

Additive Effects of Cellulase, *L*-Carnitine, and Fish Oil on Growth Performance and Intestinal Morphology of Male Tegal ducks

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Abstract | Feed is a significant expense in duck production, especially in Indonesia, where local feed ingredients often have high fiber content that can limit nutrient utilization. This study examined the effects of supplementing cellulase, *L*-carnitine, and fish oil on body weight gain and intestinal morphology in male Tegal ducks during the grower phase. A total of 100 day-old male ducks were assigned to five dietary treatments in a completely randomized design with four replicates of five birds each. The treatments consisted of a basal diet without additives (T0), basal diet with 0.1% cellulase (T1), T1 plus 0.003% *L*-carnitine (T2), T2 plus 2% tuna fish oil (T3), and T2 plus 2% lemuru fish oil (T4). After a common brooding period from day 1 to day 14, the experimental diets were fed from day 15 to day 70. The T2, T3, and T4 treatments increased body weight gain, digestive tract length, and relative digestive organ weights compared with T0 and T1 ($p < 0.01$). Intestinal morphology also improved, as indicated by lower crypt depth and a higher villus height-to-crypt depth ratio, with the strongest response in T4. Feed intake, feed conversion ratio, protein efficiency ratio, and energy efficiency ratio were not significantly affected by the treatments ($p > 0.05$). In conclusion, supplementation with cellulase and *L*-carnitine increased body weight gain and improved specific gut characteristics, whereas fish oil had limited additional effects, primarily on ileal morphology.

Keywords | dietary additive, digestive efficiency, omega-3 lipid source, poultry nutrition, tropical feed resources

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INTRODUCTION

Tegal ducks represent one of Indonesia's important indigenous ducks, with male Tegal ducks being particularly recognized for their potential as meat-producing birds (Ismoyowati *et al.*, 2019). As a locally adapted genotype commonly reared in Indonesian production systems, male Tegal ducks provide a relevant model for evaluating nutritional strategies for practical field conditions, especially when fibrous local feed ingredients are incorporated into diets. Research involving

this breed is therefore essential for developing feeding interventions that are not only effective under controlled experimental settings but also applicable to commercial and smallholder production environments. Although the growth performance of male Tegal ducks is generally lower than that of modern commercial broiler strains, this breed remains economically important within Indonesia's duck production sector due to its adaptability, local availability, and market acceptance. Improving feed utilization efficiency and growth performance within the constraints of local production systems is a more relevant objective for

this genotype. Previous studies have demonstrated that the growth performance of male Tegal ducks is highly dependent on management and production systems (Rahayu *et al.*, 2020; Ramadhana *et al.*, 2019). Under intensive rearing conditions, male Tegal ducks achieved approximately 994 g live weight at 42 days of age, whereas birds raised under semi-intensive systems reached only around 766 g at the same age (Ramadhana *et al.*, 2019). These variations highlight the substantial influence of environmental and nutritional management on production outcomes and indicate that reported growth values should be interpreted as context-dependent observations rather than absolute indicators of the breed's commercial potential.

A significant challenge in Indonesian duck farming is the inclusion of fibrous feedstuffs, such as rice bran, which may be difficult to digest. Ducks lack the endogenous enzymes necessary to break down these fibrous components effectively (Sudibya *et al.*, 2023). To improve nutrient utilization in such high-fiber feeding conditions, current strategies have increasingly focused on exogenous enzymes, nutritional additives, and functional fatty acids. The use of exogenous enzymes represents an important nutritional approach for optimizing nutrient digestibility (Bedford and Apajalahti, 2022). Cellulase is an exogenous enzyme to address the cellulose-rich component of local fibrous feed ingredients. Studies showed that the incorporation of fiber-degrading enzymes into poultry diets enhances the availability of energy and nutrients, especially when feeds contain elevated fiber levels (Pirgozliev *et al.*, 2025). Specifically, cellulase improved nutrient digestibility in poultry by breaking down cellulose through hydrolysis, producing glucose that serves as an accessible energy substrate (Perim *et al.*, 2024).

Furthermore, *L*-carnitine plays a central role in lipid metabolism by facilitating the transport of long-chain fatty acids into the mitochondria for β -oxidation, thereby improving energy utilization efficiency in poultry (Ahmadipour *et al.*, 2025; Alzamly *et al.*, 2024). Recent studies have shown that dietary *L*-carnitine supplementation enhanced growth performance, lipid metabolism, and physiological resilience in poultry, particularly under nutritionally or environmentally challenging conditions (Ahmadipour *et al.*, 2025; Musazadeh *et al.*, 2023; Azizi-Chekosari *et al.*, 2021; Akhoondzadeh *et al.*, 2023; Xu *et al.*, 2003). Carnitine is present in limited quantities in plant-derived ingredients but is abundant in animal-derived feedstuffs. Therefore, poultry diets formulated primarily from cereals may supply insufficient carnitine, potentially resulting in suboptimal carnitine status (Azizi-Chekosari *et al.*, 2021).

Long-chain unsaturated fatty acids are generally more digestible than saturated fatty acids in poultry. Essential

fatty acids not only support poultry health and productivity but also facilitate the production of nutritionally enhanced animal products that meet consumer preferences (Farahiyaha *et al.*, 2025; Rahbari *et al.*, 2025). Incorporating fish oils, including tuna and lemuru oil, into poultry diets represents an effective method for increasing the unsaturated fatty acid content of poultry-derived products (Sudibya and Purnomo, 2013). Fish oils are notable for the high levels of omega-3 fatty acids. Specifically, lemuru fish oil contains approximately 34.17% eicosapentaenoic acid (EPA) and 17.40% docosahexaenoic acid (DHA), with a total fat content of 6%. In comparison, tuna fish oil provides approximately 33.6% EPA, 14.64% DHA, and has a fat content of 5.8% (Sudibya *et al.*, 2017). It is important to note that the EPA and DHA composition were not analyzed for the specific batches used in this study. Substantial evidence indicates that dietary inclusion of unsaturated fatty acid sources positively influences growth performance and nutrient digestibility in poultry (Farahiyaha *et al.*, 2025; Nur Mahendra *et al.*, 2023).

Previous studies have generally evaluated exogenous enzymes, *L*-carnitine, or dietary lipid sources as separate interventions, mostly in broiler chickens and commercial poultry strains (Agboola *et al.*, 2021; Perim *et al.*, 2024; Ahmadipour *et al.*, 2025). Less information is available regarding their combined use, especially in local duck genotypes. This represents an important knowledge gap because additive responses among fiber-degrading enzymes, lipid metabolism modulators, and omega-3-rich lipid sources may differ across poultry species and genotypes. These additives may improve digestive efficiency and growth through interconnected effects on nutrient release, lipid metabolism, and gut health. Therefore, this study aimed to evaluate the effects of supplementation with cellulase, *L*-carnitine, and fish oil on growth performance and intestinal morphology in male Tegal ducks.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN AND DUCK MANAGEMENT

This study involved 100 day-old male Tegal ducks, with an initial body weight of 41.63 ± 4.76 g. The experimental groups were organized as follows: the control group (T0) received a basal diet; group T1 received the basal diet supplemented with 0.1% cellulase enzyme; group T2 received the basal diet containing 0.1% cellulase enzyme and 0.003% *L*-carnitine; group T3 received the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% tuna fish oil; and group T4 received the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% lemuru fish oil. Each treatment included four replicates, with five ducks per replicate. A schematic overview of the experimental design, including the brooding period, treatment groups,

replication, and measured parameters, is shown in Figure 1. Cellulase (Nutrizyme® CEA, Sunson Industry Group Co., Ltd., Guangzhou, China) was used at an inclusion level of 0.1% of the diet, with an activity range of 2,800–10,000 U/g. The manufacturer provided the declared activity; however, independent verification of the enzyme activity was not performed in this study. *L*-carnitine was included at 0.003% (30 ppm) as part of a combined additive strategy evaluated in this study. While higher inclusion levels have been reported in some poultry studies, this trial aimed to assess the use of *L*-carnitine in combination with cellulase and fish oil rather than as a high-dose standalone intervention. No additional antioxidants were included; however, the diets were prepared and handled to minimize oxidative deterioration during storage and feeding.

The feed was formulated according to the recommendations of the National Research Council (1994) for ducks in the grower phase, as outlined in Table 1.

The ducks were raised in 1 m × 1 m cages from day 1 to day 70. During the first 14 days, all the ducks were given a common starter diet. At day 14, the birds were weighed individually and then randomly assigned to different treatment groups. From day 15 to day 70, the ducks were

fed experimental diets based on their assigned treatments. Throughout the 70-day trial, all birds completed the study without any mortality or health issues.

PERFORMANCE AND DIGESTIVE ORGAN MEASUREMENT

Feed intake (FI) was measured daily, and weight gain (WG) was measured weekly during the treatment period from days 15 to 70. WG was expressed as average daily gain (ADG). The feed conversion ratio (FCR) was calculated as WG divided by FI. The protein efficiency ratio (PER) was calculated as WG divided by protein consumption, and the energy efficiency ratio (EER) was calculated as WG divided by energy consumption (Dono, 2012). On day 70, one duck from each cage that was closest to the average weight was selected for measurement of the digestive tract length and weight as described by Fandi *et al.* (2019).

INTESTINAL MORPHOLOGY

Upon completion of the experimental period, tissue specimens from the duodenum, jejunum, and ileum were harvested following euthanasia. From each intestinal segment, tissue samples (approximately 2 cm in length) were obtained and promptly preserved in 10% neutral buffered formalin solution, following the protocol of Kpomasse *et al.* (2023).

Table 1: Composition and nutrient contents of the diets.

Ingredients	T0	T1	T2	T3	T4
Yellow corn (%)	47.5	47.5	47.5	47.5	47.5
Rice bran (%)	29.8	29.8	29.8	29.8	29.8
Soybean meal (%)	20.2	20.2	20.2	20.2	20.2
Coconut oil (%)	1.03	1.03	1.03	1.03	1.03
Minerals (%)	1.4	1.4	1.4	1.4	1.4
<i>DL</i> -methionine (%)	0.07	0.07	0.07	0.07	0.07
Cellulase enzyme (%)	0	0.1	0.1	0.1	0.1
<i>L</i> -carnitine (%)	0	0	0.003	0.003	0.003
Tuna fish oil (%)	0	0	0	2	0
Lemuru fish oil (%)	0	0	0	0	2
Nutrient contents					
Metabolizable energy (kcal/kg)	3018.33	3015.31	3015.22	3117.96	3118.35
Crude protein (%)	16.02	16.01	16.01	15.76	15.77
Crude fat (%)	4.76	4.75	4.75	5.76	5.80
Crude fiber (%)	5.67	5.67	5.67	5.57	5.57
Calcium (%)	0.62	0.62	0.62	0.61	0.61
Phosphorus (%)	0.16	0.16	0.16	0.15	0.15
Lysine (%)	0.84	0.84	0.84	0.83	0.83
Methionine (%)	0.36	0.36	0.36	0.35	0.35

Basal diet with no additives served as the control (T0); the basal diet supplemented with 0.1% cellulase enzyme (T1); the basal diet containing 0.1% cellulase enzyme and 0.003% *L*-carnitine (T2); the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% tuna fish oil (T3); and the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% lemuru fish oil (T4). Mineral refers to a mineral premix used in the diet formulation. The detailed composition of the premix was not fully documented in the original formulation record.

In the present study, hematoxylin and eosin staining were used to facilitate visualization of the mucosal architecture for villus and crypt measurements. Histological preparations were examined and documented using a Leica microscope equipped with a digital camera. Intestinal morphology was evaluated in three intestinal segments, namely the duodenum, jejunum, and ileum. For each segment, 20 well-oriented villi and their associated crypts were measured from four histological sections. Measurements of villus height (VH), villus width (VW), and crypt depth (CD) were performed using Image Raster 3.0 software (Samanya and Yamauchi, 2001). The villus surface area (VSA) was calculated using the formula $2\pi \times VW \times VH$, as described by Moita *et al.* (2021). This formula serves as a geometric approximation for comparative analysis across different treatments. However, since it assumes a simplified shape of the villus, the VSA values should be interpreted with caution and not regarded as exact measurements of the true three-dimensional villus surface area. This calculation was used as a standardized geometric approximation for comparative purposes among treatments, and no additional correction factor was applied.

STATISTICAL ANALYSIS

Prior to statistical analysis, the data were assessed for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test, and the results indicated that the data met the assumptions of normality and homogeneity. No data transformation was applied. The replicate cage was considered the experimental unit for statistical analysis. Therefore, the variability in the tables reflects differences among replicate cages or cage-level observations rather than variation among individual birds within each treatment. Growth performance variables were recorded on a cage basis, whereas organ traits, and intestinal morphology were measured using one representative bird from each replicate cage to represent that replicate. Data analysis was conducted using a one-way analysis of variance (ANOVA), followed by orthogonal contrasts to assess the effects of different treatments. These orthogonal contrasts were predetermined based on the sequential structure of the dietary treatments and the specific biological comparisons that informed the experimental design. No additional multiple-comparison corrections were applied to the orthogonal contrasts, as they were established *a priori* in accordance with the experimental design. Superscript letters included in the tables indicate significant differences among treatment means based on the ANOVA results and should be interpreted descriptively. Statistical significance was determined at $P < 0.05$ (Steel and Torrie, 1995). The following adjusted contrasts were applied:

T0 vs T1, T2, T3, T4 (to compare treatments with and without the addition of cellulase), T1 vs T2, T3, T4 (to

compare treatments between cellulase enzyme and *L*-carnitine), T2 vs T3, T4 (to compare treatments between *L*-carnitine and fish oil), and T3 vs T4 (to compare treatments between tuna fish oil and lemuru fish oil).

The comparison between T2 and T3-T4 assesses the impact of adding fish oil within the sequential supplementation design. However, this comparison does not isolate the specific effects of omega-3 fatty acids, as it also reflects the impact of increased dietary energy density. This is because the fish oil-containing diets were not isoenergetic with T2.

RESULTS

GROWTH PERFORMANCE

Dietary supplementation significantly affected total ADG of male Tegal ducks during the experimental feeding period ($P < 0.01$), as presented in Table 2. Orthogonal contrast analysis (Table 3) showed that cellulase alone increased ADG compared with the unsupplemented control. Furthermore, the addition of *L*-carnitine to cellulase-supplemented diets (T2) resulted in higher ADG compared to cellulase alone (T2-T4; $P < 0.01$). However, further supplementation with fish oil did not produce additional improvements in ADG beyond those achieved with *L*-carnitine (T2 vs. T3, T4; $P > 0.05$). Comparisons between the two fish oil types (tuna and lemuru) revealed no significant differences for any of the measured parameters ($P > 0.05$). FI, FCR, PER, and EER were unaffected by the dietary treatments ($P > 0.05$).

GASTROINTESTINAL TRACT LENGTH

Cellulase supplementation increased ($P < 0.01$) the length of all digestive tract segments (Table 4). Orthogonal contrast analysis (Table 5) demonstrated a stepwise pattern of improvement. All supplemented groups had longer duodenal length, jejunum, ileum, and caecum compared to the control (T0 vs. T1-T4; $P < 0.01$). Groups receiving *L*-carnitine showed further increases in digestive tract length compared to cellulase alone (T1 vs. T2-T4; $P < 0.01$). Supplementation with fish oil generated additional increases compared to *L*-carnitine alone (T2 vs. T3, T4; $P < 0.01$). However, no difference was observed between tuna and lemuru fish oil sources (T3 vs. T4; $P > 0.05$).

DIGESTIVE TRACT AND ORGAN WEIGHTS

Treatments affected the relative weights of digestive organs ($P < 0.05$), as presented in Table 6. Orthogonal contrast results (Table 7) revealed that all cellulase-supplemented groups had heavier organs compared to the control (T0 vs. T1-T4; $P < 0.01$). Groups receiving *L*-carnitine exhibited heavier ventriculus, liver, and caecum compared to cellulase alone (T1 vs. T2-T4; $P < 0.01$). Fish oil supplementation further increased these organ weights compared to

Table 2: Average production performance of male Tegal ducks in the grower phase.

Parameters	Treatments					p-value
	T0	T1	T2	T3	T4	
FI (g/day)	184.80±6.60	183.47±8.64	185.76±2.23	186.11±2.42	185.14±2.15	0.957
ADG (g/day)	19.26±0.18 ^c	19.50±0.12 ^b	19.78±0.17 ^a	19.80±0.16 ^a	19.90±0.12 ^a	0.000
FCR	3.43±0.15	3.36±0.14	3.36±0.04	3.36±0.05	3.32±0.05	0.668
PER	1.82±0.08	1.86±0.08	1.86±0.03	1.89±0.03	1.91±0.03	0.281
EER (g/kcal)	0.10±0.01	0.10±0.01	0.10±0.00	0.10±0.01	0.10±0.01	0.252

^{a,b,c} Different superscript letters within a row indicate differences based on orthogonal contrast analysis ($P < 0.05$). Basal diet with no additives served as the control (T0); the basal diet supplemented with 0.1% cellulase enzyme (T1); the basal diet containing 0.1% cellulase enzyme and 0.003% *L*-carnitine (T2); the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% tuna fish oil (T3); and the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% lemuru fish oil (T4); FI: feed intake, ADG: average daily gain, FCR: feed conversion ratio, PER: protein efficiency ratio, EER: energy efficiency ratio.

Table 3: Orthogonal contrast set for the production performance of male Tegal ducks in the grower phase.

Parameter	Treatments			
	T0 vs T1, T2, T3, T4	T1 vs T2, T3, T4	T2 vs T3, T4	T3 vs T4
ADG (g/day)	**	**	ns	ns

Note: ** ($p < 0.01$) significantly different. ns ($p > 0.05$) not significantly different.

Table 4: Average length of the digestive tract of male Tegal ducks in the grower phase.

Parameters	Treatments					p-value
	T0	T1	T2	T3	T4	
Duodenum length (cm)	22.75±0.96 ^c	24.50±0.58 ^b	25.25±0.96 ^b	27.25±0.96 ^a	27.50±0.58 ^a	0.000
Jejunum length (cm)	65.25±0.58 ^c	67.75±0.96 ^b	68.00±0.82 ^b	71.50±2.38 ^a	71.75±2.06 ^a	0.000
Ileum length (cm)	60.75±0.96 ^d	63.00±1.15 ^c	65.00±1.63 ^b	66.25±1.50 ^a	68.00±0.82 ^a	0.000
Caecum length (cm)	14.25±0.5 ^{0c}	15.00±0.82 ^c	16.75±0.96 ^b	17.75±0.50 ^a	18.00±0.0 ^{0a}	0.000

^{a,b,c,d} Different superscript letters within a row indicate differences based on orthogonal contrast analysis ($P < 0.05$). Basal diet with no additives served as the control (T0); the basal diet supplemented with 0.1% cellulase enzyme (T1); the basal diet containing 0.1% cellulase enzyme and 0.003% *L*-carnitine (T2); the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% tuna fish oil (T3); and the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% lemuru fish oil (T4).

Table 5: Orthogonal contrast set for the digestive tract length of male Tegal ducks in the grower phase.

Parameters	Treatments			
	T0 vs T1, T2, T3, T4	T1 vs T2, T3, T4	T2 vs T3, T4	T3 vs T4
Duodenum length (cm)	**	**	**	ns
Jejunum length (cm)	**	**	**	ns
Ileum length (cm)	**	**	**	ns
Caecum length (cm)	**	**	**	ns

Note: ** ($p < 0.01$) significantly different. ns ($p > 0.05$) not significantly different.

Table 6: Relative weight of the digestive organs of male Tegal ducks in the grower phase.

Parameters	Treatments					p-value
	T0	T1	T2	T3	T4	
Ventricular weight (%)	2.38±0.06 ^c	2.48±0.06 ^c	2.77±0.25 ^b	3.17±0.03 ^a	3.19±0.03 ^a	0.000
Liver weight (%)	1.55±0.07 ^c	1.66±0.14 ^{bc}	1.77±0.15 ^b	1.97±0.10 ^a	1.99±0.12 ^a	0.000
Small intestine weight (%)	1.80±0.12 ^b	1.90±0.05 ^{ab}	1.98±0.13 ^a	2.02±0.11 ^a	2.05±0.09 ^a	0.027
Caecum weight (%)	0.75±0.04 ^c	0.79±0.05 ^c	0.89±0.12 ^b	0.99±0.04 ^a	1.01±0.03 ^a	0.000

^{a,b,c,d} Different superscript letters within a row indicate differences based on orthogonal contrast analysis ($P < 0.05$). Basal diet with no additives served as the control (T0); the basal diet supplemented with 0.1% cellulase enzyme (T1); the basal diet containing 0.1% cellulase enzyme and 0.003% *L*-carnitine (T2); the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% tuna fish oil (T3); and the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% lemuru fish oil (T4).

Table 7: Orthogonal contrast set of the digestive organ weights of male Tegal ducks in the grower phase.

Parameters	Treatments			
	T0 vs T1, T2, T3, T4	T1 vs T2, T3, T4	T2 vs T3, T4	T3 vs T4
Ventricular weight (%)	**	**	**	ns
Liver weight (%)	**	**	**	ns
Small intestine weight (%)	**	ns	ns	ns
Caecum weight (%)	**	**	**	ns

Note: ** ($p < 0.01$) significantly different. ns ($p > 0.05$) not significantly different.

Table 8: Morphology of the small intestine of male Tegal ducks in the grower phase.

Parameters	Treatments					p-value
	T0	T1	T2	T3	T4	
Duodenum						
Villus height (μm)	29.03±3.71	33.28±2.26	33.97±1.85	34.15±3.78	36.72±4.03	0.056
Villus width (μm)	5.67±1.05	5.41±0.56	5.60±2.22	6.14±2.60	4.08±1.54	0.556
Crypt depth (μm)	12.33±0.55 ^a	11.23±1.16 ^{ab}	11.14±1.97 ^{ab}	9.49±1.23 ^{bc}	8.08±1.30 ^c	0.003
VH:CD ratio	2.35±0.20 ^c	2.98±0.29 ^{bc}	3.14±0.64 ^{bc}	3.70±0.97 ^b	4.58±0.38 ^a	0.001
VSA (μm ²)	1018.14±108.02	1137.97±193.49	1203.72±524.03	1363.44±743.47	931.79±361.87	0.699
Jejunum						
Villus height (μm)	24.78±6.61	29.28±7.50	29.56±7.69	36.44±6.15	39.67±9.69	0.089
Villus width (μm)	6.39±1.89	4.91±1.72	4.70±1.31	4.33±1.77	5.83±1.23	0.382
Crypt depth (μm)	10.97±0.35 ^a	8.67±0.60 ^b	8.06±0.96 ^{bc}	7.22±2.01 ^{bc}	6.44±0.38 ^c	0.000
VH:CD ratio	2.27±0.62 ^c	3.44±1.14 ^{bc}	3.73±1.16 ^{bc}	5.29±1.52 ^{ab}	6.14±1.44 ^a	0.003
VSA (μm ²)	938.78±124.71	877.77±274.47	828.17±116.28	941.08±228.10	1307.61±589.65	0.273
Ileum						
Villus height (μm)	28.78±2.67	30.59±4.83	30.67±4.21	31.84±6.25	38.19±1.93	0.059
Villus width (μm)	6.49±1.31	5.38±1.94	7.18±2.58	6.31±2.06	4.17±1.33	0.254
Crypt depth (μm)	9.60±2.14	8.23±0.84	7.38±1.09	7.06±0.86	6.45±1.96	0.071
VH:CD ratio	3.10±0.70 ^b	3.74±0.67 ^b	4.27±1.08 ^b	4.61±1.26 ^{ab}	6.34±1.94 ^a	0.021
VSA (μm ²)	1165.85±220.55	1042.77±463.92	1336.26±314.18	1274.63±537.11	1012.37±380.16	0.736

^{a,b,c} Different superscript letters within a row indicate differences identified based on orthogonal contrast analysis ($P < 0.05$). Basal diet with no additives served as the control (T0); the basal diet supplemented with 0.1% cellulase enzyme (T1); the basal diet containing 0.1% cellulase enzyme and 0.003% *L*-carnitine (T2); the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% tuna fish oil (T3); and the basal diet with 0.1% cellulase enzyme, 0.003% *L*-carnitine, and 2% lemuru fish oil (T4). VH:CD: villus height-to-crypt depth. VSA: villus surface area.

L-carnitine alone (T2 vs. T3, T4; $P < 0.01$). A significant increase in the relative weight of the small intestine was observed in ducks receiving *L*-carnitine compared to those supplemented with cellulase alone (T1 vs. T2–T4; $P < 0.05$), but fish oil did not provide additional improvement beyond *L*-carnitine (T2 vs. T3, T4; $P > 0.05$). No difference was observed between tuna and lemuru fish oil sources for any organ weight parameter (T3 vs. T4; $P > 0.05$).

INTESTINAL MORPHOLOGY

Dietary supplementation exerted marked effects on small intestinal architecture, with differential responses observed across the duodenum, jejunum, and ileum (Table 8). Orthogonal contrast analysis (Table 9) revealed that the inclusion of cellulase, *L*-carnitine, and fish oil induced distinct histological modifications, particularly CD and

the villus height-to-crypt depth (VH:CD) ratio. In the duodenum, ducks receiving supplemented diets (T1–T4) exhibited lower CD ($P < 0.01$) and elevated VH:CD ratio ($P < 0.01$) relative to unsupplemented control (T0 vs T1–T4). The incorporation of *L*-carnitine, either independently or in combination with fish oil (T2–T4), further enhanced the VH:CD ratio beyond levels achieved with cellulase alone (T1 vs. T2–T4; $P < 0.05$). Additionally, fish oil supplementation (T3 and T4) promoted more pronounced CD attenuation ($P < 0.01$) and superior VH:CD ratio ($P < 0.01$) compared to *L*-carnitine alone (T2 vs. T3, T4). A source-specific effect was also evident, as lemuru oil (T4) yielded a greater VH:CD ratio than tuna oil (T3 vs. T4; $P < 0.05$). No treatment effects were detected for VH, VW, or VSA in the duodenum ($P > 0.05$).

Table 9: Orthogonal contrast set of the small intestine morphology of male Tegal ducks in the grower phase.

Parameters	Treatments			
	T ₀ vs T ₁ , T ₂ , T ₃ , T ₄	T ₁ vs T ₂ , T ₃ , T ₄	T ₂ vs T ₃ , T ₄	T ₃ vs T ₄
Duodenum				
Crypt depth (μm)	**	*	**	ns
VH:CD ratio	**	*	**	*
Jejunum				
Crypt depth (μm)	**	*	ns	ns
VH:CD ratio	**	*	**	ns
Ileum				
VH:CD ratio	*	ns	ns	ns

Note: ** (p < 0.01) significantly different. * (p < 0.05) significantly different. ns (p > 0.05) not significantly different.

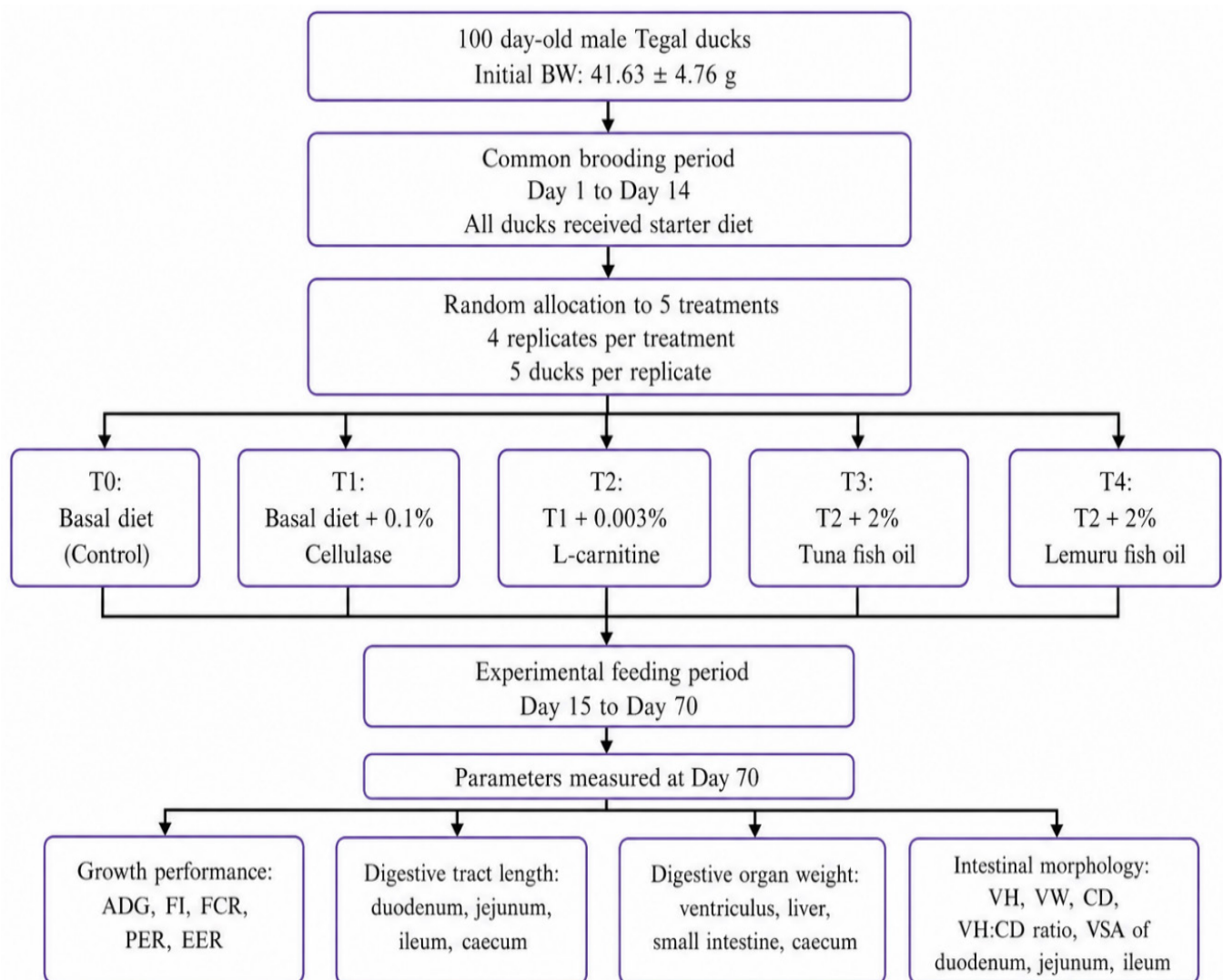


Figure 1: Schematic diagram of the experimental design.

In the jejunum, all supplementation (T1–T4) resulted in decreased CD ($P < 0.01$) and increased VH:CD ratio ($P < 0.01$) compared with the control group (T0 vs. T1–T4). The addition of *L*-carnitine and fish oil (T2–T4) produced further reductions in CD ($P < 0.05$) and improvements in VH:CD ratio ($P < 0.05$) relative to cellulase supplementation alone (T1 vs. T2–T4). Moreover, diets

containing fish oil (T3 and T4) enhanced both VH:CD ratio ($P < 0.01$) compared to the diet supplemented exclusively with *L*-carnitine (T2 vs. T3, T4). No statistically significant differences emerged between the two fish oil sources in the jejunum (T3 vs T4; $P > 0.05$). Additionally, VH, VW, and VSA remained unaffected by dietary treatments ($P > 0.05$).

In the ileum, the supplemented groups (T1–T4) showed a higher VH:CD ratio ($P < 0.05$) than the control (T0 vs. T1–T4). Lemuru oil supplementation (T4) was associated with an increased VH:CD ratio ($P < 0.05$) relative to tuna oil (T3 vs. T4). As observed in other segments, VH, CD, VW, and VSA showed no significant variation across treatments ($P > 0.05$). The ileal morphology response should be interpreted cautiously because histological measurements were obtained from only one representative bird per replicate. Thus, the possibility that isolated differences were influenced by individual biological variation cannot be completely excluded.

DISCUSSION

GROWTH PERFORMANCE

Supplementation with cellulase alone resulted in increased body weight gain compared with the unsupplemented control group (T0 vs T1, T2, T3, T4; $P < 0.01$), but did not significantly affect FI, FCR, PER, or EER (Tables 2 and 3). Exogenous enzymes, such as cellulase, are typically added to enhance nutrient digestibility in diets containing high-fiber ingredients, such as rice bran (Al-Arif *et al.*, 2025). The observed improvement in weight gain without changes in feed intake or FCR suggests that cellulase increases the availability of nutrients from the fibrous components of the basal diet. This allows ducks to allocate more energy and amino acids to growth rather than maintenance. Supporting this finding, previous studies have demonstrated that supplementation with cellulase can improve nutrient digestibility and overall performance in poultry without necessarily altering feed intake (Perim *et al.*, 2024; Yaqoob *et al.*, 2022).

Carnitine has the potential to positively impact poultry performance due to its crucial role in lipid metabolism. In this study, adding *L*-carnitine to a diet that already included cellulase enzyme (T2) resulted in a greater ADG compared to the diet supplemented with cellulase alone (T1 vs. T2, T3, T4; $P < 0.01$). This improvement aligns with carnitine's role in facilitating the transport of long-chain fatty acids across the inner mitochondrial membrane for β -oxidation, thereby enhancing the utilization of energy from fats (Ahmadipour *et al.*, 2025). As a result, carnitine supplementation may reduce reliance on protein-derived energy, allowing more dietary amino acids to be allocated to muscle synthesis and weight gain (Ahmadipour *et al.*, 2025; Azizi-Chekosari *et al.*, 2021). This finding corroborates previous studies indicating that carnitine supplementation enhances growth performance in poultry (Eskandani *et al.*, 2022).

Supplementing the diet with fish oil (from tuna or lemuru), along with the cellulase enzyme and *L*-carnitine (T3 and T4), did not lead to any significant improvement

in ADG beyond that achieved with *L*-carnitine alone (T2 compared to T3 and T4; $P > 0.05$). Fish oil is known to provide energy and is rich in unsaturated fatty acids, particularly omega-3 fatty acids such as EPA and DHA (Sudibya *et al.*, 2017). While the inclusion of fish oil increased the dietary metabolizable energy content from approximately 3015 kcal/kg in T2 to about 3118 kcal/kg in T3 and T4, this increase in energy density did not result in any significant improvements in ADG. The similar effects observed among T2, T3, and T4 suggest that the combination of cellulase and *L*-carnitine was already effective in optimizing energy utilization from the basal diet. Because fish oil supplementation also increased calculated dietary energy density, the lack of additional ADG improvement cannot be attributed solely to omega-3 fatty acids. This finding aligns with the idea that once the energy requirements for optimal growth are met, any extra dietary energy may contribute to fat deposition rather than increasing lean tissue growth and body weight gain. This has been previously demonstrated in broiler chickens fed high-energy, low-crude-protein diets (Wang *et al.*, 2025). The results from this experiment further confirm earlier observations that dietary supplementation with fish oil does not consistently enhance growth performance in poultry, especially when the basal diet already provides sufficient energy and nutrients (Agboola *et al.*, 2021).

Additionally, the lack of difference between the growth performance parameters of tuna and lemuru fish oil sources (T3 vs. T4; $P > 0.05$) is likely due to the similar nutrient compositions of the two fish oils, particularly their metabolizable energy content, which is 8.260 kcal/kg for tuna fish oil and 8.280 kcal/kg for lemuru fish oil (Sudibya *et al.*, 2015). The comparable fatty acid profiles, including EPA and DHA levels, may also contribute to the similar growth responses observed between groups T3 and T4.

The WG, FI, FCR, PER, and EER were not significantly affected by the dietary treatments ($P > 0.05$). The stable feed intake suggests that the additives did not alter the diet's palatability or feed intake behavior. Similarly, the lack of significant differences in FCR, PER, and EER indicates that the increase in weight gain did not result in proportional improvements in nutrient utilization efficiency. This finding aligns with previous poultry nutrition studies, where enhanced growth performance can occur without corresponding changes in feed efficiency parameters. The impact of diet composition, physiological condition, and the duration of supplementation may influence these outcomes (Oketch and Heo, 2025; Zakria *et al.*, 2025).

GASTROINTESTINAL TRACT LENGTH

Supplementation with the cellulase enzyme significantly

increased ($P < 0.01$) the length of all segments of the digestive tract, including the duodenum, jejunum, ileum, and caecum, when compared to the unsupplemented control group (T0 vs. T1, T2, T3, T4; [Tables 4 and 5](#)). Exogenous enzymes, such as cellulase, are primarily used to enhance nutrient digestibility by breaking down fibrous components, particularly in diets containing high-fiber ingredients like rice bran ([Al-Arif et al., 2025](#)). The increase in digestive tract length following cellulase supplementation likely indicates improved substrate availability for microbial fermentation in the hindgut. The degradation of cellulose and hemicellulose by cellulase produces oligosaccharides and short-chain fatty acids (SCFAs), which serve as energy sources for intestinal epithelial cells, thereby stimulating intestinal growth and development ([Borda-Molina et al., 2021](#); [Liu et al., 2021](#)). Supporting this finding, previous research has shown that dietary fiber-degrading enzyme supplementation increased the length of the small intestine and caecum in poultry by modulating digesta viscosity and fermentation patterns ([Liu et al., 2021](#); [Perim et al., 2024](#)).

Carnitine has the potential to impact gastrointestinal development due to its essential role in energy metabolism. In the current study, adding *L*-carnitine to a diet that included the cellulase enzyme (T2) resulted in a greater length of all digestive tract segments compared to the diet supplemented with cellulase alone (T1 vs. T2, T3, T4; $P < 0.01$). This response aligns with carnitine's role in facilitating the transport of long-chain fatty acids across the inner mitochondrial membrane for β -oxidation, which enhances cellular energy availability ([Ahmadipour et al., 2025](#)). Improved energy metabolism in intestinal tissues may support epithelial cell proliferation and differentiation, thereby contributing to increased intestinal length ([Ahmadipour et al., 2025](#); [Tang et al., 2025](#)). As a result, carnitine supplementation reduces the energy demand for intestinal cell turnover, allowing more energy for tissue growth and elongation. This finding supports earlier observations that *L*-carnitine supplementation positively influences intestinal morphology and development in poultry ([Eskandani et al., 2022](#); [Mirzaei et al., 2022](#)).

Supplementing the diet with fish oil (from tuna or lemuru) in conjunction with cellulase enzyme and *L*-carnitine (T3 and T4) led to a significant increase in the length of the digestive tract compared to *L*-carnitine alone (T2 vs. T3 and T4; $P < 0.01$). Fish oil is a rich source of long-chain omega-3 polyunsaturated fatty acids (PUFAs), specifically EPA and DHA ([Sudibya et al., 2017](#)). These omega-3 PUFAs are known to improve intestinal health by reducing inflammatory responses and enhancing intestinal barrier integrity ([Thanabalan and Kiarie, 2021](#); [Tompkins et al., 2024](#)). These fatty acids are incorporated into cell

membranes, altering membrane fluidity and influencing signaling pathways that regulate cell proliferation and differentiation ([Ali and Szabó, 2023](#)). The increase in caecal length associated with fish oil supplementation is particularly significant because the caeca are key sites for fiber fermentation. A longer caecum provides a greater capacity for microbial digesting fiber in the hindgut, which may enhance the production of SCFAs and overall energy extraction from fibrous feed ingredients ([de Sousa et al., 2025](#)). This finding is consistent with [Agboola et al. \(2021\)](#) who reported that dietary fish oil supplementation in broiler chickens was linked to improved intestinal morphology and gut health. The results of this experiment support previous studies indicating that supplementation with unsaturated fatty acids positively affects gastrointestinal development in poultry ([Thanabalan and Kiarie, 2021](#)). However, because intestinal length was not normalized to body weight, these differences should be interpreted cautiously.

The lack of effect of tuna and lemuru fish oil on digestive tract length (T3 vs. T4; $P > 0.05$) reflects the similar nutrient compositions of the two fish oil sources, particularly their metabolizable energy content, which are 8.260 kcal/kg for tuna fish oil and 8.280 kcal/kg for lemuru fish oil ([Sudibya et al., 2015](#)). Additionally, comparable fatty acid profiles, including EPA and DHA content, may contribute to the similar effects on intestinal elongation observed between T3 and T4. Notably, the progressive improvements from T0 to T4 suggest cumulative and potentially additive effects of these supplements on intestinal growth. The gradual lengthening of intestinal segments with each successive supplement indicates that the combination of cellulase, *L*-carnitine, and fish oil has progressive positive effects on intestinal development, likely through complementary mechanisms that enhance nutrient availability, improve energy metabolism, and reduce inflammatory responses.

DIGESTIVE TRACT AND ORGANS WEIGHTS

The supplementation of cellulase enzyme increased ($P < 0.01$) the relative weights of the ventriculus, liver, and caecum without affecting small intestine weight ([Tables 6 and 7](#)). Exogenous enzymes, such as cellulase, are primarily used to improve nutrient digestibility by breaking down fibrous components, especially in diets containing high-fiber ingredients like rice bran ([Al-Arif et al., 2025](#)). The observed increase in ventricular weight after cellulase supplementation likely indicates adaptive hypertrophy in response to changes in digesta characteristics. The cellulase-mediated degradation of dietary fiber (cellulose) reduces digesta viscosity and disrupts the structure of plant cell walls, potentially altering the physical properties of the digesta and affecting muscular activity during mechanical digestion ([Rosyada et al., 2026](#)). Supporting this finding,

previous studies have shown that dietary enzyme supplementation, which influences digesta viscosity and particle size, can also affect gizzard development in poultry (Novotný *et al.*, 2023). Additionally, the increase in liver weight may indicate heightened metabolic activity associated with improved nutrient absorption and processing (Chen *et al.*, 2022).

Carnitine plays a significant role in lipid metabolism and energy homeostasis, which may affect the development of digestive organs. In this study, the addition of *L*-carnitine to a diet that included the cellulase enzyme (T2) resulted in increased relative weights of the ventriculus, liver, and caecum compared to the diet supplemented with cellulase alone (T1 vs T2, T3, T4; $P < 0.01$). This finding is consistent with carnitine's function in transporting long-chain fatty acids across the inner mitochondrial membrane for β -oxidation, thereby enhancing cellular energy availability (Ahmadipour *et al.*, 2025; Wahid *et al.*, 2023). Improved energy metabolism in digestive tissues may promote tissue growth and functional development (Huang *et al.*, 2022). In the ventriculus, enhanced development can improve feed efficiency by reducing the metabolic costs of mechanical digestion, ultimately supporting greater tissue growth. In the liver, carnitine supplementation may improve lipid metabolism and reduce fat accumulation, thereby improving liver function (Eskandani *et al.*, 2022; Mirzaei *et al.*, 2022). This finding aligns with previous research indicating that *L*-carnitine supplementation affects organ weights and tissue development in poultry (Akhoondzadeh *et al.*, 2023).

Supplementation with fish oil to a diet containing cellulase enzyme and *L*-carnitine (T3 and T4) resulted in greater increases in the relative weights of the ventriculus, liver, and caecum compared to *L*-carnitine alone (T2 vs T3 and T4; $P < 0.01$). Fish oil is rich in long-chain omega-3 PUFAs, particularly EPA and DHA (Sudibya *et al.*, 2017). These omega-3 PUFAs have been shown to influence metabolic processes and tissue development. In the ventriculus, these fatty acids are incorporated into muscle cell membranes, which may affect tissue growth and function (Thanabalan and Kiarie, 2021). Additionally, in the liver, omega-3 fatty acids provide hepatoprotective effects by reducing inflammation and oxidative stress while supporting normal liver metabolism (Rahbari *et al.*, 2025). The increase in caecal weight associated with fish oil supplementation corresponds with the longer caecal length observed in these treatments (Table 4) and suggests an enhanced capacity for microbial fermentation. Omega-3 fatty acids are known to influence the composition of the cecal microbiota, promoting beneficial bacteria that support mucosal health and tissue development (Jadhav *et al.*, 2025).

The results of this experiment confirmed previous observations indicating that dietary supplementation with unsaturated fatty acids affects organ weights and tissue development in poultry (Tompkins *et al.*, 2024). Notably, *L*-carnitine supplementation led to an increase in small intestine weight (T1 vs. T2, T3, T4; $P < 0.05$). However, the addition of fish oil did not provide any improvement beyond that already achieved with *L*-carnitine (T2 vs. T3, T4; $P > 0.05$). This pattern suggests that *L*-carnitine has a significant impact on small intestinal tissue development, and adding fish oil, while beneficial for other organs, did not further stimulate small intestinal growth under the conditions of this study. The increase in small intestinal weight likely reflects both hypertrophy and hyperplasia in response to increased nutrient absorption demand, as supported by enhanced energy availability from *L*-carnitine-mediated fatty acid oxidation (Ahmadipour *et al.*, 2025; Perim *et al.*, 2024; Tompkins *et al.*, 2024).

The lack of difference in the effects of tuna and lemuru fish oils on digestive organ weight (T3 vs. T4; $P > 0.05$) likely reflects their similar nutrient compositions, particularly their metabolizable energy content (Sudibya *et al.*, 2015). Furthermore, the comparable fatty acid profiles, including EPA and DHA contents, may also contribute to the similar effects on organ weights observed between T3 and T4. The stepwise pattern of improvement from T0 to T4 indicates the cumulative effects of these additives on digestive organ development. The increased relative weights of the ventriculus, liver, and cecum, along with the longer digestive tract length previously observed, suggest that the combined supplementation of cellulase, *L*-carnitine, and fish oil promotes comprehensive gastrointestinal development in male Tegal ducks.

INTESTINAL MORPHOLOGY

Supplementation with cellulase enzyme alone reduced CD and increased the VH:CD ratio in the duodenum and jejunum compared to the unsupplemented control (T0 vs T1, T2, T3, T4; Tables 8 and 9). Exogenous enzymes, such as cellulase, are primarily used to improve nutrient digestibility by breaking down fibrous components, particularly in fiber-rich diets like rice bran (Al-Arif *et al.*, 2025; Bedford and Apajalahti, 2022). The reduction in CD following cellulase supplementation suggests a decrease in epithelial cell turnover. CD reflects the proliferative activity of the intestinal epithelium; shallower crypts indicate reduced cell loss and better maintenance of villus integrity. This allows energy to be redirected toward absorptive functions rather than rapid cellular regeneration (Xu *et al.*, 2024). The lack of effect on VH, VW, and VSA implies that the primary impact of cellulase on intestinal morphology was to stabilize the crypt compartment rather than elongate the villi. Supporting this finding, previous

research has shown that dietary fiber-degrading enzyme supplementation in poultry improves gut health primarily by modulating microbial fermentation and the production of short-chain fatty acids (SCFAs), which influence crypt dynamics and epithelial turnover (Borda-Molina *et al.*, 2021; Liu *et al.*, 2021).

Carnitine has the potential to modify intestinal structure due to its role in energy metabolism and cellular protection. In this study, supplementing *L*-carnitine to a diet containing a cellulase enzyme (T2) resulted in a further reduction in CD and an increase in the VH:CD ratio in the duodenum and jejunum compared to the diet with only cellulase (T1 vs. T2, T3, T4; $P < 0.05$). This response aligns with carnitine's role in facilitating the transport of long-chain fatty acids across the inner mitochondrial membrane for β -oxidation, thereby improving cellular energy availability and reducing oxidative stress (Ahmadipour *et al.*, 2025). Enhanced energy metabolism in intestinal epithelial cells may decrease the need for compensatory cell proliferation in response to cellular stress. This leads to shallower crypts and a more favorable VH:CD ratio (Azizi-Chekosari *et al.*, 2021; Eskandani *et al.*, 2022). These findings support previous observations that *L*-carnitine supplementation positively influences intestinal morphology in poultry, increasing VH and the VH:CD ratio while reducing CD (Bahrapour *et al.*, 2024; Tang *et al.*, 2025). Overall, carnitine supplementation appears to enhance the functional capacity of the small intestine by promoting more efficient epithelial renewal and maintaining a larger absorptive surface area at a lower energy cost.

Supplementation with fish oil in a diet containing the cellulase enzyme and *L*-carnitine (T3 and T4) further improved intestinal morphology. Specifically, there were notable reductions in CD and an increase in the VH:CD ratio in both the duodenum and jejunum. Additionally, the lemuru oil resulted in a specific increase in the ileal VH:CD ratio compared to the use of *L*-carnitine alone (T2 vs. T3 and T4; $P < 0.01$ for duodenal CD and VH:CD ratio; $P < 0.01$ for jejunal VH:CD ratio; $P < 0.05$ for ileal VH:CD ratio in T4). The PUFAs in fish oil, particularly EPA and DHA (Sudibya *et al.*, 2017), are known to enhance intestinal health by reducing pro-inflammatory responses, improving tight junction integrity, and supporting the structural and functional integrity of the intestinal epithelium (Tompkins *et al.* (2024); Thanabalan and Kiarie, 2021). The SCFAs are absorbed by enterocytes as a primary energy source and activate signaling pathways, such as GPR signaling and HDAC inhibition, which regulate intestinal epithelial cell proliferation, immune cell differentiation, and gut homeostasis in poultry (Liu *et al.*, 2021). The results of this experiment confirm earlier findings that dietary fish oil supplementation improves intestinal morphology in

poultry. This is particularly evident in the increases of VH and the VH:CD ratio, alongside reductions in CD (Rahbari *et al.*, 2025).

A source-specific effect was observed in the ileum, where lemuru fish oil (T4) produced a significantly greater VH:CD ratio than tuna fish oil (T3 vs. T4; $P < 0.05$). This difference may be linked to the slightly higher DHA content in lemuru fish oil, which is approximately 17.40%, compared to tuna fish oil at around 14.64%. Both oils, however, contain similar levels of EPA, with lemuru fish oil at 34.17% and tuna fish oil at 33.6% (Sudibya *et al.*, 2017). The higher DHA content may confer enhanced anti-inflammatory and membrane-stabilizing effects in the distal small intestine, where the epithelial barrier is more susceptible to luminal challenges (Li *et al.* 2026). The absence of significant effects on VW and VSA across all treatments indicates that supplementation primarily influences the VH rather than the VW. This observation aligns with the notion that VH is a key indicator of absorptive surface area, more responsive to nutritional interventions, whereas VW is likely more tightly regulated by developmental and structural constraints (Wang *et al.*, 2025). Two-dimensional measurements may not fully capture the three-dimensional improvements in villus architecture, such as increased villus density or enhanced microvillus development (Eskandani *et al.*, 2022). The segment-specific patterns observed indicate more pronounced effects in the proximal segments (duodenum and jejunum) for some parameters, while distal effects (ileum) were noted for others. The duodenum and jejunum, being the primary sites for nutrient digestion and absorption, showed consistent improvements in both CD and the VH:CD ratio. In contrast, the ileum, which plays a more significant role in immune surveillance and the absorption of specific nutrients like bile acids and vitamin B12, exhibited selective responses. Improvements in the VH:CD ratio were mainly seen in T4. Notably, although the VH:CD ratio increased across all segments with supplementation, the lack of significant changes in VH or CD alone in some segments underscores the VH:CD ratio's value as an integrated indicator of intestinal health. This ratio captures the balance between absorptive capacity (VH) and tissue turnover costs (CD).

In addition, this study had several limitations. The EPA and DHA contents of the fish oil were not directly analyzed, which means the exact omega-3 profile of the diets could not be confirmed. Additionally, the sample size for the intestinal morphology analysis was relatively small, potentially limiting the statistical power of the findings. Moreover, the diets were not fully isoenergetic, as the inclusion of fish oil increased the dietary energy density, which may have influenced some of the observed responses.

To address these issues, future studies should analyze the EPA and DHA content of the fish oil sources, use larger sample sizes for evaluating intestinal morphology, and formulate isoenergetic diets to better differentiate the effects of omega-3 fatty acids from those of dietary energy.

CONCLUSIONS

Combined supplementation led to greater body weight gain than in the control group, primarily due to the inclusion of cellulase and *L*-carnitine. However, fish oil supplementation did not provide additional growth benefits beyond those of *L*-carnitine, with only limited effects on specific ileal morphology parameters. The responses included improvements in the length of the digestive tract, relative weights of digestive organs, and several intestinal histomorphological indices, particularly the depth of crypts and the ratio of villus height to crypt depth. It is important to interpret these findings cautiously, as diets containing fish oil had higher metabolizable energy and crude fat contents than those without it. This makes it challenging to differentiate the specific effects of omega-3 fatty acids from those of increased nutrient density. Feed intake, feed conversion ratio, protein efficiency ratio, and energy efficiency ratio were not significantly affected by the treatments. In summary, supplementation with cellulase and *L*-carnitine may promote growth and intestinal morphology in male Tegal ducks. However, further studies using isoenergetic diets, larger sample sizes, and verified fatty acid profiles are needed to confirm the specific contributions of fish oil and its practical applications.

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NOVELTY STATEMENT

The primary novelty of this study lies in being the first investigation to evaluate the combined effects of cellulase, *L*-carnitine, and fish oil (tuna and lemuru) on growth performance and intestinal morphology in male Tegal ducks. Unlike previous research that examined these feed additives in isolation or in other poultry species, this study demonstrates that supplementation with 0.1% cellulase and 0.003% *L*-carnitine significantly improves body weight gain, digestive tract length, relative organ weights, and intestinal morphology, as indicated by reduced crypt depth and increased villus height-to-crypt depth ratio. Furthermore, the inclusion of 2% fish oil (either tuna or

lemuru) offers no additional improvement in growth performance beyond the combination of cellulase and *L*-carnitine, exerting only limited effects on ileal morphology. These findings contribute to the development of more efficient and cost-effective feeding strategies for local duck production systems in Indonesia that rely on fibrous feed ingredients.

AUTHORS CONTRIBUTIONS

Neldino Soares Sarmento: Conceptualization; Methodology; Investigation; Formal analysis; Writing – original draft; Visualization.

Sudibya: Conceptualization; Methodology; Supervision; Project administration; Funding acquisition; Resources; Writing – review and editing.

Adi Ratriyanto: Validation; Data curation; Formal analysis; Writing – review and editing; Visualization.

ANIMAL ETHICS

The experimental procedures in this study have been approved by the Animal Research Ethics Committee of Sebelas Maret University (333/UN27.14/TA.00.03/2025).

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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