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The Effect of Feeding Duration on Omega Fatty Acid Accumulation in Muscle of Village Chicken Fed Diet Supplemented with Flaxseed Oil

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ABSTRACT

Village chicken is an indigenous chicken which is known to have lean meat and now becoming one of the important protein sources available in Malaysia. The demand for this chicken has also increased in recent years as people has become more health conscious, and value-adding the meat product may be an advantage to improve its functional properties. Thus, an experiment was conducted to determine the optimum feeding duration of village chicken fed with diet supplemented with flaxseed oil on the accumulation of omega fatty acids in the breast and thigh muscles. A total of 120 male village chickens were randomly assigned to 4 treatments with 5 replications and were fed omega supplemented feed for 3 different duration lengths. The chickens were fed isonitrogenous and isocaloric corn-soy based diets supplemented with 2% flaxseed oil as omega source for 5 weeks (T5), 3 weeks (T3), 1 week (T1), and a control diet (T0) without any supplementation of flaxseed oil for 5 weeks. The omega fatty acids, linoleic acid (omega-6) and alpha-linolenic acid (omega-3) were present in all breast and thigh muscles except for control breast muscle. Chickens fed with flaxseed oil for 5 weeks showed highest accumulation of alpha-linolenic acid in thigh muscle (85.74mg/100g). Longer duration of feeding with flaxseed oil resulted with higher accumulation of alpha-linolenic acid in thigh muscle. In the breast muscle however, accumulation of alpha-linolenic acid was found to be abundant in the 3 weeks (42.12mg/100g) and 5 weeks (40.39mg/100g) treatment. The linoleic acid content in both thigh (360.22–440.95mg/100g) and breast (177.78–221.55mg/100g) muscles however were higher in all feeding durations compared to alpha-linolenic acid. In this study, it was found that 3 weeks of flaxseed oil supplementation is sufficient to accumulate the alpha-linolenic acid into the breast and 5 weeks supplementation for thigh muscle of village chicken.

Keywords: Alpha-linolenic Acid, Breast, Flaxseed oil, Omega-3, Thigh

INTRODUCTION

Omega-3 fatty acid is one of the nutrients which have become of great interest due to its good functional properties for health. The intake of omega especially omega-3 is however lacking and low among Malaysian (Ng *et al.,* 2012). Currently, worldwide ratio intake of omega-6 to omega-3 reaches 20:1, however recommendation for these omega intakes is 4:1 (Cartino Mancinelli *et al.,* 2022). Mostly, omega-3 has been taken into human through direct consumption of sources rich with omega such as fish, algae and plants like flaxseed and canola (Rymer & Givens, 2005). Intake through oral supplementation is also available, however this method is somehow greatly affected by the pricey cost of the supplement which may be unaffordable to some people. Enrichment or fortification of food products such as chicken meat and eggs through manipulation of fatty acids/lipid profile of the poultry diet (Bhalerao et al., 2014) seems to be another applicable way in which it helps to deliver the nutrients to the consumers body without changing the feeding habit (Konieckza *et al*., 2017; Rymer *et al*., 2010). Flaxseed oil is known to contain high polyunsaturated fatty acids which is 73% of the total fatty acids in the oil (Kajla *et al.,* 2014). It is one of the richest plant sources of omega-3 fatty acids (Gebauer *et al.,* 2006), mainly the short chain alpha-linolenic acid (ALA). The ALA in flaxseed oil ranges from 39 to 60.42% (Goyal et al., 2014). The use of this flaxseed oil in poultry diet may be an excellent way to deliver its high functional omega-3 into the chicken meat for human needs (Moghadam & Cherian, 2017).

The consumption of chicken meat as main protein source in Malaysia has increased steadily, which was recorded to raise from 40.09kg per capita in 2009 to 49.30kg per capita after a decade, and will hike up to 51.28kg by 2025 as forecasted by the Department of Veterinary Services Malaysia (DVS, 2020). This high intake of poultry meat by Malaysian has made this country to be ranked third in world and the first in Asia for poultry consumption (Ferlito, 2020). Native chicken or commonly described as village chicken and free-range chicken is a slow growing chicken which recently creating high demand and is favoured by the Malaysians as a healthier choice of chicken meat. The production of village chicken has also increased in past years from 5.14% population in 2015 to 8.22% from whole chicken population in the year 2020 (DVS, 2020). MARDI village chicken is one of the village chicken's breeds that has the advantageous of good egg production (3-4 folds more than the common village chicken), with more meat and less fat. Besides that, it has a good FCR of 1.5 and could reach harvesting weight of 1.2-1.23 kg in 12 weeks of rearing making it to be a potential local village chicken for commercialization purpose (Muhammad et al., 2019).

As to author's knowledge, there are very few or limited reports on the absorption duration and accumulation of Omega in free-range or village chicken. In commercial broilers, however, Konieczka *et al.,* (2017) has reported that a week of feeding is sufficient to enrich breast meat with Omega 3 fatty acids with two weeks for the thigh parts. Prolonged feeding of chicken with this high polyunsaturated fatty acid (PUFA) may also be attributed to the decrease of the meat storage stability (Alagawany *et al.,* 2019) and increasing the production cost. Hence, this study was conducted to determine the optimum feeding duration for the ALA omega-3 to be deposited in the muscles (breast and thigh) of village chicken.

MATERIALS AND METHODS

Animal ethics approval

This experiment was conducted accordingly under the approval of MARDI Animal Ethics (2020827/R/MAE00092).

Animal preparation and feeding trial

This experiment was conducted using a 7-week MARDI male village chicken. The chicken was reared from day old chick (obtained from MARDI Muadzam Shah) until it reached the age for the experiment at MARDI Serdang farm. A total of 120 chickens were then randomly assigned to 4 treatments with 5 replications and 6 birds in each cage. The birds were reared in steel battery cages equipped with a water drinker and feeder and is available *ad libitum*. The feeding duration was conducted for 5 weeks according to the scheduled feeding regime (Table 1). The treatment consists of different durations of 2% Flaxseed oil (FO) supplementation at 1 week (T1), 3 weeks (T3) and 5 weeks (T5) before slaughter and a control treatment (T0) without any FO supplementation at all. Diets were formulated to contain optimum nutritional requirements as recommended by MARDI breed nutritional requirement guide of at least 19% of crude protein and 11 MJ/kg of energy.

Table 1. Feeding regime and duration of birds fed with supplemented Flaxseed oil (FO).

Birds age \Treatment	7-8wk	8-9wk	9-10wk	10-11wk	11-12wk
T0					
T1					
T2					
T3					

T0-Control, T1- 1 week FO supplementation, T2- 3 weeks FO supplementation, T3- 5 weeks FO supplementation

Basal Diet
Experimental Diet (Omega feed)

Chickens weight and feed intake were measured fortnightly to record their body weight gain and their feed intake efficiency. Daily routine (maintenance) as of feeding the bird was done twice in the morning and evening, and feces cleaning every alternate day. Daily temperature and humidity were monitored everyday using a data logger.

Sample preparation

At the age of 12 weeks, three birds from each cage were randomly sampled, weighed, and slaughtered to collect muscle tissue consist of the pectoralis major and minor (breast) and bicep femoris (thigh) to evaluate the fatty acids component. Only the right-side muscle's part was collected for the lipid and fatty acid analysis. Analysis of fatty acid methyl esterification (FAMEs) using direct methylation was performed to quantify the component in the muscle samples. The experimental feeds were also analysed for proximate content and fatty acids composition. All samples were dried in the oven at 60°C until it was fully dried with constant weight. The dried samples were ground to a fine smooth texture using pestle and mortar and homogenized using a homogenizer.

Chemical analysis

Proximate composition analysis

The feeds were analyzed for proximate, energy and fatty acid composition. Crude protein, fat, fiber, moisture, and ash were analyzed following the AOAC method with crude protein using Kjedahl method (Gerhardt brand). Energy was quantified using Bomb Calorimeter (3000, IKA brand) for the determination of gross energy of the feed. Dry matter was determined after drying the feed in oven at 135°C for 2 hours until it reaches constant weight (AOAC, 1999). The proximate composition of the control and flaxseed oil feed is as presented in Table 2.

Table 2. Proximate and omega fatty acid compositions of feeds.

Components	Control Feed	Flaxseed Oil Feed
Dry matter (%)	88.72	88.96
Crude protein (%)	18.96	19.86
Gross energy (MJ/kg)	16.33	16.36
Crude fat (%)	2.79	3.99
Crude fiber (%)	3.24	3.27
Ash (%)	4.93	6.22
Fatty acids (mg/100g) in feed		
Linoleic acid	97.61	106.42
Alpha-linolenic acid	11.39	110.25
Palmitic acid	156.33	98.91
Stearic acid	16.40	24.21
Oleic acid	179.48	142.14

Lipid analysis

Sample of muscles and the feeds were analysed for lipid composition using the common chloroform: methanol (2:1) Folch solution and method as described by Wang *et al.,* (2000). A 3.0g sample was weighed in a 50ml beaker and was soaked with 40ml chloroform: methanol solution and shaken well using shaker for 2 minutes. The prepped beaker was then left soaked overnight. The soaked samples were then poured into a separating funnel with 40mm Whatman filter paper, and 10ml of 0.88% NaCl was added into the separating funnel. After 4 hours, the solution in the funnel will separate, and the bottom layer of the funnel were siphoned into the rotavapor flask. The weight of lipids in the samples were determined after evaporating under vacuum in a rotary evaporator at 40°C until dried.

Fatty Acid Methyl Esterification (FAME) analysis

As for fatty acid analysis, 3ml of methanolic-HCL (3N) was added to the dried lipid to resolubilize it. The soluble lipid was then transferred to a screw capped 50ml test tube, capped tightly, and incubated in water bath at 95 °C for 1 hour following the method by Wang *et al*., (2000). After cooling at room temperature, 5ml of water and 3 ml of hexane was added, and the tube was shaken vigorously for complete mixing and then placed on the rack for 5 minutes to allow solution to separate. The top layer aliquot containing clear liquid (FAME) was then pipetted into 1.5mL vial and kept in -18° prior to analysis using gas chromatography.

Gas chromatography analysis

The fatty acid methyl esters (FAME) were isolated and quantified using a gas chromatograph (Agilent Technologies Gas Chromatograph, 7890B series), equipped with an automatic column injector and autosampler. The column used was the Durabond, DB-Fast FAME (Agilent Technologies Brand) capillary column with its measurement of 30m length, diameter 0.250mm Narrowbore and 0.25 μm film thickness was used to separate the FAMEs. Flame Ionization Detector (FID) was used in this GC and was set at 280°C and inlet temperature of 225°C. The oven temperature was set to initially start at 70°C to 245°C with a post run of 2 minutes and flowrate of 0.25ml/min. The parameter setup for the column used in this gas chromatography follows the one recommended by Agilent Technologies with a slight modification done by the author to optimize the fatty acid determination. The standard used for identifying all the 37 peaks of fatty acids were a component FAME mixture (Supelco 37) from Sigma Aldrich. After all peaks from the standard were identified in the chromatograph, it was then used to compare and identify the peaks appeared based on the retention time of the standard for each sample. All results of fatty acids were expressed as mg/100g.

Statistical analysis

All data collected were analyzed using General Linear Model (GLM) analysis of variance and significant differences ($p<0.05$) between means were determined by Tukey test using the SAS Version 9.4.

RESULTS AND DISCUSSION

Diet fatty acid profile

The addition of flaxseed oil at 2g/100g in the experimental feed demonstrated a 10-fold ALA concentration compared to the control feed (Table 2). The ratio of omega-6 to omega-3 in the experimental diets was somehow around 1:1 (106.42mg/100g : 110.25mg/100g). The addition of flaxseed oil seems to reduce the proportion of palmitic and oleic acid as less palm oil was used in the experimental feed, and slightly increased the proportion of stearic acid.

Lipid composition in thigh and breast muscle

The lipid composition of thigh and breast muscle is presented in Table 3. There was no significant difference ($p>0.05$) in the lipid weight and percentage of village chicken breast muscle when fed with supplemented flaxseed oil at 1 week, 3 weeks and 5 weeks. In the thigh muscle however, higher ($p<0.05$) lipid composition was found in birds fed with the supplemented flaxseed oil at 3 weeks of supplementation and was reduced as the birds were fed longer with flaxseed oil.

Lipid composition of the village chicken breast and thigh muscle in this study is within the range reported as in broiler chicken (Milićević *et al.,* 2014), but is slightly higher compared to other village chicken breeds as reported by Haunshi *et al.,* (2013) with the Indian native chicken breeds slaughtered at 20 weeks. Lipid concentration is always higher in the thigh part compared to breast as this cut is mainly composed of triglycerides (Rymer & Givens, 2005).

Table 3. Lipid weight and percentage in breast and thigh muscle of village chicken (Mean ± SEM).

Treatment	Breast		Thigh	
	Lipid Wt. (g)	Lipid%	Lipid Wt. (g)	Lipid%
T0	$0.1626^{a}\pm0.02$	$5.4188^{a}\pm0.57$	$0.3816^{ab}\pm0.05$	$13.4844^{ab}\pm1.00$
T1	$0.1663^{a}\pm0.01$	$5.5380^{a}\pm0.48$	$0.4176^{ab}\pm0.04$	$13.9106^{ab}\pm1.23$
T2	$0.1511^{a}\pm0.01$	$5.0346^{a}\pm0.27$	$0.5000^{b}\pm0.04$	$16.6562^{b}\pm1.39$
T3	$0.1465^{a}\pm0.01$	$4.8808^{a}\pm0.46$	$0.3327^{a}\pm0.03$	$11.9122^{a}\pm1.07$
p-value	0.7311	0.7319	0.0383	0.0480

a,b Means with different superscript within the same column differ significantly at $P<0.05$.
T0-Control, T1- 1 week FO supplementation, T2- 3 weeks FO supplementation, T3- 5 weeks FO supplementation

Polyunsaturaed Fatty acids concentration in breast and thigh muscle of village chicken

Omega-3 fatty acids

Table 4 and 5 shows the omega-3 fatty acids composition (mg/100g) of fresh weight in breast and thigh muscle of village chicken fed supplemented flaxseed oil for 3 different feeding durations (1, 3 and 5 weeks of supplementation with flaxseed oil). Both ALA accumulation in breast and thigh muscle becomes higher as the feeding duration becomes longer. This suggest that the longer the birds were fed with flaxseed oil, the ALA accumulation in the muscle becomes higher, especially those in the thigh muscle. The ALA composition in the breast and thigh muscles in this study showed that the absorption of ALA is directly from the gut into the muscle. This is in accordance with other findings using broilers that discovered the same effect of longer feeding with flaxseed oil (Lopez Ferrer et al., 2001; Rymer & Givens, 2005).

In the control feed, the ALA concentration was low, which is 11.39 mg in 100g diet. Therefore, in the breast muscle, no ALA was present which is certainly due to the lower concentration of ALA in the control feed. However, in the thigh muscle of control treatment, there was some ALA present which is due to that the ALA is usually associated more to the dark meat (thigh). When the birds were fed longer with flaxseed oil up to 5 weeks (40.39 mg/100g), the accumulation of the ALA in the breast muscle did not differ significantly (p>0.05) compared to the 3 weeks (42.12 mg/100g) of supplementation suggesting that the latter feeding duration was found to be sufficient for the ALA to be administered in the breast muscles of this village chicken.

Table 4. Omega fatty acids composition (mg/100g) of fresh weight in breast muscle of village chicken fed supplemented flaxseed oil for 3 feeding durations (Mean ± SEM).

Fatty acids	**Treatment**				
	T0	T1	T2	T3	p-value
Linoleic (LA)	178.83 ± 16.97	219.49 ± 21.41	221.55 ± 18.33	177.78 ± 8.95	0.1271
Alpha-Linoleneic (ALA)	0.00	$15.79^{b} \pm 0.96$	$42.12^{a} \pm 5.50$	$40.39^{a} \pm 4.32$	<0.0000
Arachidonic (AA)	$100.03^{ab} \pm 5.27$	$128.90^{c} \pm 7.77$	$116.20^{ac} \pm 8.10$	$81.34^{b} \pm 4.72$	<0.0001

^{a,b} Means with different superscript within the same row differ significantly at P<0.05.
T0-Control, T1- 1 week FO supplementation, T2- 3 weeks FO supplementation, T3- 5 weeks FO supplementation

Table 5. Omega fatty acids composition (mg/100g) of fresh weight in thigh muscle of village chicken fed supplemented flaxseed oil for 3 feeding durations (Mean ± SEM).

Fatty acids	**Treatment**				
	T0	T1	T2	T3	p-value
Linoleic (LA)	440.95 ± 41.19	364.37 ± 28.31	414.05 ± 36.93	360.22 ± 28.83	0.3928
Alpha-Linoleneic (ALA)	$12.31^{a} \pm 1.85$	$26.33^{a} \pm 2.82$	$62.58^{c} \pm 4.82$	$85.74^{b} \pm 7.55$	<0.0001
Arachidonic (AA)	$144.43^{a} \pm 17.19$	$119.59^{ab} \pm 10.08$	$99.31^{ab} \pm 5.30$	$90.55^{b} \pm 10.31$	0.0051

^{a,b} Means with different superscript within the same row differ significantly at P<0.05.
T0-Control, T1- 1 week FO supplementation, T2- 3 weeks FO supplementation, T3- 5 weeks FO supplementation

In the thigh muscle, ALA level showed a significant increased ($p<0.05$) as the feeding duration increases with its concentration is higher than the breast muscle. At 5 weeks of supplementation, the ALA concentration recorded the highest accumulation in the thigh with 85.74mg in 100g meat. Tougan *et al.,* (2018) has also discovered that the proportion of ALA was way up in the thigh muscle compared to breast muscle of some native chickens. Thigh muscles which were considered as dark meat accumulated more ALA as it is a richer source of the triaglycerols than the breast muscle or also known as white meat (Rymer & Givens, 2005; Cortinas *et al.,* 2004). In broiler, 2 weeks and 1 week is needed to enrich the breast and thigh meat respectively as reported by Konieczka et al., (2017). Mirshekar *et al.,* (2021) reported that supplementation of flaxseed oil in Japanese quail a week before slaughter (28 days of age) resulted in a 4.97 folds of omega 3 fatty acid increment in the breast muscle. The same outcome was documented by Gonzalez-Esquerra and Leeson (2000) that a week of feeding with 10% of flaxseed oil significantly accumulated the omega 3 into the chicken tissues.

Commercial broilers however have a shorter period to achieve the harvesting weight which recently around 35 days, but for village chicken it may take longer time around 12-14 weeks due to its slow growth. The addition of ALA source in the feed did gave a positive accumulation of this nutrient into this chicken meat as has been reported by many authors (Konieczka *et al.*, 2017; Lopez-Ferrer *et al.,* 2002; Carragher *et al.,* 2016). The accumulation of fatty acids in chicken has been said to be affected by many factors (Tougan *et al.,* 2018), which one of them is age factor. Older birds and slow growing chicken were said to have higher composition of the long chain PUFA and the total PUFA (Bosco *et al.,* 2014). The concentration of flaxseed oil addition however in this study is only 2% and could be increased more to attain higher accumulation in the meat. Addition of up to 10% of flaxseed oil in the diet of a broiler showed an increase of ALA content. However, prolonged feeding of chicken with flaxseed oil will elevate the deposition of the omega fatty acids in the meat, whilst causing problem in the meat quality and storage (Mirshekar *et al.,* 2021). Omega 3 is more prone to oxidation than omega 6, thus, higher administration could impair the oxidation stability of the meat (Rymer *et al.,* 2010).

Omega-6 fatty acids

Linoleic acid (LA) which is from the family of omega-6 on the other hand showed that 1 week of supplementation is sufficiently enough to accumulate this fatty acid in both thigh and breast muscle ($p>0.05$). Konieczka *et al.,* (2017) had also discovered the same finding with only 1 week of time needed for the LA to be accumulated in the breast and thigh of a broiler chicken. However, its concentration was higher in the thigh muscle ranging from 360.22mg to 440.95mg in a 100mg of muscle (Table 3). Arachidonic acid (AA), which is a derivative fatty acid from LA is also an essential fatty acid which sometimes can be found in some natural sources such as eggs and certain plants. The conversion of AA from LA was somehow reported to be low in animals (Tallima & Ridi, 2018), nevertheless, in this experiment, it was found that the AA presented in the meat muscles was fully derived from the LA available in the feed, as no AA was detected in the feed. In this study, the birds were found to be inefficient in converting ALA to its longer chain fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In contrast, it efficiently synthesis the LA to AA.

Conversion of omega fatty acids to its longer derivative

Chicken is known to have the ability to elongate and desaturate the fatty acids ALA and LA through the activity of enzyme, but this ability is somehow limited. The biosynthesis of lipid and fatty acids occurs in the liver for avian species (In this study, the ALA to LA ratio was 1:1, thus, it is possible that the enzyme has selected to metabolize the LA into AA rather than ALA to its longer chain fatty acids, as these both fatty acids (LA and ALA) shared the same enzymes related to their metabolism (El-Zenary *et al.,* 2020). The transformation or metabolism of fatty acids omega-6 and omega-3 to its derivatives, which is AA, EPA and DHA is done through the elongation and desaturation processes involving the desaturase and elongases enzymes (Kajla *et al.,* 2014). The pathway and factors in which why the LA was selected is still being studied in this experiment, and will be further discussed in the next experiment. Increasing ALA to LA ratio may be a way to increase the chances of ALA to be elongated as reported by El-Zenary *et al.,* 2020, and this factor will be further studied with this chicken. However, the concentration of AA significantly decreases as the length of feeding with supplemented flaxseed oil increases, which contradicts with the concentration of ALA. Omega-3 is known to have many beneficial impacts especially those for human consumption and health. The intake however is very low and did not actually meet the requirement suggested for human compared to omega-6. Higher intake of unsaturated fatty acids has shown that it reduces the risk of cardiovascular diseases, cancers asthma and diabetic in human (Milićević *et al.,* 2014).

CONCLUSION

Fron the findings, it was found that increasing the length of feeding duration with flaxseed oil rich in omega-3 fatty acid does increase the deposition of the ALA in both breast and thigh muscle of the village chicken. However, three weeks of supplementing the diet of the village chicken with flaxseed oil was deemed to be sufficient for the ALA to be deposited in the breast muscle, whereas longer period of 5 weeks is needed for the thigh muscle.

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