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The Effect of Canola Oil Supplementation at Different Feeding Duration on Local Chicken, Ayam Saga Growth Performance, Carcass Composition and Omega-3 Fatty Acid Content

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Abstract

A total of 160 MARDI village chicken breed (Ayam Saga) at the age of 9 weeks were fed feed supplemented with or without 2% of Canola oil containing approximately 10mg/g of Alpha-linolenic Acid (ALA) for 3 feeding durations; 3 weeks (T3), 2 weeks (T2) and 1 week (T1) before slaughter to evaluate its effect on the bird's performance and accumulation of ALA in the breast and thigh meat. The control treatment (T0) was not supplemented with canola oil. All birds showed no negative effect on the growth performances with no significant difference when compared to the control ($p>0.05$). Birds fed with canola oil during T2 and T1 showed better feed intake and weight gain compared to the control (T0) during the same period. Carcass performance for all treatments showed similar results. Treatment with no addition of canola oil has the highest final weight gain (1378.53g/bird) at 12 weeks, however, it was not significantly different ($p>0.05$) with the other treatments. This indicates that the addition of Canola oil did not suppress nor giving an adverse effect on the total performance of the birds. ALA was present in the breast and thigh meat of all treatments except T1 of canola oil supplementation in the breast meat. In conclusion, from this study, it is found that two weeks of Canola oil supplementation is needed to enrich the breast and thigh meat of SAGA chicken with Omega-3 ALA.

Keywords: Alpha-linolenic Acid, Canola oil, Omega-3, Performance, Ayam Saga

Introduction

Omega-3 is one of the essential fatty acids which are unable to be synthesized by the animal body and has to be taken through the diet. There are 3 main types of Omega-3, which are Alpha-linolenic acid (ALA), Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) with its chemical structure of C18:3, C22:6 and C20:5 respectively. ALA is the precursor of Omega-3, in which the enzymatic process of desaturation and elongation of this fatty acid will produce EPA and DHA (El-Zenary *et al.*, 2020). This Omega can be found in animal and plant sources, whereas ALA is more abundantly and naturally found in terrestrial plants such as canola and flaxseed. EPA and DHA on the other hand are commonly found in marine fatty fishes, algae and other seafood products (Rymer & Givens, 2005). Fortification or enrichment of poultry products such as eggs and meat has now become a trend to produce available Omega-3 sources for human consumption (Bhalerao *et al.*, 2014; Rymer & Givens, 2005; Alagawany *et al.*, 2019). Omega-3 is one of the important fatty acids which have many health benefits for human health, especially in the cardioprotective functions, and development of the brain and nervous system (Simopoulos and DiNocalantonio, 2016) However, its intake and consumption are reported to be below the recommendations for human (Ng *et al.*, 2000; Rymer *et al.*, 2010). Most of the studies reported however involve the application of the common broilers and layers breed, with very few reports on indigenous species of a specific country.

Among the poultry in Malaysia, chicken has become the most important type of meat consumed by Malaysians these days with the consumption in Malaysia has increased steadily from 40.09 kg per capita to 49.3kg per capita and is forecasted to increase to 51.28kg in the year 2025 by the Department of Veterinary Services, Malaysia. Malaysia was ranked third in the world and first in Asia for its high intake of poultry (Ferlito, 2020). Chicken meat in Malaysia came from two different types which is the common broiler breed such as Cobb and Ross, and the indigenous chicken breed or village chicken. The differences between these breeds are basically in their growth rates, meat textures, rearing system and morphological characteristics. Village chicken is mostly reared in a free-range system and is known to have a muscular and lean body resulting in low-fat composition in its meat and tougher texture. The growth rate of this chicken is also relatively low compared to commercial broiler chicken. Apart from that, the village chicken is known to be a hardy type of chicken and is also resistant to disease (Engku Azahan *et al.*, 1990).

Ayam Saga is a breed produced by MARDI (Malaysian Agricultural Research & Development Institute) with the hybridization of a few native Malaysian chickens. This breed has been improved genetically through the genetic selection which was successful and its distribution is now being expanded to the whole country as one of the village chicken breeds which has dual purposes, egg and meat production. It has been reported to have good egg production (3-4 folds more than the common village chicken), with more meat and less fat (Azlina & Noraziah, 2013). Besides that, it has a good FCR of 1.5 and could reach a harvesting weight of 1.2-1.23 kg in 12 weeks of rearing making it to be a potential local village chicken for commercialization purposes (Muhammad *et al.*, 2019).

There are not many studies found on the absorption and accumulation of Omega in free-range or village chicken. In commercial broilers, however, it is found that a week of feeding trial is sufficient to enrich breast meat with Omega 3 fatty acids and two weeks of feeding for the thigh parts (Konieczka *et al.*, 2017). However, prolonged feeding of high PUFA may also be attributed to the decrease in the storage stability of the meat (Alagawany *et al.*, 2019) and will increase the cost of production. Canola oil is one of the commodity vegetable oils that has been used widely in human consumption. It has the highest ALA content among other commodity vegetable oils (Dunford, 2018). Therefore, this study was designed to evaluate the feeding duration of Ayam Saga with Canola oil at three (3) different durations (weeks) on Omega 3 composition in the meat (breast and thigh) together with its effect on feed intake, weight gain and carcass performance.

Materials and methods

Animal and feed preparation

The birds used in this study were all male Ayam Saga (7 weeks of age) which were obtained from the hatchery in MARDI Muadzam Shah Station. A total of 160 birds were randomly assigned to 4 treatments with 5 replications and 8 birds in each cage. The birds were reared in 2-tier steel battery cages equipped with 2 sets of feeder and water drinker which was available *ad libitum.* The experiment was conducted in an open house system farm with proper ventilation, light supply and temperature monitoring for 24 hours.

The treatment consists of different duration of Omega-3 supplemented feed with 2% (10mg/g ALA) Canola oil at 1 week (T1- 11 weeks of birds age), 2 weeks (T2- 10 weeks of birds age) and 3 weeks (T3- 9 weeks of birds age) before slaughter and a control treatment (T0) without any Omega supplementation at all (Table 1). All diets were formulated to contain optimum nutritional requirements as recommended by the MARDI breed nutritional requirement guide of at least 19% of crude protein and 11 MJ/kg of energy (Table 2). Birds in the control group (T0) were fed with the control diet until slaughter, whereas in the experimental groups, one group (T3) consumed the experimental diet from the beginning (3 weeks), and the other two groups (T2) and (T1) were fed control feed until it was substituted with the experimental diet at two or one weeks before slaughter.

Table 1. Feeding regime and duration of birds fed with supplemented Canola oil

Birds age \Treatment	Weeks			
	8-9	9-10	10-11	11-12
Control (T0)				
T1				
T2				
T3				

Basal Diet
Experimental Diet (Omega feed)

Data collection and sample preparation

Birds were weighed every week according to treatments to evaluate their growth performance through the collection of body weight gain and feed intake data. The mortality of the birds was also recorded and measured daily. Daily routine (maintenance) as of feeding the birds, cleaning the faeces and disposing of any dead birds were done twice in the morning and evening. At the age of 12 weeks, two birds from each cage were slaughtered to collect meat samples consisting of breast and thigh for lipid and fatty acid analysis.

All meat samples were dried in the oven at 60°C until it is fully dried with constant weight. The dried samples were ground to a fine smooth texture, homogenized and kept in -5°C for Omega 3 fatty acid composition using the direct methylation method by Wang *et al.* (2000). A sample of 0.3g was weighed into a screw-capped 50mL test tube and added with 1 mL of hexane to be left overnight. Tricosanoic acid (2mg which is dissolved in 1 mL of hexane) was prepared and served as an internal standard and was added to the sample the following day followed by the addition of 3mL of 3N Methanolic-HCl and 1mL methanol. The test tubes were then capped tightly and refluxed in a water bath at 95°C for 1 hour. After the sample is cool down, 8mL of 0.88% NaCl solution and 3mL of hexane were added and mixed well. The sample was then centrifuged at 3000 rpm (Brand Kubota 2010) for 5 minutes to allow complete mixing. The top layer containing clear liquid (FAME) was pipetted into a 1.5 mL vial and analysed using gas chromatography.

Table 2. Formulation and proximate composition of the experimental feed.

Ingredients	Control	Canola Oil Supplement
Corn	402.00	402.00
Soybean meal	250.00	250.00
Wheat pollard	110.00	110.00
Corn Gluten	50.00	50.00
Rice bran	110.00	110.00
Crude palm oil	25.00	5.00
Canola oil	0.00	20.00
Limestone	16.50	16.50
DCP	22.00	22.00
Methionine	2.00	2.00
Lysine	4.20	4.20
NaCl	0.30	0.30
Choline chloride	6.70	6.70
Mineral	1.00	1.00
Vitamin	0.30	0.30
Total	100.00	1000.00
Nutrients (calculated basis)		
Crude Protein (%)	19.00	19.00
Crude Lipid (%)	6.13	6.14
Fibre (%)	3.93	3.93
ALA (%)	2	2
Energy (MJ/kg)	11	11

Gas chromatography analysis

The fatty acid methyl esters (FAME) were separated and quantified following the method by Tang *et al.* (2015) using a gas chromatograph (Clarus® 500 Gas Chromatograph (GC); PerkinElmer) which was equipped with an on-column injector and autosampler. A fused-silica capillary column (30 m long, 0.32 mm internal diameter, 0.25 µm thickness, Crossbond® polyethylene glycol, FAMEWAX column; Restek Corporation) was used to separate the FAMEs. The detector used was a Flame Ionization Detector (FID) set at 250 °C and the injector temperature was set at 225 °C. The oven temperature was programmed for temperature adjustment from 130 °C to 225 °C at a heating rate of 6 °C/min. Helium was used as the carrier gas at a flow rate of 1 mL/min and a standard FAME mixture (Supelco) was used to identify all 37 fatty acid peaks.

Statistical Analysis

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

Results and Discussion

Performance of birds fed Canola Oil

The birds fed with feed supplemented Canola oil did not show any adverse effects when compared to control on final body weight and weight gain in all three feeding durations. Birds fed for 3 weeks with Canola oil showed slightly poorer performance with lower weight gain (15.29%) and feed intake (4.38%) compared to the control treatment (Table 3) but is not significantly different ($p>0.05$). The longer the bird was introduced to Canola oil (3 weeks of supplementing with Canola oil), the lesser the feed intake was. The effect of feeding the birds with Canola for a longer period showed the same pattern as reported by Konieczka *et al.* (2017) in the broiler.

Table 3. Performance of birds from age 9-12 weeks fed with or without supplemented Canola oil for 3 weeks (T3).

Treatment	Control		2% Canola oil	
	Mean	SEM	Mean	SEM
Initial Weight (g)	1124.45[a]	8.44	1081.58[b]	7.3
Final Weight (g)	1328.82	53.06	1258.83	34.47
Weight gain (g)	204.37	57.53	177.26	32.3
Feed Intake (g)	1740.04	68.28	1667.07	123.74

[a,b] Means with different superscripts within the same row differ significantly at $P<0.05$.

Feed intake was much better with birds fed supplemented Canola oil during 2 weeks and 1 week of supplementation. In 2 weeks (Table 4), the birds fed Canola increased in weight by about 14.14% (186.40g) than the control, with a feed intake of 8.91% higher than the control ($p>0.05$).

Table 4. Performance of birds from age 10-12 weeks fed with or without supplemented Canola oil for 2 weeks (T2).

Treatment	Control		2% Canola oil	
	Mean	SEM	Mean	SEM
Initial Weight (g)	1225.55	15.68	1186.78	22.23
Final Weight (g)	1328.82	53.06	1291.91	51.69
Weight gain (g)	163.31	19.37	186.40	26.77
Feed Intake (g)	1038.90	62.24	1131.42	97.70

[a,b] Means with different superscripts within the same row differ significantly at $p<0.05$.

The same trend was also noticed in birds fed with Canola oil for 1-week length. They have better performances in terms of weight gain and feed intake. Weight gain for birds fed with Canola oil increases to 95.73g within a week, which is 27.54% more than the control with 75.06g. The feed intake was also higher with Canola fed birds by 37.91% than the birds without Canola supplementation (Table 5). Lopez-Ferrer *et al.* (2001) reported that the addition of PUFA to a broiler diet will increase weight gain and feed intake which was also seen in this study.

Table 5. Performance of birds from age 11-12 weeks fed with or without supplemented Canola oil for 1 week (T1).

Treatments/ Parameters	Control		2% Canola oil	
	Mean	SEM	Mean	SEM
Initial Weight (g)	1294.45	15.38	1241.79	19.33
Final Weight (g)	1328.82	53.06	1307.36	42.8
Weight gain (g)	75.06	20.63	95.73	10.97
Feed Intake (g)	485.28[a]	27.23	669.25[b]	25.74

[a,b] Means with different superscripts within the same row differ significantly at $P<0.05$.

However, all birds whether it was fed with diets containing Canola oil at different periods or fed with zero Canola showed a similar final weight when measured and slaughtered at 12 weeks of rearing (Table 6). The control treatment which was not supplemented with canola oil shows the highest body weight at 12 weeks of age (1378.53g/bird), followed by the addition of 1 week of canola oil, 2 weeks and 3 weeks respectively. From the result, it can be seen that the longer the birds were fed with feed containing Canola, the final body weight is reduced slightly. This was also seen in Japanese quail where a longer period of feeding the birds with flaxseed oil has impaired the FCR (Mirshekar *et al.,* 2021). All treatments, however, showed no significant differences ($P>0.05$) indicating that the addition or supplementation of Canola oil has the same effect on the final weight of the birds.

Table 6. The final weight of birds fed with or without Canola oil at 12 weeks of age (Mean±SEM)

Treatment	Control (T0)	T3	T2	T1	P-value
Final Weight (g)	1378.53±53.06	1288.22±34.47	1291.91±51.68	1307.36±42.80	0.7495

T0 - No Canola oil (Control); T1 - 2% Canola oil supplemented (1 week before slaughter); T2 - 2% Canola oil supplemented (2 weeks before slaughter); T3 - 2% Canola oil supplemented (3 weeks before slaughter)

Carcass composition

The present report demonstrates that the carcass performance of birds for all treatments was not affected by the diet and length of Canola oil supplementation (Table 7). There were no significant differences found ($p>0.05$) for live weight, dressing weight, thigh and breast weight in all treatments. The addition of canola oil did not have any effect on the carcass performances of the birds. The dressing percentage is in the range of around 67 - 68% of the whole body for all treatments. The thigh weight is in the range of 80g to 97g, whereas for the breast meat, it is around 115g to 128g. This was also supported by a study done by Bostami *et al.* (2017), where the addition of different fat sources did not have an impact on the carcass weight of the birds.

Table 7. The carcass of birds at 12 weeks of age fed with supplemented Omega-3 at different feeding duration (Mean ± SE).

Treatment	T0	T3	T2	T1
Live Wt. (g)	1320.56 ± 59.32	1225.47 ± 36.33	1289.59 ± 74.13	1214.73 ± 59.07
Dressing Wt. (g)	904.56 ± 42.66	825.36 ± 14.94	884.65 ± 48.18	818.34 ± 48.66
Dressing %	68.79 ± 0.97	67.36 ± 0.89	68.64 ± 0.75	67.12 ± 0.83
Thigh Wt. (g)	88.46 ± 6.65	80.52 ± 5.66	96.80 ± 8.49	84.80 ± 6.00
Breast Wt. (g)	128.10 ± 5.70	115.00 ± 3.13	124.88 ± 8.74	117.36 ± 11.91

^{a,b} Means with different superscripts within the same row differ significantly at $P<0.05$. T0 - No Canola oil (Control); T1 - 2% Canola oil supplemented (1 week before slaughter); T2 - 2% Canola oil supplemented (2 weeks before slaughter); T3 - 2% Canola oil supplemented (3 weeks before slaughter)

Breast and thigh meat Omega -3 fatty acid

Alpha-linolenic acid was presented in the breast tissue of all treatments except for treatment with 1 week of ALA supplementation (Table 7). Highest ALA was found in birds fed with canola oil two weeks before slaughter (T2- 0.72%) suggesting that a period of two weeks is needed to enrich the breast meat of this bird. Within one week time, ALA was not presented at all which might be due to the shorter time of feeding therefore, the accumulation was not efficient enough. In broiler birds, some reports mentioned that a week of feeding is sufficient to enrich breast meat with Omega 3 fatty acids (Konieczka *et al.*, 2017). However, this does not happen to the village chicken, which may be related to the slow growth rate of this bird species compared to the commercial broiler (Engku Azahan *et al.*, 1990). The percentage of ALA in this study, however, showed an increasing trend as the duration of feeding decreased which was in contrast with what has been reported by Konieczka *et al.* (2017). Rymer & Givens (2005) also note that the addition of ALA in the diet will increase the accumulation of ALA in the meat.

Table 7. The percentage of Omega-3 fatty acids in breast and thigh meat of Ayam Saga at 12 weeks of age.

Parameters	Treatments			
	T0	T3	T2	T1
Breast meat				
ALA %	0.49	0.56	0.72	nd
EPA%	nd	nd	nd	nd
DHA%	nd	nd	0.41	nd
Thigh meat				
ALA%	0.67	0.98	1.06	0.93
EPA%	1.48	nd	nd	nd
DHA%	0.81	0.48	0.12	nd

T0 - No Canola oil (Control); T1 - 2% Canola oil supplemented (1 week before slaughter); T2 - 2% Canola oil supplemented (2 weeks before slaughter); T3 - 2% Canola oil supplemented (3 weeks before slaughter)

The ALA was presented in the thigh tissue for all treatments (Table 7). From the result, it is seen that the thigh meat has a higher ALA percentage than the breast meat which is similar to what has been reported in the broiler (Cortinas *et al.*, 2004). Two weeks of supplementation showed the highest ALA accumulation in the thigh meat with 1.06%. The lowest percentage of ALA in birds fed 1 week of supplemented Canola oil may also be subjected to the shorter time of accumulation such as in the breast tissue. Thigh meat usually contains higher fatty acids due to the high lipid content of this meat part than breast meat (Rimer & Givens, 2005). It is also reported that dark meat is a richer source of ALA than white meat. Breast meat normally is abundant with phospholipids type fatty acids such as EPA and DHA. Whereas thigh meat, it contains more Omega 3 PUFA in triacylglycerols fraction such as ALA (Konieczka *et al.*, 2017; Rymer & Givens, 2005). In this current study, however, EPA was undetected in the breast meat for all treatments and was only present in the thigh tissue of the control.

Conclusion

The Ayam Saga breed (native village chicken in Malaysia) showed no differences in terms of performance and carcass when being fed with Canola oil as a source of Omega-3. The addition of this oil however does have an effect on the accumulation of the Omega-3 in the meat (breast and thigh) of the bird. From the finding, it can be concluded that the ALA was present in both meat when Canola oil is added, with 2 weeks of feeding is efficiently enough to deposit this fatty acid into the meat.

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