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Performance, Lipid, and Omega Fatty Acids Composition of Village Chickens Fed Diet Supplemented with Flaxseed Oil

I. J. Farahiyah[a,b,*], I. Lokman Hakim[a], M. Mardhati[b], A. B. Nurulhayati[b], A. Hafandi[a], & S. Noraini[b]
[a]Department of Veterinary Pre-Clinical Science, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
[b]Livestock Science Research Centre, Malaysian Agricultural Research & Development Institute (MARDI), Persiaran MARDI-UPM, Serdang, Selangor, Malaysia.
*Corresponding author: filyana@mardi.gov.my
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ABSTRACT

Flaxseed oil is a rich source of omega fatty acids and has gained attention as a functional feed additive in the poultry industry. While the use of flaxseed oil in poultry has been widely reported, its application in village chickens remains limited, particularly regarding its physiological and metabolic effects. Thus, this study aimed to determine the effect of flaxseed oil (FO) supplementation on growth performance, carcass yields, and omega fatty acid levels in village chickens. A total of 120 village chickens (with average body weight 606.80 g) were randomly divided into four treatment groups with five replicates. Three levels of FO inclusion were experimented to replace palm oil used in the control treatment, where T0-basal diet with no FO, T1-2.5% FO inclusion, T2-3.0% FO inclusion, and T3-4.0% FO inclusion. Chickens were fed these experimental diets for five weeks, after which they were slaughtered. Breast and thigh muscles were collected for lipid and fatty acid analysis. Weight gain was slightly compromised as higher incorporation of FO was added to the diet of this village chicken (p<0.05). No significant differences were observed in carcass yield (p>0.05) between the treatments. Lipid composition in breast muscle increased with increasing addition of FO (p>0.05), but all FO treatments were found to be lower than control (p<0.05). However, in the thigh muscle, chickens in treatment T2 (3.0% FO inclusion) had the highest composition among FO diets but much lower compared to the control (p<0.05). Alpha-linolenic acids (ALA) in both breast and thigh muscles showed an increasing trend as a higher inclusion of FO was added (p<0.05) compared to the control and slightly reduced at 4.0% inclusion. In conclusion, the supplementation of flaxseed oil had no negative impact on the growth performance and carcass yields of village chickens while significantly enhancing the omega-3 fatty acid content in both breast and thigh muscles.

Keywords: flaxseed oil; lipid; omega 3 fatty acid; performance; village chicken

INTRODUCTION

Flaxseed, or linseed, is an annual blue flower herb containing yellowish to reddish and brownish flat seeds (Kajla *et al.*, 2015). It has been cultivated commercially across the world for many purposes: as a nutritious medicinal supplement for humans (Parikh *et al.*, 2019), for industrial purposes, for extraction of its oil as edible oil, and incorporated into many food products as a functional food (Goyal *et al.*, 2014). This plant has recently gained interest due to its various functional properties that benefit human health and animal well-being. Flaxseed is among the oil plants that contain high short-chain polyunsaturated fatty acids (PUFA) in the form of alpha-linolenic acid (ALA). The ALA content is around 53% (Nowak & Jeziorek, 2023) to 39%-60% (Goyal *et al.*, 2014) of the total fatty acids. The rich fiber and lignan content, coupled with a good source of essential polyunsaturated fatty acids in this plant, has demonstrated flaxseed to be known as one of the superfoods (Nowak & Jeziorek, 2023). Emerging interest in this plant in poultry feeds as a source of omega-3 to enrich animal products such as eggs and meat is also rather on the rise (Alagawany *et al.*, 2019; Beheshti Moghadam & Cherian, 2017). Flaxseed is usually consumed in two forms: directly as the seed or as an oil (flaxseed oil).

The addition of flaxseed meal to poultry diets was reported to have a positive impact by increasing omega fatty acids (Yaseen, 2020) in layer hens, increasing the weight gain of broiler chickens (Ló Pez-Ferrer *et al.*, 2001), and improving the production of breeder chickens by enhancing the hatching rates and weight of the chicks (Whittle *et al.*, 2024). On the contrary, Al-Hilali (2018) reported that supplementation with flaxseed meal reduced the body weight gain of broiler chickens, egg weight, and egg production in layer hens (Yaseen, 2020). This reduction in body weight gain, egg weight, and egg production could be attributed to the anti-nutritional factor (Ali *et al.*, 2023) and non-starch polysaccharides (Alagawany *et al.*, 2019; Apperson & Cherian, 2017) present in the flaxseed which hinder the absorption of some nutrients in the chicken. On the other hand, flaxseed oil has lower anti-nutrients and the ALA bioavailability is better in the oil form than in the seed form (Goyal *et al.*, 2014). Apart from that, the main source of energy for chickens are from oils added in the diet, as it possesses higher caloric values than other available ingredients in a formulated diet (Alagawany *et al.*, 2019). The addition of flaxseed oil also helps to elevate the ALA content in the chicken meat, as reported by Carragher *et al*. (2016). However, supplementing flaxseed oil in quail seems to have had no significant effect on feed intake and weight gain (Mirshekar *et al.*, 2021).

The omega-3 intake of Malaysians is still low and inadequate (Ng *et al*., 2012), and there is a lack of awareness of its importance for health. The recommended intake ratio of omega-6 to omega-3 is 4:1, but it is still unachievable to date due to the feeding intake and lifestyle that we are possessing now, which contains more omega-6 (Alagawany *et al.*, 2019; Cartoni Mancinelli *et al.*, 2022). Alternatively, individuals can meet this requirement by consuming high-omega foods. Enriching meat product of poultry, which is the main meat protein consumed by Malaysians (Drewnowski *et al.*, 2020), may be an effective way to accumulate this nutrient in humans without compromising their feeding habits (Farahiyah *et al.*, 2024). With increasing awareness among consumers about healthier food choices (Al-Mamun *et al.*, 2020), the demand for village chicken in Malaysia's poultry market is increasing, as village chicken is deemed to have lower fat composition and higher omega-3 (Katekhaye, 2019) and is a healthier option compared to the commercial broiler chicken (Nematbakhsh *et al.*, 2021). The demand and consumption of village chickens are currently increasing, and enhancing the meat by fortifying it with flaxseed oil appears to be a promising option for facilitating an increase in omega-3 intake among humans. So far, no study has been reported on the supplementation of flaxseed oil in village chickens. Therefore, this study aims to determine the effect of flaxseed oil (FO) supplementation on the growth performance, carcass yields, and omega fatty acid levels in village chickens.

MATERIALS AND METHODS

This study was conducted following the guidelines listed and was approved by MARDI Animal Ethics with the approval number 20230622/R/MAEC00125. The Ayam Saga chickens were obtained from the MARDI Muadzam hatchery in Muadzam Shah, Pahang. One hundred twenty (120) one-day-old chicks were transported to the MARDI Serdang, Selangor, poultry house and raised in two-tier stainless-steel battery cages. Water and feed were supplied *ad libitum*, and light was provided 24 hours. The cages were placed in an enclosed poultry house system equipped with proper ventilation and cooling pads to maintain the temperature and humidity. The study utilized seven-week-old male village chickens fully acclimated to the environmental and rearing conditions. The duration of this study was 5 weeks (35 days), where the birds were fed with the experimental diets from the age of 7 weeks to 12 weeks. A completely randomized design (CRD) was used with four treatments and five replications, each containing six chickens. The treatments consist of different levels of flaxseed inclusion, where T0 was denoted as the control treatment of a basal corn-soy diet without any flaxseed oil inclusion, and three levels of flaxseed inclusion in the basal diet: T1-2.5% flaxseed oil, T2-3.0% flaxseed oil, and T3-4.0% flaxseed oil (Table 1). All feeds were formulated according to the nutritional requirements of this Ayam Saga breed as proposed by MARDI, and they were all isonitrogenous and isocaloric.

Chickens were reared in steel two-tier battery cages equipped with a feeder and water drinker. Feed and water were provided *ad libitum*. Chickens and the feed intake were weighed and recorded fortnightly. The chickens were fed twice daily (morning and evening), and their feces were cleaned every other day. The relative humidity and temperature were also closely monitored in the poultry house to avoid any sudden changes and to identify if environmental factors had an effect. At week 12, three birds were randomly sampled from each cage, weighed, and slaughtered. Warm carcass weights and dressed weights were taken to carcass and dressing percentages. The breast muscle (pectoralis major and minor) and thigh muscle (bicep femoris) were sampled to analyze lipid and fatty acid contents. Fatty acid analysis was conducted by methylating the fatty acids into FAMEs (fatty acid methyl esterification) using the direct methylation method. The experimental feeds were analyzed for their proximate content and fatty acid composition.

The muscle samples were dried in a conventional oven at 60 °C until the samples were fully dried and had a constant weight. Dried samples were then ground to a fine particle using a pestle and mortar. Samples were then subjected to lipid analysis following the usage of the common Folch solution of a ratio of 2 chloroform: 1 methanol method (Wang *et al.*, 2000). For lipid extraction, 3 g of the sample was weighed into a 50 mL beaker, soaked with 40 mL of the Folch solution, and shaken well for 2 minutes using a shaker. The beaker containing the sample and solution was then left soaked overnight. Soaked samples were poured into a glass separating funnel and filtered using filter paper (40 mm Whatman) and rinsed with 10 mL of 0.88% NaCl. In about 4 hours, the solution in the funnel will separate into two layers, with the bottom layer of the solution being siphoned and collected into a rotavapour flask for drying.

The solvent in the flask was then evaporated using a vacuum rotary evaporator at 40 °C until yellowish-thick lipid was formed. The weight of the lipid was then measured by weighing the dried lipid. As for the analysis of fatty acids, the dried lipid was then resolubilized by adding 3 mL of methanolic-HCL (3N) and transferred into a capped test tube, then incubated

Table 1. Composition and nutrient profile of experimental diets with varying levels of flaxseed oil inclusion (g/100g)

Feed ingredients	Experimental diets			
	Control (T0)	T1	T2	T3
Soybean meal	20.67	20.67	20.74	20.87
Corn	56.79	56.79	56.21	55.04
Wheat pollard	5.00	5.00	5.00	5.00
Corn gluten	7.00	7.00	7.00	7.00
Palm oil	2.50	0.00	0.00	0.00
Flaxseed oil	0.00	2.50	3.00	4.00
Rice bran	3.50	3.50	3.50	3.50
DiCaP	0.48	0.48	0.48	0.47
Limestone	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50
Lysine	0.69	0.69	0.70	0.73
Methionine	0.13	0.13	0.13	0.13
Vitamin E	0.02	0.02	0.02	0.02
Choline chloride	0.60	0.60	0.60	0.60
Vitamin premix[a]	0.03	0.03	0.03	0.03
Mineral premix[b]	0.10	0.10	0.10	0.10
Nutrient composition (calculated)				
Metabolizable enegy (MJ/kg)	12.90	12.91	12.99	13.14
Crude protein (%)	19.00	19.00	19.00	19.00
Crude fat (%)	4.12	4.11	4.60	5.57
Methionine (%)	0.59	0.59	0.59	0.58
Cysteine (%)	0.84	0.84	0.84	0.83
Lysine (%)	1.50	1.50	1.51	1.53
Arginine (%)	1.27	1.27	1.27	1.27
Threonine (%)	0.78	0.78	0.78	0.78
Calcium (%)	1.00	1.00	1.00	1.00
P. Available (%)	0.45	0.45	0.45	0.45

Note: [a]=Vitamin premix provided per kilogram of diet: vitamin A, 50 miu/kg; vitamin D3, 10 miu/kg; vitamin E, 130 g/kg; vitamin K3, 10g/kg; vitamin B1, 10 g/kg; vitamin B2; 25 g/kg, vitamin B6; 16 g/kg; vitamin B12, 0.1 g/kg; biotin, 0.5 g/kg; folic acid, 8 g/kg; niacin, 200 g/kg; and pantothenic acid, 56 g/kg. [b]= Mineral premix provided per kilogram of diet: iron, 70 g/kg; copper, 8 g/kg; zinc, 80 g/kg; iodine, 1.3 g/kg; cobalt, 0.55 g/kg; manganese, 90 g/kg; and selenium, 0.25 g/kg.

in a water bath for 1 hour (95 °C temperature) and cooled down at room temperature afterward following the method described by Wang *et al*. (2000). Once cooled, the mixture was added with 5ml of water and 3 ml of hexane and vigorously shaken by hand for complete mixing. The mixture was then left on for 5 minutes to allow the solution separation. The top layer of the separated solution, which contained clear liquid, was then collected using a pipette into a 1.5 mL amber vial and stored at -20 °C prior to analysis. Fatty acid analysis was performed using a Gas Chromatograph-Flame Ionisation Detector (GC-FID) from Agilent Technologies Gas Chromatograph, model 7890B series. All data were analyzed using the General Linear Model Model's (GLM) analysis of variance with significant differences between means separated by the Tukey test using SAS Version 9.4 software.

RESULTS

The Ayam Saga chicken appeared to show comparable results in its weight gain and average daily gain when palm oil was replaced with flaxseed oil in its diet at levels lower than 4% (Table 2). The control treatment (T0), which contains palm oil at 2.5%, had lower weight gain than T1 (2.5% FO) and T2 (3.0% FO). Even though the oil level of the control (palm oil) and T1 (flaxseed oil) of 2.5% were at the same values (Table 1), chickens fed with flaxseed oil supplements showed slightly higher weight gain than palm oil-fed (p>0.05). However, as the inclusion of flaxseed oil increased, the weight gain was found to decrease and significantly reduced as 4.0% flaxseed oil was administered.

Feed intake was not compromised when flaxseed oil was used to replace palm oil in the diet of this village chicken, showing that it is palatable and did not affect its intake (p>0.05). The feed efficiency ratio (FCR), which measures the efficiency of the feed taken to transform it into meat muscle, was also highest (3.00) in chickens fed with the highest inclusion of flaxseed oil (4.0%) compared to other treatments. Lower flaxseed oil inclusion resulted in lower FCR due to the better weight gain demonstrated. However, researchers deemed an FCR of 3 and below sufficient for slow-growing type chickens.

Table 3 presents the carcass composition of village chickens fed with different levels of flaxseed oils. The dressing percentage for all treatments showed a similar pattern with no significant differences (p>0.05). No differences (p>0.05) in the muscle weight were

Table 2. Growth performance of Ayam Saga fed with different levels of flaxseed oil inclusion in 5 weeks (7 to 12 weeks of age) of rearing (Mean±SEM)

Variables	Experimental diets				
	T0-Control	T1- 2.5% FO	T2- 3.0% FO	T3- 4.0% FO	p-values
Initial weight (g)	605.53 ± 17.68	605.66 ± 20.02	606.56 ± 10.65	606.57 ± 6.95	0.999
Final weight (g)	1363.67 ± 9.27	1408.39 ± 32.21	1380.17 ± 16.04	1320.23 ± 26.76	0.1009
Weight gain (g)	760.53 ± 27.22[ab]	820.73 ± 23.91[a]	775.21 ± 8.03[ab]	711.51 ± 21.23[b]	0.0257
Feed intake (g)	2234.44 ± 49.70	2215.58 ±57.54	2207.88 ± 64.85	2322.11 ± 61.42	0.5152
ADG (g/d)	21.73 ± 0.78[ab]	23.45 ± 0.68[a]	22.15 ±0.23[ab]	20.33 ± 0.61[b]	0.0257
FCR	2.89 ± 0.09	2.71 ± 0.10	2.78 ± 0.07	3.00 ± 0.11	0.1905

Note: Means in the same row with different superscripts differ significantly (p<0.05). T0-Control= basal diet with no flaxseed oil (FO), T1= 2.5% FO inclusion, T2= 3.0% FO inclusion, T3= 4.0% FO inclusion, ADG= average daily gain, FCR= feed conversion ratio.

Table 3. Carcass composition of the Ayam Saga at 12 weeks of age, after fed diet with flaxseed oil inclusion in 5 weeks (35 days) of rearing (Mean±SEM)

Carcass composition	Experimental diets				
	T0-Control	T1-2.5% FO	T2-3.0% FO	T3-4.0% FO	p-value
Dressing %	69.61 ± 0.90[a]	68.92 ± 0.37[a]	69.05 ± 0.55[a]	69.86 ± 0.36[a]	0.6699
Thigh weight (g)	127.71 ± 3.55[a]	135.50 ± 3.09[a]	118.77 ± 9.61[a]	114.00 ± 13.40[a]	0.4117
Breast weight (g)	147.19 ± 2.80[a]	139.15 ± 7.35[a]	142.02 ± 6.70[a]	140.28 ± 9.89[a]	0.8632
Drumstick weight (g)	94.07 ± 2.04[a]	101.27 ± 3.07[a]	96.04 ± 3.89[a]	100.17 ± 3.57[a]	0.3514
Wing weight (g)	47.85 ± 2.37[a]	50.82 ± 2.58[a]	44.27 ± 1.88[a]	48.53 ± 1.69[a]	0.2058
Meat to bone ratio	3.21 ± 0.17[a]	3.22 ± 0.23[a]	2.88 ± 0.18[a]	2.99 ± 0.14[a]	0.4846

Note: Means in the same row with different superscripts differ significantly (p<0.05). T0-Control= basal diet with no flaxseed oil (FO), T1= 2.5% FO inclusion, T2= 3.0% FO inclusion, T3= 4.0% FO inclusion.

also observed in all edible parts (cut-up parts) of the chicken, which are the breast, thigh, drumstick, and wing. All these parts had almost similar weightage in all treatments, indicating that the addition of flaxseed oil did not compromise the yield of the muscles and meat. Even though the weight of the breast muscle in chickens fed with lesser flaxseed oil (2.5%) is higher than the 3.0% and 4.0%, the differences between them were not significant (p>0.05). The breast muscle comprises the biggest cut, weighing heavier than others. T1, with a 2.5% flaxseed oil inclusion, showed better carcass yield in terms of breast, drumstick, and wing muscle weight compared to other treatments. Similarly, for the meat-to-bone ratio, chickens fed with 2.5% flaxseed oil had the highest ratio, and it was reduced when more flaxseed oil was added, but this was not significant (p>0.05).

Table 4 describes the means of lipid composition in terms of percentage and lipid weight of the breast and thigh muscles of the village chicken. Supplementing the diet with flaxseed oil significantly reduced the lipids in the thigh muscles. The thigh muscle showed significantly higher lipid compositions compared to the breast muscle, which was 4 times higher in the control and 2 times higher in the experimental diets (Table 4). In the breast muscle, lower flaxseed oil insertion (2.5%) resulted in lower lipid composition compared to the control (p<0.05). T1 demonstrated a lower lipid weight and percentage despite both T0 (control palm oil) and T1 (flaxseed oil) having the same lipid composition. The higher lipid values in T0 are likely due to its palm oil content, which is richer in saturated fats, whereas T1 contains more unsaturated fats. The lipid content, however, increases as the amount of flaxseed oil increases, but it is not significantly affected (p>0.05). Synonymy, the lipid composition in chickens fed a control diet (T0), showed a significantly higher lipid percentage in the thigh muscle compared to those diets that included flaxseed oil (p=<0.0001). T2 accumulated the highest concentration within the flaxseed oil diet, with 15.86% lipid in the thigh muscle. The lipid composition of the control was double that of the ones with a flaxseed oil addition.

Tables 5 and 6 summarise the omega fatty acid compositions of the breast and thigh muscles (fresh weight) of the Ayam Saga chicken. In both muscles, the concentration of linoleic acid (LA), an omega-6 fatty acid, was significantly higher than that of alpha-linolenic acid (ALA), an omega-3 fatty acid. This is because the feed in this experiment was mainly composed of corn and soybeans, which in hand these ingredients contain LA abundantly. These corn-soy-based diets, however, tend to have the same amount of protein and lipid composition, hence providing the same energy for the growth of the chickens. In the breast muscle (Table 5), it is worth noting that the concentration for both LA and ALA increases as the levels of flaxseed oil increase, with a slight reduction at 4.0% FO, but it was not significant (p>0.05). As for arachidonic acid (AA), the concentration becomes lower as a higher FO is inserted (p<0.0001). AA is a derivative of the metabolization of LA to its longer-chain fatty acid. The lowest AA concentration in the breast was recorded when 4.0% FO was inserted into the diet. Although the concentration of LA and ALA statistically did not show any significant difference in all chickens fed FO diets, its value was highest in the 3.0% FO inclusion with concentrations of 342.22 mg/100g and 69.63 mg/100g of fresh muscle, respectively.

Table 4. Lipid weight and percentage in breast and thigh muscle of Ayam Saga at 12 weeks of age, after fed diet with flaxseed oil inclusion in 5 weeks (35 days) of rearing (Mean ± SEM)

Variables		Experimental diets				
		T0-Control	T1-2.5% FO	T2-3.0% FO	T3-4.0% FO	p-value
Breast	Lipid weight (g)	0.1863 ± 0.01[a]	0.1392 ± 0.01[b]	0.1590 ± 0.01[ab]	0.1673 ± 0.01[ab]	0.0144
	Lipid %	6.19 ± 0.34[a]	4.63 ± 0.35[b]	5.29 ± 0.28[ab]	5.55 ± 0.34[ab]	0.0146
Thigh	Lipid weight (g)	0.7440 ± 0.03[a]	0.3315 ± 0.02[c]	0.4765 ± 0.02[b]	0.4188 ± 0.03[c]	<0.0001
	Lipid %	24.73 ± 0.96[a]	11.04 ± 0.71[c]	15.86 ± 0.58[b]	13.95 ± 1.13[bc]	<0.0001

Note: Means in the same row with different superscripts differ significantly (p<0.05). T0-Control= basal diet with no flaxseed oil (FO), T1= 2.5% FO inclusion, T2= 3.0% FO inclusion, T3= 4.0% FO inclusion.

Table 5. Omega fatty acids composition (mg/100g) of fresh weight in breast muscle of Ayam Saga at 12 weeks of age, after rearing and fed diets supplemented with flaxseed oil at different inclusion levels (Mean ± SEM)

Omega fatty acids	Experimental diets				p-value
	T0-Control	T1-2.5% FO	T2-3.0% FO	T3-4.0% FO	
Linoleic acid (LA)	293.92 ± 26.05	333.39 ± 8.50	342.44 ± 27.05	301.96 ± 31.04	0.4794
Alpha linolenic acid (ALA)	NA	59.32 ± 3.66	69.63 ± 5.25	65.22 ± 7.94	0.4726
Arachidonic acid (AA)	282.55 ± 25.03[a]	306.30 ± 5.44[a]	298.36 ± 24.07[a]	89.77 ± 8.95[b]	<0.0001

Note: Means in the same row with different superscripts differ significantly (p<0.05). T0-Control= basal diet with no flaxseed oil (FO), T1= 2.5% FO inclusion, T2= 3.0% FO inclusion, T3= 4.0% FO inclusion. NA= Not available.

Table 6. Omega fatty acids composition (mg/100g) of fresh weight in thigh muscle of Ayam Saga at 12 weeks of age, after rearing and fed diets supplemented with flaxseed oil at different inclusion levels (Mean ± SEM)

Omega fatty acids	Experimental diets				p-value
	T0-Control	T1-2.5% FO	T2-3.0% FO	T3-4.0% FO	
Linoleic acid (LA)	476.62 ± 23.66	431.46 ± 13.59	439.85 ± 16.01	407.78 ± 23.42	0.1098
Alpha linolenic acid (ALA)	55.48 ± 1.30[b]	279.80 ± 9.70[a]	289.62 ± 10.50[a]	281.84 ± 16.79[a]	<0.0001
Arachidonic acid (AA)	317.02 ± 10.10[a]	289.06 ± 9.74[ab]	279.16 ± 10.65[ab]	257.68 ± 13.63[b]	0.0043

Note: Means in the same row with different superscripts differ significantly (p<0.05). T0-Control= basal diet with no flaxseed oil (FO), T1= 2.5% FO inclusion, T2= 3.0% FO inclusion, T3= 4.0% FO inclusion. NA= Not available.

In the thigh muscle (Table 6), the ALA concentration showed an incredibly significant increase (p=<0.0001) compared to the control, as FO was added to the diet by four folds. However, it was not significantly different among the diets with flaxseed oil treatment (T1, T2, and T3). In contrast, a negligible reduction in LA concentration was observed when flaxseed oil was included in the diet of this village chicken. The ALA's association with the thigh may have increased its concentration and decreased the LA's.

DISCUSSION

The final weight of the Ayam Saga is in the range of 1320 g to 1408 g, with an average of 1368 g within 12 weeks of rearing, which was expected to be as such, as reported by Muhammad *et al*. (2019). The chickens showed no adverse effect in terms of growth performances when being fed with flaxseed oil, replacing palm oil for 5 weeks until slaughtered. Chickens fed with flaxseed oil seem slightly better but insignificantly different weight gain than the control feed. Improvement in weight gain for chickens fed an equivalent 2.5% oil inclusion was also noted when chickens were fed an unsaturated fat diet (T1-flaxseed oil) rather than the control saturated fat diet (T0-palm oil). This was aligned with a study by Uchewa (2015), where he found that unsaturated fatty acids' inclusion in the diet of broilers improved the growth rate and feed intake of the birds significantly (p<0.05) compared to a saturated fatty acid diet. Carragher *et al*. (2016) noted the same outcome of higher body weight when fed broilers with higher ALA (2.5% flaxseed oil inclusion) compared to the control feed without flaxseed oil. Unsaturated fats are easy to digest and absorb; hence, feed absorption is more efficient. The weight gain within the groups of flaxseed oil-fed chickens was slightly reduced, though, as more flaxseed oil was inserted. This may be because flaxseed oil contains more unsaturated fatty acids (Kajla *et al*., 2015) than palm oil, which is richer in saturated fatty acids (palmitic acids). Therefore, chickens fed with flaxseed oil at higher concentrations will have lower body weight gain compared to chickens fed with palm oil (control), even though higher ascertainment of flaxseed oils was demonstrated. This assumption was true, as studied by Timmers *et al*. (2011), where mice fed with feed containing saturated fatty acids gained higher weight than those fed with polyunsaturated fatty acids.

According to the data presented in Table 2, chickens in all treatments, including the control feed, which contains higher saturated fatty acid proportions, were all growing well. No adverse effects were noted on the growth performance parameters, as well as the efficiency of chickens in converting feed to muscle when an additional omega source (flaxseed oil) was added to the diet. This finding is also supported by some studies that looked at using flaxseed oil in other species of poultry, such as broilers (Ló Pez-Ferrer *et al*., 2001; Uchewa, 2015), quails (Mirshekar *et al*., 2021), and turkeys (Jankowski *et al*., 2015). However, no comparison can be made to village chickens as there is currently no report on the addition of flaxseed oil to their diets. Mirshekar *et al*. (2021) reported that the substitution of sunflower oil in Japanese quail feed with flaxseed oil had no significant effect on weight gain, as well as its feed intake. They also noted that adding fats, especially from vegetable oil sources, increases the digestibility of feed ingredients by reducing the digesta passage rate in the gastrointestinal tract. In fact, this study found that adding flaxseed oil increased the palatability of the feed, leading to higher feed consumption by chickens when a greater amount of flaxseed oil was included. The weight gain significantly impacted the FCR's value, demonstrating a proportionate relationship. Al-Hilali (2018) also reported similar findings, indicating that the FCR values in Hubbar broilers increased as the amount of flaxseed oil increased. The increase in FCR value in this study (2.71–3.00) is due to the lowering of the chicken's weight gain with the increase of the dietary flaxseed oil concentration. Nevertheless, these values were within the reported values for this specific Ayam Saga breed, which were 2.8-3.2 (unpublished data). A similar range of FCR was reported by Doğan *et al*. (2019) in their study, where the slow-growing chicken exhibited an FCR of 2.67 with a higher feed intake to achieve a harvest weight of 2 kg. The higher range of FCR in slow-growing chickens compared to commercial broilers is because a longer period is needed for the chickens to achieve a marketable size; thus, more feed is needed to attain that weight (Doğan *et al.*, 2019; Zidane *et al.*, 2018). Here, however, chickens in all treatments had an FCR lower than 3, which was considered good for slow-growing chickens.

One criterion for determining the slaughter value of a broiler carcass depends on the dressing percentage of the chicken (Nematbakhsh *et al.*, 2021). Commercial broilers usually have a higher dressing percentage (Nematbakhsh *et al.*, 2021), which is around 75%, compared to village chickens or slow-growing chickens, which have a lower dressing percentage of around 65%-70%. This finding was also reported by Lokman *et al*. (2011), who compared the carcass and muscle weight of the broiler with village chickens reared in the same environmental condition and found that commercial broilers tend to have higher meat weight than village chickens. In this study, the dressing percentage ranges from 68.92% to 69.86%, which is within the range for slow-growing birds (Lokman *et al.*, 2011). Ayam Saga in this study also showed a parallel trend of dressing percentage, and the weight of all the edible cut-up parts was within the same range amongst the treatments, indicating that there is no negative effect on the use of flaxseed oil in the diet of this chicken, similarly as being reported in another study (Kumar *et al.*, 2019).

Based on the lipid composition result tabulated in Table 4, the thigh muscle possesses higher lipid weight and percentage compared to the breast muscle. This higher lipid composition in the thigh meat (dark meat) than the breast meat (white meat) is based on the lipid composition results tabulated in Table 4. The thigh muscle possesses higher lipid weight and percentage compared to the breast muscle. This higher lipid composition in the thigh meat (dark meat) than in the breast meat (white meat) is attributed to the fact that it is composed mainly of triglycerides (Rymer & Givens, 2005). The thigh and drumstick, known as the whole leg parts, are the most active parts of the chicken body and are used for movements and locomotion. Therefore, this muscle retains and uses more energy, resulting in a higher lipid composition. Thigh also acts as the primary reserve part of fat, where chickens use lipids as their main energy source because it is more concentrated and has twice the energy value of carbohydrates (Oketch *et al.*, 2023). This explains the threefold difference in lipid composition between the thigh and breast muscles. These muscles were known to be oxidative-type muscles, meaning that more oxygen was transported to support their function and activity, hence higher usage of energy compared to breast muscles, which contain glycolytic-type muscles (Head *et al.*, 2019).

Palm oil contains a relatively high amount of saturated fatty acids, with more than 50% of its composition comprising this type compared to other edible oils (Mancini *et al.*, 2015). This attribute may be the reason higher fats were accumulated in the muscle for both breast and thigh fed with palm oil compared to flaxseed oil, as flaxseed oil contains higher polyunsaturated fatty acids. Kumar *et al*. (2019) also reported that the addition of flaxseed in broiler diets has decreased the fat composition in the breast and thigh muscles, similar to what we have found in this study. Unsaturated fatty acids are easily and efficiently absorbed faster than saturated fatty acids (Karki & Sharma, 2020) as they have lower density and are more beneficial for chicken health. Apart from that, unsaturated fatty acid addition boosts the chicken's productivity, as well as generating more energy at a relatively lower cost (Nur Mahendra *et al.*, 2023; Whittle *et al.*, 2024). The findings revealed that chickens fed with flaxseed oil had a lower lipid content than those fed with palm oil.

Omega fatty acids are known to be indispensable fatty acids, which cannot be synthesized by the body and must be taken orally from other sources (Simopoulos & Dinicolantonio, 2016). It is normally divided into two main groups, which are omega-3 (alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA)) and omega-6 (linoleic acid (LA) and arachidonic acid (AA)). In chickens, these fatty acids are limited; however, they are also vital for enhancing the chicken's immune system and promoting its growth (Cherian, 2015). ALA is the precursor for the longer-chain polyunsaturated fatty acids EPA and DHA, whereas LA is the precursor for AA.

In this study, omega-3 (ALA) and omega-6 (LA and AA) were found in the muscles of Ayam Saga, and their concentrations increased when flaxseed oil replaced palm oil in the diet. The higher LA concentrations compared to ALA in all diets were due to the main ingredients of the basal diet being soybean meal and corn, which are high in LA (Table 1). The composition of fatty acids in chicken meat depends entirely on the feed supplied (Bostami *et al*., 2017). Omega-3 in the form of ALA, which is highly abundant in this flaxseed oil, was found in both the breast (Table 5) and thigh (Table 6) muscles of the chicken, except for the control breast, with the thigh muscle having ALA concentrations that are 4 times higher than those in the breast muscle. ALA is associated more with triglycerides, which can be found mainly in the thigh, whereas EPA and DHA are related more to the phospholipids, which accumulate at the breast (Cortinas *et al*., 2004; Rymer & Givens, 2005), which explains the higher ALA accumulation in the thigh muscle.

The ALA concentration increases in both muscles as a higher percentage of FO is added, whilst the LA concentration decreases and is similar to what has been reported by Ali et al. (2023) due to the ALA being directly absorbed into the muscle. Ló Pez-Ferrer *et al*. (2001) have also reported that the addition of flaxseed oil to the diet of broilers does accumulate this vital fatty acid in their muscles. Other reports have also been published proving that using flaxseed oil in chicken diets increased the ALA composition in the muscle tissue (Konieczka *et al*., 2017). The addition of flaxseed oil to the diets of this Ayam Saga has been shown to increase the ALA concentration by 5 folds in the thigh muscle when compared to the control diet. Supplementing the diet of this village chicken with an ALA source relatively increases the concentration of these fatty acids in the chicken muscle (Rymer & Givens, 2005). Carragher *et al*. (2016) reported the same event where the addition of 2.5% flaxseed oil in the diet of broilers increased the ALA concentration in the muscle by 4 folds.

This finding was also consistent with the study conducted by Kartikasari *et al*. (2012), whereby they observed that increasing ALA concentration in a broiler diet did increase the ALA concentration in the meat tissue. They also found that the broiler chicken could convert the ALA to its longer chains, EPA and DHA when the birds were fed with an ALA source throughout the rearing period, which took around 28 days in total. In this experiment, EPA and DHA were not detected in both thigh and breast muscles in all treatments. According to an earlier study on this same chicken breed, feeding them flaxseed oil for five weeks was deemed sufficient to accumulate ALA in the thigh, while three weeks suffice for the breast muscle (Farahiyah *et al*., 2024). However, in both the previous and current studies, no EPA and DHA were detected in the chicken muscles, which may be due to the breed's inability to convert the ALA to its longer chain derivatives.

In this study, it was found that the LA concentration decreased as higher FO was inserted. The declining concentration of LA may be attributed to its use in metabolizing its derivative fatty acids. Arachidonic acid (AA) is also one of the essential fatty acids from the family of omega-6 and was derived from the metabolism of linoleic acid. The LA undergoes elongation and desaturation processes through the available hepatic enzymes in the liver. In this study, the AA detected in the meat muscles was fully derived from the available LA contained in the feed since no AA was detected in the feed. Conversion of AA from its precursor, LA, was somehow observed to be low in animals (Tallima & El Ridi, 2018), but in this case, it was successfully converted. The imbalance of 18:2n (LA) to 18:3n6 (ALA) in the diet also caused the higher AA fatty acids found in the muscles, as pointed out by Pérez *et al*., 2021. Although chickens can transform and synthesize the fatty acids ALA and LA to their longer chains (El-Zenary *et al*., 2020), this village chicken was unable to transform ALA to EPA and DHA but successfully elongate LA to AA.

CONCLUSION

The growth performance and carcass yield of village chickens were unaffected by the addition of FO; nevertheless, there was a notable rise in the amount of omega-3 fatty acid (ALA) in the thigh and breast muscles. Therefore, the permissible range for increasing the content of omega-3 fatty acids (ALA) in chicken muscles and preserving their performance is 2.5%–3.0% of FO in the diet.

CONFLICT OF INTEREST

We certify that there is no conflict of interest in any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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