Original paper

LINSEED OIL AS A SOURCE OF POLYUNSATURATED FATTY ACIDS IN BROILER CHICKEN NUTRITION

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Abstract

The aim of the paper was to examine the effect of polyunsaturated fatty acids from linseed oil on productive performances and fatty acid composition of lipids of broiler chicken abdominal fat, as well as correlation and regression between consumed amount of oil and possibilities of their incorporation in the tissues. For the need of experimental research, three groups of 40 day old chicken, hybrid line Cobb 500 were formed. Every group had five replicates, in total amount of 200 chickens per treatment. Control group were fed with diet in addition of 4% soybean oil, while the experimental groups were fed with addition of 4 and 8% of linseed oil in the diet. Addition of linseed oil had a statistically high ($P<0.01$) influence on chicken body weight reduction in treatments T2 and T3, compared to the body weights of chicken in control group T1. Feed conversion ratio in chicken in experimental groups T2 and T3 was 1.84 and 1.82 kg of feed/kg of gain. Introduction of linseed oil in amounts of 4 and 8% in chicken nutrition led to statistically high ($P<0.01$) differences in content of linoleic acid (C18:2) in treatments T2 and T3, compared to control group T1, which leads to a conclusion that linseed oil significantly reduces linoleic acid in tissue. Compared to linoleic acid, content of α-linolenic acid (C18:3) was statistically significantly increased ($P<0.05$) in treatment T2 and highly significantly ($P<0.01$) in treatment T3, compared to control. Negative values of regression (b) and correlation (r) also confirm the fact that introduction of linseed oil in the diet significantly reduces content of linoleic acid in fat tissue of chickens (b=-0.551; r=-0.79), while the same coefficients show positive dependence between added linseed oil in the diet and deposited linolenic acid in tissues (b=1.081 and r=0.87). Based on the gained results it can be concluded that addition of linseed oil in the amount of 4 and 8% could be successfully used in chicken nutrition for fatty acid composition improvement and for lowering the ratio between n-6/n-3 polyunsaturated fatty acid in tissues of broilers.

Key words: *fatty acids, linseed oil, regression, correlation, nutrition, chickens*

Introduction

Poultry production is the fastest way for obtaining high-quality food of animal origin which meets the high demands of modern human nutrition. Improving the quality of meat is reflected among other things in a favorable balance of fatty acids and a reduced content of undesirable and increased participation of desirable fatty acids in muscle lipids of

broilers. For normal metabolic processes in the human body essential fatty acids are necessary (Ryan et al., 2010; Wall et al., 2010). Since body is unable to synthesize them, they have to be inserted in sufficient quantity, usually by consuming food of animal origin. Studies have shown that the composition of lipids in food can significantly affect the fatty acid composition of lipids in chicken meat that can be used freely in the diet of cardiovascular patients and people with high blood cholesterol levels (Adkins and Kelley, 2010; Stanaćev et al., 2013; Puvača et al., 2013). The main sources of polyunsaturated long chain fatty acids are oils of sea and river fish (Ljubojević et al., 2013; Ljubojević et al., 2013a). However, the addition of fish oil in feed for chickens violates the organoleptic characteristics of the product, so we resorted to the enrichment of chicken meat in polyunsaturated fatty acids with 18 carbon atoms by the addition of vegetable oils rich in these acids. Linseed (*Linum usitatissiumum* L.) belongs to this nutrient because it contains oil rich in n-3/n-6 polyunsaturated fatty acid which is used in animal nutrition to improve the fatty acid composition of meat and eggs (Hall et al., 2007; Bassett et al., 2009; Grashorn, 2013) and thus provide a functional food for consumers (Vukelić et al., 2012). Linseed oil contains a high level of α-linolenic acid of the total amount of polyunsaturated fatty acids contained in the oil (Ahmad et al., 2013). Since the poultry is unable to synthesize the same acid it can be successfully incorporated in the edible tissues of the chicks by introducing the linseed oil diet (Shen et al., 2005; Vukmirović et al., 2012). It is well known that the quality of broiler meat is a complex concept that can be evaluated from several aspects. Thus, from the point of slaughter industry and consumer interests it is very important that fattened chickens have a good yield, the preferred conformation, as much meat on the carcass, the optimal distribution of adipose tissue rich in polyunsaturated fatty acids and the appropriate skin color. Representation of certain tissues in more valuable parts of the carcass is an important element that determines the quality of meat (Grashorn and Serini, 2006; Eleroglu et al., 2013; Moujahed and Haddad, 2013). For proper interpretation of the results we used the correlation and regression analysis of the data showing the extent and severity of dependence between variables, which is of great importance when it comes to fatty acids and their possible incorporation into tissue lipids depending on their level in feed mixtures (Vidović, 2009; Vidović, 2013; Lukač and Vidović, 2013).

The aim of this study was to investigate the effect of polyunsaturated fatty acids originating from linseed oil in the diet of broiler chickens on production performance and fatty acid composition of lipids in adipose tissue, as well as correlation and regression dependence between the entered quantity of oil through the diet and the possibility of their incorporation in the tissues of chickens.

Material and methods

Biological tests were carried out under production conditions at the experimental farm "Pustara" in property of the Faculty of Agriculture, Department of Animal Science. At the beginning of experiment, three groups of 40 day-old broiler chickens of Cobb 500 line in five reps on a total of 200 chickens per treatment were formed. For the chicks nutrition three mixtures were used, with 21, 20 and 18% of crude protein (Table 1). The first 14 days, during the preparatory period, the chicks were fed starter mixture. Following the preparation period, the chicks were divided into three groups and fed the next 21 days with Grover mixture, and then the last 7 days of fattening period with finisher mixture. The control group (T1) was fed a standard commercial feed mixture for chicken, while the experimental groups were fed a diet supplemented with 4% (T2) and 8% (T3) of linseed oil. During the experiment, which lasted 42 days, the chicks were fed and watered ad

libitum, and microclimate conditions were regularly monitored. Chickens were on the floor holding system. Control of body weight and feed consumption was performed every 7 days.

Table 1. *The structure and chemical composition of feeds mixtures for broiler chickens, %*

Feedstuffs	Starter	Grover		Finisher	
	T1	T2	T3	T2	T3
	0%	4%	8%	4%	8%
Corn flour	50.00	56.50	44.00	60.60	51.00
Bran	4.00	1.00	6.00	5.00	5.50
Linseed oil	0.00	4.00	8.00	4.00	8.00
Soybean oil	4.00	0.00	0.00	0.00	0.00
Soybean meal	17.40	34.00	33.00	26.00	23.00
Sunflower meal	0.00	0.00	4.50	0.00	8.00
Soybean grits	21.00	0.00	0.00	0.00	0.00
Yeast	3.00	0.00	0.00	0.00	0.00
Monocalcium phosphate	1.50	1.50	1.40	1.50	1.50
Salt	0.30	0.30	0.30	0.25	0.30
Limestone	1.50	1.50	1.50	1.50	1.50
Lysine	0.10	0.00	0.10	0.00	0.00
Methionine	0.20	0.20	0.20	0.15	0.00
Premix	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
ME, MJ/kg	12.88	13.13	13.59	13.30	13.73

For the purpose of determining the fatty acid composition of lipids, at the end of the experiment, 10 chicks of each group, 5 male and 5 female of an average body weight were sacrificed. After sacrifice, all supporting operation was carried out, and then samples of the abdominal fat were taken for analysis of fatty acid content. For a proper interpretation of the results analysis of variance (ANOVA), LSM and Fisher's LSD post-hoc test were performed and also analysis of the regression coefficient and Pearson's correlation coefficient within the statistical software STATISTICA 12.

Results and discussion

Based on the obtained results it can be concluded that the addition of linseed oil at a concentration of 4 and 8% in the diet of broiler chickens did not statistically significantly ($p>0.05$) affected the achieved body weight of chickens in the first fattening period, until the end of the experiment when was observed a statistically highly significant ($p<0.01$) decrease in body weight of chicks in experimental treatments T2 and T3 compared to the final weights of the chickens on control treatment T1, which amounted to a row 2636.06 g 2648.51 g and 2701.44 g (Table 2).

In their research, Lopez-Ferrer et al. (2001) have come to similar conclusions that the addition of linseed oil in the amount of 2 to 4% in the mixture for feeding broiler chickens affected slightly a depression in the final body weight of chickens, as well as the addition of 8% fats of animal origin. In later studies, addition of linseed oil in the amount of 6% by Leonard et al. (2004) also demonstrated a depressing effect on the body weight of chicks at the end of the experiment. When it comes to the efficiency of feed utilization, it can be

seen that the chickens in the experimental treatment had lower feed conversion ratio, compared to chickens of the control group, which was statistically highly significant difference ($p<0.01$). The achieved conversion ratio of chickens in the control treatment T1, at the end of the sixth week of the experiment amounted to 1.87 kg of feed per kg of gain, while the conversion ratio in the experimental groups T2 and T3 was 1.84 and 1.82 kg feed/kg gain (Table 3).

Table 2. *Body weight of chickens (LSM), g*

Treatments and weeks of experiment	Control (T1) 0%		Linseed oil (T2) 4%			Linseed oil (T3) 8%		
	LSM	SE_{Lsm}	LSM	SE_{Lsm}	p	LSM	SE_{Lsm}	p
End of preparatory period	468.00	5.91	468.12	5.91	0.98^{ns}	468.00	5.91	1.00^{ns}
End of III week	994.72	10.04	970.33	10.04	0.75^{ns}	984.44	10.04	0.25^{ns}
End of IV week	1552.11	18.49	1599.11	18.49	0.00**	1607.33	18.49	0.00**
End of V week	2139.57	18.80	2164.78	18.86	0.00**	2082.17	18.31	0.06^{ns}
End of VI week	2701.44	28.48	2636.06	28.56	0.00**	2648.51	27.73	0.00**

$p<0.05$ - *; $p<0.01$ - **; $p>0.05$ - ^{ns}

Table 3. *Feed conversion ratio ($\bar{x}$), kg feed/kg gai*n.

Treatments and weeks of experiment	Control (T1) 0%	Linseed oil (T2) 4%		Linseed oil (T3) 8%	
	$\bar{x}$	$\bar{x}$	p	$\bar{x}$	p
End of preparatory period	1.34	1.29	0.00**	1.34	0.76^{ns}
End of III week	1.37	1.39	0.02*	1.42	0.00**
End of IV week	1.48	1.41	0.00**	1.49	0.04*
End of V week	1.62	1.59	0.00**	1.68	0.00**
End of VI week	1.87	1.84	0.00**	1.82	0.00**

$p<0.05$ - *; $p<0.01$ - **; $p>0.05$ - ^{ns}

By analyzing the fatty acid composition of the linseed oil lipids and adipose tissue lipids using gas chromatography, 6 to 11 fatty acids in total were detected (Table 4 and 5).

Table 4. *Fatty acid composition of linseed oil (LSM), g/100 g*

Oil source	Fatty acids											
	C16:0		C18:0		C18:1		C18:2		C18:3		C20:0	
Lan	LSM	SE_{Lsm}	LSM	SE_{Lsm}	LSM	SE_{Lsm}	LSM	SE_{Lsm}	LSM	SE_{Lsm}	LSM	SE_{Lsm}
	2.63	28.3	4.94	21.4	19.97	14.5	16.74	26.8	55.53	15.9	0.18	18.3

From the results shown in Table 4 it can be seen that the most common member of the oil is α-linolenic acid (C18: 3 n-3), with 55.53%, followed by oleic (C18: 1 n-9) to 19.97%, and linoleic acid (C18: 2 n-6) to 16.74%.

Table 5. *Fatty acid composition of abdominal fat (LSM), g/100 g*

Fatty acids		Control (T1) 0%		Linseed oil (T2) 4%			Linseed oil (T3) 8%		
		LSM	SE_{Lsm}	LSM	SE_{Lsm}	p	LSM	SE_{Lsm}	p
myristic	C14:0	0.04	0.01	0.06	0.01	0.27^{ns}	0.06	0.01	0.31^{ns}
palmitic	C16:0	17.74	0.38	18.39	0.38	0.26^{ns}	16.35	0.38	0.02*
palmitoleic	C16:1	3.27	0.25	3.83	0.25	0.14^{ns}	3.45	0.25	0.61^{ns}
stearic	C18:0	5.23	0.17	5.06	0.17	0.52^{ns}	4.71	0.17	0.06^{ns}
oleic	C18:1	35.45	1.01	35.72	1.01	0.85^{ns}	34.36	1.01	0.46^{ns}
linoleic	C18:2	29.22	0.55	25.13	0.55	0.00**	24.80	0.55	0.00**
linolenic	C18:3	5.88	0.96	9.61	0.96	0.01*	14.53	0.96	0.00**
arachidic	C20:0	0.07	0.02	0.07	0.02	0.97^{ns}	0.08	0.02	0.73^{ns}
gadoleinic	C20:1	0.45	0.06	0.42	0.06	0.70^{ns}	0.32	0.06	0.20^{ns}
behenic	C22:0	0.06	0.02	0.01	0.02	0.18^{ns}	0.08	0.02	0.52^{ns}
lignoceric	C24:0	0.00	0.00	0.00	0.00	0.24^{ns}	0.00	0.00	0.24^{ns}

$p<0.05$ - *; $p<0.01$ - **; $p>0.05$ - ^{ns}

In Table 5, the results of fatty acid composition of abdominal fat show statistically significant and highly significant differences in the content of the individual fatty acids in the experimental treatment compared to the control treatment. Introduction of linseed oil at concentrations of 4 and 8% in the diet of chickens has led to a statistically significant difference ($p<0.01$) in the content of linoleic acid (C18:2) in the treatment T2 (25.13 g/100 g), and T3 (24.80 g/100 g) compared to the control treatment T1 (29.22 g/100 g), what indicates a significant reduction of linoleic acid in the tissue. The content of α-linolenic acid (C18:3) was significantly increased ($p<0.05$) in the treatment T2 (9.61 g/100 g), and highly significantly ($p<0.01$) in the treatment T3 (14.53 g/100 g) in response than the content of α-linolenic acid in the chickens tissue of treatment T1, which amounted to 5.88 g/100 g. From the results shown in the same table it can be seen that the addition of linseed oil at both concentrations was not statistically significant ($p>0.05$) affected on the presence of oleic acid (C18:1). The research of Nyquist et al. (2013) with the linseed oil in the diet of chickens showed an increased content of n-3 EPA, DPA and DHA and reduced content of arachidonic acid (AA) of the n-6 family of fatty acids. These authors noted that the relationship between AA/EPA to 19/1 in the group with addition of the soybean oil in the mixture decreased to 1.7/1 in the treatment of chickens with the linseed oil in the feed. A number of authors (Bou et al., 2005; Haug et al., 2007; Smink et al., 2008; Wongsuthavas et al., 2011) in their studies with the addition of linseed oil to feed for chickens came to similar results with increasing concentrations of α -linolenic acid, EPA, DPA and DHA in comparison with the group with addition of the soybean oil, which had higher concentrations of linoleic and arachidonic acid in the tissues.

In the case of regression and correlation coefficients, from the results in Table 6, it can be observed dependence with statistically significant differences.

By the means of the regression coefficient and Pierson correlation coefficient we came to the conclusion that there is a positive and a negative correlation between the added linseed oil to the chicken diet and deposit of fatty acids in lipids. The negative value of the

regression (b) and correlation (r) show that the introduction of linseed oil statistically ($p<0.01$) significantly affected the content of linoleic acid in adipose tissue of chickens (b=-0.551, r=-0.79), while the regression coefficients and correlations show a positive correlation between the added linseed oil to diet and deposited α-linolenic acid in the tissues (b=1.081 and r=0.87) with a statistically high significance ($p<0.01$). Table 7 also shows the regression equation which shows, or predicts, how the content of certain fatty acids in adipose tissue of chickens will increase or decrease by a certain amount of linseed oil (x) supplement in the diet mixture.

Table 6. *The coefficient of regression and correlation for the observed properties of fatty acid composition*

Fatty acids		a	b	The regression equation	r	r^2	p
myristic	C14:0	0.048	0.002	y=0.048+0.002 x	0.28	0.08	0.30^{ns}
palmitic	C16:0	18.196	-0.174	y=18.196-0.174 x	-0.49	0.25	0.06^{ns}
palmitoleic	C16:1	3.431	0.023	y=3.431+0.023 x	0.14	0.02	0.63^{ns}
stearic	C18:0	5.262	-0.064	y=5.262-0.064 x	-0.51	0.26	0.05^{ns}
oleic	C18:1	35.723	-0.135	y=35.723-0.135 x	-0.21	0.04	0.45^{ns}
linoleic	C18:2	28.595	-0.551	y=28.595-0.551 x	-0.79	0.62	0.00**
linolenic	C18:3	5.686	1.081	y=5.686+1.081 x	0.87	0.77	0.00**
arachidic	C20:0	0.071	0.001	y=0.071+0.001 x	0.09	0.01	0.72^{ns}
gadoleinic	C20:1	0.467	-0.016	y=0.467-0.016 x	-0.36	0.13	0.19^{ns}
behenic	C22:0	0.043	0.003	y=0.043+0.003 x	0.16	0.02	0.57^{ns}
lignoceric	C24:0	0.005	-0.000	y=0.005-0.000 x	-0.33	0.11	0.23^{ns}

$p<0.05$ - *; $p<0.01$ - **; $p>0.05$ – ^{ns}; x – variable amount of oil

Conclusion

Based on the obtained results it can be concluded that the introduction of linseed oil in the amounts of 4% and 8% had mild depressant effect on the final body mass of fattening chickens and a positive effect on the reduction of feed conversion ratio. Regarding the fatty acid composition of lipids in adipose tissue, it can be concluded that the introduction of the oil in diet affects highly significantly the increased incorporation of n-3 polyunsaturated fatty acids, in particular α-linolenic fatty acids, and highly significantly the reduction of the content of n-6 fatty acid, linoleic acid. From the obtained results it can be concluded that linseed oil in the amount of 4% and 8% can be successfully used in the diet of broiler chickens to improve fatty acid composition of edible tissues of chickens and balance the ratio of n-6/n-3 polyunsaturated fatty acids, which would also improve products and make them suitable in cardiovascular patients nutrition and in the prevention of atherosclerosis and other diseases related to disorders of the digestive tract. Also, the application of these regression and corelation models may be one of the practical solutions on the ground in the formulation of diet mixtures for poultry when it comes to improving the fatty acid composition of the final product.

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