

CHAPTER IV

OPTIMIZING N-3 PUFA DIETARY ENRICHMENT IN SLOW-GROWING KORAT CHICKENS USING LIPID-BASED NANOPARTICLES: EFFECTS ON GROWTH PERFORMANCE, CARCASS TRAITS, MEAT QUALITY, MEAT FATTY ACID COMPOSITION, AND BLOOD BIOCHEMICAL PARAMETERS

4.1 Abstract

This research aimed to investigate the optimization of lipid-based nanoparticles to improve the utilization of n-3 PUFA source in chicken diets. Three groups of chickens were reared under the same conditions and fed a diet containing 6% rice bran oil (RBO, control group), 3% tuna oil (3%TO) and 3% tuna oil in lipid-based nanoparticles (3%TO_TNP). The growth performance, carcass composition, meat quality, fatty acid (FA) profile of breast and thigh meat, hematological and plasma biochemical parameters were evaluated. The dietary lipid source had no effect on the growth performance, carcass composition neither on breast and thigh meat quality ($P > 0.05$). By comparison with the control group, 3%TO and 3%TO_TNP groups had lower levels of C18:2 n-6 (linoleic acid) and n-6 PUFA ($P < 0.05$). There was no significant difference between groups for C18:3 n-3 (ALA) level in breast and thigh meat whereas the levels of C20:5 n-3 (EPA) and C22:6 n-3 (DHA) were higher in both TO groups. However, due to the nanoparticle's synthesis method requiring high temperature during the drying step the enrichment in n-3 PUFA was more efficient with TO directly incorporated in feed than using targeted nanoparticles. However, there was no difference between 3%TO and 3%TO_TNP groups for the n-6/n-3 ratio ($P > 0.05$). The hemoglobin and hematocrit values were higher in 3%TO_TNP than in the other groups ($P < 0.05$). In the plasma, HDL level was reduced in the TO groups compared to the control group ($P < 0.05$) suggesting a lower cholesterol hepatic synthesis and export. The TO groups had a higher activity of alanine amino transferase but a lower creatinine content than the

control group ($P < 0.05$). The uric acid content was higher in 3%TO_TNP group than in the 3%TO group ($P < 0.05$) but no different compared with the control group. However, the values obtained for the hematological and biochemistry parameters measured in blood and plasma were within the normal range for chickens. To conclude, the supplementation of lipid-based nanoparticles allowed to change the fatty acid profile of chicken meat without any negative effect on health status of chickens.

Keywords: Korat chicken, feed, nanoparticles, n-3 PUFAs, meat quality.

4.2 Introduction

In the last decades, there has been an increasing interest for functional foods for human health such as n-3 PUFAs enrichment in meat products, which can be achieved with n-3 oil source supplementation in animal feed formulation. However, the n-3 oil source exhibits numerous challenges such as disagreeable taste and aroma, low storage ability, and decreasing bioavailability of important polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Improving the stability of n-3 PUFAs by using lipid-based nanoparticles could increase the use of these n-3 PUFA sources.

Korat chicken is a crossbreed between a male Thai indigenous chicken (Lueng Hang Khao) and a female of a breeder line from Suranaree University of Technology (SUT). Korat chicken is a slow-growing chicken that could be used to produce functional meat enriched with n-3 PUFAs. The previous studies showed that dietary 4% tuna oil supplementation increased n-3 PUFAs content in Korat chicken meat (Hang et al., 2018). By the way, the literature research proved that n-3 PUFA sources are susceptible to lipid oxidation affecting the feed quality (Sanguansri et al., 2013). However, these lipids can be protected with encapsulation, but this tool is insufficient to improve the utilization of n-3 PUFA sources as a part of these fatty acids will be also distributed in non-targeted organs. Until now, the research was mainly focused on the enrichment of meat with n-3 PUFA rather than the large-scale production of feed supplemented with stabilized sources of n-3 PUFA.

The incorporation of n-3 PUFAs into skeletal muscle is in vesicle form. The FA are first transferred to hepatocytes (Niot et al., 2009) also known as “hepatic first-pass metabolism” (Poonia et al., 2016). According to Keegan et al. (2020), the accumulation of n-3 PUFAs mostly occurred in the liver, adhering fat, and skin followed by breast and thigh muscles. Therefore, feed formulation requires higher n-3 PUFAs supplementation to allow meat enrichment with n-3 PUFAs to reach the recommended level. To reduce accumulation in non-target organs, nanotechnology could therefore be a more appropriate technology (Lu et al., 2021). The lipid-based nanoparticles are suitable carriers for improving bioavailability. They are nontoxic for the cells (Saedi et al., 2018) and the use of modified surfactant respects gastrointestinal tolerance (Zhang et al., 2011) and to reach directly skeletal muscles (Yeh et al., 2014). Therefore, this technology might become an opportunity to enhance the precision of utilization of the bioactive compound, and nutritional value of meat.

Several researchers claim the safety of applying lipid-based nanoparticles (Das and Chaudhury, 2011; Zhou et al., 2015; Rodriguez-Ruiz et al., 2018). Moreover, the research studies about nanotoxicity attended mostly metals and polymers. By the way, the lipid nanoparticles showed a lower toxicity response due to the biodegradable and harmless properties (Winter et al., 2016). However, a few studies showed that the material of lipid nanoparticle affected some hematological parameters such as decreased erythrocytes or immunogenicity (Lim et al., 2012). Therefore, in order to use nanoparticles at an appropriate level, it is important to study the safety of its use to develop feed additives and consider the level of supplementation without inducing toxic effect.

Therefore, this research study aimed to generate lipid-based nanoparticles, which might reduce the utilization of n-3 oil sources in feed formulation and increase the efficiency of n-3 PUFAs accumulation in Korat chicken meat. The effects of n-3 PUFAs loaded lipid-based nanoparticles in diet formulation will be investigated on growth performance, carcass and meat quality, FA composition of muscles, and hematological and biochemical parameters in blood of Korat chickens. Finally, the experimental results will be analyzed and discussed to optimize the production of nanocarriers.

4.3 Materials and methods

4.3.1 Preparation of targeted lipid nanoparticles

Tuna oil loaded lipid-based nanoparticles were prepared as described in chapter III, section 3.4.2.

4.3.2 Ethics statement

The experiment was conducted at farm of Suranaree university of technology. All procedures in the present study were approved by the Ethics Committee on Animal Use of the Suranaree University of technology, Nakhon Ratchasima, Thailand (SUT-IACUC-002/2022).

4.3.3 Birds and housing

The experiment was conducted at SUT farm. One-day old female Korat chickens were reared on floor pens with a density of 8 birds/m². The birds were maintained at a brooding temperature of 30°C to 35°C during the first 21 d of age. Then, they were housed in an open-sided facility with natural ventilation, exposed to ambient conditions. The temperature ranged from 14.1°C to 32.2°C, with an average relative humidity of 70.68% (National Agricultural Big Data Center, Nakhon Ratchasima, Thailand). They were vaccinated against Marek's disease at the hatchery, followed by Newcastle disease, and infectious bronchitis at 7 and 21 d of age. Additionally, a Gumboro disease was administered at 14 d of age. The chickens were reared together from hatching until 49 d of age, reaching an average body weight (BW) of 903.14 ± 17.71 g.

4.3.4 Experimental design

The experimental model was a completely randomized design, which included three dietary treatments, 5 replications and twenty-five 49-d-old female chickens per replication. The experimental chickens were maintained in an open indoor housing with the temperature ranged from 12.6°C to 35.1°C, with an average relative humidity of 69.60% (National Agricultural Big Data Center, Nakhon Ratchasima, Thailand). An automatic ventilation fan maintained a temperature range of 30°C to 35°C within the housing. A basal diet was formulated based on corn-soybean containing 6% of rice bran oil (Thai Ruam Jai Korat Co., Ltd., Nakhon Ratchasima, Thailand) as the control. In the other dietary treatments, 3% of the RBO oil content were replaced with 3% tuna oil (TO) or 3% tuna oil in targeted nanoparticles (TO-TNP). All experimental

diets were formulated to provide 3,100 Kcal/Kg ME and 19% CP for the finisher (d 49 to 70) (Table 4.1). Feed-grade oils were used in all diets. The fatty acids profiles of oils used for supplementation are showed in Table 4.2.

4.3.5 Data and sample collection

4.3.5.1 Growth performance

The chickens were weighed, and the feed intake was recorded weekly to calculate the average daily gain (ADG) and feed conversion ratio (FCR) of each group. The mortality rate was also recorded.

4.3.5.2 Carcass and meat quality

Slaughter procedure and carcass measurements

At 70 days of age, 60 chickens (4 females per replicate x 5 replicates per treatment x 3 groups) randomly chosen were slaughtered after 12 hours feed withdrawal period. The chickens were transported to the slaughterhouse within one hour and then rested for about 30 min. All chickens were stunned by electricity (current parameters, V and Hz), scalding at 60°C, then de-feathering by machine and eviscerating manually. They were washed with clean water and put in a chilled room (4°C, 24 h), before cutting into portions. The hot carcass (without viscera, head, neck, feet, and shanks), abdominal fat (considered as the fat extending within the ischium, surrounding the cloaca, and adjacent to the abdominal muscle), and edible giblets were weighted. The weight of abdominal fat and edible giblets were then calculated as the proportion of the hot carcass of the chicken. The portions of carcass after chilled including breast and thigh meats were expressed as the percentage of the chilled carcass weight. The pH and meat color of the breast and thigh meats were measured before being stored at -20°C for subsequent evaluation of meat quality, including drip loss, cooking loss, and shear force measurement.

4.3.5.3 Meat quality

pH measurement, drip loss, and cooking loss

The pH value was measured at 24 h post-mortem in the Pectoralis major muscle at a depth of 0.5 to 1.0 cm. The breast fillet pH was measured by inserting a probe into the muscle and using a pH meter (Testo 206-pH2, Testo SE & Co. KGaA, Titisee-Neustadt, Germany) with precision of 0.01 pH unit. After chilling for

24 h, the water holding capacity was determined as drip loss (hung as airtight container, chilled for 72 h at 4°C) and cooking loss (boiled in a water bath in open plastic bags until as internal temperature of 85°C was reached). The drip loss and cooking loss were expressed as a percentage of the initial weight.

$$\% \text{ Drip loss} = \left(\frac{\text{weight before storage} - \text{weight after storage}}{\text{weight before storage}} \right) \times 100$$

$$\% \text{ Cooking loss} = \left(\frac{\text{weight before cooking} - \text{weight after cooking}}{\text{weight before cooking}} \right) \times 100$$

The meat color was measured using a Hunter Lab ColorQuest XE spectrophotometer, with QC Software (Reston, VA, USA) calibrated against black and white reference tiles. L* (lightness), a* (redness), and b* (yellow) values were obtained by an illuminant/observer D65/10°. The measurements were conducted through the transparent packaging material. Three random readings were taken, and subsequently averaged on a sample surface for the purpose of statistical analysis.

Shear force measurement

Cooked meat samples were used to determine shear force value. At least 3 subsamples will be removed (2.0×1.0×0.5 cm) by cutting parallel to the muscle fibers from each cooked sample. A Texture Analyzer (TA-XT2, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a Warner-Bratzler shear force apparatus was used to determine the peak shear force values of subsamples. The crosshead speed was set at 20 cm/min. Peak WBSF values will be determined by averaging the values of three subsamples (Tasoniero et al., 2016).

4.3.5.4 Fatty acid composition

Samples of breast and thigh meat were collected from 10 females per group and stored at -20°C until analysis fatty acid profiles.

Table 4.1 Ingredients and nutrient composition of the experimental diets (as-fed basis).

Ingredients ¹	49 to 70 days		
	Control	3% TO	3% TO_TNP
Corn	58.10	58.10	58.10
Soybean meal 44%	32.42	32.42	32.42
Tuna oil	-	3.00	-
Tuna oil TNP	-	-	3.00
Rice bran oil	6.00	3.00	3.00
DL-methionine	0.16	0.16	0.16
Salt	0.35	0.35	0.35
CaCO ₃	1.27	1.27	1.27
P21 (monocalcium phosphate)	1.20	1.20	1.20
Premix ²	0.50	0.50	0.50
Total (%)	100.00	100.00	100.00
Calculated nutrients			
Metabolizable energy (kcal/kg)	3180.00	3180.00	3180.00
Crude proteins	18.80	18.80	18.80
Crude fiber	3.72	3.72	3.72
Crude fat	8.47	8.47	8.47
Calcium	0.80	0.80	0.80
Total phosphorus	0.64	0.64	0.64
Available phosphorus	0.40	0.40	0.40
Lysine	0.94	0.94	0.94
Methionine	0.45	0.45	0.45
Analyzed nutrient levels (%)²			
Total lipids	6.81	6.76	6.51
C18:2n-6, Linoleic acid	35.88	27.51	31.59
C18:3n-3, ALA	0.50	0.95	0.87
C20:5n-3, EPA	0.00	2.83	1.19

Table 4.1 (continued).

Ingredients	49 to 70 days		
	Control	3% TO	3% TO_TNP
C22:6n-3, DHA	0.00	8.66	3.11
SFA	24.86	26.04	27.14
MUFA	38.70	32.55	35.11
PUFA	36.44	41.41	37.75
n-3 PUFA	0.50	12.44	5.16
n-6 PUFA	35.94	28.97	32.58
n-6/n-3	72.26	2.33	6.31

¹ Premix contained the following nutrients (units are expressed per kg of diet): vitamin A, 15,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B1, 2 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; vitamin B12, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg

² ALA, Alpha-linolenic acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acid.

The lipids were extracted from approximately 5 g of each samples using 90 mL of chloroform:methanol (2:1, v/v) (Folch et al., 1957).. Then, 20 to 25 mg of extracted fat were methylated (Metcalfe et al., 1966). The fatty acid methyl esters (FAME) were analyzed using gas chromatography (Hewlett-Packard 7890A; Agilent Technologies, Santa Clara, CA, USA) with a capillary column (SP 2560, Supelco Inc., Bellefonte, PA, USA, 100 m × 0.25 mm i.d., 0.20-µm film thickness) and a flame ionization detector. The carrier gas was helium at a flow rate of 0.95 mL/min. The temperatures of the injector and detector were 260°C. The initial column temperature was 70°C, which was then raised to 175°C at a rate of 13°C/min, and finally raised to 240°C at a rate of 4°C/min. The identified and quantified of compound were performed by using Masshunter software (v10.0.707.0, Agilent, USA) (Zhang et al., 2022).

Table 4.2 Fatty acid composition of rice bran oil, tuna oil and lipid-based nanoparticles used in the diets.

Fatty acids (g/100 g total FA) ¹	Rice bran oil	Tuna oil	Lipid-based nanoparticles
C14:0, Myristic acid	0.45	4.65	6.06
C15:0, Pentadecanoic acid	-	16.57	-
C16:0, Palmitic acid	21.75	20.28	29.43
C16:1, Palmitoleic acid	0.21	4.73	5.93
C18:0, Stearic acid	2.36	5.28	8.46
C18:1n-9, Oleic acid	41.48	14.16	22.64
C18:2n-6, Linoleic acid	33.13	2.84	7.69
C18:3n-3, ALA	0.57	1.68	2.40
C20:4n-6, Arachidonic acid	0.05	2.52	4.30
C20:5n-3, EPA	-	6.98	3.60
C24:1, Nervonic acid	-	0.28	0.25
C22:6n-3, DHA	-	20.03	9.24
SFA	24.56	46.78	43.95
MUFA	41.69	19.17	28.82
PUFA	33.75	34.05	27.23
n-3 PUFA	0.57	28.69	15.24
n-6 PUFA	33.18	5.36	11.99
n-6/n-3	58.09	0.19	0.79

¹ ALA, Alpha-linolenic acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acid.

4.3.6 Blood parameters

4.3.6.1 Blood sample collection

At 70 days of age, before the feed withdrawal period, blood samples were collected on 10 chickens per group at jugular and cutaneous ulnar veins. Blood samples were kept into lithium heparin tubes for hematological analysis and

ethyl diamine tetra acetic acid (EDTA) tube for further analysis of plasma biochemical parameters. All blood parameter analysis were measured by a veterinary laboratory (Vet Central Lab, Nakhon Ratchasima, Thailand).

Heparinized whole blood samples were used to determine hemoglobin (Hb), hematocrit (packed cell volume, PCV), erythrocytes (red blood cell, RBC), leucocytes (white blood cells, WBC), platelets (thrombocytes), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), leucocytes differential count including monocytes, lymphocytes, eosinophils according to Abdulazeez et al. (2016).

The value of Hb, RBC and PCV were used to calculate MCV $[(PCV \div RBC) \times 10]$, MCHC $[(Hb \div PCV) \times 100]$ (Davies et al., 1984) and MCH $[(Hb \div RBC) \times 10]$ (Jain, 1986).

Plasma samples were used to quantify concentrations of clinical chemistry biomarkers including glucose, total proteins, albumin, lipoproteins, globulin, creatinine, cholesterol, urea, electrolytes and liver enzymes, including aspartate aminotransferase (AST), creatine phosphokinase (CPK), and glutamate dehydrogenase (GLDH) (Akinola and Etuk, 2015).

4.3.7 Statistical analysis

Analysis of variance (ANOVA) will be performed using the GLM procedure for a completely randomized design in SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA; University Edition) with the following statistical model:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij},$$

where y_{ij} = the dependent variable, μ = the overall mean, τ_i = the treatment effect, and ϵ_{ij} = the random residual error. Significant differences between treatment means were assessed by Tukey's multiple comparison tests after a significant F-test. The level of statistical significance will establish at $P < 0.05$. All values were expressed as means $\pm$ standard error of the mean (SEM), which represents the pooled SEM for the model.

4.4 Results

4.4.1 Growth performance

The dietary treatments, differing in the delivery system, were evaluated for their effects on growth performance including body weight gain (BWG), Feed intake (FI) and Feed conversion ratio (FCR). There was no statistically significant difference ($P < 0.05$) were observed between the group consuming tuna oil (3% TO) and the control group, as shown in Table 4.3.

Table 4.3 Growth performance of Korat chickens fed diets containing rice bran oil (control), tuna oil (3% TO) or tuna oil in lipid-based nanoparticles (3% TO_TNP).

Item ¹	Treatments			SEM	P-value
	Control	3% TO	3% TO_TNP		
Initial BW (d 49)	894.61	908.00	906.80	4.57	0.449
Final BW (d 70)	1342.00	1333.20	1344.02	5.95	0.761
BWG	447.39	425.20	437.22	6.69	0.430
FI	1624.24	1593.82	1681.35	23.25	0.318
FCR	3.64	3.76	3.85	0.07	0.487
Mortality rate	0.00	0.00	1.67	0.38	0.110

¹ BW, Body weight; BWG, Body weight gain; FI, Feed intake; FCR, Feed conversion ratio; SEM, Standard error of the mean.

4.4.2 Carcass composition

The carcass composition of Korat chickens is detailed in Table 4.4. The 3% TO group had a higher dressing percentage than the control group ($P < 0.05$). Both TO groups had higher percentages of gizzard than the control group ($P < 0.05$) For all the other cut parts, there was no significant differences between groups ($P > 0.05$).

4.4.3 Meat quality

The average values of meat quality traits are presented in Table 4.5. The dietary treatments had no significant effect ($P > 0.05$) on the quality traits measured on breast and thigh meat.

Table 4.4 Carcass composition of Korat chickens fed diets containing rice bran oil (control), tuna oil (3% TO) or tuna oil in lipid-based nanoparticles (3% TO_TNP)¹.

Items	Treatments ²			SEM	P-value
	Control	3% TO	3% TO_TNP		
Live weight (g)	1389.89	1379.87	1351.22	3.24	0.087
Hot carcass weight (g)	903.40	913.47	889.71	4.25	0.071
Chilled carcass weight (g)	899.01	906.39	885.35	3.87	0.067
Dressing ³	65.01 ^a	66.18 ^b	65.85 ^{ab}	0.19	0.021
% of hot carcass weight					
Heart	0.60	0.53	0.60	0.01	0.084
Spleen	0.48	0.43	0.50	0.04	0.761
Liver	2.47	2.39	2.58	0.04	0.245
Gizzard	2.20 ^b	2.58 ^a	2.56 ^a	0.07	0.034
Abdominal fat	1.97	1.27	1.82	0.14	0.104
% of chilled carcass weight					
Breast meat	18.93	18.69	18.96	0.17	0.795
Thigh meat	14.84	14.48	14.21	0.17	0.335

^{a, b} Means within row carry on common superscript are significantly different at $P < 0.05$.

¹ Carcass composition represents values from 20 chickens per group (5 replicates $\times$ 4 chickens/ replicate).

² Treatment consisted of 6% rice bran oil (control), 3% rice bran oil + 3% tuna oil (3% TO), 3% rice bran oil + 3% tuna oil in lipid-based nanoparticles (3% TO-TNP).

³ Without viscera, head, neck, feet and shank, calculated as a percentage of live weight.

Table 4.5 Meat quality traits of Korat chickens fed diets containing rice bran oil (control), tuna oil (3% TO) or tuna oil in lipid-based nanoparticles (3% TO_TNP)¹.

Items	Treatments ²			SEM	P-value
	Control	3% TO	3% TO_TNP		
Breast meat					
pH 24 h	5.54	5.53	5.53	0.01	0.832
Drip loss (%)	8.14	7.27	8.52	0.32	0.274
Cooking loss (%)	17.65	17.46	16.66	0.23	0.170
Breast WBS ³	2.88	3.07	2.50	0.14	0.235
L* (lightness)	52.35	53.40	53.60	0.38	0.376
a* (redness)	5.80	5.12	5.26	0.14	0.126
b* (yellowness)	15.87	16.20	15.96	0.17	0.749
Thigh meat					
pH 24 h	6.13	6.09	6.11	0.03	0.854
Drip loss	5.25	5.71	6.23	0.22	0.192
Cooking loss	19.70	20.13	20.81	0.31	0.371
Thigh WBS ³	2.83	2.53	2.59	0.07	0.175
L* (lightness)	41.46	41.54	41.26	0.32	0.944
a* (redness)	11.89	11.63	11.89	0.16	0.772
b* (yellowness)	12.17	12.68	12.15	0.22	0.583

¹ Each mean represents values from 20 chickens per group (5 replicates × 4 chickens/replicate).

² Treatment consisted of 6% rice bran oil (control), 3% rice bran oil + 3% tuna oil (3% TO), 3% rice bran oil + 3% tuna oil in lipid-based nanoparticles (3% TO-TNP).

³ WBS: Warner-Bratzler shear force expressed as kgf/0.5 cm².

4.4.4 Lipid content and fatty acid composition

The dietary effect on the fatty acid composition of breast and thigh meat is presented in Tables 4.6 and 4.7. The dietary treatment had no effect on the lipid content of breast and thigh meat. In the breast meat, both TO groups had lower levels of C18:2n-6, C20:4n-6, n-6 PUFA and C24:1 but higher levels of EPA, DHA, PUFA and n-

3 PUFA than the control group ($P < 0.06$). The ratio n-6/n-3 was 4 to 5-fold lower for the TO groups compared to the control group ($P < 0.05$). The enrichment of breast meat with EPA, DHA and n-3 PUFA was higher in the 3% TO group than in 3% TO_TNP group ($P < 0.06$). There were no significant differences between groups for the other fatty acids ($P > 0.05$).

In the thigh meat, both TO groups had lower levels of C18:2n-6, n-6 PUFA, C24:1 and MUFA but higher levels of C14:0, EPA, DHA and n-3 FA than the control group. The n-6/n-3 ratio was 4 to 8 folds lower for the TO groups compared to the control group ($P < 0.05$). The 3% TO group had higher levels of EPA, DHA, PUFA and n-3 PUFA than the 3% TO-TNP group ($P < 0.05$) but lower levels of MUFA than the control group ($P < 0.05$). There were no significant differences between groups for the other fatty acids.

Table 4.6 Total lipids (g/100 g fresh meat) and fatty acid composition (g/100 g total FA) of breast meat of Korat chickens fed diets containing rice bran oil (control), tuna oil (3% TO) or tuna oil in lipid-based nanoparticles (3% TO_TNP)¹.

Fatty acid composition	Treatments			SEM	P-value
	Control	3% TO	3% TO_TNP		
Total lipids	0.85	0.91	0.89	0.02	0.620
C14:0, Myristic acid	0.26	0.41	0.43	0.04	0.153
C15:0, Pentadecanoic acid	4.38	4.64	5.08	0.21	0.425
C16:0, Palmitic acid	22.26	22.05	21.90	0.14	0.625
C18:0, Stearic acid	10.68	10.25	11.27	0.21	0.144
C16:1, Palmitoleic acid	0.59	0.92	0.80	0.07	0.162
C24:1, Nervonic acid	3.11 ^a	0.87 ^b	1.61 ^b	0.28	< 0.001
C18:1n-9, Oleic acid	24.85	23.96	23.76	0.43	0.578
C18:2n-6, Linoleic acid	19.62 ^a	17.01 ^b	17.56 ^b	0.49	0.059
C20:4n-6, Arachidonic acid	11.14a	8.23b	9.31b	0.52	0.053
C18:3n-3, ALA	0.30	0.32	0.40	0.03	0.395
C20:5n-3, EPA	0.23c	1.01a	0.65b	0.10	< 0.001
C22:6n-3, DHA	2.05c	10.80a	7.21b	0.99	< 0.001

Table 4.6 (continued).

Fatty acid composition ³	Treatments ²			SEM	P-value
	Control	3% TO	3% TO_TNP		
SFA	37.58	37.37	38.68	0.33	0.234
MUFA	28.55	25.76	26.18	0.58	0.097
PUFA	33.87 ^b	36.88 ^a	35.14 ^a	0.54	0.059
n-3 PUFA	2.47 ^c	12.24 ^a	8.62 ^b	1.09	< 0.001
n-6 PUFA	30.76 ^a	25.24 ^b	26.88 ^b	0.80	0.004
n-6/n-3	13.97 ^a	2.65 ^b	3.42 ^b	1.59	< 0.001

^{a, b, c} Means within row carry on common superscript are significantly different at $P < 0.06$.

¹ Each mean represents values from 10 chickens per group (5 replicates $\times$ 2 chickens/replicate).

² Treatment consisted of 6% rice bran oil (control), 3% rice bran oil + 3% tuna oil (3% TO), 3% rice bran oil + 3% tuna oil in lipid-based nanoparticles (3% TO-TNP).

³ ALA, Alpha-Linolenic acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acid.

Table 4.7 Total lipids (g/100 g fresh meat) and fatty acid composition (g/100 g total FA) of thigh meat of Korat chickens fed diets containing rice bran oil (control), tuna oil (3% TO) or tuna oil in lipid-based nanoparticles (3% TO_TNP)¹.

Fatty acid composition ³	Treatments ²			SEM	P-value
	Control	3% TO	3% TO_TNP		
Total lipids	1.30	1.08	1.15	0.58	0.415
C14:0, Myristic acid	0.38 ^b	0.64 ^a	0.62 ^a	0.037	0.001
C15:0, Pentadecanoic acid	2.66	3.77	3.35	0.229	0.133
C16:0, Palmitic acid	22.12	21.92	22.26	0.199	0.814
C16:1, Palmitoleic acid	10.54	11.39	12.16	0.348	0.167
C18:0, Stearic acid	0.94	1.28	1.27	0.094	0.255
C18:1n-9, Oleic acid	2.37 ^a	0.64 ^b	1.18 ^b	0.213	< 0.001

Table 4.7 (continued).

Fatty acid composition ³	Treatments ²			SEM	P-value
	Control	3% TO	3% TO_TNP		
C18:2n-6, Linoleic acid	26.15	21.97	23.48	0.846	0.119
C18:3n-3, ALA	25.31 ^a	21.04 ^b	22.56 ^b	0.588	0.002
C20:4n-6, Arachidonic acid	7.90	6.61	6.76	0.399	0.379
C20:5n-3, EPA	0.31	0.33	0.32	0.013	0.730
C24:1, Nervonic acid	0.08 ^c	0.91 ^a	0.41 ^b	0.094	< 0.001
C22:6n-3, DHA	1.25 ^c	9.52 ^a	5.64 ^b	0.964	< 0.001
SFA	35.70	37.71	38.39	0.586	0.150
MUFA	29.45 ^a	23.88 ^b	25.92 ^{ab}	0.928	0.031
PUFA	34.85 ^b	38.41 ^a	35.69 ^b	0.556	0.010
n-3 PUFA	1.64 ^c	10.76 ^a	6.37 ^b	1.053	< 0.001
n-6 PUFA	33.21 ^a	27.65 ^b	29.32 ^b	0.715	< 0.001
n-6/n-3	22.55 ^a	2.66 ^b	5.09 ^b	2.441	< 0.001

^{a, b, c} Means within row carry on common superscript are significantly different at $P < 0.05$.

¹ Each mean represents values from 10 chickens per group (5 replicates $\times$ 2 chickens/replicate).

² Treatment consisted of 6% rice bran oil (control), 3% rice bran oil + 3% tuna oil (3% TO), 3% rice bran oil + 3% tuna oil in lipid-based nanoparticles (3% TO-TNP).

³ ALA, Alpha-linolenic acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acid.

4.4.5 Hematology measurements

The hematology observations of blood samples are presented in Table 4.8. There was no significant effect of dietary treatments on hematology measurements, except for hemoglobin and hematocrit that were higher in 3% TO_TNP group compared to the other groups ($P < 0.05$) and the level of white blood cell in TO group was lower than that the control group ($P < 0.05$).

Table 4.8 Hematology parameters of Korat chickens fed diets containing rice bran oil (control), tuna oil (3% TO) or tuna oil in lipid-based nanoparticles (3% TO_TNP)¹.

Parameters ³	Treatments ²			SEM	P-value
	Control	3% TO	3% TO_TNP		
RBC (x10 ⁶ /mm ³)	2.23	2.28	2.48	0.06	0.131
Hb (g/dL)	9.46 ^b	10.06 ^b	11.32 ^a	0.27	< 0.05
PCV (%)	28.40 ^b	30.20 ^b	34.00 ^a	0.80	< 0.05
MCV (fL)	13.00	13.50	13.92	0.20	0.171
MCH (Pg)	43.00	44.88	45.88	0.61	0.152
MCHC (g/dL)	33.08	33.10	33.18	0.04	0.619
WBC (x10 ⁶ /mm ³)	3872 ^a	1837 ^b	2310 ^b	268.90	< 0.05
Heterophils (%)	64.40	55.60	69.40	2.82	0.125
Basophils (%)	3.80	2.80	2.00	0.43	0.253
Eosinophil (%)	1.80	2.00	1.40	0.36	0.809
Lymphocyte (%)	28.20	37.60	25.60	2.66	0.154
Monocytes (%)	1.80	2.00	1.60	0.22	0.790

^{a, b} Means within row carry on common superscript are significantly different at P < 0.05.

¹ Each mean represents values from 5 chickens per group (5 replicates x 1 chicken/replicate).

² Treatment consisted of 6% rice bran oil (control), 3% rice bran oil + 3% tuna oil (3% TO), 3% rice bran oil + 3% tuna oil in lipid-based nanoparticles (3% TO-TNP).

³ RBC, Red blood cell; Hb, Haemoglobin; PCV, Packed cell volume; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin.; MCHC, Mean corpuscular haemoglobin concentration; WBC, White blood cells

4.4.6 Plasma biochemistry

The average values of biochemical parameters measured in plasma are presented in Table 4.9. Both TO groups had lower HDL concentration than the control group. The 3% TO_TNP group had lower creatinine plasma concentration but higher alanine amino transferase activity than the control group (P < 0.05). The 3% TO group had a higher aspartate amino transferase activity but a lower uric acid plasma concentration than the other groups (P < 0.06). For all the other measured parameters there were no significant differences between groups.

Table 4.9 Biochemical parameters in plasma of Korat chicken fed diets containing rice bran oil (control) of control, tuna oil (3% TO) or tuna oil in lipid-based nanoparticles (3% TO_TNP)¹.

Parameters ²	Treatment			SEM	P-value
	Control	3% TO	3% TO_TNP		
Glucose (mg/dL)	169.60	167.80	157.60	3.41	0.323
Cholesterol (mg/dL)	116.40	101.60	108.00	3.62	0.261
Triglycerides (mg/dL)	50.8 ^a	27.40 ^b	27.20 ^b	4.17	0.015
LDL (mg/dL)	26.40	30.00	28.40	1.03	0.392
HDL (mg/dL)	53.20 ^a	45.40 ^{ab}	41.20 ^b	1.83	0.011
Total proteins (g/dL)	4.22	4.28	4.16	0.06	0.775
Albumin (g/dL)	2.26	2.40	2.40	0.04	0.275
Total bilirubin (mg/dL)	0.74	0.86	0.96	0.05	0.268
Direct bilirubin (mg/dL)	0.02	0.01	0.02	0.00	0.493
Globulin (g/dL)	1.96	1.88	1.76	0.07	0.486
Blood urea nitrogen (mg/dL)	1.60	2.20	1.80	0.17	0.344
CPK (U/L)	2628.20	2944.40	2453.40	123.67	0.277
LDH (U/L)	845.00	898.00	820.00	33.68	0.661
ALT (U/L)	5.40 ^b	6.20 ^{ab}	7.00 ^a	0.26	0.029
AST (U/L)	244.20 ^b	277.80 ^a	246.60 ^b	6.66	0.058
Creatinine (mg/dL)	0.42 ^a	0.36 ^{ab}	0.32 ^b	0.02	0.021
Uric acid (mg/dL)	7.46 ^a	4.18 ^b	7.18 ^a	0.60	0.034
Na (mmol/L)	153.00	154.40	151.20	0.68	0.160
K (mmol/L)	5.30	5.88	5.28	0.13	0.084
Mg (mg/dL)	2.06	2.14	2.00	0.04	0.331
Ca (mg/dL)	10.20	10.28	10.78	0.17	0.341
Inorganic phosphorus (mg/dL)	8.34	8.42	8.86	0.24	0.663

^{a, b} Means within row carry on common superscript are significantly different at $P < 0.06$.

¹ Each mean represents values from 5 chickens per group (5 replicates x 1 chicken/replicate).

² Treatment consisted of 6% rice bran oil (control), 3% rice bran oil + 3% tuna oil (3% TO), 3% rice bran oil + 3% tuna oil in lipid-based nanoparticles (3% TO-TNP).

³ LDL, Low density lipoprotein; HDL, High density Lipoprotein; CPK, Creatine phosphokinase; LDH, Lactate dehydrogenase; ALT, Alanine amino transferase; AST, Aspartate amino transferase; Na, Sodium; K, Potassium; Cl, Chloride; Mg, Magnesium; Ca, Calcium.

4.5 Discussion

4.5.1 The enrichment n-3 PUFA in slow-growing chicken meat with targeting lipid nanoparticle

According to our previous study, Hang et al. (2018) able to produce the chicken meat with n-3 PUFAs by increasing the proportion of n-3 PUFA oil source in the diet similarly with this study. However, the synthesis process of oil into nanoparticles and dried may losing the important fatty acids. At present is difficult to concluded that the accumulation of n-3 PUFA in the breast and thigh muscles by 3%TO_TNP group caused by nanoparticle directly into muscle cell or through the same pathways of 3%TO. The description of the discovery process will help to understand the deposition of these important fatty acids in the target organs.

The fatty acids in thigh meat found that the content of C14:0 was high in TO groups due to this fatty acid mostly found in tuna oil. Moreover, the content of C18:2n-6 is lowest in 3%TO_TNP group because this fatty acid mainly exists in rice bran oil. The C24:1 content in breast and thigh meat is mostly composed in the control group while found in the formula diet of 3%TO and 3%TO_TNP as well, but not in the rice bran oil and the diet of the control group. There are indicated that the production of intracellular fatty acids (de novo lipogenesis) within the muscles to produce C24:1 and the process require C18:1n-9 as precursors of fatty acid synthesis (Liu et al., 2021).

The different in fatty acid profile in lipid nanoparticles have no changes in the n-6 PUFA and n-3 PUFA in the breast but thigh meat compared to the 3%_TO group. Meanwhile the ratio of n-6/n-3 no significant different. Some fatty acids in the n-6 PUFA group including C18:2n-6 in thigh meat are lowest in the 3%TO_TNP while this fatty acid content in the diet is equal to the control group (Table 4.1).

This research is the first to use targeted nanotechnology to increase the accumulation of n-3 PUFAs in poultry. However, the nanoparticles did not meet expectations in terms of increasing fatty acid accumulation. This is clearly due to the

difference in the fatty acid profile between the lipid nanoparticles and pre-synthesized oils. The drying process is one factor that should be given more attention. Additionally, the direct penetration of nanoparticles filled with n-3 PUFAs into the muscles may stimulate the fat metabolism process.

The present research, it is not yet possible to clearly conclude the efficiency of fatty acid accumulation. However, if it can be demonstrated that the nanoparticles can enter the organs directly, this would be a significant step towards understanding their function. This information is crucial for improving the efficiency of nanoparticles in the future. The fatty acid accumulation does not adequately address whether the nanoparticles can directly enter the muscles. The results of blood sample analysis may help explain the link between nanoparticle use and fatty acid accumulation.

The production of red blood cells depends on the oxygen demand used in the process of requiring oxygen molecules to receive electrons (Stark and Schuster, 2012). This study showed increasing of red blood cell and hemoglobin content in the 3%TO_TNP group. The possible process is such as oxidative phosphorylation in mitochondria (Mokhova and Khailova, 2005). This process can be enhanced by intake of n-3 PUFAs. The fatty acids can stimulate the proliferation of mitochondria (mitochondrial biosynthesis) which can be found in both muscle cells (Vaughan et al., 2012; McGlory et al., 2019) and liver cells (López-Vicario et al., 2023) lead to rapid breakdown the fatty acid through oxidative phosphorylation. This phenomenal requires more oxygen supply (Vaughan et al., 2012; Lepretti et al., 2018). Therefore, it is necessary to produce red blood cells and hemoglobin to transport sufficient oxygen for the reaction (Moneva et al., 2016). This study indicates that supplementing oils rich in n-3 PUFA in form of nanoparticles affects increase the metabolic changes of mitochondria.

Regarding plasma concentrations of cholesterol and LDL in this study, no significant differences were observed between the groups. However, plasma triglyceride and HDL concentrations were lower in the TO groups than in the control group. These results contrast with the findings of Ibrahim et al. (2018), Abbasi et al. (2019), and Avi et al. (2023), who reported that cholesterol and triglyceride levels decreased while HDL levels increased with higher consumption of oils rich in n-3 PUFAs, particularly EPA and DHA. The increase in HDL and triglyceride levels in the

control group in this study may be attributed to its high content of oleic and linoleic acids, as reported by Viveros et al., (2009) and Ibrahim et al. (2018). The reduction in plasma HDL levels in both TO groups is likely explained by the major role of n-3 PUFAs as agonists of peroxisome proliferator-activated receptor-alpha (PPAR α). Activation of PPAR α upregulates the expression of cholesteryl ester transfer protein (CETP), facilitating the transfer of cholesteryl esters from HDL to other lipoproteins. This triglyceride-enriched HDL particles becoming susceptible to hydrolysis by hepatic lipase led to increasing HDL clearance from circulation (Raposo et al., 2014). Although a reduction in HDL levels may appear unfavorable, supplementation with EPA and DHA-rich sources has been shown to reduce triglyceride accumulation while potentially enhancing cholesterol clearance. This process may help prevent excessive cholesterol buildup, which is associated with cardiovascular diseases (Sherratt et al., 2023). Thus, these findings suggest that the TO groups contribute to improved cholesterol homeostasis and support a cardioprotective effect.

The reduction of HDL content in the 3%TO_TNP group is due to the process mentioned above. However, this may be due to the specific transport pathway of nanoparticles and the transport ability of lipid-based nanoparticles with targeted and non-targeted can enter the blood through lymphatic vessel, thus avoiding liver metabolism (Ali Khan et al., 2013). By the ways, the development of lymphatic vessel in poultry is not very complete. Triglycerides are transported into the blood in the form of portomicrons (Alvarenga et al., 2011). So, the obtain of nanoparticles in poultry may mainly be through the blood pathway. This means that nanoparticles may be transported to the portal-mesenteric system and enter the liver, leading to the metabolic process of fat.

4.5.2 The evaluation of the safety of nanocarrier used in Korat chicken feed formulation

4.5.2.1 Targeted lipid nanoparticles have no effect on growth performance and production performance except the visceral organs weight

Supplementing poultry diets with n-3 PUFAs has shown mixed effects on growth performance. While some studies report weight gain (López-Ferrer et al., 2001; Farhoomand & Checaniazer, 2009), others found no significant impact

(Chen et al., 2012; Hang et al., 2018), similar to this experiment. This consistency is likely due to equalized dietary components across all groups, ensuring balanced energy, protein, and nutrients. However, factors such as supplementation duration, starting from day one (López-Ferrer et al., 2001), and the type of oil blended with fish oil including poultry fat (Farhoomand & Checaniazer, 2009), tallow (López-Ferrer et al., 2001), or soybean oil (Abbasi et al., 2019) can influence growth. Additionally, the fatty acid profile balance plays a crucial role in determining growth responses (Poorghasemi et al., 2013).

From a physical and production perspective, evaluating carcass traits provides insights into muscle yield and processing efficiency. In this study, no significant statistical differences were observed in the percentage of chilled parts after refrigeration, indicating that the application of lipid nanoparticles does not negatively impact carcass composition or post-chilling weight retention. This suggests that while nanoparticles may enhance muscle performance at the physiological level, their influence on overall carcass yield remains stable. This means that supplementing nanoparticles in the diet does not have enough impact on muscle function to change the carcass weight and dressing percentage and other relevant observations include heart, spleen, abdominal fat, and meat, but can affect changes in visceral weight including gizzard which the weight of the 3%TO and 3% TO_TNP groups were statistically significantly different ($P=0.034$) compared with the control group.

However, the percentage of liver by live BW was found only in the 3%TO_TNP group with a statistically significant difference ($P=0.005$) compared to the control group. The supplantation oil in nanoparticles form have been shown an effect on the physiological characteristics of the gizzard and liver organs with the increase in muscle function and in term of molecular function, respectively, changes in weight are caused by the following factors: Food ingredients have large particle sizes or roughness. Grind food into large particles size, making it more difficult to pass through organs with enzymatic functions. The effectiveness of lipid-based nanoparticles was studied to demonstrate their resistance to destruction under the conditions in the digestive system. In vitro digestion laboratories, lipid-based nanoparticles exhibit tolerance to simulated gastric fluid (SGF). The particle size remains unchanged. However, these nanoparticles are ruptured in the intestinal environment (simulated small intestinal,

SIF) (Aditya et al., 2013; Park et al., 2017; Alam et al., 2018; Hunthayung et al., 2019; Soleimanian et al., 2020).

In this experiment, the used of TNP in the formular diet is agglomerated to a micrometer particle size and dried. So, that is convenient to be mixed in the diet. That is the reason the effect of increase muscle movement in gizzard. It is possible that micrometer-scale particles can remain stable for long periods of time. Therefore, the muscles have to work more to reduce the particle size. However, there are no experimental results showing to support that reason. Therefore, the function of the gizzard muscles and the fragmentation of nanoparticles should be further studied.

The increasing weight of liver organ in the 3%TO_TNP group may be due to the presence of n-3 PUFA in the diet as found in a study by Attia et al. (2020), high levels can lead to weight gain in liver organs. In addition, it may be related to toxicity response, leading to the accumulation of toxins lead to Increase the detoxification process. The high level of detoxification process might affect the normality of liver cells, thereby increasing the overall organ weight of the liver (ŚLiżewska et al., 2019)

However, the studies of nanotoxicity response are based on hematology and serum biochemical values in blood samples. Serum biochemistry can be used to indicate organ function that may be caused by cellular abnormalities (such as enzymes). The creatinine phosphokinase found in the blood can indicate liver injury (Navidshad et al., 2019) and muscle tissue activity (Sandercock et al., 2009, Baird et al., 2012). In addition, AST, ALT, uric acid, and creatine Functional indicators related to liver and renal toxicity reactions (Campion et al, 2013; Mohammadi et al., 2016).

Measuring pH at 24h, meat color (L *, a *, b *), drip loss, cooking loss, and shear force is essential for understanding the effects of lipid nanoparticles on muscle metabolism, water-holding capacity, and structural integrity. However, in this study, the 3% TO_TNP group did not significantly affect meat quality traits, implying that the incorporation of lipid nanoparticles does not alter the fundamental physicochemical properties of chicken meat. This finding is critical, as it suggests that while nanoparticles may improve muscle performance at the cellular level, they do not compromise meat texture, color stability, or tenderness, which are key factors for consumer acceptance and industrial processing. The white meat after slaughter is a saccharification process caused by the accumulation of glycogen and the stress during the slaughter process

(Baéza et al., 2022). However, some research has found that PUFAs also have an effect on changes in meat quality, such as the percentage of cooking loss increased with the group supplemented with n-3 PUFA sources from linseed oil and tuna oil. The cooking loss was increased due to the oxidation of long-chain polyunsaturated fatty acids (Hang et al., 2018). But the results of this experiment show that the amount of n-3 PUFA oil used in the formular diet did not affect the change in meat quality. Although oil supplementation in the form of nanoparticles did not affect the change in meat quality but responses at the cellular level can be different or involve other processes. However, cellular responses from different organs can be determined by considering biochemicals present in animal blood samples which can help to identify the processes that may be affected by nanoparticle augmentation.

4.5.2.2 Targeted lipid nanoparticles effect on hematological and plasma biochemical parameters

Based on the toxicity response study from blood samples. The differences in blood counts, hemoglobin and hematocrit, were observed in 3%TO_TNP supplementation group, indicating that the animal body responded to the nanoparticle supplementation. As hematocrit is represents the amount of red blood cells produced per total blood volume while red blood cells also contain hemoglobin molecules in red blood cells (Billett, 1990) thus the hemoglobin and hematocrit amounts are found to be in the same direction.

Red blood cell density and hemoglobin in all three experimental groups were within the standard of poultry (Abdulazeez et al., 2016; Soualio et al., 2021). Thus, it can be indicated that oil supplementation in the form of nanoparticles have no negative effect on red blood cell and white blood cell activity in Korat chickens. However, the study of blood parameter is only show the final product that response of the cells stimulated with lipid nanoparticles. Therefore, all the pathways involved should be studied to demonstrate the working mode that occurs when receiving lipid-based nanoparticles.

The determination of CPK, ALT, AST, and LDH in serum can be used to indicate liver and muscle damage. CPK and LDH are used as indicators of muscle injury and myopathy, respectively (Kong et al., 2021; Meloche et al., 2018). ALT and AST can indicate liver dysfunction, including cell damage, and the loss of liver cell wall function

(Kumar et al., 2005), as this enzyme is sensitive to reactions with contamination of toxic substances in food (Ahamefule et al., 2006).

The results indicated that cell activity showed no significant difference in the group that received a diet supplemented with n-3 PUFA. However, the ALT levels observed in this study ranged from 9 to 43 U/L, which is within the normal range reported by Gudiso et al. (2019), suggesting that enzyme function remained stable. In contrast, AST levels are typically expected to fall within the normal range of 70 to 220 U/L (Odunitan-Wayas et al., 2018). However, in the present study, all treatment groups showed AST levels ranging from 244 to 277 U/L. This discrepancy may be due to species differences, as Alam et al. (2020) reported that indigenous chickens tend to exhibit higher AST levels. Additionally, the increase in AST levels in the 3% TO group may be attributed to lipid oxidation, which could potentially alter liver function.

The level of creatinine and uric acid can be used as indicators of protein metabolism in liver organs (Qasem et al., 2016) and able to excreted by renal organ (Al-Garadi et al., 2022). Moreover, creatinine level can indicate the occurrence of muscle metabolism as well (Ostermann et al., 2016). Therefore, the amount of creatine and uric acid could be analyzed from the blood sample is an indicator of protein's metabolism to the derivatives production. In this study, there was found that the creatinine content was lower with 3%TO_TNP group than that the control group, but no significant difference with the 3%TO group.

According to Keegan et al. (2020), supplementing with n-3 PUFA sources have no affect the creatinine level. In contrast, the present study found that the creatinine level was decreased in the 3%TO group compared to the control group. The use of nanoparticles in high dose could lead to excreted the waste by the liver and kidneys into the blood circulation, including increase the level of creatine and creatinine and decreased some metabolites such as citrate, alpha-ketoglutarate, succinate and betaine indicated that the oxidative stress and toxicity is appear in liver and kidney organs (Valentini et al., 2019). However, the creatinine level found in the 3%TO_TNP group was lower compared with the control group. The result indicating that the supplementation of nanoparticles in dietary does not negative effect on liver or renal function.

Anyway, the decrease of creatinine content in the blood is could associated with a decrease in muscle mass (Wyss and Kaddurah-Daouk, 2000; Thongprayoon et al., 2016; Ostermann et al., 2016) or caused by muscle cell damage, leading to the release of enzymes or cellular waste into the bloodstream (Kong et al., 2021). However, damaged muscle cells can be detected by increasing the amount of CK enzyme (Kong et al., 2021). In this study, the amount of the CK have no statistically significant difference, which means that supplementing the nanoparticles did not affect change the muscle cells mass. However, nanoparticles that directly enter muscles may lead to high intake of n-3 PUFAs.

The high in n-3 PUFA stimulate energy generation through oxidative phosphorylation (Lepretti et al., 2018), thereby improving the performance of oxygen muscle (Veras et al., 2021; Young et al., 2021) leading to increase the activity of oxidative related genes, proteins and enzyme, that act on fat breakdown and inhibit fat synthesis processes (Philp et al., 2015). By using of fat as the main fuel source which increase the function of slow twitch oxidative muscle types whereas decrease in glycolytic skeletal muscles function, could be affects the decrease in muscle mass (Philp et al., 2015). The possibility reason in reduced the creatinine level due to the changes in muscle functional processes.

The uric acid content in the 3%TO_TNP group was higher than that 3%TO group but no different result compared with the control group. It could be indicated that the nanoparticles supplementation can affect renal function, but it is unclear as a same pathway by the control group or not. By the way, an rise in uric acid can indicate an increase in renal function or responding to the toxicity by nanoparticles supplementation could lead to renal dysfunction (Mahmoud et al., 2019).

The present study can be concluded in health status of animals in this study received the lipid nanoparticles have no negative effect on liver, muscles and kidney function. The amount of metabolites is within the normal range, creatinine is normal in the range of 0.1- 0.4 mg/dl (Fitri et al., 2021) and uric acid is in the range of 1.9-12.5 mg/dl (Odunitan-Wayas et al., 2018).

4.6 Conclusion

This research is the first demonstration that it is possible to enrich the chicken meat with n-3 PUFAs by using diets containing targeted lipid nanoparticles made with tuna oil. In the present study, the enrichment with n-3 PUFAs was less efficient than that obtained with crude tuna oil directly incorporated in the diet. However, this result could be improved by optimizing the synthesis process of targeted lipid nanoparticles modifying the temperature levels for heating and drying steps for example or increasing the initial amount of tuna oil. Further research is needed to optimize this synthesis process but also to understand how the targeted lipid nanoparticles cross the intestinal epithelium barrier, are transported into the blood and/or lymphatic circulation and how the n-3 PUFAs are delivered into the muscles. The present study also showed that targeted lipid nanoparticles had no negative effects on liver, muscles and kidney functions. Nevertheless, as the final consumer of chicken meat is the human, the safety of poultry meat enriched with n-3 PUFAs lipid nanoparticles should be controlled. Finally, when the synthesis process of targeted lipid nanoparticles will be stabilized, it will be interesting to realize an economical evaluation of the feed cost.

4.7 References

- Abbasi, F., Samadi F., Jafari S. M, Ramezanzpour S., & Shams-Shargh M. (2019). Production of omega-3 fatty acid-enriched broiler chicken meat by the application of nanoencapsulated flaxseed oil prepared via ultrasonication. **Journal of Functional Foods**, 57, 373-381.
- Abdulazeez, H., Adamu, S., Igwebuike, J. U., Gwayo, G. J., & Muhammad, A. I. (2016). Haematology and serum biochemistry of broiler chickens fed graded levels of baobab (*Adansonia digitata* L.) seed meal. **IOSR Journal of Agriculture and Veterinary Science**, 9(10), 48–53.
- Aditya, N. P., Shim, M., Lee, I., Lee, Y., Im, M. H., & Ko, S. (2013). Curcumin and genistein coloaded nanostructured lipid carriers: In vitro digestion and antiproliferative cancer activity. **Journal of Agricultural and Food Chemistry**, 61(8), 1878–1883.
- Ahamefule, F. O., Eduok, G. O., Usman, A., Amaefule, K. U., Obua, B. E., & Oguike, S. A. (2006). Blood biochemistry and haematology of weaner rabbits fed sundried,

- ensiled, and fermented cassava peel-based diets. **Pakistan Journal of Nutrition**, 5, 248–253.
- Akinola, L. A. F., & Etuk, M. O. (2015). Haematological and serum biochemical responses of broilers fed varying levels of Indomie waste-based diets. **IOSR Journal of Agriculture and Veterinary Science**, 8(3), 66–70.
- Alam, M., Ullah, M., Umme, S., Malik, F., Shahidul, M., & Islam, M. S. (2020). Broiler and Indigenous Chickens: A Comparison through Biochemical Parameters. **International Journal of Sustainable Agricultural Research**, 7.
- Alam, T., Khan, S., Gaba, B., Haider, M. F., Baboota, S., & Ali, J. (2018). Adaptation of quality by design-based development of isradipine nanostructured-lipid carrier and its evaluation for in vitro gut permeation and in vivo solubilization fate. **Journal of Pharmaceutical Sciences**, 107(11), 2914–2926.
- Al-Garadi, M. A., Al-Baadani, H. H., & Alqhtani, A. H. (2022). Growth performance, histological changes, and functional tests of broiler chickens fed diets supplemented with Tribulus terrestris powder. **Animals**, 12, 1930.
- Ali Khan, A., Mudassir, J., Mohtar, N., & Darwis, Y. (2013). Advanced drug delivery to the lymphatic system: Lipid-based nanoformulations. **International Journal of Nanomedicine**, 8, 2733–2744.
- Alvarenga, R., Zangeronimo, M., Pereira, L., Rodrigues, P., & Gomide, E. (2011). Lipoprotein metabolism in poultry. **World's Poultry Science Journal**, 67(3), 431–440.
- Attia, Y. A., Al-Harhi, M. A., & Abo El-Maaty, H. M. (2020). The effects of different oil sources on performance, digestive enzymes, carcass traits, biochemical, immunological, antioxidant, and morphometric responses of broiler chicks. **Frontiers in Veterinary Science**, 7.
- Avi, A. C., Islam, M. T., Bari, M. S., Khandoker, R. S. S., & Das, G. B. (2023). Effects of fish oil on growth performance, carcass characteristics, blood parameter, and cost efficiency of broiler chicken. **Advances in Animal and Veterinary Sciences**, 11(7), 1083-1089.
- Baéza, E., Guillier, L., & Petracci, M. (2022). Review: Production factors affecting poultry carcass and meat quality attributes. **Animal**, 16, 100331.

- Baird, M. F., Graham, S. M., Baker, J. S., & Bickerstaff, G. F. (2012). Creatine-kinase- and exercise-related muscle damage: Implications for muscle performance and recovery. **Journal of Nutrition and Metabolism**, 2012, 960363.
- Billett, H. H. (1990). **Hemoglobin and hematocrit**. In: Walker, H. K., Hall, W. D., & Hurst, J. W. (Eds.), *Clinical Methods: The History, Physical, and Laboratory Examinations* (3rd ed.). Boston: Butterworths. Chapter 151. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK259/>
- Campion, S., Aubrecht, J., & Boekelheide, K. (2013). The current status of biomarkers for predicting toxicity. **Expert Opinion on Drug Metabolism & Toxicology**, 9(11), 1–28.
- Chen, W., Wang, J. P., & Huang, Y. Q. (2012). Effects of dietary n-6:n-3 polyunsaturated fatty acid ratio on cardiac antioxidative status, T-cell and cytokine mRNA expression in the thymus, and blood T lymphocyte subsets of broilers. **Livestock Science**, 150, 114–120.
- Das, S., & Chaudhury, A. (2011). Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. **AAPS PharmSciTech**, 12(1), 62–76.
- Davies, E. T., Benson, J. A., Bicknel, S. R., Grey, D. E., Hewitt, S. G., Lloyd, Morrison, J. R. S., ... Purvis, C. M. (1984). MAFF, **Ministry of Agriculture, Fisheries and Food Manual of Veterinary Investigation Techniques** (3rd ed., Volume 2). MAFF/ADASS. (Reference Book 1984), 390.
- Farhoomand, P., & Checaniazar, S. (2009). Effects of graded levels of dietary fish oil on the yield and fatty acid composition of breast meat in broiler chickens. **Journal of Applied Poultry Research**, 18, 508–513.
- Fitri, A., Fitriana, I., Rosetyadewi, A., Pratama, A., Septiana, A., Setiawan, D. C., & Wijayanti, A. (2021). The effect of colistin administration as medicated feed on alanine aminotransferase and creatinine level in broiler infected with *Escherichia coli*. **BIO Web of Conferences**, 33, 03002.
- Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. **Journal of Biological Chemistry**, 226(1), 497–509.
- Gudiso, X. C., Hlatini, V. A., Ncobela, C. N., Chimonyo, M., & Mafongoya, P. L. (2019). Serum metabolites and weights of internal organs of broilers fed on varying levels

- of *Acacia angustissima* leaf meal. **Canadian Journal of Animal Science**, 99, 475–481.
- Hang, T. T. T., Molee, W., & Khempaka, S. (2018). Linseed oil or tuna oil supplementation in slow-growing chicken diets: Can their meat reach the threshold of a “high in n-3 polyunsaturated fatty acids” product? **Journal of Applied Poultry Research**, 27, 389–400.
- Hunthayung, K., Klinkesorn, U., Hongsprabhas, P., & Chanput, W. (2019). Controlled release and macrophage polarizing activity of cold-pressed rice bran oil in a niosome system. **Food Function**, 10(6), 3272–3281.
- Ibrahim, D., El-Sayed, R., Khater, S. I., Said, E. N., & El-Mandrawy, S. A. M. (2018). Changing dietary n-6:n-3 ratio using different oil sources affects performance, behavior, cytokines mRNA expression and meat fatty acid profile of broiler chickens. **Animal Nutrition**, 4, 44-51.
- Jain, N. C. 1986. Schalm’s veterinary hematology. Pages 140-175 in Schalm’s Veterinary Hematology. 4th ed. Lea and Febiger, Philadelphia, USA.
- Keegan, J. D., Fusconi, G., Morlacchini, M., & Moran, C. A. (2020). Comparing docosahexaenoic acid supplementation strategies in terms of broiler tissue enrichment, productivity, and cost. **Journal of Applied Poultry Research**, 29, 636–652.
- Kong, F., Zhao, G., He, Z., Sun, J., Wang, X., Liu, D., ... Wen, J. (2021). Serum creatine kinase as a biomarker to predict wooden breast in vivo for chicken breeding. **Frontiers in Physiology**, 12, 711711.
- Kumar, G., Banu, G. S., Kannan, V., & Pandian, M. R. (2005). Antihepatotoxic effect of beta-carotene on paracetamol-induced hepatic damage in rats. **Indian Journal of Experimental Biology**, 43(4), 351–355.
- Lepretti, M., Martucciello, S., Burgos Aceves, M. A., Putti, R., & Lionetti, L. (2018). Omega-3 fatty acids and insulin resistance: Focus on the regulation of mitochondria and endoplasmic reticulum stress. **Nutrients**, 10, 350.
- Lim, S. B., Banerjee, A., & Önyüksel, H. (2012). Improvement of drug safety by the use of lipid-based nanocarriers. **Journal of Controlled Release**, 163(1), 34–45.

- Liu, F., Wang, P., Xiong, X., Zeng, X., Zhang, X., & Wu, G. (2021). A review of nervonic acid production in plants: Prospects for the genetic engineering of high nervonic acid cultivars. **Frontiers in Plant Science**, 12, 626625.
- López-Ferrer, S., Baucells, M. D., Barroeta, A. C., & Grashorn, M. A. (2001). n-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: Fish oil. **Poultry Science**, 80, 741–752.
- López-Vicario, C., Sebastián, D., Casulleras, M., Duran-Güell, M., Flores-Costa, R., Aguilar, F., ... Clària, J. (2023). Essential lipid autacoids rewire mitochondrial energy efficiency in metabolic dysfunction-associated fatty liver disease. **Hepatology**, 77(4), 1303–1318.
- Lu, H., Zhang, S., Wang, J., & Chen, Q. (2021). A review on polymer and lipid-based nanocarriers and its application to nano-pharmaceutical and food-based systems. **Frontiers in Nutrition**, 8, 783831.
- Mahmoud, U. M., Mekawy, I. A. A., Naguib, M., & Sayed, A. E. H. (2019). Silver nanoparticle-induced nephrotoxicity in *Clarias gariepinus*: Physio-histological biomarkers. **Fish Physiology and Biochemistry**, 45(6), 1895–1905.
- McGlory, C., Calder, P. C., & Nunes, E. A. (2019). The influence of omega-3 fatty acids on skeletal muscle protein turnover in health, disuse, and disease. **Frontiers in Nutrition**, 6, 144.
- Meloche, K. J., Fancher, B. I., Emmerson, D. A., Bilgili, S. F., & Dozier, W. A. (2018). Effects of reduced digestible lysine density on myopathies of the Pectoralis major muscles in broiler chickens at 48 and 62 days of age. **Poultry Science**, 97(9), 3311–3324.
- Metcalfe, L. D., Schmitz, A. A., & Pelka, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. **Analytical Chemistry**, 38(3), 514–515.
- Mohammadi, F., Noori, A., & Mohammadi, A. (2016). Effects of titanium dioxide nanoparticles toxicity on the kidney of male rats. **International Journal of Life Sciences**, 10, 65.
- Mokhova, E. N., & Khailova, L. S. (2005). Involvement of mitochondrial inner membrane anion carriers in the uncoupling effect of fatty acids. **Biochemistry (Moscow)**, 70, 159–163.

- Moneva, P., Yanchev, I., Dyabolova, M., & Gudev, D. (2016). Hematocrit as a potential marker of acclimatization capacity and stress sensitivity in sheep exposed to transport and high altitude. **Journal of Veterinary Medicine and Animal Health**, 22, 999–1005.
- Navidshad, B., Maghsoodi, Z., Nikbin, S., & Kalantari Hesari, A. (2019). Dietary garlic powder supplementation could ameliorate unfavorable effects of choline deficiency on the liver health and immune system of broiler chickens. **Iranian Journal of Applied Animal Science**, 9, 337–346.
- Niot, I., Tran, T. T., & Besnard, P. (2009). Intestinal absorption of long-chain fatty acids: Evidence and uncertainties. **Progress in Lipid Research**, 48(2), 101–115.
- Odunitan-Wayas, F., Kolanisi, U., & Chimonyo M. (2018). Haematological and Serum Biochemical Responses of Ovambo Chickens Fed Provitamin A Biofortified Maize. **Brazilian Journal of Poultry Science**, 20, 425-434.
- Ostermann, M., Kashani, K., & Forni, L. G. (2016). The two sides of creatinine: Both as bad as each other? **Journal of Thoracic Disease**, 8, E628–E630.
- Park, S. J., Garcia, C. V., Shin, G. H., & Kim, J. T. (2017). Development of nanostructured lipid carriers for the encapsulation and controlled release of vitamin D3. **Food Chemistry**, 225, 213–219.
- Philp, L., Heilbronn, L., Janovska, A., & Wittert, G. (2015). Dietary enrichment with fish oil prevents high fat-induced metabolic dysfunction in skeletal muscle in mice. **PLOS ONE**, 10, e0117494.
- Poonia, N., Kharb, R., Lather, V., & Pandita, D. (2016). Nanostructured lipid carriers: Versatile oral delivery vehicle. **Future Science OA**, 2(3), FSO135.
- Poorghasemi, M., Seidavi, A., Qotbi, A. A., Laudadio, V., & Tufarelli V. (2013). Influence of dietary fat source on growth performance responses and carcass traits of broiler chicks. **Asian-Australasian Journal of Animal Sciences**. 26:705-710.
- Qasem, M., Alhajj, M., Nabi, A. R., & Al-Mufarrej, S. (2016). Effects of dietary supplement of turmeric powder (*Curcuma longa*) on blood biochemistry parameters and antioxidant activity in chickens. **South African Journal of Animal Science**, 46, 204–213.

- Raposo, H. F., Patrício, P. R., Simões, M. C., & Oliveira, H. C. (2014). Fibrates and fish oil, but not corn oil, up-regulate the expression of the cholesteryl ester transfer protein (CETP) gene. **Journal of Nutritional Biochemistry**, 25, 669-674.
- Rodriguez-Ruiz, V., Salatti-Dorado, J. Á., Barzegari, A., Nicolas-Boluda, A., Houaoui, A., Caballo, C., ... Pavon-Djavid, G. (2018). Astaxanthin-loaded nanostructured lipid carriers for preservation of antioxidant activity. **Molecules**, 23(10), 2601.
- Saedi, A., Rostamizadeh, K., Parsa, M., Dalali, N., & Ahmadi, N. (2018). Preparation and characterization of nanostructured lipid carriers as drug delivery system: Influence of liquid lipid types on loading and cytotoxicity. **Chemistry and Physics of Lipids**, 216, 65-72.
- Sandercock, D. A., Barker, Z. E., Mitchell, M. A., & Hocking, P. M. (2009). Changes in muscle cell cation regulation and meat quality traits are associated with genetic selection for high body weight and meat yield in broiler chickens. **Genetics Selection Evolution**, 41(1), 8.
- Sanguansri, L., Day, L., Shen, Z., Fagan, P., Weerakkody, R., Cheng, L. J., ... Augustin, M. A. (2013). Encapsulation of mixtures of tuna oil, tributyrin and resveratrol in a spray dried powder formulation. **Food Function**, 4(12), 1794-1802.
- Sherratt, S. C. R., Libby, P., Budoff, M. J., Bhatt, D. L., & Mason, R. P. (2023). Role of Omega-3 Fatty Acids in Cardiovascular Disease: the Debate Continues. **Current Atherosclerosis Reports**, 25, 1-17.
- Śliżewska, K., Cukrowska, B., Smulikowska, S., & Cielecka-Kuszyk, J. (2019). The effect of probiotic supplementation on performance and the histopathological changes in liver and kidneys in broiler chickens fed diets with aflatoxin B1. **Toxins**, 11(2), 112.
- Soleimanian, Y., Goli, S. A. H., Varshosaz, J., Di Cesare Mannelli, L., Ghelardini, C., Cirri, M., & Maestrelli, F. (2020). Beta-sitosterol loaded nanostructured lipid carrier: Physical and oxidative stability, in vitro simulated digestion and hypocholesterolemia activity. **Pharmaceutics**, 12(4), 386.
- Soualio, K., Paul, Y., Angoua Baudouin, K., & Bleyere, M. (2021). Hematological profile of broilers and local chickens in Korhogo, Cote d'Ivoire. **International Journal of Agriculture, Environment and BioResearch**, 6, 2456-2864.

- Stark, H., & Schuster, S. (2012). Comparison of various approaches to calculating the optimal hematocrit in vertebrates. **Journal of Applied Physiology**, 113(3), 355–367.
- Tasoniero, G., M. Cullere, M. Cecchinato, E. Puolanne, and A. Dalle Zotte. 2016. Technological quality, mineral profile, and sensory attributes of broiler chicken breasts affected by White Striping and Wooden Breast myopathies. *Poult. Sci.* 95:2707-2714.
- Thongprayoon, C., Cheungpasitporn, W., & Kashani, K. (2016). Serum creatinine level, a surrogate of muscle mass, predicts mortality in critically ill patients. **Journal of Thoracic Disease**, 8(5), E305–E311.
- Valentini, X., Rugira, P., Frau, A., Tagliatti, V., Conotte, R., Laurent, S., ... Nonclercq, D. (2019). Hepatic and renal toxicity induced by TiO₂ nanoparticles in rats: A morphological and metabonomic study. **Journal of Toxicology**, 2019, 5767012.
- Vaughan, R. A., Garcia-Smith, R., & Bisoffi, M. (2012). Conjugated linoleic acid or omega-3 fatty acids increase mitochondrial biosynthesis and metabolism in skeletal muscle cells. **Lipids in Health and Disease**, 11, 142.
- Veras, A. S. C., Gomes, R. L., Almeida Tavares, M. E., Giometti, I. C., Cardoso, A. P. M. M., da Costa Aguiar Alves, B., ... Teixeira, G. R. (2021). Supplementation of polyunsaturated fatty acids (PUFAs) and aerobic exercise improve functioning, morphology, and redox balance in prostate obese rats. **Scientific Reports**, 11(1), 6282.
- Viveros, A., Ortiz L. T., Rodríguez M. L., Rebolé A., Alzueta C., Arija I., ... Brenes A. (2009). Interaction of dietary high-oleic-acid sunflower hulls and different fat sources in broiler chickens. **Poultry Science**. 88, 141-151.
- Winter, E., Dal Pizzol, C., Locatelli, C., & Crezkynski-Pasa, T. B. (2016). Development and evaluation of lipid nanoparticles for drug delivery: Study of toxicity in vitro and in vivo. **Journal of Nanoscience and Nanotechnology**, 16(2), 1321–1330.
- Wyss, M., & Kaddurah-Daouk, R. (2000). Creatine and creatinine metabolism. **Physiological Reviews**, 80, 1107–1213.
- Yeh, Y. C., Kim, S. T., Tang, R., Yan, B., & Rotello, V. M. (2014). Insulin-based regulation of glucose-functionalized nanoparticle uptake in muscle cells. **Journal of Materials Chemistry B**, 2, 4610–4614.

- Young, K. G., Vanderboor, C. M., Regnault, T. R. H., & Guglielmo, C. G. (2021). Species-specific metabolic responses of songbird, shorebird, and murine cultured myotubes to n-3 polyunsaturated fatty acids. **American Journal of Physiology-Regulatory, Integrative and Comparative Physiology**, 320, R362–R376.
- Zhang, J., H. Zhuang, J. Cao, A. Geng, H. Wang, Q. Chu, Z. Yan, X. Zhang, Y. Zhang, and H. Liu. 2022. Breast meat fatty acid profiling and proteomic analysis of Beijing-You chicken during the laying period. *Front. Vet. Sci.* 9:908862.
- Zhang, T., Chen, J., Zhang, Y., Shen, Q., & Pan, W. (2011). Characterization and evaluation of nanostructured lipid carriers as a vehicle for oral delivery of etoposide. **European Journal of Pharmaceutical Sciences**, 43(3), 174–179.
- Zhou, X., Zhang, X., Ye, Y., Zhang, T., Wang, H., Ma, Z., & Wu, B. (2015). Nanostructured lipid carriers used for oral delivery of oridonin: An effect of ligand modification on absorption. **International Journal of Pharmaceutics**, 479, 391–399.