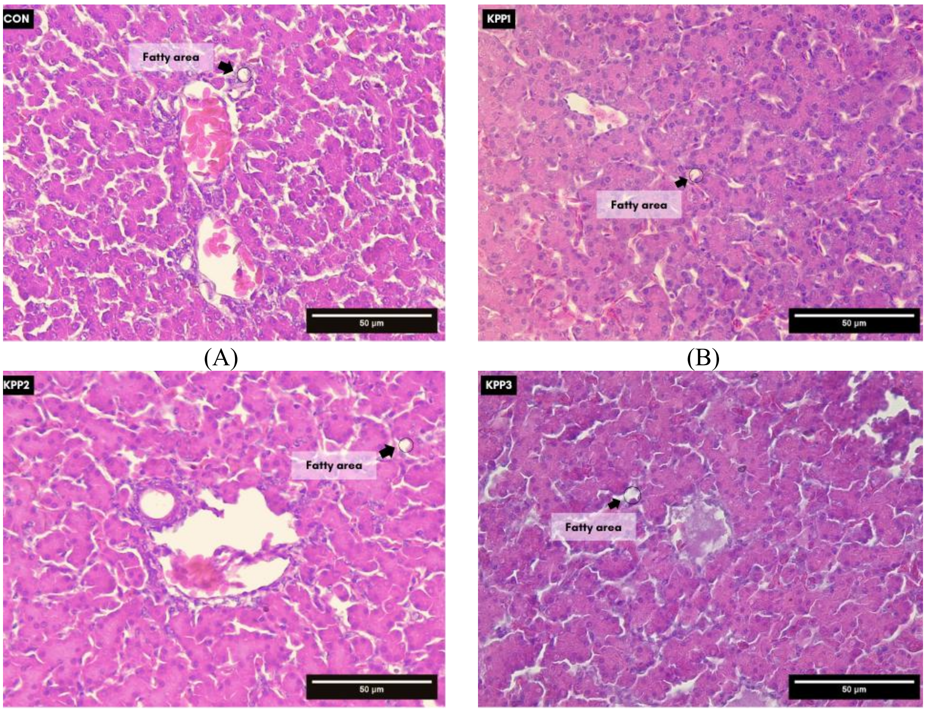
Note: Control (CON): Basal feed (BF) (without supplementation of *Katsuwonus pelamis* liver powder (KPP)); KPP1: 1% KPP supplemented/kg BF; KPP2: 3% KPP supplemented/kg BF; KPP3: 5% KPP supplemented/kg BF. Data are shown as mean ± standard error of the mean (SEM). Different superscript letters ($^{a–b}$) within the same row denote statistically significant differences among values ($p<0.05$).



(A) (B)

(C) (D)

Figure 2. Transverse histological sections of the pectoralis major muscle showing fascicles (Fas) and myofibers (Myo) in 18-day-old broilers treated with *Katsuwonus pelamis* liver powder (KPP) using 10 × 10 magnification. A. Control (CON): Basal feed (BF) (without KPP); B. KPP1: 1% KPP supplemented/kg BF; C. KPP2: 3% KPP supplemented/kg BF; D. KPP3: 5% KPP supplemented/kg BF. This figure shows that the KPP group had a larger size of fascicle compared to the CON groups.



(A) (B)

(C) (D)

Figure 3. Fatty livers in 18-day-old broilers after supplementation of *Katsuwonus pelamis* liver powder liver powder (KPP) under 40x10 microscope magnification. Hematoxylin-Eosin (HE) staining. A. Control (CON): Basal feed (BF) (without KPP); B. KPP1: 1% KPP supplemented/kg BF; C. KPP2: 3% KPP supplemented/kg BF; D. KPP3: 5% KPP supplemented/kg BF. Administration of KPP1, KPP2, and KPP3 to 18-day-old broilers did not cause fatty livers.

Table 10. Broiler thoracic bones length on day 18 after administration of *Katsuwonus pelamis* liver powder

Variables	Treatments				p-value
	CON	KPP1	KPP2	KPP3	
Os clavicle length (mm)	28.54 ± 0.913^{a}	30.91 ± 1.036^{ab}	32.68 ± 0.502^{b}	33.52 ± 0.969^{b}	0.005
Os coracoid length (mm)	26.24 ± 0.340^{a}	27.14 ± 0.499^{ab}	27.90 ± 0.313^{bc}	29.13 ± 0.541^{c}	0.002
Os sternum length (mm)	18.42 ± 0.493^{a}	20.88 ± 0.292^{b}	21.76 ± 0.548^{b}	25.04 ± 1.982^{c}	0.002

Note: CON=Control, BF=Basal feed, KPP: *Katsuwonus pelamis* liver powder, KPP1: 1% KPP supplemented/kg BF, KPP2: 3% KPP supplemented/kg BF, KPP3: 5% KPP supplemented/kg BF. Data are shown as mean ± standard error of the mean (SEM). Different superscript letters ($^{a–c}$) within the same row denote statistically significant differences among values (p<0.05).

Table 11. Broiler growth performance after administering *Katsuwonus pelamis* liver powder at 17 days old

Variables	Day	Treatments				p-value
		CON	KPP1	KPP2	KPP3	
Body weight (g)	0	46.12 ± 0.587	45.56 ± 0.539	45.32 ± 0.442	44.32 ± 0.574	0.126
	4	55.55 ± 2.160	56.10 ± 3.059	56.65 ± 3.216	57.85 ± 2.434	0.062
	7	74.64 ± 0.955^{a}	81.96 ± 1.202^{b}	93.12 ± 2.327^{c}	96.200 ± 1.713^{cd}	≤ 0.001
	10	126.80 ± 3.611^{a}	145.84 ± 2.850^{b}	155.64 ± 3.102^{c}	167.92 ± 3.223^{d}	≤ 0.001
	14	192.48 ± 3.799^{a}	237.28 ± 4.028^{b}	259.28 ± 5.132^{c}	288.12 ± 5.496^{d}	≤ 0.001
	17	322.40 ± 4.943^{a}	362.36 ± 6.357^{b}	399.04 ± 9.545^{c}	438.04 ± 8.008^{d}	≤ 0.001
Thigh weight (g)		31.80 ± 0.970^{a}	37.200 ± 1.625^{b}	42.00 ± 2.121^{c}	48.40 ± 1.470^{d}	≤ 0.001
Weight gain (g/day)		20.43 ± 0.607^{a}	21.66 ± 0.672^{a}	24.06 ± 0.827^{b}	26.23 ± 0.775^{b}	≤ 0.001
Feed intake (g/day)		43.70 ± 2.695	42.99 ± 1.223	41.49 ± 1.801	40.19 ± 1.792	0.266
Feed conversion ratio		2.15 ± 0.170^{a}	1.99 ± 0.045^{ab}	1.73 ± 0.057^{bc}	1.54 ± 0.075^{c}	0.002

Note: CON=Control, BF=Basal feed, KPP: *Katsuwonus pelamis* liver powder, KPP1: 1% KPP supplemented/kg BF, KPP2: 3% KPP supplemented/kg BF, KPP3: 5% KPP supplemented/kg BF. Data are shown as mean ± standard error of the mean (SEM). Different superscript letters ($^{a–d}$) within the same row denote statistically significant differences among values (p<0.05).

DISCUSSION

K. pelamis is abundant in Indonesia, and liver has a high potential as a protein source for broiler feed. Moreover, the proximate protein content found in this study was 51.87%, exceeding the value reported (47.95%) by Jeerakul *et al*. (2024). Liver is rich in essential amino acids (EAAs) such as leucine, valine, and lysine, which are crucial for muscle metabolism and energy provision (Sarojnalini & Hei, 2019). Adequate protein and EAA intake during the pre-starter stage is essential for optimizing broiler growth performance, as nutrient availability in early growth stages determines subsequent muscle and skeletal development (Nogueira *et al*., 2019; Qaid *et al*., 2021). The 17-day experimental period was intentionally selected to focus on the pre-starter (0–7 days), starter (8–14 days), and early grower (14–17 days), which represent the most dynamic stages of broiler development. During this period, rapid intestinal maturation, muscle fiber formation, and high metabolic turnover occur, making broilers highly responsive to nutritional interventions, such as dietary amino acid levels (Qaid *et al*., 2021; Sampath *et al*., 2023; Zhang *et al*., 2021; An & Kong, 2024).

This study found that KPP supplementation positively improved the histological structure of the small intestine, enhancing nutrient absorption and general broiler growth. The significant rise in villus length, crypt depth, and goblet cell number/area suggested greater epithelial proliferation and mucus secretion (Birchenough *et al*., 2015; Kaunitz & Akiba, 2019). Longer villi expand the absorptive surface area, while deeper crypts signify active cell turnover, and a higher villi-to-crypt ratio is associated with optimal nutrient absorption (Blatama *et al*., 2023; Bondar *et al*., 2023). Goblet cells produce mucus, which helps to maintain microbial homeostasis and protect the villous mucosa against pathogenic attack as well as serve the purpose of nutrient transportation (Duangnumsawang *et al*., 2021). These are consistent with previous studies showing that dietary protein sources, such as *Spirogyra jaoensis* (18% protein) and *Hornstedtia scottiana* fruit (12.15% protein), significantly enhance small intestinal morphology, including villi length, crypt depth, villus-to-crypt ratio, and goblet cell morphology in the duodenum, jejunum, and ileum (Saragih *et al*., 2019; Blatama *et al*., 2023). Amino acid content of the proteins may facilitate epithelial cell proliferation, villus-crypt morphogenesis, and signal transduction, thereby improving nutrient absorption efficiency in broilers (Ensari & Marsh, 2018; Wang *et al*., 2020; Tran *et al*., 2023).

Muscle growth in broilers serves as a critical indicator of feed supplementation efficacy, and this study showed that protein-rich liver powder from *K. pelamis* affected skeletal muscle histomorphology by positively increasing pectoralis weight, fasciculus area, as well as myofiber number and size. Recent studies confirm that higher dietary protein and amino acid supplementation enhances muscle fiber development, fasciculus area, and general muscle mass in broilers (Lee *et al*., 2023; Zhou *et al*., 2024). Protein supplementation promotes muscle hypertrophy by enhancing protein synthesis, activating satellite cells, and stimulating the release of insulin-like growth factor-1 (IGF-1), which regulates myogenesis through Akt/mTOR pathway

(Perdamaian *et al*., 2017; Mohammadigheisar *et al*., 2020; Saragih *et al*., 2024). These results are consistent with previous studies showing that increasing protein content in feed, such as macroalgae *Chaetomorpha linum* with 15.48% protein and Goloba kusi *Hornstedtia scottiana* 5% with a protein content of 12.15%, accelerates muscle growth by expanding fasciculi and increasing myofiber size and number (Saragih *et al*., 2019; Saragih *et al*., 2024).

The evaluated broilers were provided with KPP-supplemented feed, which, according to proximate analysis, had normal fat content in the physiological range. At the histological level, fat was absent in the broiler liver after supplementation with KPP and the normal liver had no fatty storage vacuole. This result is consistent with a study in which hepatic steatosis was not experienced by broilers fed a diet containing fish by-products, specifically *K. pelamis* viscera, including liver. Recent studies further support that fish-derived protein and lipid sources can be safely incorporated into broiler diets without pathological lipid deposition in the liver, provided that general dietary fat levels remain balanced (Alshamy *et al*., 2019; Luo *et al*., 2023). Contemporary evidence shows that the use of fish oil or other marine by-products as dietary lipid sources does not predispose broilers to fatty liver, compared to diets rich in saturated fat or excessive n-6 polyunsaturated fatty acids (Wang *et al*., 2024). These results confirm that KPP is a protein-rich ingredient with a physiologically safe fat profile that does not overload hepatic metabolism or induce fatty liver in broilers.

Thoracic bone measurement is a reliable indicator of broiler growth performance (Abdelmoniem & Mona, 2023). Adequate dietary protein, particularly from KPP, which also provides sufficient carbohydrate and fat, enhances collagen synthesis and bone mineralization, thereby strengthening the skeletal framework that supports body growth (Boontiam *et al*., 2017). The hormonal control by growth hormone (GH) and insulin-like growth factors (IGFs) stimulates osteoblast proliferation, increasing osteogenesis as it directly promotes skeletal growth (Pagala *et al*., 2018). Carbohydrate provides the metabolic energy needed for rapid bone formation, ensuring sustained growth during high-demand stages (Panda *et al*., 2015), while fat supplies essential fatty acids that maintain cell integrity and signaling processes critical for skeletal development (Cetıngul *et al*., 2022). The synergy of nutritional and hormonal factors drives effective osteogenesis, leading to optimal bone strength and general growth in broilers (Bai *et al*., 2021).

Growth performance in broilers was improved by KPP supplementation, which enhanced the parameters of water, ash, fat, protein, carbohydrate, and caloric contents in feed. Among these nutrients, protein served as the primary factor directly supporting muscle development and tissue synthesis. Carbohydrates acted as the substrates of energy to support the metabolic need for growth, and fat supplied essential fatty acids, playing a crucial role in cell membrane integrity, tissue composition, and metabolism. The presence of ash supplied minerals that contributed to bone and structural development, water maintained physiological balance, and total caloric content ensured sufficient energy availability. These integrated nutritional contributions were reflected in significant increases in thigh weight, body weight, and total weight gain (Son *et al*., 2024). The results are consistent with previous studies showing that diets containing 19 to 21% crude protein can improve broiler growth (Srilatha *et al*., 2018). Additionally, tilapia (*Oreochromis niloticus*) gill powder containing 18.42% protein was proven to increase broiler body weight (Saragih *et al*., 2024). Protein- and carbohydrate-rich feed, such as *Spirogyra jaoensis* with 16% protein, has been found to increase body weight and general growth performance (Saragih *et al*., 2019). FCR decline observed in this study represents better feed efficiency due to improvement in nutrient absorption at the small intestinal level and the use of a high-protein diet supplemented with essential amino acids (Boontiam *et al*., 2017; Muñoz *et al*., 2025). Efficient amino acid utilization is crucial for promoting muscle accretion and supporting skeletal development, thereby enhancing general growth performance in broilers.

CONCLUSION

In conclusion, dietary supplementation with KPP improved intestinal and muscle histomorphology, promoted bone growth, enhanced broiler performance, and did not induce fatty liver, thereby suggesting it as an ideal protein-rich feed supplement.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used *Grammarly* to check grammar and *Turnitin* to check for plagiarism. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

REFERENCES

Abdelmoniem, A. M., M. Y., M., S. A., E.-S., & Mona A. M., H. (2023). Prediction models for carcass traits and bone geometrics using live body weight in broiler chickens related to strain, sex and its interactions. Egyptian Poultry Science Journal, 43(4), 595–615. https://doi.org/10.21608/epsj.2023.332122

Adugna, A. A., Habib, K. M., Odier, O., & Gebresilase, T. (2020). Effects of fish by-product meal inclusion in broiler feeds on growth performance. Journal of Experimental Agriculture International, 42(7), 76–81. https://doi.org/10.9734/jeai/2020/v42i730556

Alshamy, Z., Richardson, K. C., Harash, G., Hünigen, H., Röhe, I., Hafez, H. M., Plendl, J., & Masri, S. A.. (2019). Structure and age-dependent growth of the chicken liver together with liver fat quantification: A comparison between a dualpurpose and a broiler chicken line. PLoS ONE, 14(12), 1–18. https://doi.org/10.1371/journal.pone.0226903

An, S. H., & Kong, C. (2024). Growth performance of male broiler chickens in different growth phases in response to amino acid concentrations in the pre-starter diet. Journal of Animal Science and Technology. https://doi.org/10.5187/jast.2024.e55

AOAC. (2006). Official methods of analysis of AOAC International (16th ed). Association of Official Analytical Chemists.

Bai, L., Ding, X., Sun, C., Zhou, J., & Lu, J. (2021). Effects of gallus epidermal growth factor gEGF from chicken embryos on growth performance, serum biochemical indices, immune function and intestinal morphology of broilers. Italian Journal of Animal Science, 20(1), 1645–1655. https://doi.org/10.1080/1828051X.2021.1976684

Birchenough, G. M. H., Johansson, M. E. V., Gustafsson, J. K., & Hansson, G. C. (2015). New developments in goblet cell mucus secretion and function. Mucosal Immunology, 8(4), 712–719. https://doi.org/10.1038/mi.2015.32

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. https://doi.org/10.14202/vetworld.2023.329-340

Bondar, A., Slencu, B. G., Popovici, I., & Solcan, C. (2023). Effect of turmeric (*Curcuma longa*) on duodenal structure in broiler chickens. Brazilian Journal of Poultry Science, 25(3), 1–8. https://doi.org/10.1590/1806-9061-2022-1738

Boontiam, W., Jung, B., & Kim, Y. Y. (2017). Effects of lysophospholipid supplementation to lower nutrient diets on growth performance, intestinal morphology, and blood metabolites in broiler chickens. Poultry Science, 96(3), 593–601. https://doi.org/10.3382/ps/pew269

Cetıngul, I. S., Inal, F., Gultepe, E. E., Uyarlar, C., & Bayram, I. (2022). The effects of different dietary oil sources on broiler chicken bone mineralization. Biological Trace Element Research, 200(5), 2321–2328. https://doi.org/10.1007/s12011-021-02833-9

Duangnumsawang, Y., Zentek, J., & Goodarzi Boroojeni, F. (2021). Development and functional properties of intestinal mucus layer in poultry. Frontiers in Immunology, 12(October), 1–18. https://doi.org/10.3389/fimmu.2021.745849

Ensari, A., & Marsh, M. N. (2018). Exploring the villus. Gastroenterology and Hepatology from Bed to Bench, 11(3), 181–190.

Haritadi, H., Reksohadiprodjo, S., & Tilma, A. D. (2017). Feed composition table for Indonesia (5th Ed.). Gadjah Mada University Press.

Hutama, A. A., Hadiaty, R. K., & Hubert, N. (2016). Biogeography of Indonesian Freshwater Fishes: Current Progress. Treubia, 43, 17–30. https://doi.org/10.14203/treubia.v43i0.2969

Jeerakul, C., Kitsanayanyong, L., Pansawat, N., Boonbumrung, S., Klaypradit, W., & Tepwong, P. (2024). Effects of different preparation methods on the physical, chemical, and functional properties of protein powders from Skipjack tuna (*Katsuwonus pelamis*) liver. Food Research, 8(2), 64–77. https://doi.org/10.26656/fr.2017.8(2).465

Kaunitz, J. D., & Akiba, Y. (2019). Control of intestinal epithelial proliferation and differentiation: the microbiome, enteroendocrine L cells, telocytes, enteric nerves, and GLP, too. Digestive Diseases and Sciences, 64(10), 2709–2716. https://doi.org/10.1007/s10620-019-05778-1

Kementerian Pertanian. (2022). Livestock commodity outlook for broiler meat. Pusat Data dan Sistem Informasi Pertanian Sekretariat Jenderal, 75.

Lee, B., Kim, D. H., Lee, J., Cressman, M. D., Choi, Y. M., & Lee, K. (2023). Greater numbers and sizes of muscle bundles in the breast and leg muscles of broilers compared to layer chickens. Frontiers in Physiology, 14(October), 1–5. https://doi.org/10.3389/fphys.2023.1285938

Luo, C., Chen, Y., Yin, D., & Yuan, J. (2023). Effects of different dietary starch sources and digestible lysine levels on carcass traits, serum metabolites, liver lipid and breast muscle protein metabolism in broiler chickens. Animals, 13(13). https://doi.org/10.3390/ani13132104

Maulidiah, I. R. (2020). Pemanfaatan limbah ikan cakalang (*Katsuwonus pelamis*) sebagai alternatif tepung ikan pada pembuatan pakan ikan lele dumbo (*Clarias gariepinus*). Fakultas Teknologi Pertanian Universitas Jember.

Mohammadigheisar, M., Shouldice, V. L., Sands, J. S., Lepp, D., Diarra, M. S., & Kiarie, E. G. (2020). Growth performance, breast yield, gastrointestinal ecology and plasma biochemical profile in broiler chickens fed multiple doses of a blend of red, brown and green seaweeds. British Poultry Science, 61(5), 590–598. https://doi.org/10.1080/00071668.2020.1774512

Muñoz, J. A., Martins, T. da S., Garbossa, P. L. M., Pimentel, L. B. F., Barbalho, C. B., da Silva, M. M., de Arruda, A. F., Baraldi-Artoni, S. M., da Silva Araújo, C. S. & Pereira, A. S. C. (2025). Bone development of broiler chickens supplemented with chondroitin sulfate and manganese. Veterinary and Animal Science, 29(July). https://doi.org/10.1016/j.vas.2025.100485

Nogueira, B. R. F., Reis, M. P., Carvalho, A. C., Mendoza, E. A. C., Oliveira, B. L., Silva, V. A., & Bertechini, A. G. (2019). Performance, growth curves and carcass yield of four strains of broiler chicken. Revista Brasileira de Ciencia Avicola / Brazilian Journal of Poultry Science, 21(4), 1–8. https://doi.org/10.1590/1806-9061-2018-0866

Oke, O. E., Alo, E. T., Oke, F. O., Oyebamiji, Y. A., Ijaiya, M. A., Odefemi, M. A., Kazeem, R. Y., Soyode, A. A., Aruwajoye, O. M., Ojo, R. T., Adeosun, S. M., & Onagbesan, O. M. (2020). Early age thermal manipulation on the performance and physiological response of broiler chickens under hot humid tropical climate. Journal of Thermal Biology, 102517. https://doi.org/10.1016/j.jtherbio.2020.102517

Pagala, M. A., Aku, A. S., Badaruddin, R., & Has, H. (2018). Karakteristik fenotip dan genotip gen GH (growth hormon) pada ayam tolaki. Jurnal Ilmu dan Teknologi Peternakan Tropis, 5(2), 1. https://doi.org/10.33772/jitro.v5i3.4705

Panda, A. K., Bhanja, S. K., & Shyam Sunder, G. (2015). Early post hatch nutrition on immune system development and function in broiler chickens. World's Poultry Science Journal, 71(2), 285–296. https://doi.org/10.1017/S004393391500029X

Perdamaian, A. B. I., Saragih, H. T. S. S. G., & Daryono, B. S. (2017). Effect of varying level of crude protein and energy on insulin-like growth factor-I expression level in Indonesian hybrid chicken. International Journal of Poultry Science, 16(1), 1–5. https://doi.org/10.3923/ijps.2017.1.5

Qaid, M. M., Al-Mufarrej, S. I., Azzam, M. M., Al-Garadi, M. A., Albaadani, H. H., Alhidary, I. A., & Aljumaah, R. S. (2021). Growth performance, serum biochemical indices, duodenal histomorphology, and cecal microbiota of broiler chickens fed on diets supplemented with cinnamon bark powder at prestarter and starter phases. Animals, 11(1), 1–18. https://doi.org/10.3390/ani11010094

Sala, R., Siahaan, T. M. B., Bawole, R., Mudjirahayu, M., & Patanda, M. (2023). Growth and exploitation status of skipjack tuna (*Katsuwonus pelamis*) in waters around Manokwari. Jurnal Sumberdaya Akuatik Indopasifik, 7(1), 41–50. https://doi.org/10.46252/jsai-fpik-unipa.2023.Vol.7.No.1.251

Sampath, V., Gao, S., Park, J. H., & Kim, I. H. (2023). Exogenous phytase improves growth performance, nutrient retention, tibia mineralization, and breast meat quality in Ross-308 broilers. Agriculture (Switzerland), 13(10). https://doi.org/10.3390/agriculture13101906

Santoso, U. (2023). Upaya peningkatan konsumsi protein hewani asal ternak di Indonesia. Buletin Peternakan, 47(3), 155–166. https://doi.org/10.31186/bpt.3.2.89-95

Saragih, H. T., Fauziah, I. N., Saputri, D. A., & Chasani, A. R. (2024). Dietary macroalgae *Chaetomorpha linum* supplementation improves morphology of small intestine and pectoral muscle, growth performance, and meat quality of broilers. Veterinary World, 17(2), 470–479. https://doi.org/10.14202/vetworld.2024.470-479

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. https://doi.org/10.14202/vetworld.2019.1233-1239

Saragih, H. T. S. S. G., Salsabila, N., Deliaputri, R., Firdaus, A. B. I., & Kurnianto, H. (2024). Growth morphology of the gastrointestinal tract, pectoralis thoracicus muscle, lymphoid organ and visceral index of kampong chicken. Journal of the Saudi Society of Agricultural Sciences, 23(1), 34–41. https://doi.org/10.1016/j.jssas.2023.08.005

Saragih, Hendry T., Alifiah, A. R., Vikasari, O. A., Sayidinar, A., & Sofyantoro, F. (2024). Nile tilapia (*Oreochromis niloticus*) gill powder as a novel feed additive for enhanced histological structure and performance in broilers. Acta Veterinaria Eurasia, 50(2), 94–102. https://doi.org/10.5152/actavet.2024.23037

Sarojnalini, C., & Hei, A. (2019). Fish as an important functional food for quality life. Functional Foods, 1–19. https://doi.org/10.5772/intechopen.81947

Son, J., Lee, W.-D., Kim, C.-H., Kim, H., Hong, E.-C., & Kim, H.-J. (2024). Effect of dietary crude protein reduction levels on performance, nutrient digestibility, nitrogen utilization, blood parameters, meat quality, and welfare index of broilers in welfare-friendly environments. Animals, 14(21), 3131. https://doi.org/10.3390/ani14213131

Srilatha, T., Reddy, V. R., Preetam, V. C., Rao, S. V. R., & Reddy, Y. R. (2018). 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, 52(4), 559–563. https://doi.org/10.18805/ijar.B-3255

Tabassum, F., Hoque, M., Islam, F., Ritchil, C., Faruque, M., & Bhuiyan, A. (2015). Phenotypic and morphometric characterization of indigenous chickens at Jhenaigati Upazila of Sherpur District in Bangladesh. SAARC Journal of Agriculture, 12(2), 154–169. https://doi.org/10.3329/sja.v12i2.21927

Tran, S., Juliani, J., Fairlie, W. D., & Lee, E. F. (2023). The emerging roles of autophagy in intestinal epithelial cells and its links to inflammatory bowel disease. Biochemical Society Transactions, 51(2), 811–826. https://doi.org/10.1042/BST20221300

Wang, Min, Yang, C., Wang, Q., Li, J., Huang, P., Li, Y., Ding, X., Yang, H. & Yin, Y. (2020). The relationship between villous height and growth performance, small intestinal mucosal enzymes activities and nutrient transporters expression in weaned piglets. Journal of Animal Physiology and Animal Nutrition, 104(2), 606–615. https://doi.org/10.1111/jpn.13299

Wang, M., Li, K., Jiao, H., Zhao, J., Li, H., Zhou, Y., Cao, A., Wang, J., Wang, X., & Lin, H. (2024). Dietary bile acids supplementation decreases hepatic fat deposition with the involvement of altered gut microbiota and liver bile acids profile in broiler chickens. Journal of Animal Science and Biotechnology, 15(1), 1–23. https://doi.org/10.1186/s40104-024-01071-y

Zhang, Q., Zhang, S., Cong, G., Zhang, Y., Madsen, M. H., Tan, B., & Shi, S. (2021). Effects of soy protein concentrate in starter phase diet on growth performance, blood biochemical indices, carcass traits, immune organ indices and meat quality of broilers. Animals, 11(2), 1–13. https://doi.org/10.3390/ani11020281

Zhou, M., Wei, Y., Feng, Y., Zhang, S., Ma, N., Wang, K., Tan, P., Zhao, Y., Zhao, J., & Ma, X. (2024). Arginine regulates skeletal muscle fiber type formation via mTOR signaling pathway. International Journal of Molecular Sciences, 25(11). https://doi.org/10.3390/ijms25116184