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THE EFFECT OF DIET CONTAINING HIGH ALPHA-LINOLENIC ACID ON OMEGA-3 FATTY ACIDS AND HEALTH STATUS OF THE HEART IN BROILERS

PENGARUH PAKAN YANG MENGANDUNG ASAM ALPHA-LINOLENAT TINGGI TERHADAP ASAM LEMAK OMEGA-3 DAN STATUS KESEHATAN JANTUNG PADA BROILER

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ABSTRACT

The objective of the study was to examine the effects of diet containing high alpha-linolenic acid (ALA) on omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) and the health status of heart in broilers. Diets were based on a commercial starter diet, with a low level of fat. The experimental diets contained two levels of ALA (2.23 and 19.37%), with the total fat content was approximately 5%. Pure or blended vegetable oils were included at a level of 2.8% in order to produce diets with the desired levels of linoleic acid (LA) and ALA. The ratio of LA to ALA of the diets was 9.75:1 for low ALA diet and 1.37:1 for high ALA diet. Each diet was provided *ad libitum* for the duration of the 28-d growth period. A total of 32 one-day-old chicks housed in four pens (n=8 birds/pen). At 28 days of age, six selected birds from each pen were weighed individually and hearts were collected for analysis. A ratio of right ventricle mass (RV) to total ventricle mass (TV) was used to indicate the health status of the heart. Results showed that dietary treatment increased the level of ALA in heart tissue from 0.1% (low ALA diet) to 0.5% ($P<0.01$). Increasing levels of dietary ALA raised the level of n-3 LCPUFA and total n-3 in heart tissues by 4-5-fold. There was no significant difference observed in the level of heart LA, arachidonic acid (AA) and total n-6. The increased levels of dietary ALA did not cause changes in the ratio of RV to total TV. In conclusion, it appears clear that based on our data, increasing ALA content in the diet of chickens could potentially be beneficial for the health of the birds; however, further work is necessary.

(Keywords: Alpha-linolenic acid, Chicken heart, Health status, Omega-3 fatty acids)

INTISARI

Penelitian dilakukan untuk mengetahui pengaruh pakan yang kaya akan kandungan asam alpha-linolenat (alpha-linolenic acid, ALA, asam lemak n-3) terhadap kandungan asam lemak tak jenuh omega-3 rantai panjang (n-3 LCPUFA) dan status kesehatan jantung ayam. Pakan yang diberikan didasarkan pada pakan starter komersial yang mengandung lemak rendah. Pakan perlakuan terdiri dari dua aras ALA (2,23 and 19,37%), dengan kandungan total lemak sekitar 5%. Minyak murni dan campuran minyak sayuran ditambahkan dengan aras 2,8% dengan tujuan menghasilkan pakan dengan tingkat asam linoleat (LA, asam lemak n-6) dan ALA yang diharapkan. Rasio LA terhadap ALA pakan adalah 9,75:1 untuk pakan yang mengandung ALA rendah dan 1,37:1 untuk pakan yang mengandung ALA tinggi. Sejumlah 32 ekor ayam umur satu hari dikandangkan ke dalam empat kandang (n=8 ekor/kandang). Pada umur 28 hari, enam ayam broiler yang terpilih dari masing-masing kandang ditimbang secara individu dan sampel jantung diambil untuk dianalisa. Rasio dari massa ventrikel kanan terhadap massa ventrikel total digunakan untuk menunjukkan status kesehatan dari jantung. Hasil-hasil penelitian menunjukkan bahwa pakan perlakuan meningkatkan kandungan ALA dari 0,1% (pakan yang mengandung ALA) sampai 0,5% (P<0,01). Peningkatan aras ALA pakan meningkatkan kandungan asam lemak tak jenuh n-3 rantai panjang dan total asam lemak n-3 jaringan jantung ayam hingga 4-5 kali lipat. Tidak terdapat perbedaan yang nyata pada kandungan LA, asam arakhidonat (AA) dan total asam lemak n-6. Aras ALA pakan yang ditingkatkan tidak menyebabkan perubahan pada rasio RV terhadap total TV. Berdasarkan data yang diperoleh dapat disimpulkan bahwa peningkatan kandungan ALA pada pakan ayam mempunyai potensi memberikan manfaat kesehatan bagi ayam.

(Kata kunci: Asam alfa-linolenat, Asam lemak omega-3, Jantung ayam, Status kesehatan)

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Introduction

Ascites and sudden death syndrome (SDS) are the most common heart-related conditions and highly susceptible to heart failure that affects mainly fast-growing broiler chickens. Frequently, birds which die of SDS to be in good condition without any discernible preexisting clinical signs and all birds were well-fleshed and had general pulmonary congestion (Olkowski, 2007). However, birds with history of cardiac rhythm disturbances and showing serious ventricular arrhythmia are at high risk of sudden death. Male birds have higher in the incidence of arrhythmia than females, and the prevalence of SDS is much higher in male meat type poultry (Olkowski and Classen, 1998).

Julian (1998) noted that right ventricular failure following right ventricular hypertrophy (RVH) from pulmonary hypertension causes ascites, which has become a prominent cause of illness and death in broiler chickens. An early sign of right ventricle hypertrophy in the pathogenesis of ascites is higher right ventricle weight to total ventricular weight ratio (Wideman, 2001). The rate of sudden death ranges from 1.3 to 9.6% depending on the conditions and age of the birds (Gardiner *et al.*, 1988). The sudden death in chickens could have a similarity to sudden cardiac death in humans which has been related to low levels of n-3 fats in heart membranes (Albert *et al.*, 1998).

Therefore, the accumulation of heart omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) in chickens was measured to examine whether diets high in ALA were potentially beneficial for the health of birds by increasing the level of n-3 LCPUFA, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in cardiac membranes. Support for this comes from previous reports that feeding chickens diets containing 5% linseed oil reduced right ventricle hypotrophy (Walton *et al.*, 1999) and pulmonary hypertension was reduced in birds fed a diet containing 10% linseed oil (Bond *et al.*, 1996). The primary objective of the research was to examine the effects of diet containing high ALA on heart n-3 LCPUFA levels and the health status of the heart.

Materials and Methods

Experimental design

The design of this study was a one-way classification. The variable factor was the ALA level in the diet. The dietary treatments were based on the ratio of LA to ALA of the diets. There was a total of two diets comprised of a low ALA diet and a high ALA. These diets were provided to a total of 32 one-day-old chicks housed in four pens (n=8 birds/pen). Each dietary treatment was assigned to two pens.

Birds

A total of 32 one-day-old mixed sex broiler chickens (Cobb 500) obtained from the Baiada hatchery (Willaston, SA, Australia) were randomly placed in four pens and distributed between two dietary treatments. The birds were housed for 28 days and reared at PPPI, SARDI, Roseworthy Campus under controlled environmental conditions. Upon arrival from the hatchery, the chickens were immediately weighed in groups of 8 (Libror EB-32KS SHIMADZU) and placed on brown paper in raised rearing pens (1.2 x 0.9 m each pen). Feed was provided in a plastic hopper and scattered over the paper to encourage the chickens to eat immediately after placement. Fresh water was placed in the splash cup under the drinking nipples to encourage the chickens to drink soon after placement. Both feed and water were provided *ad libitum* for the duration of the experiment.

Room temperature was maintained at 27°C for 4 days and gradually reduced to 20°C during the experimental period. All birds were subjected to a 24 h light program throughout the growth period. At the same time, the pens were also given heat from infrared lamps (175 watts) for 21 days. During the first few days, the chickens were observed at frequent intervals to ensure that they were comfortable with the environmental conditions and that all had access to adequate feed and water. The room temperature was maintained by a logic controller (Tempron 606) which managed air flow, cooling and heating. Fresh wood shavings were put on the pen floor at 3 days old. During the experimental period fresh shavings were added three times.

Diets

Diets were based on a commercial starter diet, with a low level of fat (Table 1). The experimental diets were formulated by varying the levels of ALA, which was from 2.23 (diet low in ALA) to 19.37% (diet high in ALA), with the total fat content was approximately 5%. Pure (macadamia oil) or blended vegetable oils (macadamia and flaxseed oil) were included at a level of 2.8% to produce diets with the desired levels of LA and ALA.

The dietary treatments had the same nutritional values as the basal diet except for the fatty acid composition. This resulted in the ratio of LA to ALA of the diets 9.75:1 (low in ALA) and 1.37:1 (high in ALA). All diets met or exceeded National Research Council guidelines for broiler chickens. The fatty acids content of the experimental diets is presented in Table 2. Each diet was provided *ad libitum* for the duration of the 28-d growth period.

Sample collection and fatty acid analysis

At 28 days of age, six selected birds from each pen (12 birds per dietary treatment group) were weighed individually and hearts were collected for analysis. A ratio of right ventricle mass (RV) to total ventricle mass (TV) was used to indicate the health status of the heart. The heart analysis of birds followed the procedure described by Julian (1987). After dissection, the heart was removed and placed on a paper plate. Auricles, major vessels and fat were stripped from the heart. The RV including the valve was carefully removed from the left ventricle (LV) and septum. The RV was weighed and recorded. The LV and septum were also weighed. These data were used to calculate the percentage values of RV/TV, where TV is

Table 1. Ingredient composition and nutrient content of basal diet

Ingredients[1]	kg/100 kg
Wheat fine	43.91
Wheat mil vits	0.80
Barley	10.00
Triticale fine	10.00
Peas fine	10.00
Meat meal	4.60
Blood meal	1.40
Soybean meal	15.00
Millrun	2.00
Limestone small	0.79
Salt	0.18
Sodium bicarbonate	0.27
Choline chloride 75%	0.07
Potassium carbonate	0.01
L-threonine	0.09
Alimet	0.35
Standard broiler starter premix	0.20
Lysine sulphate	0.29
Phyzyme XP5000L broiler	0.01
Feed enzyme premix	0.03
Nutrient content (%)	
Metabolizable energy, kcal/kg	2,787
Crude protein	22.99
Crude fat	2.20
Crude fiber	3.82
Calcium	0.98
Phosphorus	0.74
Available phosphorus	0.5
Na	0.2
K	0.71
Cl	0.2
Lysine	1.3
Methionine	0.59
Methionine + Cystine	0.99

[1]A standard commercial starter diet (Ridley Agriproducts Pty Ltd, Murray Bridge, Australia).

Table 2. Fatty acid content of the diets

	Experimental diets	
	Low ALA Diet	High ALA Diet
LA (% energy, %en)	2.34	2.90
ALA (% en)	0.24	2.12
LA:ALA ratio	9.75:1	1.37:1
Fat (%)	5.05	5.11
Fatty acid[1] (% of total fatty acids)		
Total SFA[1]	21.37	19.41
Totals Trans	0.39	0.42
18:1n-9	40.25	27.11
18:1n-7	2.53	1.58
Total MUFA[1]	53.85	33.80
Total n-9	42.40	28.34
Total n-7	11.31	5.36
18:2n-6	21.63	26.52
Total n-6	21.85	26.76
18:3n-3	2.23	19.37
Total n-3	2.47	19.52
Total PUFA[1]	24.32	46.28

[1]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid

total ventricle and septum weight. A ratio of RV/TV of <0.25 (25%) was classified as a normal broiler (Julian, 1987). Hearts were stored for fatty acid analysis. The fatty acid profile were analysed followed procedures of Kartikasari *et al.* (2012).

Results and Discussion

Heart tissue fatty acids

The fatty acid composition of heart tissues is presented in Table 3. The two diets contained similar levels of dietary LA (around 2%) but high ALA group contained 10 times the low level of ALA. Dietary treatment increased the level of ALA in heart tissue from 0.1% (low ALA diet) to 0.5%. This aspect of the current project was conducted to examine whether increasing the level of dietary ALA was potentially beneficial for the health of fast growing broilers. Our study found that by increasing dietary level of ALA, heart membrane n-3 LCPUFA (EPA, DPA and DHA) and total n-3 increased 4 to 5 fold. The level of EPA was found in the highest amount (2.2%) among n-3 LCPUFA and appears as the major n-3 LCPUFA in heart tissues while DHA was less than 1% of the total fatty acids. This might be because DHA has a limited ability to accumulate in cardiac tissue in chickens. This contrasts with the fatty acid patterns in human hearts where the level of EPA is normally around 0.5% and DHA is nearly 5% of the total fats (Metcalf *et al.*, 2007). Feeding fish oil to human subjects has been shown to increase EPA and DHA levels markedly but feeding ALA rich flax oil had no effect (Metcalf *et al.*, 2007).

The results show that the response to saturated fatty acid (SFA) content of heart samples was not different between low ALA diet and the high ALA diet whereas the concentration of monounsaturated fatty acid (MUFA) was reduced (P<0.05). There was no significant difference observed in the level of heart LA, AA and total n-6.

Heart analysis

We observed that there was no difference in the percentage of right ventricle (RV) to total ventricle (TV) of birds with increasing dietary ALA level in the diet using 1.7% flaxseed oil (Table 3). These results are supported by other investigators. Walton *et al.* (1999) conducted a study in broiler chickens fed diets containing 2.5 or 5% flaxseed oil or control diets with equivalent amounts of animal/vegetable blend oil for 4 weeks using hypobaric chambers and control pens. They found that the inclusion level of 5% flaxseed oil in the diet reduced right ventricle hypertrophy in birds exposed to hypobaric conditions compared to the birds fed with control diets. However, there was no change observed in the birds fed with 2.5% flaxseed oil. Thus our inability to demonstrate a change in ventricular size may have been due to the lower level of flaxseed oil tested (1.7%).

The ratio of RV to TV both in the low ALA diet (19.12%) and high ALA diet (21.39%) was <0.25 (25%). It indicates that

Table 3. Ventricular characteristics and fatty acid composition of heart phospholipids from chickens fed experimental diets varying in LA to ALA ratio for 28 days[1]

Nutrient content	Experimental diets			
	Low ALA diet	High ALA diet		
LA (% en)	2.34	2.90		
ALA (% en)	0.24	2.12		
LA:ALA ratio	9.75:1	1.37:1		
Fat content (%)	5.05	5.11		
Fatty acids[2]	(% of total fatty acids)[1]		PSEM	P value
16:0	16.83	16.97	0.601	NS
18:0	20.68	21.44	0.481	NS
SFA	41.59	42.97	0.323	NS
18:1n-9	13.74[b]	10.31[a]	0.260	*
18:1n-7	3.98	3.02	0.196	NS
MUFA	19.88[b]	14.59[a]	0.446	*
20:3n-9	1.55[b]	0.78[a]	0.063	*
18:2n-6	25.43	25.93	0.729	NS
20:3n-6	1.50	1.33	0.109	NS
20:4n-6	7.46	8.22	0.684	NS
Total n-6	35.57	36.63	0.408	NS
18:3n-3	0.08[a]	0.48[b]	0.017	**
20:3n-3	0.06[a]	0.21[b]	0.010	**
20:5n-3	0.44[a]	2.21[b]	0.090	**
22:5n-3	0.25[a]	1.15[b]	0.053	**
22:6n-3	0.14[a]	0.64[b]	0.012	**
Total n-3	0.99[a]	4.71[b]	0.018	**
Total PUFA	36.56[a]	41.34[b]	0.410	*
RV/TV ratio (%)				
RV/TV	19.12	21.39	1.225	NS

[a,b] Values in the same row with no common superscript are significantly different
[1]Fatty acid values are means of 12 observations per treatment
[2]SFA= saturated fatty acid; MUFA= monounsaturated fatty acid; PUFA= polyunsaturated fatty acid; RV= right ventricle; TV= Total ventricle; PSEM = pooled standard error of the mean; NS=not significant; *P<0.05; **P<0.01.

the birds were in a normal range as noted by Julian (1987). Studies conducted by other investigators show that increasing dietary ALA seems to be a potential way to reduce RVH leading to ascites (Walton *et al.*, 1999), mortality and the incidence of ascites at a high altitude (Bond *et al.*, 1996) and the incidence of pulmonary hypertension syndrome (Walton *et al.*, 2001). Potential of diets rich in ALA to prevent sudden death needs to be fully evaluated in a large-scale commercial setting and in environment more conducive to causing sudden death syndrome.

Conclusion

Increasing levels of dietary ALA enhanced the level of n-3 LCPUFA in heart tissues by 4 to 5-fold but did not cause change in the percentage of RV to TV of birds. It appears clear that based on our data and the work of others, increasing ALA content in the diet of chickens could potentially be beneficial for the health of the birds; however, further work is necessary.

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