

Morphometry of Body and Organ Indices of Broiler Chickens [*Gallus gallus gallus* (Linnaeus, 1758)] After Administration of Blue-spotted Maskray Liver Extract [*Neotrygon caerulopunctata* (Last, White & seret, 2016)]

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Abstrak

Industri ayam broiler merupakan penyedia utama protein bagi masyarakat Indonesia. Agar pertumbuhannya optimal, ayam broiler membutuhkan pakan dengan kandungan protein tinggi yang saat ini masih memiliki harga mahal karena bahan bakunya sebagian besar merupakan produk impor. Limbah hasil pengolahan ikan, seperti hati ikan pari totol biru (*Neotrygon caerulepunctata*), kaya akan protein dan dapat menjadi alternatif pakan yang ekonomis untuk ayam broiler. Penelitian ini bertujuan untuk mengevaluasi dampak pemberian ekstrak hati ikan pari totol biru terhadap struktur morfometri boiler dan Indeks organ. Sebanyak 300 ayam broiler jantan dan betina strain Cobb 500 DOC dipelihara hingga umur 16 hari dengan menggunakan Rancangan Acak Lengkap (RAL) dan 4 perlakuan, masing-masing dengan 5 ulangan yang terdiri dari 15 ekor ayam. Perlakuan pemberian ekstrak hati pari biru (EHP) diberikan per kg pakan basal (PB) dengan variasi Kontrol (0% EHP/kg PB), T1 (0,5% EHP/kg PB), T2 (1% EHP/kg PB), dan T3 (2% EHP/kg PB). Parameter yang diamati mencakup morfometri, indeks organ seperti berat usus, panjang usus, panjang otot, luas otot, berat bursa, dan berat lien. Hasil penelitian menunjukkan bahwa kelompok perlakuan T3 dengan pemberian Ekstrak Hati Pari sebesar 2,0% menunjukkan peningkatan signifikan pada morfometri, indeks organ seperti berat usus, panjang usus, panjang otot, luas otot, berat bursa, dan berat lien dibandingkan dengan kelompok Kontrol (0% EHP). Kelompok T3 juga menunjukkan performa pertumbuhan yang lebih baik dibandingkan dengan kelompok Kontrol. Dengan demikian, dapat disimpulkan bahwa pemberian EHP pada konsentrasi optimal 2,0% mampu meningkatkan struktur morfometri dan indeks organ usus halus ayam broiler.

Kata kunci— ayam broiler, ekstrak hati ikan pari totol biru

Abstract

The broiler chicken industry is a primary provider of protein for the Indonesian population. For optimal growth, broiler chickens require feed with a high protein content, which currently remains expensive due to the majority of its raw materials being imported

products. Waste from fish processing, such as the liver of the blue-spotted stingray (*Neotrygon caerulepunctata*), is rich in protein and can serve as an economical alternative feed for broiler chickens. This study aims to evaluate the impact of blue-spotted stingray liver extract on the morphometric structure and organ indices of broilers. A total of 300 male and female Cobb 500 DOC broiler chickens were raised until 16 days old using a Completely Randomized Design (CRD) with 4 treatments, each with 5 replications consisting of 15 chickens. The treatments of blue-spotted stingray liver extract (EHP) were administered per kg of basal feed (PB) with variations of Control (0% EHP/kg PB), T1 (0.5% EHP/kg PB), T2 (1% EHP/kg PB), and T3 (2% EHP/kg PB). Parameters observed included morphometry and organ indices such as intestinal weight, intestinal length, muscle length, muscle area, bursa weight, and spleen weight. The results showed that the T3 treatment group with 2.0% stingray liver extract demonstrated significant improvements in morphometry and organ indices such as intestinal weight, intestinal length, muscle length, muscle area, bursa weight, and spleen weight compared to the Control group (0% EHP). The T3 group also exhibited better growth performance compared to the Control group. Therefore, it can be concluded that administering EHP at an optimal concentration of 2.0% can enhance the morphometric structure and organ indices of the small intestine in broiler chickens.

Keywords— broiler chicken, liver extract, blue spotted stingray

1. INTRODUCTION

Demand for poultry products such as broiler chicken meat increases annually. According to the Central Statistics Agency (BPS), broiler chicken production in Indonesia reached 3.77 million tons in 2022, marking an 18.20% increase compared to the previous year's production of 3.18 million tons (BPS, 2022). This rising demand reflects growing public awareness of the importance of health and nutritious food consumption. Therefore, poultry farmers must prioritize the quality of chickens raised to meet the public's demand for high-quality chicken meat. One factor influencing chicken quality is the composition of the feed provided.

High-quality feed must meet the nutritional needs of chickens, including protein, carbohydrates, fats, vitamins, minerals, and other essential elements. Additionally, the feed composition should align with the chickens' age development and the desired rearing objectives. Stingrays are frequently targeted by fishermen (Tirtadanu *et al.*, 2019), reflecting increased demand for processed stingray products (Hermansyah *et al.*, 2022). Stingray liver is notably high in fat content (Sellami *et al.*, 2018), with around 54% fat content in *Dasyatis* sp. stingray liver (Joesidawati, 2022). Similar proportions of fat content are found in *Himantura bleekeri* stingray liver (Le Néchet *et al.*, 2007). Therefore, research is needed to understand the impact of blue-spotted maskray liver extract [*Neotrygon caerulepunctata* (Last, White, and Secrét, 2016)] on the morphometric structure and organ indices of broiler chickens [*Gallus gallus gallus* (Linnaeus, 1758)]. The findings of this study will provide valuable insights for developing more environmentally friendly poultry feed and enhancing the performance of broiler chickens [*Gallus gallus gallus* (Linnaeus, 1758)].

2. RESEARCH METHODOLOGY

2.1 Time and Location of Research

This research was conducted from December 2023 to March 2024 in the Laboratory of Animal Structure and Development, Faculty of Biology, Universitas Gadjah Mada, Sleman Yogyakarta.

2. 1.1 Steps of Research

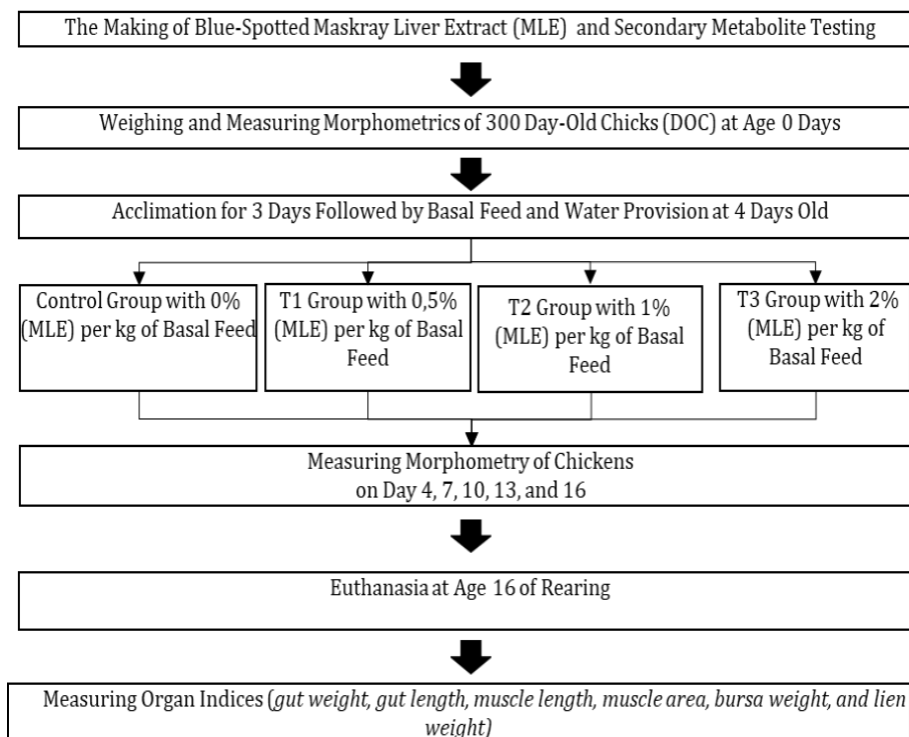


Figure 1.Flowchart of the Research Study

2. 2 Research Procedure

The process of making blue-spotted maskray liver extract involves first drying the liver with tissue paper and then grinding it using a blender. After blending, the liver is extracted using the maceration method with acetone at a ratio of 1:2 (liver:acetone). The mixture is stirred, wrapped, and left for 24 hours. The next day, the mixture is squeezed by hand or further macerated using a clean filter cloth over a funnel into an Erlenmeyer flask. The liquid is filtered slowly until fully separated. The resulting macerate is then evaporated to obtain a thick extract. The thick extract is tested for saturated and unsaturated fatty acid content. After the blue-spotted maskray liver is extracted, the next steps are acclimation, maintenance, and feeding. A total of 300 day-old chicks (DOC) are divided into 4 groups: K = Control (basal feed); T1 = Treatment 1 (0.5% liver extract/kg basal feed); T2 = Treatment 2 (1% liver extract/kg basal feed); T3 = Treatment 3 (2% liver extract/kg basal feed), with 5 repetitions, each group containing 15 DOCs. The chicks are acclimated for up to 3 days with intensive care in a container measuring 82.5 x 59.5 x 45.4 cm (150 liters), equipped with an incandescent lamp to maintain a stable temperature range of 31-33°. Acclimation and maintenance are conducted at the Sawitsari Research Station, Faculty of Biology, Gadjah Mada University. Morphometric measurements are taken every 2 days until the chicks reach 16 days of age using midline. There are 7 variables for measuring chick morphometrics based on the method by Suparyanto et al. (2004), including body length, chest circumference, thigh length, body height, lung length, head length, neck length, and wing length. Each DOC is weighed and its morphometric characteristics measured on days 0 (after hatching),

4, 7, 10, 13, and 16. At 16 days old, the chicks are fasted for 6 hours before 5 chicks from each treatment group are randomly selected for termination by decapitation. The ventral part of the chicks is operated on using scissors and a scalpel to separate the small intestine, muscle, spleen, and bursa from other organs, and they are washed with physiological saline (0.9% NaCl). These organs are then cleaned, weighed, and measured for length.

3. RESULTS AND DISCUSSION

3.1 The saturated and unsaturated fatty acid content of blue-spotted maskray liver extract

The study by Widiyanto *et al.* (2015) stated that the extraction of mondol ray liver (*Himantura gerrardi*) has a high oil content (about 54% of the raw material weight) with unsaturated fatty acids such as EPA (4%) and DHA (16%). Kebir *et al.* (2007) reported that the liver of three ray species (*Rhinobatos cemiculus*, *Rhinoptera marginata*, and *Dasyatis marmorata*) caught in the Republic contains secondary metabolites in the form of omega-3 fatty acids, especially EPA and DHA. Lukas *et al.* (2011) stated that omega-3 fatty acids can increase bone mass in animals during their growth period. Body morphometrics of broilers. Omega-3 fatty acids (PUFA ω -3), such as EPA and DHA, can help maintain the strength of human intestinal epithelial cells. When omega-3 is present in sufficient quantities, the epithelial cells in the small intestine are better able to function in nutrient absorption. PUFA ω -3 also plays a role in stopping the production of inflammation-inducing compounds and triggering the production of anti-inflammatory compounds in the body, ensuring good intestinal growth and keeping inflammation under control (Durkin *et al.*, 2021). Omega-3 is also referred to as a prebiotic for gut microbiota. Prebiotics are substances that help the growth or activity of beneficial microorganisms in the human gut (Costantini *et al.*, 2017).

3.2 Body morphometrics of broilers

This study was conducted over 16 days, including a 3-day acclimation period. The first three weeks are a crucial phase in the nutritional needs of broilers (brooding phase), accounting for approximately 17% of the total growth cycle (Fatmaningsih *et al.*, 2016). During this phase, broilers experience rapid early growth, with a high demand for nutrients to support the development of muscles, bones, and vital organs. Body weight during the first 7 days significantly influences the final body weight, although there is no direct causal relationship with body weight at day 0 (Payte *et al.*, 2022). Successful management during this period can increase body weight by up to 50-fold by the age of 35 days. Digestive tract development during the first week also significantly contributes to overall body growth. The first 1–10 days are critical, covering 20–25% of the production cycle and involving significant metabolic changes and digestive tract development, which affect broiler growth and health throughout their life (Colombino *et al.*, 2023). Therefore, a 3-week study period is highly relevant for evaluating the impact of feed on broiler morphometric growth, as this period sees a significant increase in both nutrient requirements and growth.

The morphology of broiler chickens is influenced by the increase in their body weight. Therefore, one way to observe poultry growth is through morphological measurements. Morphological measurements are also commonly used as indicators to assess the quality of chicken growth performance (Willemsen *et al.*, 2008; Michalczyk *et al.*, 2011). The parameters used in measuring the morphometrics of broiler chickens refer to previous research, including body length, body height, chest circumference, wing length, neck length, and thigh length (Blatama *et al.*, 2023).

Table 1. Morphometrics of 16-Day-Old Broilers After Administration of Blue-Spotted Maskray Liver Extract

Days-	Variable (cm)	Treatment (Mean \pm SEM)				P-value
		C (Basal feed)	T1 (0,5%)	T2 (1%)	T3 (2%)	
4	Body Length	7,58 \pm 0,138 ^{ns}	7,567 \pm 0,117 ^{ns}	7,487 \pm 0,096 ^{ns}	7,607 \pm 0,132 ^{ns}	0,910
	Chest Circumference	11,8 \pm 0,293 ^{ns}	11,84 \pm 0,227 ^{ns}	11,733 \pm 0,358 ^{ns}	11,687 \pm 0,265 ^{ns}	0,983
	Thigh Length	5,44 \pm 0,205 ^{ns}	5,467 \pm 0,19 ^{ns}	5,513 \pm 0,148 ^{ns}	5,513 \pm 0,164 ^{ns}	0,989
	Body Height	15,26 \pm 0,235 ^{ns}	15,36 \pm 0,232 ^{ns}	15,38 \pm 0,22 ^{ns}	15,407 \pm 0,209 ^{ns}	0,970
	Beak Length	1,873 \pm 0,038 ^{ns}	2 \pm 0,053 ^{ns}	1,933 \pm 0,05 ^{ns}	1,94 \pm 0,038 ^{ns}	0,285
	Head Length	2,54 \pm 0,137 ^a	4,013 \pm 1,5 ^{ns}	2,827 \pm 0,067 ^{ns}	3,233 \pm 0,081 ^{ns}	0,546
	Neck Length	3,573 \pm 0,112 ^{ns}	3,593 \pm 0,094 ^{ns}	3,573 \pm 0,145 ^{ns}	3,62 \pm 0,107 ^{ns}	0,991
	Wing Length	6,673 \pm 0,224 ^{ns}	6,6 \pm 0,213 ^{ns}	6,693 \pm 0,131 ^{ns}	6,733 \pm 0,201 ^{ns}	0,970
7	Body Length	8,507 \pm 0,184 ^a	8,927 \pm 0,173 ^{ab}	9,093 \pm 0,157 ^b	9,32 \pm 0,209 ^b	0,02
	Chest Circumference	13,4 \pm 0,281 ^a	15,833 \pm 0,252 ^b	16,033 \pm 0,265 ^b	16,127 \pm 0,257 ^b	0,000
	Thigh Length	6,433 \pm 0,131 ^a	6,68 \pm 0,258 ^a	6,967 \pm 0,116 ^{ab}	7,28 \pm 0,2 ^b	0,013
	Body Height	16,4 \pm 0,276 ^a	17,647 \pm 0,257 ^b	17,74 \pm 0,161 ^b	18 \pm 0,189 ^b	0,000
	Beak Length	2,093 \pm 0,048 ^a	2,54 \pm 0,086 ^b	2,567 \pm 0,071 ^b	2,573 \pm 0,065 ^b	0,000
	Head Length	2,593 \pm 0,102 ^a	2,62 \pm 0,116 ^{ab}	2,947 \pm 0,099 ^b	3,32 \pm 0,146 ^c	0,000
	Neck Length	5,240 \pm 0,517 ^a	5,613 \pm 0,687 ^{ab}	5,867 \pm 0,561 ^b	5,940 \pm 0,526 ^b	0,007
	Wing Length	8,387 \pm 1,483 ^a	9,100 \pm 1,515 ^{ab}	9,380 \pm 0,857 ^{bc}	10,107 \pm 1,014 ^c	0,005
10	Body Length	10,087 \pm 0,2 ^a	10,473 \pm 0,272 ^{ab}	10,8 \pm 0,232 ^b	11,067 \pm 0,189 ^b	0,021
	Chest Circumference	17,027 \pm 0,255 ^a	17,4 \pm 0,184 ^{ab}	17,967 \pm 0,256 ^b	18,733 \pm 0,172 ^c	0,000
	Thigh Length	7,42 \pm 0,115 ^a	7,887 \pm 0,222 ^{ab}	7,927 \pm 0,205 ^{ab}	8,293 \pm 0,132 ^b	0,009
	Body Height	18,5 \pm 0,152 ^a	19,46 \pm 0,316 ^b	19,8 \pm 0,269 ^b	20,567 \pm 0,171 ^c	0,000
	Beak Length	2,493 \pm 0,048 ^a	2,547 \pm 0,052 ^a	2,6 \pm 0,06 ^a	2,753 \pm 0,047 ^b	0,006
	Head Length	3,113 \pm 0,076 ^a	3,393 \pm 0,055 ^b	3,433 \pm 0,113 ^b	3,473 \pm 0,104 ^b	0,027
	Neck Length	6,507 \pm 0,465 ^a	6,777 \pm 0,379 ^{ab}	6,817 \pm 0,425 ^{ab}	6,933 \pm 0,368 ^b	0,046
	Wing Length	11,073 \pm 0,993 ^a	12,073 \pm 1,676 ^b	13,140 \pm 1,093 ^c	13,580 \pm 1,170 ^c	0,000
13	Body Length	11,16 \pm 0,262 ^a	11,953 \pm 0,142 ^{ab}	11,64 \pm 0,164 ^b	13,307 \pm 0,128 ^c	0,000
	Chest Circumference	19,867 \pm 0,318 ^a	20,6 \pm 0,268 ^{ab}	21 \pm 0,272 ^b	22,467 \pm 0,165 ^c	0,000
	Thigh Length	8,753 \pm 0,195 ^a	9,133 \pm 0,15 ^{ab}	9,233 \pm 0,118 ^b	10,06 \pm 0,107 ^c	0,000
	Body Height	22 \pm 0,28 ^a	22,633 \pm 0,179 ^b	22,767 \pm 0,182 ^b	23,367 \pm 0,186 ^b	0,000
	Beak Length	2,947 \pm 0,04 ^a	3,033 \pm 0,056 ^a	3,08 \pm 0,051 ^a	3,287 \pm 0,06 ^b	0,000
	Head Length	3,327 \pm 0,09 ^a	3,46 \pm 0,097 ^a	3,507 \pm 0,069 ^a	3,907 \pm 0,073 ^b	0,000
	Neck Length	7,013 \pm 0,505 ^a	7,733 \pm 0,535 ^b	7,813 \pm 0,528 ^b	7,987 \pm 0,346 ^b	0,000
	Wing Length	12,700 \pm 0,996 ^a	13,800 \pm 1,412 ^b	14,587 \pm 1,187 ^b	16,147 \pm 1,277 ^c	0,000
16	Body Length	13,133 \pm 0,237 ^a	13,72 \pm 0,231 ^a	14,627 \pm 0,328 ^b	15,807 \pm 0,252 ^c	0,000
	Chest Circumference	22,6 \pm 0,16 ^a	23,567 \pm 0,254 ^b	25,727 \pm 0,284 ^c	26,72 \pm 0,322 ^d	0,000
	Thigh Length	10,22 \pm 0,223 ^a	10,613 \pm 0,926 ^a	10,927 \pm 0,289 ^{ab}	11,533 \pm 0,232 ^b	0,004
	Body Height	22,707 \pm 0,14 ^a	23,4 \pm 0,275 ^a	24,5 \pm 0,365 ^b	25,967 \pm 0,381 ^c	0,000
	Beak Length	3,12 \pm 0,04 ^a	3,293 \pm 0,046 ^b	3,453 \pm 0,071 ^c	3,72 \pm 0,039 ^d	0,000
	Head Length	3,3 \pm 0,051 ^a	3,587 \pm 0,083 ^b	3,727 \pm 0,079 ^{bc}	3,887 \pm 0,089 ^c	0,000
	Neck Length	7,833 \pm 0,511 ^a	8,187 \pm 0,424 ^b	8,453 \pm 0,374 ^{bc}	8,533 \pm 0,387 ^c	0,000
	Wing Length	13,600 \pm 1,901 ^a	13,987 \pm 1,382 ^a	15,880 \pm 2,185 ^b	17,513 \pm 2,385 ^c	0,000

Note: Control (C): Basal Feed (BF) (without the addition of blue-spotted maskray liver extract supplement).

T1: supplementation of 0.5% Blue-Spotted Maskray Liver Extract/kg BF; T2: supplementation of 1% Blue-Spotted Maskray Liver Extract/kg BF; T3: supplementation of 2% Blue-Spotted Maskray Liver Extract/kg BF. Mean \pm Standard Error of the Mean (SEM).

a-d Different letter superscripts indicate significant differences ($P \leq 0.05$).

Morphometric measurements were conducted to determine the effect of blue-spotted maskray liver extract on broiler morphometrics. The measurement of livestock growth performance can be used as an indicator to assess the efficiency of feed utilization on livestock products (Pakage *et al.*, 2020). Body morphometrics are important indicators of bone and muscle development (Albab *et al.*, 2020). These measurements include body length, wing length, thigh length, neck length, chest circumference, and broiler height, which showed an increase in the

treatment group given blue-spotted maskray liver extract. Measurements were taken periodically at ages 4, 7, 10, 13, and 16 days. The results of broiler measurements after the administration of blue-spotted maskray liver extract at 4 days old showed no significant difference compared to the control group. However, morphometric measurements of broilers given blue-spotted maskray liver extract at 7 days old began to show a significant increase ($P \leq 0.05$) compared to the control group. On the 16th day, the morphology of broilers in each treatment group showed greater growth compared to the control group. The differences in the rate of morphological growth were observed not only between the treatment and control groups but also among the treatment groups that received varying doses of maskray liver extract. The highest morphological growth was observed in treatment group 3, followed by treatment group 2, and lastly, treatment group 1. This trend was directly proportional to the dose of maskray liver extract administered, where treatment group 3 received the highest dose (2%), followed by treatment group 2 with 1%, and group 1 with 0.5%. Based on this data, it can be confirmed that the morphological growth of broilers in the treatment groups was indeed influenced by the administration of maskray liver extract. These findings also demonstrate that maskray liver extract has a significant effect on the morphometric growth of broilers.

3.3 Organ Index of Broiler

Table 2. Organ Index of 16-Day-Old Broilers After Administration of Blue-Spotted Maskray Liver Extract.

Variable	Treatment (Mean \pm SEM)				P-value
	C (Basal Feed)	T1 (0.5%)	T2 (1%)	T3 (2%)	
Intestine Weight (g)	21,04 \pm 0,539 ^a	25,38 \pm 0,545 ^b	27,82 \pm 0,607 ^c	30,02 \pm 0,32 ^d	0,000
Intestine Length (cm)	124,20 \pm 0,583 ^a	134,80 \pm 0,800 ^b	143,80 \pm 1,019 ^c	153,40 \pm 0,92 ^d	0,000
Muscle Weight (g)	9,83 \pm 0,29 ^a	10,68 \pm 0,52 ^{ab}	11,01 \pm 0,20 ^b	11,62 \pm 0,29 ^b	0,016
Muscle Area (μm^2)	40,979 \pm 0,509 ^a	41,577 \pm 1,287 ^a	44,487 \pm 1,154 ^b	46,522 \pm 0,648 ^b	0,003
Spleen Index (g)	0,086 \pm 0,018 ^a	0,091 \pm 0,009 ^a	0,099 \pm 0,013 ^{ab}	0,110 \pm 0,006 ^b	0,035
Bursa Index (g)	0,253 \pm 0,057 ^a	0,285 \pm 0,069 ^{ab}	0,347 \pm 0,028 ^{bc}	0,372 \pm 0,028 ^c	0,006

Note: Control (C): Basal Feed (BF) (without the addition of blue-spotted maskray liver extract supplement).

T1: supplementation of 0.5% Blue-Spotted Maskray Liver Extract/kg BF;

T2: supplementation of 1% Blue-Spotted Maskray Liver Extract/kg BF;

T3: supplementation of 2% Blue-Spotted Maskray Liver Extract/kg BF.

Mean \pm Standard Error of the Mean (SEM).

a-d Different letter superscripts indicate significant differences ($P \leq 0.05$).

Based on the results above, the organ index of the small intestine is indicated by the weight and length of the intestine. Intestinal weight showed a significantly higher difference ($P \leq 0.05$) in the P3 treatment group compared to the control group. This is consistent with the report by Kiarie & Mills (2019), which states that a larger intestinal structure indicates higher nutrient absorption. In this study, the P3 treatment group showed a greater average small intestine weight (30.02 \pm 0.32 grams) compared to the control group (21.04 \pm 0.539 grams). This aligns with the measurements of small intestine length, which were highest in the T3 treatment group (113.20 \pm 1.827 cm), while the shortest length was observed in the control group (153.40 \pm 0.92 cm). The mean (Mean \pm SEM) length and weight of the small intestine in the EHP treatment groups showed an increase with the administration of the optimal supplement dose. This is in line with the opinion of Chen *et al.* (2016), who stated that the administration of date fruit extract can increase abdominal circumference due to the enlargement of the proventriculus stomach and small intestine as a result of increased body weight in geese.

Muscle weight and area showed significantly higher differences ($P \leq 0.05$) in the P3 treatment group compared to the control group. This is in accordance with opinion Konieczka *et al.*, (2017) which states that omega-3 can enrich meat with LC-PUFA so that the resulting meat will be thicker due to thickening of the myofibers, which changes in thickness can cause

differences in the weight and area of the meat. In this research group P2 and P1 had weights that were not much different from the P3 treatment group, but group P3 treatment showed a greater mean muscle weight, namely 11.62 ± 0.29 grams, which is a very different value from the control group whose average weight was only 9.83 ± 0.29 grams. Likewise with muscle area, the P3 treatment group showed a larger mean muscle area compared to other treatments, namely: $46,522 \pm 0,648 \mu\text{m}^2$ whereas in the control group the average area produced was only $40,979 \pm 0,509 \mu\text{m}^2$. Bursa of Fabricius and spleen index increases in treatments (T1, T2, and T3) compared to control groups.

3.3 Mechanism of action of Blue-spotted maskray liver extract

In this study, significant growth could be seen in the P3 group or treatment group with the addition of 2% liver extract. The P3 group grew faster than other treatment groups as well as with the control group. This proves that the fatty acid content in stingray liver can accelerate growth in broiler chickens. Meanwhile, in the control group, the growth was still stable, although not as fast as in the treatment group with the addition of stingray liver extract. This is because the growth of living things, including broiler chickens, is not only influenced by the nutrients provided, but can also be influenced by genetics and environmental conditions (Noys *et al.*, 2017).

The significance of the growth that occurred in the treatment group proved that the administration of blue-spotted stingray liver extract up to 16 days of age was able to improve the morphometric structure and organ index of broilers. Blue-spotted maskray liver extract contains Omega-3, Omega-6, and Omega-9 fatty acids. Polyunsaturated fatty acids (PUFAs) play roles in cellular metabolism, signaling, and anti-inflammatory processes (Vaughan *et al.*, 2012). One specific polyunsaturated fatty acid, Omega-3, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), predominantly found in fish, crucially enhances muscle cell growth through several mechanisms (Calder, 2010). Docosahexaenoic acid (DHA) can increase lipid oxidation and insulin sensitivity in skeletal muscle by activating AMP-activated protein kinase (AMPK), which aids in boosting energy metabolism and muscle cell maintenance (Lam *et al.*, 2011). Moreover, the combination of Omega-3 fatty acids (EPA & DHA) can increase the expression of PGC-1 α (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1- α), a key stimulator in mitochondrial biosynthesis (Vega *et al.*, 2000). Elevated PGC-1 α not only enhances oxidative capacity within cells but also stimulates the production of uncoupling proteins in mitochondria, thereby increasing metabolic rate and energy production within cells (Esterbauer *et al.*, 1999).

Weight and size will significantly increase to facilitate digestion and nutrient absorption in growing chicks (Colombino *et al.*, 2023; Dibner & Richards, 2004). This aligns with the functional properties of PUFA as reported. Polyunsaturated Fatty Acids (PUFA) influence several biological activities, including regulation of cell membrane structure and function, intracellular signaling pathways, transcription factor activity, gene expression, and regulation of bioactive lipid mediators (Zarate *et al.*, 2017). Polyunsaturated Fatty Acids (PUFA) can be released from cell membrane phospholipids by phospholipase PLA-2 stimulation. Subsequently, PUFA can regulate gene expression directly or through cytosolic substances. Polyunsaturated Fatty Acids (PUFA) are precursors for second messenger molecules of eicosanoids and docosanoids. Thus, they play roles in inflammation, immune function, vascular health, neuronal function, and cell growth (Kalish *et al.*, 2012; Bazinet & Laye, 2014; Luchtman & Song, 2013). In muscles, PUFA plays an important role in the composition of cell membranes which helps as the body's first defense so that more myofibers are produced and make the meat thicker compared to chicken meat in general. (Konieczka *et al.*, 2017).

The balance between omega-3 (ω -3), omega-6 (ω -6), and omega-9 (ω -9) fatty acids is very important because these oils are important components for the formation of cell membranes, especially in poultry. Omega-3 functions as an anti-inflammatory and increases muscle mass by stimulating the oxidative metabolism of muscle fat (Ayubi *et al.*, 2020). In addition, omega-

3 can increase protein synthesis in muscles and improve their function (Smith *et al.*, 2011).

Omega-6 and Omega-9 also have an important role in helping growth and development. Omega-6 is needed to regulate inflammation and maintain inflammatory balance in the body. Meanwhile, Omega-9 functions to increase levels of good cholesterol (HDL) and reduce levels of bad cholesterol (LDL) in the body. Omega-6 and Omega-9 play a role in maintaining heart health, regulating blood sugar levels, and preventing stress and depression. In mammalian fetuses, omega-6 plays a role in the growth and development of the brain and vision (Diana, 2012). Apart from that, omega-9 also functions as a main component in the formation of cell membranes, supports cell integrity and optimizes cell function, so that it can improve poultry growth performance (Minich, 1997).

In immune function, dietary n-3 PUFA enrichment and conjugated linoleic acid (CLA) supplementation can modulate immune response and performance in broiler chickens, but finding the optimal balance is key. Moderate levels of n-3 PUFA enhance both performance and immune response, while CLA promotes immune tissue growth and alters immune function (He *et al.*, 2007). However, high levels of fish oil can negatively affect performance despite improving certain health markers (Saleh *et al.*, 2009). Additionally, while n-3 PUFA-rich diets may suppress some immune responses crucial for disease defense, the overall enrichment of broiler diets with PUFAs offers potential benefits for both poultry health and human consumption (Al-Khalaifah & Al-Nasser, 2021). Further research is needed to fully understand the implications of these dietary adjustments on infection risk and overall poultry health.

4. CONCLUSION

Based on the research conducted, the following conclusions can be drawn:

1. The administration of blue-spotted stingray liver extract can increase morphometric structure of broilers, specifically on body length, chest circumference, thigh length, height, lung length, head length, neck length, and wing length.
2. The administration of blue-spotted stingray liver extract can increase the growth of broiler organ indices, including intestinal weight, intestinal length, muscle weight, muscle area, spleen weight, and bursa weight.

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