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Cholesterol, fatty acid, and micronutrient profiles of IPB D1 chicken meat fed diets supplemented with lemuru fish oil and microminerals

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ABSTRACT

Chicken meat is an important source of animal protein widely consumed worldwide. The IPB D1 chicken is a crossbreed between local chickens and broilers, characterized by rapid growth, high adaptability, and desirable meat characteristics. This study aimed to analyze the effects of different feed formulations on the micronutrient content and lipid profile of IPB D1 chicken meat. A completely randomized design was used with four treatments and three replications. The dietary treatments consisted of control feed (P0), control feed supplemented with Zn and Cr (P1), feed containing lemuru fish oil (P2), and a combination of lemuru fish oil with Zn and Cr (P3). The results indicated that feed formulation significantly affected mineral content, vitamin E concentration, cholesterol level, and fatty acid composition. Treatment P3 produced the most favorable micronutrient and lipid profile, notably increasing mineral content and omega-3 polyunsaturated fatty acids while improving the omega-6 to omega-3 ratio. The P3 feed formulation was the most effective in enhancing micronutrient content and improving lipid profile characteristics of IPB D1 chicken meat.

Keywords: Chicken meat, IPB D1 chicken, Lipid profile, Micronutrients.

INTRODUCTION

Chicken meat is one of the most widely consumed sources of animal protein due to its affordability and high nutritional value. Global poultry meat consumption continues to increase, with average per capita consumption reaching approximately 19.4 kg per year in 2022, and demand projected to grow further, particularly in Asia (Worldmetrics, 2022). In Indonesia, per capita chicken meat consumption increased by 4.3% compared to the previous year, reaching 7.46 kg in 2023 (Bapenas, 2023). This rising demand reflects diverse consumer preferences for different chicken types, particularly indigenous Kampung chicken, esteemed for its unique flavour and traditional characteristics (Mahmud *et al.* 2017). Despite their superior taste, Kampung chickens generally exhibit low production and slow growth rates (Riyanti *et al.*, 2023). To address these limitations, breeding programs have been developed to produce the IPB D1 chicken, a

crossbreed between F1 Kampung × Cobb (KM) females and F1 Pelung × Sentul (PS) males (Habib *et al.*, 2020).

IPB D1 chicken is a newly established breed of superior local broiler chicken officially designated by the Decree of the Minister of Agriculture of the Republic of Indonesia No. 693/KPTS/PK.230/M/9/2019. This breed boasts several advantages, including as rapid growth, good environmental adaptability, and resistance to diseases like Tetelo or Newcastle Disease (ND) (Nepa *et al.*, 2023). IPB D1 chicken meat has been reported to have a high protein content (17.97%–18.35%) and a very low-fat content (0.15%–0.32%) (Pangestu, 2019). Additionally, IPB D1 chicken meat has a low cooking loss, a tender texture, high mineral content, and low cholesterol levels (Adelta *et al.*, 2023). Considering this potential, improving the nutritional quality of IPB D1 chicken, particularly through feed formulation, is a crucial step to produce meat with a better micromineral content and lipid profile.

Strategies for enhancing the nutritional quality of chicken meat can be implemented through the inclusion of specific lipid and micromineral sources in feed formulations. Lemuru fish oil is rich in long-chain omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have been reported to improve fatty acid composition, increase omega-3 deposition, and enhance the nutritional value of poultry meat (Wibawaningrum *et al.*, 2021; Nurnadia *et al.*, 2011). The incorporation of these bioactive fatty acids into muscle tissue may contribute to improved carcass lipid quality and a more favourable omega-6 to omega-3 ratio. However, the high degree of unsaturation of omega-3 fatty acids makes them susceptible to oxidative degradation, which may impact meat stability and shelf life.

Therefore, the inclusion of supporting microminerals such as zinc (Zn) and chromium (Cr) is essential, as these elements play roles in antioxidant defense and lipid metabolism. Zinc functions as a cofactor for superoxide dismutase, an essential antioxidant enzyme (El-Deep *et al.*, 2020), while chromium is involved in glucose and lipid metabolism and may enhance resistance to oxidative stress (Abdel *et al.*, 2019). Previous studies have suggested that the combined supplementation of omega-3 sources and antioxidant-related minerals may improve nutrient deposition and oxidative stability in poultry meat (Surai, 2020). Moreover, dietary inclusion of lemuru fish oil, particularly when balanced with other lipid sources, has been reported to influence carcass traits and reduce fat deposition in broiler meat (Nadia *et al.,* 2023), hence supporting the potential role of lemuru oil-based formulations in enhancing meat quality. Thus, the combination of lemuru fish oil with these essential minerals may provide a synergistic effect in enhancing lipid profile characteristics while maintaining oxidative stability.

Improving the nutritional quality of IPB D1 chicken meat through dietary manipulation remains an important area of research, particularly in relation to micronutrient enrichment and lipid profile modification. Lemuru fish oil has been reported to enhance omega-3 polyunsaturated fatty acid deposition in poultry meat; however, its high susceptibility to oxidation may compromise lipid stability and nutrient retention. Therefore, the inclusion of antioxidant-supporting microminerals such as zinc (Zn) and chromium (Cr) may provide additional benefits. Zinc functions as a cofactor for antioxidant enzymes such as superoxide dismutase, while chromium contributes to glucose and lipid metabolism and may help reduce oxidative stress, thereby supporting nutrient deposition in muscle tissue. Despite these potential interactions, studies evaluating the combined supplementation of lemuru fish oil with Zn and Cr in IPB D1 chicken diets remain limited. Hence, the present study aimed to investigate the effects of these dietary treatments on the mineral and vitamin contents, cholesterol concentration, and fatty acid composition of IPB D1 chicken meat.

MATERIALS AND METHODS

Preparation and Maintenance

This study used 100 IPB D1 chickens obtained from Sinar Harapan Farm, located in the Jampang Tengah Subdistrict of Sukabumi Regency, West Java, Indonesia. The chickens were raised from the 4th to the 12th week in Cage C, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University. During the rearing period, all standard operating pro-

Table 1. Nutritional Content of Experimental Diets for IPB D1 Chickens at 5–8 Weeks

Nutrient contents (DM %)	P0	P1	P2	P3
Metabolizable Energy (ME)	3153	3153	3162	3162
Crude Protein (CP)	19.10	19.10	19.22	19.22
Crude Fat (CF)	7.00	7.00	6.00	6.00
Crude Fiber (CrF)	1.11	1.11	1.14	1.14
Calcium (Ca)	1.13	1.13	1.13	1.13
Available Phosphorus	0.57	0.57	0.57	0.57

Different superscripts in the same row indicate a significant difference (P<0.05). P0 = Control diet; P1 = P0 diet supplemented with 120 ppm Zn and 400 ppb Cr; P2 = P0 diet and lemuru fish oil supplementation; P3 = P2 diet supplemented with 120 ppm Zn and 400 ppb Cr.[1]Nutrient contents were analyzed at the Feed Science and Technology Division Laboratory, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University.

Table 2. Nutritional Content of Experimental Diets for IPB D1 Chickens at 9–12 Weeks

Nutrient contents (DM %)	P0	P1	P2	P3
Metabolizable Energy (ME)	3200	3200	3208	3208
Crude Protein (CP)	17.10	17.10	17.20	17.20
Crude Fat (CF)	8	8	7	7
Crude Fiber (CrF)	1.13	1.13	1.15	1.15
Calcium (Ca)	1.07	1.07	1.07	1.07
Available Phosphorus	0.54	0.54	0.54	0.54

Different superscripts in the same row indicate a significant difference (P<0.05). P0 = Control diet; P1 = P0 diet supplemented with 120 ppm Zn and 400 ppb Cr; P2 = P0 diet and lemuru fish oil supplementation; P3 = P2 diet supplemented with 120 ppm Zn and 400 ppb Cr.[1]Nutrient contents were analyzed at the Feed Science and Technology Division Laboratory, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University.

cedures (SOPs) were implemented, including proper lighting, ventilation, and appropriate stocking density. The vaccines used were Newcastle Disease (ND) vaccine strain La Sota and Gumboro (Infectious Bursal Disease/IBD) vaccine. Vaccines were reconstituted using a commercial vaccine solvent (LD, 500 doses) and administered according to the manufacturer's instructions following standard poultry vaccination practices. In addition, vitamin supplementation was provided through drinking water with a commercial multivitamin product (Vitacik). All vaccines and supplements were obtained from licensed commercial suppliers and administered under standard veterinary guidelines.

The chickens were randomly assigned to four dietary treatment groups, each with three independent replicates , resulting a total of 12 experimental units (cages) containing five birds per cage. A completely randomized design was used, with four treatments and three replications. The experimental diets were formulated as in house rations using common feed ingredients, such as corn, soybean meal, palm oil or lemuru fish oil (depending on the treatment), a vitamin and mineral premix, and additional supporting components to meet nutrient requirements. Each cage was equipped with feeders, drinkers, and heating lamps. Feed and water were provided ad libitum throughout the experimental period.

The experimental diets were formulated based on isoenergetic and isoprotein principles to ensure a balanced nutritional intake during each growth phase (SNI, 2022; Leeson and Summers, 2005). Lemuru fish oil was used as a replacement for palm oil due to its high content of polyunsaturated fatty acids (PUFAs), particularly omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The lemuru fish oil used in this study was obtained as a byproduct of lemuru fish processing and was collected and stored under controlled conditions prior to incorporation into the diets. Micromineral supplementation, consisting of zinc (Zn) and chromium (Cr), was added following the guidelines of the NRC (1994) and the Cobb Guidebook . Lemuru fish oil was thoroughly mixed into the feed prior to feeding, while zinc and chromium were weighed and evenly blended at the specified concentrations. Feed ingredients were sourced from PT Jendela Fauna Indonesia, while lemuru fish oil and mineral

supplements were obtained from PT Nutricell Pacific. The dietary treatments consisted of a control diet (P0), P0 supplemented with 120 ppm Zn and 400 ppb Cr (P1), P0 with the addition of lemuru fish oil (P2), and P2 supplemented with 120 ppm Zn and 400 ppb Cr (P3). The nutritional composition of the experimental diets for IPB D1 chickens at 5 to 8 weeks and 9 to 12 weeks is presented in Tables 1 and 2.

All experimental procedures involving animals were approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University (Approval No. 369/KEH/SKE/IX/2025).

Slaughter and Sample Preparation

The slaughter process was conducted in accordance with SNI 3932:2008 at the end of the rearing period, when the chickens were 56 days old. Prior to slaughter, the chickens were fasted for 12 hours to reduce the contents of the digestive tract, thereby facilitating the slaughter process and maintaining carcass cleanliness. Slaughter was carried out following hygienic procedures and halal principles. After slaughter, the thigh portions were selected for analysis because they represent one of the major commercial cuts and contain a relatively higher proportion of muscle and fat, making them suitable for evaluating meat quality characteristics and nutrient composition. The thigh samples were separated from the carcass and stored at –18 °C. Before analysis, the meat samples were thawed for approximately 24 hours at room temperature and then finely ground using a blender until homogeneous, according to AOAC (2005).

Mineral Content Analysis of Thigh Meat

Mineral content (Mn, Se, Zn, Fe, and Cr) was determined using an Atomic Absorption Spectrophotometer (AAS; Shimadzu AA7000, Kyoto, Japan) according to AOAC (2005). Approximately 0.5 g of chicken meat was digested with 5 mL of concentrated HNO_3 in a microwave digester (Milestone Ethos UP, Italy) at 180–200 °C until a clear solution was obtained. The digest was subsequently diluted to 50 mL with distilled water, filtered, and analyzed by AAS at element-specific wavelengths using standard calibration curves.

Vitamin Analysis of Thigh Meat

Vitamins A and E were determined using High-Performance Liquid Chromatography (HPLC, Shimadzu Prominence LC-20AD, Japan) with a PDA detector following AOAC (2001). About 1 g of meat was saponified with ethanol, antioxidant, and KOH at 80 °C for 45 min, neutralized with acetic acid, diluted with THF: ethanol (1:1), filtered (0.45 µm), and analysed at 325 nm (vitamin A) and 290 nm (vitamin E).

Vitamin D_3 was analysed using LC-MS/MS (Shimadzu LCMS-8040, Japan) according to AOAC (2016). One gram of sample was mixed with internal standard, ethanol, pyrogallol, methanol, and KOH, homogenized, saponified, extracted, evaporated under N_2, reconstituted, filtered (0.2 µm), and injected into the LC-MS/MS.

Vitamin K_1 was determined using LC-MS/MS following AOAC (2001). One gram of sample was hydrolysed with lipase, extracted with hexane, evaporated, reconstituted with mobile phase, added with internal standard, filtered (0.2 µm), and analysed with LC-MS/MS.

Cholesterol Analysis

Cholesterol was analysed using GC-FID (Agilent 7890B, USA) according to the ASEAN Manual of Nutrient Analysis (2011) method. A cholesterol standard (≥99%, Sigma-Aldrich, USA) was prepared for the calibration curve. The sample was saponified, extracted with an organic solvent (Merck, Germany), then dried and reconstituted with dimethylformamide (DMF, HPLC grade, Merck, Germany). Derivatization was performed before injection with the addition of an internal standard. Analysis was conducted using an HP-5MS UI capillary column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies, USA), in split injection mode, with UHP nitrogen (99.999%, PT Aneka Gas Industri, Indonesia) as the carrier gas, an oven temperature of 190–315 °C, and detection using a flame ionization detector (FID).

Fatty Acid Analysis

The fatty acid profile was analysed using Gas Chromatography (GC) (Agilent 7890B, USA) equipped with a Flame Ionization Detector (FID), following the AOAC (2000) method. Lipids were extracted with methyl tert-butyl ether (MTBE) (Merck, Germany) and then trans esteri-

fied using sodium methoxide-methanol (Sigma-Aldrich, USA) to produce fatty acid methyl esters (FAME). The FAME were separated with hexane (Merck, Germany). The GC oven temperature was programmed to increase from 50 to 230 °C. A split injection mode was used, with ultra-high purity nitrogen (PT Aneka Gas Industri, Indonesia) serving as the carrier gas. Fatty acids were identified by comparing their retention times to a FAME standard mix (C4–C24, Supelco, USA) and quantified by peak area normalization (%).

Statistical Analysis

Data were expressed as means ± standard deviations (SD) from three independent replications. Prior to analysis, the assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk test and Levene's test, respectively. Differences among treatment means were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected at $P < 0.05$, the means were further compared using Tukey's post hoc test. All statistical analyses were performed using RStudio (version 2025.09).

RESULTS AND DISCUSSION

Manganese (Mn) Content of Thigh Meat

The manganese (Mn) content in the thigh meat of IPB D1 chickens showed clear variations across treatments (Figure 1). The lowest Mn content was found in the control group (P0) at 0.115 ± 0.005 mg kg^{-1}. The P1 treatment was slightly higher at 0.122 ± 0.009 mg kg^{-1}, but this was not significantly different from P0. The Mn content increased significantly in P2 to 0.160 ± 0.011 mg kg^{-1} and reached its highest value in P3 at 0.175 ± 0..017 mg kg^{-1}. Statistical analysis showed that the Mn content in P2 and P3 was significantly different ($P<0.05$) compared to P0 and P1, while P0 and P1 were not significantly different from each other.

The increase in Mn content from P0 (0.115 mg kg^{-1}) to P2 (0.160 mg kg^{-1}) represents an increase of approximately 39.1%. A further increase was seen in P3 (0.175 mg kg^{-1}), which was 52.2% higher than the P0 control. The difference between P2 (0.160 mg kg^{-1}) and P3 (0.175 mg kg^{-1}) was relatively small, at about 9,4%, although both remained higher than P0 and P1. The increased Mn content observed in P3 may be attributed to the combined effects of polyunsaturated fatty acids (PUFAs) from lemuru fish oil and Zn and Cr supplementation, which may enhance mineral bioavailability by improving intestinal absorption (Al-Khalifah *et al.*, 2020). Although the Mn concentration in fish oil is relatively low, its bioactive compounds may still contribute to mineral deposition in muscle tissue (Nurnadia *et al.*., 2011).

The higher Mn content observed in P2 (0.160 mg kg^{-1}) and P3 (0.175 mg kg^{-1}) may be associated with enhanced mineral deposition in diets containing lemuru fish oil. Although the Mn content of fish oil itself is relatively low, treatments supplemented with lemuru fish oil exhibited greater Mn accumulation than P0 (0.115 mg kg^{-1}) and P1 (0.122 mg kg^{-1}). This pattern suggests that dietary modification influenced Mn retention in muscle tissue. In P3, the combined inclusion of lemuru fish oil with Zn and Cr supplementation may have supported a more favourable metabolic environment for mineral deposition. Zinc contributes to antioxidant enzyme function, while chromium plays a role in nutrient metabolism, and together they may enhance trace mineral utilization in muscle tissue. This is reflected in the clear difference between the groups without supplementation (P0 at 0.115 mg kg^{-1} and P1 at 0.122 mg kg^{-1}) and the treatments containing lemuru fish oil (P2 and P3, above 0.160 mg kg^{-1}).

The Mn content in this study remains well below the maximum threshold set by EFSA (2023), which is 8 mg kg^{-1}. Even at the highest value in treatment P3, at 0.175 mg kg^{-1}, the content was still very low compared to this limit. The Mn levels obtained in this study are higher than the results from the USDA (2019), which reported an Mn content in chicken thighs of 0.130 mg kg^{-1}. The contribution to the recommended daily allowance is also relatively small, at only about 0,5% of the daily RDA for P0 (0.115 mg kg^{-1}) and increasing to about 0.82% for P3 (0.175 mg kg^{-1}). Thus, despite the significant increase in Mn content in P2 and P3, IPB D1 chicken meat remains safe for consumption and has the potential to be an additional source of micronutrients for consumers.

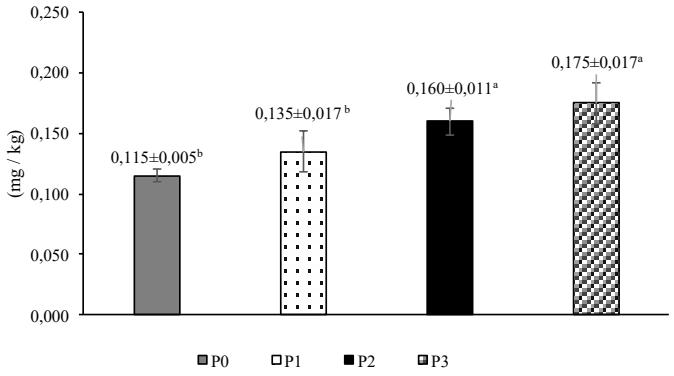

Figure 1. Manganese (Mn)

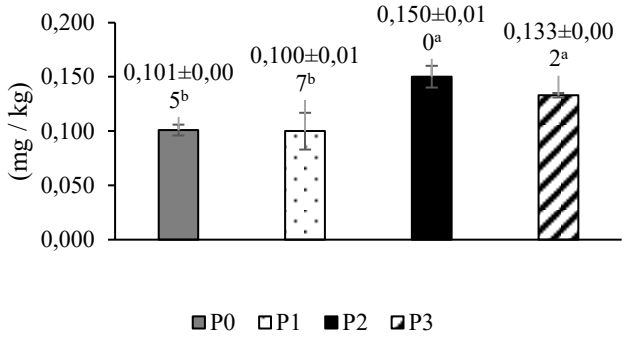

Figure 2. Selenium (Se)

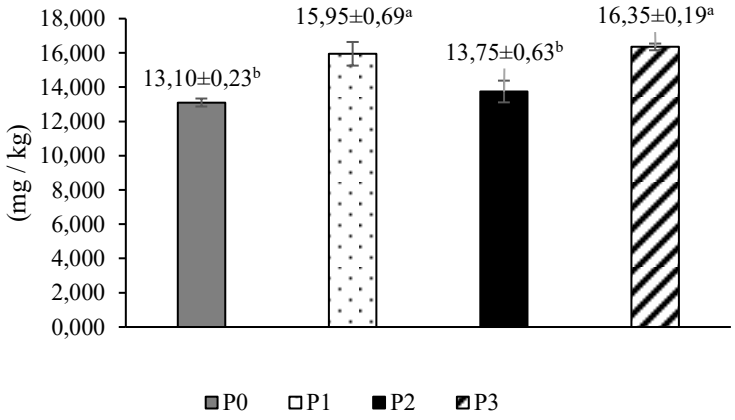

Figure 3. Zinc (Zn)

Selenium (Se) Content of Thigh Meat

The selenium (Se) content in the thigh meat of IPB D1 chickens showed variation among treatments (Figure 2). The lowest Se content was found in P0 at 0.101 ± 0.005 mg kg^{-1}, followed by P1 at 0.110 ± 0.008 mg kg^{-1}. Se content increased in P2 to 0.150 ± 0.010 mg kg^{-1}, then slightly decreased in P3 to 0.128 ± 0.007 mg kg^{-1}. Statistical analysis showed that P2 was significantly different (P<0,05) from P0 and P1, while P3 was not significantly different from the other treatments. This indicates that the addition of lemuru fish oil was more effective in increasing the Se content in the meat compared to supplementation with Zn and Cr alone. Lemuru fish oil contains PUFAs and minerals like Se, Mn, and Zn that increase mineral bioavailability (Nurnadia *et al.*, 2011; Al-Khalifah *et al.*, 2020).

The increase in Se content from P0 (0.101 mg kg^{-1}) to P2 (0.150 mg kg^{-1}) represents a rise of approximately 48.5%. The difference in Se content between P1 (0.110 mg kg^{-1}) and P2 (0.150 mg kg^{-1}) was also quite noticeable, at 36.4%. Meanwhile, the Se content in P3 (0.128 mg kg^{-1}) decreased by about 14.7% compared to P2 but remained 26.7% higher than P0. This pattern indicates that the PUFAs and minerals present in lemuru fish oil play a crucial role in enhancing the bioavailability of Se in chicken muscle tissue.

The higher Se content in P2 is likely related to the synergy between PUFAs and the essential minerals found in fish oil, including Se, Mn, and Zn. The bioactive compounds in lemuru fish oil can improve intestinal membrane permeability, thereby facilitating mineral absorption and increasing Se accumulation in tissues (Al-Khalaifah *et al.,* 2020). This explains why the P2 treatment (0.150 mg kg^{-1}) resulted in a higher Se content compared to the treatment with Zn and Cr supplementation but without fish oil (P1 at 0.110 mg kg^{-1}).

The Se content across all treatments remained well below the safe limit of 0.50 mg kg^{-1} set by the WHO (1996). In the treatment with the highest value, P2 at 0.150 mg kg^{-1}, the content only reached 30% of that threshold. Therefore, IPB-D1 chicken meat is safe for consumption. Consuming 100 g of this meat can provide about 0.010–0.015 mg of Se, equivalent to 33–50% of the daily nutritional adequacy for an adult. Thus, the increased Se content in IPB D1 chicken meat has the potential to make a significant contribution to meeting consumers micronutrient needs without posing a risk of toxicity.

Zinc (Zn) Content of Thigh Meat

The zinc (Zn) content in the thigh meat of IPB D1 chickens showed variation among treatments (Figure 3). The highest Zn content was found in P3 at 16.35 ± 0.19 mg kg^{-1}, followed by P1 at 15.35 ± 0.69 mg kg^{-1}. Lower Zn content was found in P0 and P2, with values of 13.45 ± 0.38 mg kg^{-1} and 13.69 ± 0.27 mg kg^{-1}, respectively. Statistical analysis showed that P1 and P3 were not significantly different, but both were significantly different (P<0.05) from P0 and P2. These results indicate that organic Zn supplementation, whether administered alone or in combination with lemuru fish oil, is effective in increasing Zn content in muscle tissue (Salim *et al.*, 2010).

The increase in Zn content from P0 (13.45 mg kg^{-1}) to P1 (15.35 mg kg^{-1}) was approximately 14.1%. A further increase was seen in P3 (16.35 mg kg^{-1}), which was about 21.6% higher than P0. The comparison between P2 (13.69 mg kg^{-1}) and P3 (16.35 mg kg^{-1}) also showed a clear difference of about 19.4%. These results confirm that the presence of organic Zn in P1 and the combination of organic Zn with lemuru fish oil in P3 played a role in improving Zn deposition in muscle tissue, while P2, which did not contain organic Zn supplementation, did not show a significant increase.

The higher Zn content in P1 (15.35 mg kg^{-1}) suggests the effectiveness of organic Zn supplementation, while the highest concentration observed in P3 (16.35 mg kg^{-1}) may indicate an additional effect when organic Zn is combined with lemuru fish oil. Although P1 and P3 were not statistically different, the numerical increase of approximately 1 mg kg^{-1} suggests a potential enhancement of Zn deposition under the combined treatment. The presence of dietary lipids rich in polyunsaturated fatty acids may influence intestinal membrane permeability and nutrient transport efficiency, thereby supporting improved mineral absorption and retention in muscle tissue. Consequently, the combined supplementation in P3 may create a more favourable

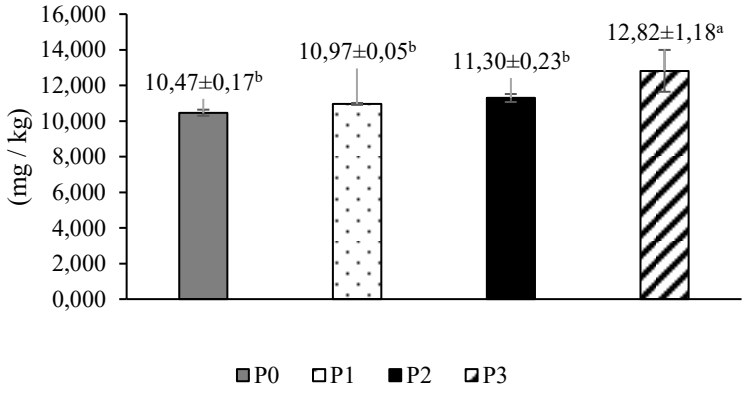

Figure 4. Iron (Fe)

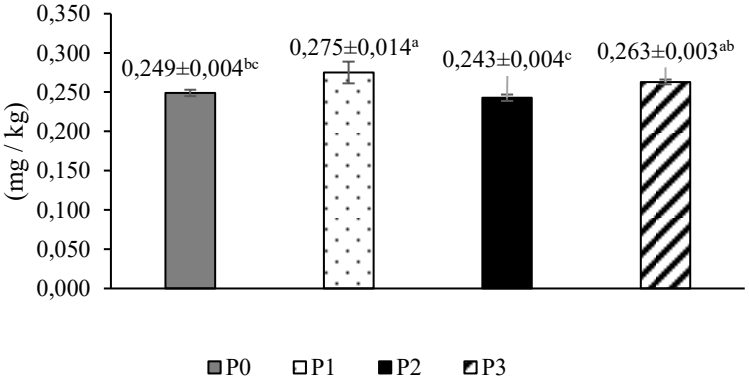

Figure 5. Chromium (Cr)

physiological environment for Zn utilization and deposition in thigh meat.

All Zn content levels in this study remained below the safe limit of 25 mg kg^{-1} set by EFSA (2019). The Zn content, ranging from 13.45 to 16.35 mg kg^{-1}, is far below the toxicity threshold. In terms of daily nutritional adequacy, consuming 100 g of IPB-D1 chicken meat can contribute about 11,9% of the RDA in P0 (13.45 mg kg^{-1}) and increase to 19% in P3 (16.35 mg kg^{-1}). Therefore, the increase in Zn content in the treatments with organic Zn supplementation provides significant additional nutritional benefits without posing any health risks.

Iron (Fe) Content of Thigh Meat

The iron (Fe) content in the thigh meat of IPB D1 chickens was significantly different among treatments (Figure 4). The highest Fe content was found in P3 at 12.82 ± 1.18 mg kg^{-1}. This value was higher than P0 (10.92 ± 0.84 mg kg^{-1}), P1 (11.05 ± 0.91 mg kg^{-1}), and P2 (11.41 ± 0.76 mg kg^{-1}). Statistical analysis showed that P3 was significantly different ($P<0.05$) from the other three treatments, while P0, P1, and P2 did not show a significant difference. The increase in Fe content from P0 (10.92 mg kg^{-1}) to P3 (12.82 mg kg^{-1}) was approximately 17.4%. The difference between P1 (11.05 mg kg^{-1}) and P3 (12.82 mg kg^{-1}) was also quite large at about 16%, while the comparison of P2 (11.41 mg kg^{-1}) with P3 showed a difference of about 12.4%. This increase indicates that the treatment combining lemuru fish oil and mineral supplementation can significantly enhance Fe accumulation in muscle tissue.

The higher Fe content in P3 may be influenced by the long-chain omega-3 polyunsaturated fatty acids (PUFAs) in lemuru fish oil, particularly eicosapentaenoic acid (EPA) and do-

cosahexaenoic acid (DHA). These bioactive fatty acids have been reported to modulate intestinal function and nutrient transport processes, potentially enhancing mineral bioavailability and absorption efficiency. Rather than acting solely through intestinal membrane integrity, omega-3 fatty acids may also influence transport mechanisms mediated by lipids and the metabolic utilization of trace minerals such as Fe in muscle tissue (Winarno, 2004).

Compared with reference data, the Fe content observed in this study was relatively high. The Fe concentration in P3 (12.82 mg kg^{-1}) and even in P0 (10.92 mg kg^{-1}) exceeded values reported by the United States Department of Agriculture (USDA, 2019) for broiler chicken meat, which range from 7 to 9 mg kg^{-1}. In addition, the Fe content of IPB D1 chicken meat was higher than that of other commercial poultry, as reported by Butt *et al.* (2016). These findings suggest that IPB D1 chickens may serve as a valuable source of dietary iron compared with conventional poultry products.

The contribution to the recommended daily allowance is also significant. Consuming 100 g of IPB-D1 chicken meat can contribute about 11.6% of the daily RDA at the lowest value treatment (P0 at 10.92 mg kg^{-1}) and increase to 13,6% at the highest value treatment (P3 at 12.82 mg kg^{-1}). Thus, the increase in Fe content in P3 is not only statistically significant but also has relevant nutritional implications for consumers.

Chromium (Cr) Content of Thigh Meat

The chromium (Cr) content in IPB D1 chicken thigh meat varied among treatments (Figure 5). The highest Cr content was found in P1, at 0.275 ± 0.014 mg kg^{-1}. The P3 treatment yielded a Cr content of 0.263 ± 0.003 mg kg^{-1}, which was not significantly different from P1 but was higher than P0 (0.198 ± 0.010 mg kg^{-1}) and P2 (0.215 ± 0.012 mg kg^{-1}). These results show that organic Cr supplementation, both alone in P1 and combined with lemuru fish oil in P3, was effective at increasing tissue Cr content. In contrast, lemuru fish oil without added Cr in P2 showed no significant increase.

The increase in Cr content from P0 (0.198 mg kg^{-1}) to P1 (0.275 mg kg^{-1}) was 38,9%, while the increase from P0 to P3 (0.263 mg kg^{-1}) was 32.8%. The difference between P1 (0.275 mg kg^{-1}) and P3 (0.263 mg kg^{-1}) was only about 4.4%, suggesting that both treatments had relatively similar effectiveness in enhancing Cr accumulation. This confirms that organic Cr supplementation is the main factor in improving Cr deposition into muscle tissue, with the fish oil playing a more supportive role.

Al-Khalifah *et al.* (2020) stated, polyunsaturated fatty acids (PUFAs) may enhance mineral absorption by modulating intestinal transport mechanisms. In addition to this effect, the lipid composition of lemuru fish oil may influence overall lipid metabolism and support antioxidant capacity, thereby creating a more favourable physiological environment for trace mineral utilization. Improved metabolic efficiency and oxidative balance may indirectly support chromium retention in muscle tissue. This mechanism may explain why the combination of Cr supplementation with lemuru fish oil in P3 (0.263 mg kg^{-1}) resulted in a relatively high Cr content, although it was slightly lower than P1 (0.275 mg kg^{-1}). Therefore, lemuru fish oil may enhance mineral bioavailability, but it does not replace the direct contribution of organic Cr supplementation.

The Cr content across all treatments in this study remained below the safe limit set by the World Health Organization (WHO 1996). The values obtained ranged from 0.198 to 0.275 mg kg^{-1}, with contributions to the daily nutritional allowance ranging from 67.8% in the lowest value treatment (P0) to 77.8% in the highest value treatment (P1). Thus, despite the variation in content among treatments, IPB-D1 chicken meat remains safe for consumption and can be an important source of organic chromium for consumer/

Vitamin A, D3, and K1 Content of Thigh Meat

Vitamins A, D_3, and K_1 were not detected in the thigh meat of IPB D1 chickens across all dietary treatments, with concentrations below the analytical detection limits (≤0,101 mg kg^{-1} for vitamin A, ≤0.003 mg kg^{-1} for vitamin D_3, and ≤0.018 mg kg^{-1} for vitamin K_1). This result is consistent with the physiological distribution of fat-soluble vitamins, which are primarily stored in the liver and adipose tissue rather than skeletal muscle (Combs, 2008; Rath *et al.*, 2021). There-

fore, their absence in thigh muscle tissue is not unexpected.

The non-detection of vitamin A may be attributed to its low natural deposition in muscle tissue and its susceptibility to degradation during handling and processing, despite the presence of provitamin A sources in palm oil-based diets (Maryuningsih *et al.*, 2021) and retinol in lemuru fish oil (Nurnadia *et al.*, 2011). Similarly, vitamin D_3 remained undetectable, likely due to the absence of fortified or metabolically active forms such as 25-hydroxycholecalciferol ($25OHD_3$) in the experimental diets, as well as limited endogenous synthesis under intensive rearing conditions with minimal ultraviolet B exposure (Avila *et al.*, 2022; Rath *et al.*, 2021). Vitamin K_1 was likewise not detected, reflecting its low deposition in muscle tissue, limited dietary availability, and sensitivity to oxidative degradation (EFSA, 2021; Shea and Booth, 2022). Overall, the absence of these fat-soluble vitamins in muscle tissue reflects their biological distribution and analytical limitations rather than nutritional inadequacy.

Vitamin E Content of Thigh Meat

The vitamin E content in the thigh meat of IPB D1 chickens showed significant variation among treatments (Table 3). The highest concentration was observed in P1 (4.65 ± 0.23 mg kg^{-1}), which included Zn and Cr supplementation and palm oil as the primary fat source. Statistical analysis indicated that P1 differed significantly ($P < 0.05$) from the other dietary treatments. In contrast, vitamin E levels in P0 (3.82 ± 0.37 mg kg^{-1}), P2 (3.47 ± 0.37 mg kg^{-1}), and P3 (3.40 ± 0.46 mg kg^{-1}) were not significantly different, although slightly lower values were recorded in the treatments containing lemuru fish oil.

The increase in vitamin E content in P1 compared with the control diet suggests that micromineral supplementation combined with palm oil may enhance vitamin E deposition in muscle tissue. Zinc and chromium are known to support antioxidant-related metabolic processes, which may contribute to improved retention of vitamin E in poultry meat. In addition, palm oil contains considerable amounts of tocopherols and tocotrienols, which are naturally occurring and relatively stable antioxidant compounds (Harlen *et al.*, 2017; Shahidi and de Camargo, 2016). This may explain why P1 produced the highest vitamin E concentration among treatments.

Meanwhile, the slightly reduced vitamin E levels in P2 and P3 may indicate increased utilization of antioxidant compounds in diets enriched with polyunsaturated fatty acids, as vitamin E plays a protective role in maintaining lipid stability (Surai, 2020). Overall, these findings suggest that the combination of Zn and Cr supplementation with palm oil represents a more favourable formulation for enhancing vitamin E content in IPB D1 chicken meat.

Cholesterol Content of Thigh Meat

Table 4 indicates, the total cholesterol content in the thigh meat of IPB D1 chicken differed significantly among treatments ($P < 0.05$). The highest cholesterol content was observed in P3 (1,060.00 ± 29.5 mg kg^{-1}), followed by P0 (1,000.40 ± 18.85 mg kg^{-1}), P1 (845.92 ± 1.71 mg kg^{-1}), and the lowest in P2 (742.00 ± 14.79 mg kg^{-1}). These differences among treatments indicate that feed formulation plays an important role in influencing lipid metabolism and cholesterol accumulation in muscle tissue.

The P3 treatment resulted in the highest total cholesterol level, approximately 5.9% higher than P0. This increase may be attributed to the inclusion of lemuru fish oil, which is rich in omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Omega-3 fatty acids have been reported to influence lipid metabolism and cholesterol regulation in poultry (Jang *et al.*, 2016). However, since high-density and low-density lipoprotein fractions were not measured in the present study, the underlying mechanism responsible for the increased total cholesterol in P3 cannot be fully explained. Therefore, this finding should be interpreted cautiously, and further studies incorporating detailed lipoprotein profiling are required to clarify the effects of lemuru fish oil and micromineral supplementation on cholesterol metabolism.

In contrast, P1 showed a significant decrease in cholesterol, with a value of 845.92 mg kg^{-1}, which is 15.4% lower than P0. This reduction reflects the positive effect of Zn and Cr supplementation in regulating lipid metabolism. According to El Husseiny *et al.* (2018) and Abdel-

Table 3. Vitamin Content of IPB D1 Chicken Meat Produced from Different Feed Formulations

Variables (mg / kg)	Treatments			
	P0	P1	P2	P3
Vitamin A	≤0.101	≤0.101	≤0.101	≤0.101
Vitamin D3	≤0.003	≤0.003	≤0.003	≤0.003
Vitamin E	3.82±0,37[b]	4.65±0,23[a]	3.47±0,37[b]	3.40±0,46[b]
Vitamin K1	≤0.018	≤0.018	≤0.018	≤0.018

Different superscripts in the same row indicate a significant difference (P<0.05). P0 = Control diet; P1 = P0 diet supplemented with 120 ppm Zn and 400 ppb Cr; P2 = P0 diet and supplemented with lemuru fish oil supplementation; P3 = P2 diet supplemented with 120 ppm Zn and 400 ppb Cr.

Table 4. Cholesterol Content of IPB D1 Chicken Thigh Meat Produced from Different Feed Formulations

Variables (mg/kg)	Treatments			
	P0	P1	P2	P3
Cholesterol	1000,40±18,85[b]	845,92±1,71[c]	742,00±14,79[d]	1060,00±29,5[a]

Different superscripts in the same row indicate a significant difference (P<0.05). P0 = Control diet; P1 = P0 diet supplemented with 120 ppm Zn and 400 ppb Cr; P2 = P0 diet and lemuru fish oil supplementation; P3 = P2 diet supplemented with 120 ppm Zn and 400 ppb Cr.

Moneim *et al.* (2019), Zn and Cr help lower low-density lipoprotein (LDL), increase insulin sensitivity, and improve fat metabolism, thereby suppressing total cholesterol accumulation.

The P2 treatment, which included lemuru fish oil without additional minerals, resulted in the lowest total cholesterol content at 742.00 mg kg^{-1}. This value was 25.8% lower than P0 and 11.7% lower than P1. This finding suggests that omega-3 fatty acids in fish oil may contribute to more favourable cholesterol regulation and reduced cholesterol accumulation in muscle tissue. However, since lipoprotein fractions such as HDL and LDL were not measured in the present study, the specific effects on cholesterol distribution cannot be determined.

Cholesterol is an essential lipid with important functions in the body, including as a component of cell membranes, a precursor to steroid hormones, and a basis for vitamin D synthesis (Rais *et al.*, 2024). Therefore, interpreting cholesterol data should not be based solely on the total content but must also consider the balance between HDL and LDL fractions. This study demonstrates that different nutrient combinations lead to varied cholesterol patterns: P3 increased total cholesterol with a likely HDL dominance, P1 suppressed total cholesterol through the role of Zn and Cr, while P2 significantly lowered total cholesterol thanks to the omega-3 from lemuru fish oil.

Fatty Acid Content of Thigh Meat

Based on Table 5, the fatty acid profile of IPB D1 chicken thigh meat shows significant differences between treatments. The highest Saturated Fatty Acid (SFA) content was found in P3 at 33.37% and P2 at 33.10%, which were higher than P0 (31.18%) and P1 (31.45%). The approximately 2% increase in P2 and P3 indicates that the addition of lemuru fish oil contributes to SFA accumulation, likely due to the presence of long-chain saturated fatty acids in the fish oil. Conversely, Zn and Cr supplementation did not significantly affect SFA, as seen by the P1 value being almost identical to P0 (Lestari *et al.* 2020).

The pattern of SFA content is inversely proportional to that of Monounsaturated Fatty Acids (MUFA). The highest MUFA content was found in P1 at 46.85% and P0 at 46.63%, while P3 and P2 were lower, at 44.40% and 43.94%, respectively. This 2-3% difference shows that lemuru fish oil contains less MUFA than palm oil. When used in the diets (P2 and P3), the chicken's meat MUFA content also decreased. Conversely, the high MUFA in P1 suggests that Zn and Cr supplementation can support lipid metabolism, allowing for more optimal endogenous MUFA synthesis.

A different trend was observed for Polyunsaturated Fatty Acids (PUFA). The highest PUFA content was found in P3 at 25.56% and P2 at 25.10%, which were higher than P0 (22,02%)

Table 5. Fatty Acid Content of IPB D1 Chicken Meat Produced from Different Feed Formulations

Variables (%)	Treatments			
	P0	P1	P2	P3
SFA	30,82±0,07^{b}	31,81±0,33^{b}	33,10±0,237^{a}	33,37±1,16^{a}
MUFA	46,63±0,31^{ab}	46,85±1,08^{a}	43,94±1,79^{c}	44,40±0,95^{bc}
PUFA	22,14±0,45^{b}	23,91±2,68^{ab}	25,10±0,43^{ab}	25,56±1,24^{a}
Omega 3	0,95±0,04^{d}	1,34±0,18^{c}	2,52±0,03^{b}	3,10±0,18^{a}
Omega 6	21,19±0,50	22,56±2,50	22,57±0,47	22,54±1,07
Omega 9	43,76±0,38^{a}	43,72±0,80^{a}	39,15±1,62^{b}	39,51±0,27^{b}
Omega 6: Omega 3 ratio	22,31:1	16,84:1	8,96:1	7,27:1
Total fat content	3,27±0,01^{a}	0,87±0,25^{c}	1,51±0,16^{b}	1,62±0,09^{b}

Different superscripts in the same row indicate a significant difference (P<0.05). P0 = Control diet; P1 = P0 diet supplemented with 120 ppm Zn and 400 ppb Cr; P2 = P0 diet and lemuru fish oil supplementation; P3 = P2 diet supplemented with 120 ppm Zn and 400 ppb Cr.

and P1 (21,70%). This 3-4% increase primarily came from the omega-3 group, specifically EPA and DHA. EPA increased dramatically from 0,11% in P0 to 0,60% in P2 and 0,92% in P3. DHA rose from 0,36% in P0 to 1,19% in P2 and 1,30% in P3. Thus, P2 yielded nearly six times more EPA than P0, while P3 had more than eight times higher EPA. Similarly, DHA increased by more than three times in P2 and nearly four times in P3. This pattern demonstrates that lemuru fish oil is the primary source of omega-3s, and the supplementation of Zn and Cr in P3 strengthens the retention of these fatty acids in tissue (Bontjura *et al.*, 2019; Wibawaningrum *et al.*, 2021).

This increase in EPA and DHA resulted in changes in the omega-6 to omega-3 ratio. In P0, the ratio was 22.31:1, but it decreased to 8.64:1 in P2 and 7.27:1 in P3. This 67,4% reduction from P0 to P3 indicates an improvement in fatty acid balance, as the ratio approaches the recommended ratio of approximately 5:1 for human health. A more balanced omega-6 to omega-3 ratio has been associated with a lower risk of chronic inflammation, cardiovascular disease, and metabolic disorders (Leeson and Atteh, 1995; Pandiangan *et al.*, 2020). In addition to the omega-6 to omega-3 ratio, the PUFA to SFA ratio is another important indicator of lipid nutritional quality. Higher PUFA/SFA values, particularly those above 0.45, are considered beneficial for reducing cardiovascular risk. Therefore, the increase in omega-3 fatty acids in P2 and P3 not only enhanced PUFA deposition but also contributed to a more favourable overall fatty acid profile with potential implications for consumer health.

Additionally, the highest omega-9 (C18:1, oleic acid) content was found in P0 (46.63%) and P1 (46.85%), while P2 and P3 were lower, at 43.94% and 44.40%, respectively. This result is consistent with the characteristics of palm oil, which is rich in oleic acid, while lemuru fish oil contains less MUFA. This fact confirms that the type of fat source in the feed directly influences the MUFA dominance in chicken meat.

Overall, the study's results show that each fatty acid group responded differently to the dietary treatments. P2 and P3 experienced an increase in SFA and PUFA (especially omega-3), while P0 and P1 were higher in MUFA, particularly oleic acid. The combination of lemuru fish oil and Zn–Cr supplementation in P3 resulted in the most balanced fatty acid profile, characterized by high EPA and DHA content, an ideal omega-6: omega-3 ratio, and still moderate SFA content. This is in line with Rajebi *et al.* (2023), who stated that fatty acids not only serve as an energy source but also as regulators of lipid metabolism and essential components of cell membranes. Thus, IPB D1 chicken meat produced with the P3 formulation can be categorized as having a healthier fatty acid profile, supporting consumer cardiovascular and metabolic health, and having potential as a functional food.

CONCLUSION

Feed formulation significantly influenced the micronutrient content and lipid profile of IPB

D1 chicken meat. Among the treatments evaluated, the P3 diet, consisting of lemuru fish oil combined with 120 ppm Zn and 400 ppb Cr supplementation, was the most effective in improving mineral content and enhancing the fatty acid composition of the meat. The highest vitamin E content was observed in the P1 treatment, indicating that mineral supplementation combined with palm oil may better support vitamin E deposition. Overall, these findings highlight the potential of targeted feed formulation strategies to improve the nutritional characteristics of IPB D1 chicken meat. However, further studies are needed to evaluate additional parameters such as lipoprotein fractions (HDL and LDL), sensory quality, shelf-life stability, and consumer acceptance in order to comprehensively assess its functional and commercial value.

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