

Growth performance, serum lipids and fatty acid profile of different tissues in chicken broilers fed a diet supplemented with linseed oil during a prolonged fattening period

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ABSTRACT

The influence of enriching the diet of chicken with 5% of linseed oil as a vegetable source of n3 fatty acids in the form of linolenic acid on the accumulation of n3 long chain polyunsaturated fatty acids in different tissues was investigated. The fatty acid profile of the different tissues reflected dietary fatty acid profile. In general, the birds fed linseed oil showed a significant difference in the summarized value of n3 fatty acid ($P < 0.001$) for thigh and adipose tissue. The increase in n3 polyunsaturated fatty acids (PUFA) ($P < 0.001$) resulted in a significant decrease in n6 fatty acids ($P < 0.001$) and n6/n3 ratio ($P < 0.001$) in thigh and adipose tissue. The observed n6/n3 ratio in the edible tissue (thigh) of linseed oil fed birds in a prolonged feeding period was in accordance with dietary recommendations for human nutrition.

Key words: linseed oil, n3/n6 ratio, polyunsaturated fatty acids, chicken broiler

Introduction

The quality and quantity of lipids and their fatty acid composition in meat are influenced by internal (age, gender, genotype and castration) and external (temperature, feeding) factors (MAŠEK et al., 2013). The oil supplement in diets is a very important

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resource of either long chain n3 polyunsaturated fatty acids (PUFA), as in fish oil, or as a form of its precursor fatty acid, α -linolenic (ALA), as in linseed oil. The exact content and ratio between ALA and linoleic acid (LA) in linseed depends on the flax variety and could range from 14% to more than 60% of ALA (ZELENKA et al., 2006). The main reason for incorporating linseed oil in mixtures for broiler chicken is the favourable effect of polyunsaturated fatty acid (PUFA) on animal and human health. The first effect of adding linseed oil is a high increase in α -linolenic acid content (ZELENKA et al., 2006) and a possible increase in other n3 PUFA (ZELENKA et al., 2008). Consequently, the ratio between competing n3 and n6 lines is also changed in favour of n3 fatty acids, which are essential for normal growth and development (SIMOPOULOS, 2000). There is increasing recognition of the health benefits of PUFA in general, and of n3 fatty acids in particular, because these fatty acids are essential for humans (CONQUER and HOLUB, 1998; SIMOPOULOS, 2001). Today we know that n3 fatty acids are important in the prevention and treatment of coronary disease, hypertension, diabetes and arthritis (SIMOPOULOS, 1991; SIMOPOULOS, 2009).

Several trials have shown that an increase in the content of long chain n3 PUFA in chicken broiler meat may be achieved by including linseed oil as a source of precursor, α -linolenic acid (ZELENKA et al., 2008). Nevertheless, possible problems arising from including linseed oil in chicken broiler diets comprise the worsening of sensory traits (LOPEZ-FERRER et al., 1999) and the high economic cost of the linseed diet. Poultry meat with an enhanced ALA content is more susceptible to oxidative damage than meat with a similar concentration of LA (KOUBA and MOURROT, 2011). The balance of volatile compounds resulting from an oxidative breakdown of n3 PUFA causes the occurrence of a fishy aroma and the off-taste, characteristic of the meat of poultry fed a higher level of n3 PUFA (RYMER and GIVENS 2005). The efficiency of fatty acid (FA) conversion in liver, which varies according to the age of the animal (BOURRE et al., 1990), could modify the differential lipid deposition. Although ALA is efficiently converted in the liver, the n3 LC-PUFA depots are not nutritionally valuable in muscle.

Therefore, the aim of this investigation was to study the lipid metabolism and muscle fatty acid content of chicken broiler meat fed linseed oil from the 25th to 55th day of the fattening period.

Materials and methods

Thirty male Ross 308 broilers were used in the feeding trial with three replicates per treatment of five birds each. The birds were divided at the 25th day of the experiment into a linseed group, which received 5% of linseed oil in diet, and a sunflower group, which received 5% of sunflower oil in the diet. The detailed composition of the experimental diets is presented in Table 1.

Table 1. Ingredients, nutrient composition and fatty acid profile of experimental diet

	Linseed oil	Sunflower oil
Ingredients (g per 100 g dry matter)		
Corn	58.5	58.5
Alfalfa. Dehydrated	3	3
Soybean meal	28.5	28.5
Mineral mixture	5	5
Sunflower oil	-	5
Linseed oil	5	-
Composition (g per 100 g dry matter)		
Crude protein	18.1	18.1
Crude fibre	3.41	3.41
Crude fat	7.64	7.59
Ash	5.92	5.92
ME, MJ/kg	16.12	16.12
Fatty acid profile ¹ (g per 100 g total fatty acids)		
16:0	11.14	7.42
18:0	5.65	2.37
18:1	21.39	41.25
18:2n6	33.08	48.66
18:3n3	28.74	0.30

¹Fatty acid composition of sunflower oil: C16:0, 5.3; C18:0, 5.7; C18:1, 29.0; C18:2, 56.0, linseed oil: C16:0, 6.7; C18:0, 4.3; C18:1, 17.9; C18:2, 18.7; C18:3, 51.4

On the 55th day of the experiment all animals were euthanized using CO₂. The body measurements, feed intake and feed conversion were recorded regularly during the experiment. After euthanasia, tissue samples were frozen in liquid nitrogen and stored at -20 °C.

Blood samples were collected on the 55th day of the experiment by jugular venepuncture. The blood serum was separated by centrifugation and stored at -20 °C until assayed. Serum glucose, triglyceride, HDL-cholesterol, LDL-cholesterol and total cholesterol values were analysed by an automatic analyser (SABA 18, AMS, Italy).

Total lipids from broiler samples (abdominal fat and thigh muscle) were extracted using a chloroform-methanol (2:1, vol/vol) mixture according to (FOLCH et al., 1957). Fatty acids were analysed as fatty acid methyl esters (FAME) and transesterification was performed according to (PETROVIĆ et al., 2010). The FAME were analysed using gas chromatography equipped with a flame-ionisation detector (Gas Chromatograph GC 2010 Plus, Shimadzu, Japan), with a capillary column ZB WAX (Phenomenex, USA)

and helium as the carrier gas. The identification was carried out by comparison of sample peak retention times with those of FAME standard mixtures (Sigma-Aldrich, Germany) and calculated as a percentage of each individual fatty acid relative to total fatty acids.

All analyses were performed using the Statistica 2009 program. Statistical significance of the differences was determined by the *t*-test.

Results

The growth performance of chickens is presented in Table 2. There were no significant differences between the groups fed linseed or sunflower oil. Increase of dietary α -linolenic acid in linseed oil diet did not influence the feed conversion, feed intake and daily weight gain.

Table 2. Growth performance of experimental birds (mean \pm standard deviation)

	Linseed (n = 15)	Sunflower (n = 15)	P value
Body mass (g)			
1 st day	41.3 \pm 0.75	41.1 \pm 1.02	0.689
25 th day	999.5 \pm 160.4	1017.0 \pm 1129.5	0.767
40 th day	2090.4 \pm 223.3	2109.1 \pm 255.4	0.853
55 th day	3456.6 \pm 224.0	3401.8 \pm 367.2	0.720
Weight gain (g)			
1 st -25 th day	958.2 \pm 160.0	976.7 \pm 129.9	0.765
25 th -40 th day	1088.1 \pm 175.6	1091.2 \pm 209.2	0.969
40 th -55 th day	1366.2 \pm 216.8	1292.6 \pm 322.0	0.524
1 st -55 th day	3415.3 \pm 223.8	3360.5 \pm 367.3	0.721
25 th -55 th day	2457.0 \pm 247.2	2383.8 \pm 328.4	0.618
Daily weight gain (g/day/bird), 25 th -55 th day	121.2 \pm 23.4	123.9 \pm 54.2	0.872
Feed intake (g/day/bird), 25 th -55 th day	218.1 \pm 43.1	230.4 \pm 66.3	0.332
Feed conversion, 25 th -55 th day	1.80 \pm 0.22	1.86 \pm 0.35	0.542

Table 3. Influence of linseed oil on serum lipids (mean \pm standard deviation)

	Linseed oil	Sunflower oil	P value
Triglycerides (mmol/L)	0.62 \pm 0.39	0.62 \pm 0.22	0.969
Total cholesterol (mmol/L)	2.36 \pm 0.21	2.72 \pm 0.45	0.017
HDL-cholesterol (mmol/L)	1.97 \pm 0.22	1.95 \pm 0.52	0.908
LDL-cholesterol (mmol/L)	0.25 \pm 0.03	0.25 \pm 0.06	0.694
Glucose (mmol/L)	13.59 \pm 0.89	13.69 \pm 0.79	0.770

Table 4. The fatty acid composition of adipose tissue total lipids (means \pm standard deviation)

	Linseed oil ¹	Sunflower oil	P value
Fatty acids (% of total fatty acids)			
C14:0	0.47 \pm 0.05	0.46 \pm 0.04	0.616
C16:0	20.56 \pm 1.54	20.50 \pm 0.82	0.947
C18:0	6.37 \pm 0.72	6.18 \pm 1.04	0.758
C18:1n9	35.73 \pm 2.39	35.97 \pm 1.10	0.865
C18:2n6	23.84 \pm 1.26	34.55 \pm 1.60	<0.001
C18:3n3	12.80 \pm 0.75	2.06 \pm 0.13	<0.001
C20:4n6	0.12 \pm 0.01	0.30 \pm 0.10	0.014
C20:5n3	0.10 \pm 0.02	0.00 \pm 0.00	0.001
C22:6n3	0.01 \pm 0.02	0.00 \pm 0.00	0.196
Summarized fatty acid profile ²			
SFA	27.40 \pm 2.27	27.13 \pm 1.49	0.848
UFA	72.60 \pm 2.27	72.87 \pm 1.49	0.848
MUFA	35.73 \pm 2.39	35.97 \pm 1.10	0.865
PUFA	36.87 \pm 1.90	36.90 \pm 1.78	0.981
SFA:UFA	0.38 \pm 0.04	0.37 \pm 0.03	0.833
Total n6	23.96 \pm 1.25	34.85 \pm 1.70	<0.001
Total n3	12.91 \pm 0.73	2.06 \pm 0.13	<0.001
n6/n3	1.86 \pm 0.06	16.98 \pm 0.84	<0.001

¹concentration of each oil supplement (linseed/sunflower) was 5%; ²SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

The serum lipids of broiler chicken are given in Table 3. The introduction of 5% of linseed or sunflower oil did not affect triglycerides, LDL and HDL cholesterol level while cholesterol level was significantly lower in bird fed with linseed oil ($P < 0.05$).

The fatty acid composition of total lipids of adipose tissue and thigh muscle is shown in Table 4 and 5. The content of saturated fatty acid (SFA) showed no significant differences among birds fed different diets. Concerning unsaturated fatty acid bird fed linseed oil showed significantly higher values for n3 fatty acid ($P < 0.001$) for thigh and adipose tissue. Furthermore the ratio of n3/n6 ratio for linseed oil fed group during prolonged feeding period was in accordance to human dietary recommendation.

Table 5. Influence of linseed oil on the fatty acid composition of thigh muscle (means \pm standard deviation)

	Linseed oil ¹	Sunflower oil	P value
Fatty acids (% of total fatty acids)			
C14:0	1.09 \pm 0.31	1.05 \pm 0.42	0.808
C15:0	0.11 \pm 0.25	0.13 \pm 0.11	0.908
C16:0	24.47 \pm 2.32	24.76 \pm 2.93	0.935
C16:1	1.71 \pm 0.33	1.89 \pm 0.93	0.828
C17:0	1.16 \pm 0.67	0.55 \pm 0.46	0.163
C17:1	0.00 \pm 0.00	0.09 \pm 0.08	0.184
C18:0	15.21 \pm 3.09	13.15 \pm 4.35	0.527
C18:1n9	23.38 \pm 3.46	23.63 \pm 4.15	0.945
C18:2n6	16.90 \pm 1.87	24.99 \pm 2.38	0.081
C18:3n6	0.00 \pm 0.00	0.11 \pm 0.10	0.185
C18:3n3	5.86 \pm 0.79	1.38 \pm 0.43	<0.001
C20:0	0.00 \pm 0.00	0.08 \pm 0.08	0.217
C20:1n9	0.00 \pm 0.00	0.20 \pm 0.02	0.003
C20:2n6	0.00 \pm 0.00	0.58 \pm 0.18	0.032
C20:3n6	0.60 \pm 0.15	0.47 \pm 0.11	0.146
C20:4n6	3.84 \pm 0.85	5.14 \pm 1.05	0.408
C20:3n3	0.00 \pm 0.00	0.00 \pm 0.00	/
C20:5n3	1.18 \pm 0.21	0.09 \pm 0.09	<0.001
C22:5n3	3.00 \pm 0.76	0.97 \pm 0.55	<0.001
C22:6n3	1.50 \pm 0.35	0.50 \pm 0.34	<0.001
Summarized fatty acid profile ²			
SFA	42.03 \pm 5.75	39.70 \pm 6.39	0.586
UFA	57.97 \pm 5.75	59.83 \pm 6.39	0.586
MUFA	25.09 \pm 3.75	25.80 \pm 4.13	0.965
PUFA	32.88 \pm 2.40	34.03 \pm 2.42	0.145
SFA:UFA	0.74 \pm 0.18	0.69 \pm 0.26	0.634
Total n6	21.34 \pm 1.18	31.28 \pm 1.48	0.015
Total n3	14.55 \pm 1.44	3.72 \pm 0.06	<0.001
n6/n3	1.47 \pm 0.31	8.47 \pm 1.41	0.004

¹concentration of each oil supplement (linseed/sunflower) was 5%; ²SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

Discussion

In our study, the inclusion of linseed or sunflower oil did not influence growth performance, as observed by different authors (CRESPO and ESTEVE-GARCIA, 2001; ZELENKA et al., 2006). Furthermore, the increase in dietary α -linolenic acid in the linseed oil diet did not influence the feed conversion, feed intake and daily weight gain. It is evident that adding 5% of linseed oil to the chicken broiler diet has no negative influence on growth performance. This is in accordance with previously described results in literature (CRESPO and ESTEVE-GARCIA, 2001; LOPEZ-FERRER et al., 1999).

The application of 5% of different oils did not affected triglycerides, LDL and HDL cholesterol level, but a lower value of total cholesterol ($P < 0.05$) was observed for the chickens fed with linseed oil. The dietary intake of n6 and n3 PUFAs is effective in lowering serum lipid levels. Nevertheless, n6 and n3 fatty acids differ in their effect on serum lipid concentration, meaning that n6 fatty acids lower serum cholesterol level, but not triglycerides, while n3 fatty acids lower serum cholesterol and serum triglyceride levels even more (BYOUNG et al., 1997). The absence of the triglyceride lowering effect in our trial could probably be explained by the lower lipogenesis capacity of the Ross 308 strain and the consequently lower triglyceride values in the serum.

The dominant unsaturated fatty acids in the adipose tissue and thigh muscle of the linseed oil group were C18:1n9, C18:2n6 and C18:3n3. In contrast, the predominant fatty acids in animals fed the sunflower diet were C18:2n6 and C18:1n9, which were present in almost identical proportions in both tissues. Oleic acid (C18:1n9) was present in the highest value in both tissues in the birds fed either linseed or sunflower oil. The basal diet used in our experiment (Table 1), contained 68% of corn, rich in monounsaturated fatty acids, oleic acid and C18:2n6, which contributed to the fatty acid composition of tissues in our experiment. Similar basal diets were also used in trials performed by AJUYAH et al. (1993) and HRDINKA et al. (1996). The addition of 5% of linseed oil, rich in C18:3n3, to the basal diet changed the relative proportions of stearic and linoleic acid in favour of the beneficial α -linolenic acid. In the experiment by CRESPO and ESTEVE-GARCIA (2001) and LOPEZ-FERRER et al. (2001) the proportions of linoleic and α -linolenic acids varied depending on the lipid supplement used, which is in agreement with our results for these fatty acids.

An increase of C18:3n3 was noted in the adipose tissue and thigh muscle of the chickens fed linseed oil. The linseed oil fed group had significantly higher values of α -linolenic acid (C18:3n3) ($P < 0.001$) for both tissues, as well as other n3 long chain PUFAs: 20:5n3 ($P < 0.001$), 22:5n3 ($P < 0.001$) and 22:6n3 ($P < 0.001$). The profile of fatty acids of the adipose tissue could be affected by the dietary fat profile (CRESPO and ESTEVE-GARCIA, 2001) However, the thigh muscle was always richer in n3 series long chain PUFA than the adipose tissue (LOPEZ-FERRER et al., 1999). The differences

in the fatty acid profile of adipose and muscle tissue could be explained by the different metabolisms of fatty acids in these tissues.

The fatty acid content of the n6 series, particularly C18:2n6, was higher in birds fed sunflower oil in both tissues. The value of linoleic acid (C18:2n6) of birds fed linseed oil supplement significantly decreased in the adipose tissue ($P < 0.001$) and tended to decrease in thigh tissue ($P = 0.081$). The lower values of n6 fatty acids in the birds fed linseed oil could be the result of the competition between n3 and n6 fatty acids for $\Delta 5$ and $\Delta 6$ desaturase. These results are in accordance with those observed by CRESPO et al. (2001). A comparison of the fatty acid profiles of thigh and adipose tissue is shown in Fig. 1.

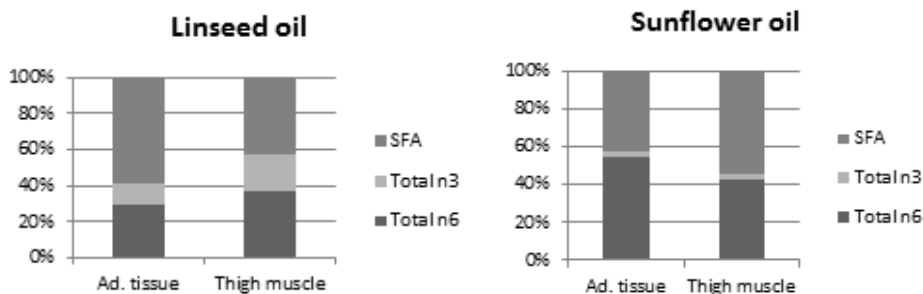


Fig. 1. Fatty acid composition of adipose tissue and thigh muscle of birds fed 5% fat. Values correspond to those in Table 4 and Table 5.

In general, the birds fed linseed oil showed significant differences in the summarized values of n3 fatty acid ($P < 0.001$) for thigh and adipose tissue. These increases in n3 PUFA ($P < 0.001$) resulted in a significant decrease in n6 fatty acids ($P < 0.001$) and decreased the ratio of n6/n3 ($P < 0.001$) in thigh and adipose tissue (Fig. 1). The n6/n3 ratio is an important determinant of human health and, therefore, the appropriate n6/n3 ratio should be from 1 to 2:1 (SIMOPOLOUS, 2002). The observed value of the n6/n3 ratio for the linseed oil fed group during the prolonged feeding period was in accordance with human dietary recommendations. Therefore, the consumption of such meat products could be beneficial for human health. The summarized values of total tissue PUFA, MUFA, SFA as well as the ratio of PUFA/UFA were not influenced by adding 5% of different vegetable oils to the basal diet.

Conclusion

The results of this experiment showed that altering the LA and ALA ratio of dietary fats could play an important role in lipid deposition and metabolism. The content of n3 PUFA in adipose and thigh tissue was elevated by increasing the ALA level of the basal

diet. Overall, our results confirm that it may be possible to increase the n3 PUFAs content of chicken meat in order to decrease the n6/n3 ratio, and thereby produce chicken meat more beneficial for human health.

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STARČEVIĆ, K., T. MAŠEK, D. BROZIĆ, N. FILIPOVIĆ, Z. STOJEVIĆ: Pokazatelji rasta, vrijednosti lipida u serumu i masnokiselinskog sastava različitih tkiva tovnih pilića hranjenih hranom obogaćenom lanenim uljem tijekom produženog tova. *Vet. arhiv* 84, 75-84, 2014.

SAŽETAK

U radu je istraživana utjecaj 5% dodatka lanenog ulja u hranu za piliće u produženom tovu na pokazatelje rasta, vrijednosti lipida u serumu te masnokiselinski sastav. U pokus je bilo uključeno 30 Ross 308 tovnih pilića, koji su bili podijeljeni u dvije skupine od po 15 jedinki. Prva je skupina pilića u periodu od 25. do 55. dana dobivala hranu obogaćenu s 5% lanenog ulja, dok je druga skupina u istom periodu dobivala hranu s 5% suncokretovog ulja. Zabilježene vrijednosti proizvodnih rezultata i vrijednosti lipida u serumu pokazale su da nema značajne razlike između pokusnih skupina pilića. Masnokiselinski sastav pojedinih tkiva podložan je promjenama ovisno o sastavu hrane što je potvrđeno i ovim istraživanjem. Kod skupine pilića hranjenih dodatkom 5% lanenog ulja utvrđena je značajna razlika ukupnih vrijednosti n3 masnih kiselina ($P < 0,001$) u mišićju batka i trbušnom masnom tkivu. Povećanje n3 višestruko nezasićenih masnih kiselina (PUFA) ($P < 0,001$) rezultira značajnim smanjenjem n6 masnih kiselina ($P < 0,001$) i n6/n3 omjerom u mišićju batka i masnom tkivu. Omjer n6/n3 koji smo utvrdili kod tovnih pilića hranjenih lanenim uljem tijekom produženog razdoblja tova u skladu je s preporukama Svjetske zdravstvene organizacije.

Ključne riječi: tovni pilić, laneno ulje, višestruko nezasićene masne kiseline, n6/n3 omjer
