Growth Performance, Carcass Composition and Alpha-Linolenic Acid Content of Ayam Saga Fed Different Dietary Sources

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Abstract

A feeding trial was conducted to determine the effect of feeding different omega-3 (alpha-linolenic acid) sources on the growth performance and accumulation of alpha-linolenic acid content in the meat of Ayam Saga. The experiment was conducted between April to May 2022 at the poultry facility in MARDI Serdang using 90 male 7-week-old Ayam Saga. These birds were randomly assigned to 3 treatments of different sources of omega-3: 0% (control), flaxseed oil and flaxseed in a complete randomized design with 5 replications of 6 birds per treatment. They received feed and water ad-lib throughout the experiment. All feeds were formulated to be isocaloric (metabolizable energy - ~12 MJ/kg) and isonitrogenous (crude protein - ~19.0%). They were raised for 5 weeks and weighed every two weeks. Feed consumption was also recorded every 2 weeks. Results showed that the inclusion of omega sources did not affect final weight and weight gain compared to the control (P>0.05). However, treatments had a significant effect on the feed intake (P<0.01) and feed conversion ratio (*P*<0.01), with birds on diets containing flaxseed and flaxseed oil were lower than the control. The increment of alpha-linolenic acid was seen in the breast and thigh meat of birds which received omega-3 diets (*P*<0.01). It can be concluded that flaxseed and flaxseed oil can be good sources of alpha-linolenic acid, however, future studies need to be done to study the optimum inclusion level of flaxseed oil and flaxseed as these omega-3 diets have better FCR and increase the alpha-linolenic acid absorption in chicken meat.

Introduction

Flaxseeds (FS) and flaxseed oil (FO) contain higher amounts of omega-3 (n-3) alpha-linolenic acid (ALA, $C_{18:3n-3}$) and omega-6 (n-6) linoleic acid (LA, $C_{18:2n-6}$) than other vegetable oils. It is also known as linseed oil (Mueed *et al.*, 2022). Typically, FO contains approximately 45-55% and 15% of ALA and LA, respectively. The content of ALA is 7 times higher, but 3 times less LA than soybean and corn oils (NRC, 1994). These fatty acids (FA) cannot be synthesized by the human body. As a result, they must come from the diet. The health benefits of ALA and LA to humans have been well studied and documented (Liu *et al.*, 2023; Naini *et al.*, 2015; Zhao *et al.*, 2004). Several studies demonstrated that diets containing ALA were proven to lower blood pressure and cholesterol to a healthy level (Fukumitsu *et al.*, 2012; Zhao *et al.*, 2004). By helping the heart to maintain its rhythm, it can also reduce the risk of heart attack (deLorgeril *et al.*, 2001) and also has been utilized to treat allergic and inflammatory conditions (Sawada *et al.*, 2021) such as psoriasis and pneumonia.

The reported beneficial effects of omega FAs have been of interest among researchers, leading to the development of many products or supplements to increase the uptake of omega FAs. Fat, particularly fatty acid consumed by the birds may alter the composition of FAs in the chicken's tissue. Many studies showed the inclusion of omega FAs sources in the chicken diet can increase the content of omega FAs in chicken meat and eggs (Bostami *et al.*, 2017; Rymer & Givens, 2005; Ayerza & Coates, 2001). This may become the alternative to fish and fish oil as potential omega sources for human consumption.

Ehr *et al.* (2016) reported that feeding flaxseeds and flaxseed oil had different effects on the deposition of FAs in eggs, as well as growth performance. Omega-3 FA in eggs from chickens fed diet containing flaxseed oil were 2 times greater than milled flaxseed (*P*<0.01). Body weight (BW) gain decreased as the inclusion of flax seeds increased, which could be due to anti-nutritional factors (**ANF**), mainly cyanogenic glycosides and phytic acid. The objectives of this study were to investigate and compare the effect of feeding ground flaxseeds and flaxseed oil as omega FA sources on the accumulation of ALA in the chicken meat of Ayam Saga. Ayam Saga is a new chicken breed introduced by the Malaysian Agricultural Research and Development Institute (MARDI) to meet the demand for chicken meat and eggs. It has potential commercial production due to its adaptability to the local environment and feedstuffs.

Materials and methods

Animal management and feed formulation

The experiment was carried out between April to May 2022, and approved by the MARDI Animal Ethics Committee (AEC Approval 20210827/R/MAEC00092) at the poultry facility in MARDI Serdang. A total of 90 male Ayam Saga aged 7 weeks old, acquired from MARDI Muadzam Shah Hatchery Farm were used in this experiment. Birds were adapted to the experimental diet (corn-soy based) and condition, 2 weeks prior to the start of the experiment. Birds were randomly allocated into 3 experimental group*s*, with 5 replications and *6* birds per replication. Birds were kept in 2-tier battery cages equipped with 2 sets of feeders and water drinkers in an enclosed house system located in MARDI Serdang. The average temperature and humidity across the trial were 29.8 °C and 64.9%, respectively. The experiment was conducted until the birds reached 12 weeks of age.

Each group of birds received 3 experimental diets (Table 1), which consisted of a corn-soy-based diet: (i) without addition of omega source as a control diet (Group A, inclusion of 2% palm oil), (ii) with 2% flaxseed oil (Group B) and (iii) with 5% flaxseed (Group C) as omega sources. Diets for Groups B and C were formulated to contain the same amount of ALA. All diets were formulated to be isocaloric (12 MJ/kg) and isonitrogenous (19% protein) to meet the minimum nutritional requirement of grower Ayam Saga, and were in mash form. Diets were assigned to the cage randomly. Diets and water were supplied ad-lib throughout the experimental period.

Data and sample collection

The initial weight (IW) of the birds was taken on the first day of the experiment by group weighing, according to the treatments and replications. Subsequently, birds were weighed according to the diet group fortnightly, as well as at the end of the experiment (final weight, FW). Feed intake (FI) per group of replications was measured fortnightly. Based on the weight and FI data, average daily weight gain (ADG) and feed conversion ratio (FCR) were calculated.

Table 1. Materials and nutrient compositions of experimental diets

Ingredients	Raw material composition, %		
	Group A	Group B	Group C
Soybean meal	25.00	25.00	25.00
Corn	55.64	54.47	52.94
Wheat pollard	11.00	11.00	11.00
Palm oil	1.00	0.00	0.00
Corn gluten	2.26	2.41	1.13
Flaxseed	0.00	0.00	5.00
Flaxseed oil	0.00	2.00	0.00
DiCaP	1.00	1.00	1.00
Limestone	1.70	1.70	1.68
NaCl	0.30	0.30	0.30
Lysine	0.31	0.31	0.29
Methionine	0.11	0.11	0.12
Rice bran	1.05	1.06	0.91
Choline Chloride	0.50	0.50	0.50
Premix (Vit-min)	0.13	0.13	0.13
Calculated nutrient compositions			
Metabolizable energy, MJ/kg	12.23	12.40	12.12
Crude protein, %	19.00	19.00	19.00
Crude fat, %	4.51	4.71	4.71
Lysine, %	1.20	1.20	1.20
Methionine + cystine, %	1.66	1.66	1.66
Arginine, %	1.21	1.21	1.24
Tryptophan, %	0.27	0.27	0.27

Group A – diet without omega-3 supplementation, Group B – diet containing 2% flaxseed oil, Group C – diet containing 5% flaxseed

The feed conversion ratio of birds was calculated as a ratio of total feed consumed to produce 1 kg of weight. These data were monitored during the grower period (7 to 12 week-old) only. Then, at the end of the experiment, one bird from each replication and treatment was randomly selected and sacrificed for carcass and fatty acid analyses. The total number of birds selected per treatment was one bird from each replicate.

Carcass analysis

The birds were de-feathered and eviscerated. The dressing percentage was calculated using Equation 1. Cleaned carcasses (without head, internal organs and shanks) were weighed individually.

$$\text{Dressing \%} = \frac{\text{Weight of carcass}}{\text{Weight of live animal}} \times 100$$

Equation 1

Then, carcasses were cut into parts. The thigh and breast were weighed separately. The yield for each part of the bird was then expressed as a percentage of the body weight. Carcasses were then dried in the oven (Memmert) at 60 °C, until drying to constant mass. Then, samples were stored in labelled plastic bags and frozen at -20 ºC for further chemical analyses.

Lipid and fatty acids analysis

Dried, deboned and ground thigh and breast samples were analysed for lipid and fatty acids. Lipid was extracted according to chloroform:methanol method as described by Wang *et al.* (2000). The dried lipid was then added with 3 ml 3N methanolic:HCl, and incubated in a water bath at 95°C for 1 h for the preparation of fatty acids methyl ester (FAME). After cooling to room temperature, 1 ml of hexane was added to the mixture, and mixed well by vigorous shaking. The clear top layer (FAME) was pipetted into a 1.5 ml vial tube and analysed and quantified using gas chromatography (GC, Clarus® 500 Gas Chromatograph GC; PerkinElmer). The GC is equipped with an on-column injector, autosampler and flame ionization detector (FID). The condition of the GC was as described by Tang *et al.* (2015).

Statistical analysis

All data were subjected to statistical analysis using one one-way analysis of variance. Then, Duncan's Multiple Range Test (DMRT) was used to compare the significant differences between treatments using SAS version 9.4. Significance was set at $P<0.05$.

Results and discussion

Growth Performance of Ayam Saga

The results for the growth performance of Ayam Saga fed diets supplemented with different omega-3 sources (0%, 2% flaxseed oil and 5% flaxseed) are summarized in Table 2. The birds in this experiment had final weights ranging from 1.52 to 1.71 kg, with the highest final weight achieved in birds from Group B, which were fed a diet containing 2% flaxseed oil, followed by Group A (control, 2% palm oil) and C (5% flaxseed). A similar trend was also noticed in the weight gain of the birds (Table 2). The highest gain was observed in birds from Group B, followed by birds from Group A which received a diet without the addition of omega-3 source and Group C (5% flaxseed), however, the differences were not significant ($P>0.05$). A significant reduction in the final weight of birds fed flaxseed diet was reported by Yannakopoulus *et al.* (1998) and Caston *et al.* (1994). However, in our study, we observed a non-significant reduction in weight because the inclusion level of flaxseed in the chicken diet was only 5%, whereas in other studies, it was included at levels of up to 20%.

The feed conversion ratio is one of the major indicators of feed efficiency, which indicates how many kilograms of feed are consumed by the birds to produce one kilogram of body weight. In our study, different dietary sources did have a significant impact on the feed intake and FCR ($P>0.05$).

The higher the FCR, the less efficient the bird is. Birds from Group C, which were fed a diet containing omega-3 sourced from flaxseed, showed the lowest feed intake ($P<0.0001$) and weight gain, although the differences in gain were not significant ($P>0.05$). Similar findings are reported by Yannakopolous *et al.* (1998) and Ayerza & Coates (2001) reported that birds which received a diet containing flaxseed produced low body weight and ate less feed. While FCR for birds from Group A (control diet) was significantly higher ($P<0.05$) than those fed omega-3 based diets (Table 2) due to high feed intake, but producing lower weight gain than Group B. Birds fed flaxseed diet (Group

C) had the lightest weight and lowest intake, however, the FCR was lower than the control diet. The reduction in gain and intake of birds fed diet containing flaxseed could be due to ANF contained in the seeds (Ayerza & Coates, 2001). Flaxseed contains ANF such as cyanogenic glycosides (CGs), cadmium, trypsin inhibitors, and phytic acid that can reduce the availability of one or more nutrients and/or decrease the maximal use of health-promoting effects have been reported (Nowak & Jeziorek, 2023). Flaxseed also contains vitamin B6 antagonist compounds (Bond *et al.*, 1996) which could affect the performance of birds and limit their intake. The presence of non-starch polysaccharides (NPS) in flaxseed decreased the nutrient digestibility of the birds (Denise Apperson & Cherian, 2017).

Table 2. Mean growth performance of Ayam Saga fed diets with and without omega-3 sources

Variable	N	Minimum	Maximum	Mean	SD	*P*-value
Initial weight						
Group A	5	0.87	0.98	0.92	0.05	0.9470
Group B	5	0.91	0.96	0.93	0.02	
Group C	5	0.91	0.93	0.92	0.01	
Final weight						
Group A	5	1.52	1.69	1.61	0.08	0.2373
Group B	5	1.62	1.71	1.66	0.03	
Group C	5	1.53	1.66	1.59	0.06	
Weight gain						
Group A	5	0.65	0.75	0.69	0.04	0.1805
Group B	5	0.68	0.79	0.73	0.04	
Group C	5	0.60	0.73	0.67	0.06	
Feed intake						
Group A	5	2.57	2.88	2.72	0.14	<0.0001
Group B	5	2.06	2.39	2.25	0.12	
Group C	5	2.09	2.23	2.17	0.05	
Feed conversion ratio						
Group A	5	3.72	4.46	3.95	0.30	0.0009
Group B	5	2.77	3.27	3.08	0.22	
Group C	5	2.93	3.59	3.27	0.30	

[a,b] Means with different superscripts within the same row differ significantly at P<0.05. SD – standard deviation; Group A – diet without omega-3 supplementation, Group B – diet containing 2% flaxseed oil, Group C – diet containing 5% flaxseed

Carcass analysis

Table 3 illustrates that birds fed the flaxseed oil diet had the highest dressing percentage (68.20%) and breast yield (16.93%) compared to the control and flaxseed diet groups. Conversely, the control diet (Group A) resulted in the highest yield of thigh (8.00%). Despite these differences, statistical analysis (as presented in Table 3) showed no significant differences among these carcass parameters. This aligns with the final weight data of the birds, as previously described. These results indicate that the addition of flaxseed oil and flaxseed in the chicken diet did not adversely affect the carcass performance. The findings were supported by Bostami *et al.* (2017), where the addition of different fat sources did not have an adverse effect on the carcass yield of the birds. On the other hand, Mridula *et al.* (2011) reported a significant reduction in the breast weight of birds which received a flaxseed meal diet. In their study, the inclusion level of flaxseed in the diet was 15%, whereas our study included 5%. This can be an indication that the higher the inclusion level of flaxseed, the more pronounced the effect, could be further investigated in future studies.

Table 3. Carcass analysis (% of body weight) of Ayam Saga fed diet with and without omega-3 sources

Treatment	Dressing percentage (%)	Thigh (%)	Breast (%)
Group A	68.20	8.00	16.29
Group B	69.75	7.80	16.93
Group C	69.51	7.64	15.91
P-value	0.6064	0.8781	0.7528

Group A – 2% palm oil (Control); Group B – flaxseed oil-based diet; Group C –flaxseed-based diet

Lipid and alpha-linolenic acid in chicken meat

The lipid content and alpha-linolenic acid (ALA) concentration in the breast and thigh muscles of Ayam Saga at week 12 are presented in Table 4. There were no significant differences in lipid content in the thigh and breast of the birds observed among the treatment groups ($P>0.05$), which indicates that lipid was not affected by the addition of flaxseed or flaxseed oil in the chicken diets. This is because all treatment diets were formulated to contain the same amount of nutrients, with lipid content ranging from 4.5% to 4.7%. Some literature has mentioned that supplementing birds with polyunsaturated fatty acid (PUFA), including omega fatty acids could lead to higher lipid peroxidation from endogenous lipogenesis, due to unstable double bonds resulting in lower fat deposition (Gallardo *et al.* 2012).

However, in this study, these effects did not occur despite flaxseed and flaxseed oil containing higher amounts of polyunsaturated fatty acids (PUFA) in comparison to monounsaturated fatty acids (MUFA), known for their greater susceptibility to oxidation. This could be due to the relatively short intake duration of 5 weeks during the grower stage. However, the optimum feeding duration to prevent oxidation has not been reported yet not observed. Future investigations can be conducted to compare various omega sources, characterized by differing proportions of MUFA and PUFA, to further explore their impact on oxidation mechanisms.

The addition of flaxseed oil and flaxseed at 2 and 5% provided experimental diets with approximately more than 50 and 100-fold increase of ALA relative to the control in breast and thigh, respectively. The control group (Group A) had significantly lower ($P<0.05$) ALA concentrations compared to the two flaxseed-based diets (Groups B and C). However, no difference in ALA contents was recorded between the flaxseed and flaxseed oil treatments in both thigh and breast meat samples. Although the difference was not significant, ALA was detected in higher quantities in the chicken thigh (0.0054 mg/g) and breast (0.018 mg/g) from the flaxseed oil group compared to the seed (Table 4). The presence of antinutritional factors in the seeds limits the availability of the nutrients to be transferred to the meat as reported by Konieczka *et al.* (2017).

Table 4. Lipid content (%) and alpha-linolenic acid (ALA, g/g lipid) (Mean ± SE) of Ayam Saga at the age of week 12 fed diet with and without omega-3 sources

Treatment	Group A	Group B	Group C	*P*-value
Breast lipid, %	5.68±1.05	4.92±0.61	5.52±0.94	0.8101
Thigh lipid, %	14.13±1.49	12.87±1.70	16.75±1.97	0.3218
ALA (breast), mg/g	0.00^{b}±0.00	0.0054^{a}±0.002	0.0053^{a}±0.003	<0.0001
ALA (thigh), mg/g	0.00^{b}±0.00	0.0180^{a}±0.001	0.0118^{a}±0.001	0.0003

[a,b] Means with different superscripts within the same row differ significantly at P<0.05.
Group A – diet without omega-3 supplementation, Group B – diet containing 2% flaxseed oil, Group C – diet containing 5% flaxseed

Instead of using flaxseed, it is recommended to incorporate flaxseed oil into chicken diets to enhance the fatty acid profile of the meat.

A greater ALA content was found in the thigh compared to the breast (Table 4). The higher ALA content is attributed to the increased lipid content in the meat (Rymer & Givens, 2005) and Cortinas *et al.* (2004), with an 85% difference in the content between the thigh and breast (Cortinas *et al.,* 2004). The omega-3, particularly ALA, can be found more abundantly in the triacylglycerols fraction of thigh meat compared to the breast. In contrast, the breast contains phospholipid-type fatty acids such as EPA and DHA (Konieczka *et al.,* 2017; Rymer & Givens, 2005).

Based on the fatty acid result, it indicates that the inclusion of flaxseed and flaxseed oil significantly increased the ALA content in the thigh and breast muscles of Ayam Saga. Alpha-linolenic acid offers greater health benefits to consumers, especially health-conscious individuals. Therefore, the addition of ALA sources like flaxseed and flaxseed oil can be considered as a strategy to enhance the ALA content in chicken meat. However, according to other studies (Yannakopoulus *et al.,* 1998; Caston *et al.,* 1994), the inclusion of flaxseed in chicken feed has been associated with a reduction in the growth performance of the birds. Hence, future research should focus on determining the optimal level of flaxseed inclusion to enhance both growth performance and alpha-linolenic acid content in birds.

Conclusion

This study concludes that both flaxseed and flaxseed oil can be used as omega-3 (ALA) sources in Ayam Saga diets. However, further investigation is needed to determine the optimum inclusion level of flaxseed and flaxseed oil in the diet. Additionally, an economic study is required to study the feasibility of omega-3 inclusion in chicken diets.

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