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FATTY ACIDS IN FEED OF LAYING HENS ON THE PRODUCTION PARAMETERS AND THE RATIO OF OMEGA-6 AND OMEGA-3 FATTY ACIDS

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Abstract: Enriching table eggs with certain nutrients such as omega-3 fatty acids is an ongoing topic. Therefore, the aforementioned research was carried out with the aim of examining the possibility of feeding laying hens with ground flax seed, flax cake and flaxseed oil and their influence on the ratio of omega-6 and omega-3 fatty acids of table eggs, as well as on certain production parameters. The experiment was set up in a production facility with 192 consuming laying hens 27 weeks old. The laying hens were divided into 4 groups: control group; group fed with flax cake (10%) + flax oil (2%); a group fed with 5% ground flax and a group fed with 10% ground flax. Each group had 8 cages with 6 laying hens, a total of 48 laying hens per group. The production parameters presented in this paper are as follows: number of eggs per laying hen , egg weight and yolk weight. Egg quality indicators were determined at the end of the 5th and 10th week from the start of the experiment, namely: pH of egg white and yolk, polyunsaturated and saturated fatty acids ratio, as well as omega-3 and omega-3 fatty acids ratio. The results of the study showed that the inclusion of flax cake, flax oil and ground flaxseed had no negative effects on the shown production parameters and that there was a positive effect of these nutrients on the ratio of omega-6 and omega-3 fatty acids in table eggs.

Key words: laying hens, production parameters, fatty acids

Introduction

Eggs are an excellent source of readily soluble proteins for humans, polyunsaturated fatty acids, phospholipids and vitamins *(Škrtić et al., 2006)*. Nowadays, people are becoming more aware of the link between food and health and they are trying to maintain and improve their health through adequate nutrition. Food can only be considered functional if its basic nutritional impact has positive effects on human health *(Perić et al., 2011)*. Intensive production of table eggs has

focused all research on determining the impact of many factors on the productivity of light line hybrids of laying hens and the quality of eggs from the cage holding system *(Škrbić et al., 2011)*. Nutrition of laying hens with nutrients of plant or animal origin results with nutritious table eggs and therefore omega-3 fatty acids. Alpha-linoleic (ALA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids belong to the group of important omega-3 fatty acids for nutrition *(Coorey et al., 2014)*. Enrichment of table eggs with omega-3 fatty acids can be achieved if the diet of the laying hens is carried out with specially selected nutrients *(Meluzzi et al., 2000)*. With the exception of the shape index, a different way of laying and feeding of laying hens may affect most indicators of egg and shell quality *(Krawczyk, 2009)*. *Polawska et al. (2013)* reports the positive effects of food enriched with polyunsaturated fatty acids in human nutrition. Large quantities of these acids are found in fish and other seafood, but in many countries the consumption of seafood is low *(Carrillo-Domínguez et al., 2005)* so the way to increase the intake of omega-3 fatty acids is to produce functional foods enriched with them. From the natural sources of unsaturated fatty acids, flax is the plant that is among the most concentrated sources available for poultry nutrition *(Caston and Leeson, 1990)*. According to some reports, a flaxseed diet can have a negative impact on the production performance of laying hens. In the studies, *Jia et al. (2008)* reported reduced egg production, increased feed consumption, increased feed conversion and less weight of laying hens flax-fed at a concentration of 150 g / kg of mixture. The production of omega-3 enriched eggs with a more desirable omega-6 / omega-3 ratio compared to standard quality eggs is achieved through the use of omega-3 fatty acids, which is why the use of these fatty acids as a nutritional supplement has a nutritional, medicinal and economic advantage *(Šefer et al., 2011)*. Adding flax to the diet of laying hens at a concentration of 10% in eggs results in increased levels of ALA, EPA and DHA and decreased levels of arachidonic acid *(Hayat et al., 2009)*.

The aim of this experiment was to investigate the effect of the addition of ground flax seed and a combination of flax cake and flax oil on the production characteristics of consumable laying hens, the ratio of polyunsaturated and saturated fatty acids as well as the ratio of omega-6 and omega-3 fatty acids.

Materials and Methods

Experimental design and feeding of laying hens

The experiment was carried out on laying hens of a light line hybrid Lohmann Brown-Classic in a production facility. The laying hens are grouped into 4 groups (control group, group fed with flax cake at a concentration of 10% + flax oil added to feed at a concentration of 2%, group fed with 5% ground flax grain and group

fed with 10% ground flax grain). During the experiment, all laying hens consumed feed with 17.30% crude protein and 11.60 MJ / kg MEn (calculative). The tested nutrients (grounded flaxseed, flax cake and flax oil) were added to the representative samples in the controled feed, reducing the percentage of certain control feed nutrients to obtain isoprotein and isocaloric mixtures. Each group had 8 cages with 6 laying hens. The age of the laying hens at the start of the experiment was 27 weeks. Egg and yolk weight were measured on an electronic scale of 0.01 g accuracy, on a sample of 10 eggs per cage.

Chemical analysis of eggs

The fatty acid composition was determined at the end of the 5th and 10th week from the start of the experiment on samples of 10 eggs per cage, and an extraction method was used according to *Folch et al. (1957).* Fatty acid composition was determined using gas chromatography. A gas chromatograph used to determine (Perkin-elmer Varian 1400) was linked to a filled column (l = 3 m, d = 3.0 mm; stationary phase - GP 10% SPTM-2330) and a flame ionization detector.
The total omega-6 PUFAs are calculated as C18:2c + C20:3 ω-6 + C20:4 ω-6
The total omega-3 PUFA are calculated as C18:3 ω-3 + C20:5 ω-3 + C22:6 ω-3
The pH values of the yolk and egg white were determined at the end of the 5th and 10th weeks of the experiment by a portable pH meter (Testo 205, Lenzkirch, Germany) equipped with a stabbed reinforced combination electrode for direct determination of pH in food products and a temperature measuring sonde.

Statistical analysis

The results obtained in the study were processed by one-factor analysis of variance (software package IBM SPSS version 22). The significance level used in all tests was 1%, while Duncan's test was used in subsequent comparisons. The lowercase letters indicate statistically significant differences between the mean values of individual treatments over time. The averages do not differ significantly when not indicated by lowercase letters.

Results and Discussion

The results presented in Table 1 show that at the end of week 1 of the trial, the lowest carrying capacity (number of eggs / housed hen) was observed in the control group, but it did not differ significantly with respect to flax cake + oil and flax treatment 10%, while the highest carrying capacity was observed in the flax treatment of 5% and it was significantly higher compared to the control group, while it did not differ significantly from the treatments of flax cake + oil and flax 10%. In the follow-up period from week 2 to week 10, there was no statistically significant difference between the control group and the treatments observed. From

the above it can be seen that there was no negative effect of the addition of PUFA omega-3 in the feed on the production results of the treated groups compared to the control group. *Caston and Leeson (1990)* and *Jiang et al. (1991)* report that there was no negative effect of the addition of flaxseed to the feed of laying hens on egg production and weight, which is in accordance with the results of this work. *Scheideler and Froning (1996)* and *Aziza et al. (2013)* report increased egg production after the addition of flaxseed, fish oil or camelin meal. From the presented it can be seen that the results obtained in this paper are in accordance with the results of other papers.

Table 1. Egg production in experimental groups (egg number/ housed hen ± standard deviation)

Week of the experiment	Treatment				p-value
	Control	Flax cake+flax oil	Flax 5%	Flax 10%	
1.	6.52 ± 0.14a	6.60 ± 0.18ab	6.81 ± 0.06b	6.63 ± 0.20ab	0.06
2.	6.69 ± 0.19	6.50 ± 0.28	6.67 ± 0.20	6.54 ± 0.21	0.28
3.	6.65 ± 0.24	6.67 ± 0.27	6.79 ± 0.12	6.60 ± 0.24	0.39
4.	6.58 ± 0.24	6.52 ± 0.30	6.65 ± 0.21	6.58 ± 0.27	0.81
5.	6.62 ± 0.23	6.56 ± 0.30	6.67 ± 0.13	6.60 ± 0.24	0.84
6.	6.65 ± 0.24	6.58 ± 0.28	6.52 ± 0.27	6.62 ± 0.31	0.81
7.	6.44 ± 0.39	6.37 ± 0.21	6.42 ± 0.30	6.40 ± 0.09	0.97
8.	6.54 ± 0.31	6.44 ± 0.31	6.58 ± 0.28	6.54 ± 0.26	0.78
9.	6.42 ± 0.41	6.46 ± 0.12	6.54 ± 0.41	6.42 ± 0.47	0.90
10.	6.44 ± 0.41	6.42 ± 0.22	6.56 ± 0.22	6.44 ± 0.41	0.80

* a-b Average values with different letters in one line differ significantly at the level of 1%

Table 2 presents the results of the measured egg weight over the duration of the experiment.

Table 2. Egg weight in experimental groups ((g) ± standard deviation)

Week of the experiment	Treatment				p-value
	Control group	Flax cake+oil	Flax 5%	Flax 10%	
1.	60.61 ± 1.95	60.35 ± 1.28	60.83 ± 1.02	61.90 ± 2.13	0.28
2.	60.19 ± 1.76	60.39 ± 2.01	61.91 ± 2.15	61.95 ± 1.88	0.15
3.	60.24 ± 2.50	61.58 ± 2.37	61.99 ± 1.71	61.88 ± 3.11	0.48
4.	60.99 ± 1.62	61.58 ± 1.99	62.48 ± 1.61	62.15 ± 2.39	0.44
5.	61.29 ± 1.58	61.90 ± 1.71	62.74 ± 1.36	62.46 ± 2.57	0.43
6.	61.60 ± 1.50	61.90 ± 1.88	62.69 ± 1.48	62.65 ± 2.63	0.60
7.	61.63 ± 1.74	62.10 ± 1.96	62.91 ± 1.33	62.95 ± 2.61	0.47
8.	61.93 ± 1.75	62.00 ± 2.58	62.84 ± 1.49	63.28 ± 2.79	0.56
9.	61.79 ± 1.99	62.01 ± 2.91	62.95 ± 1.33	63.21 ± 2.74	0.55
10.	62.20 ± 1.99	62.11 ± 2.08	62.89 ± 1.39	63.46 ± 1.93	0.44

The results presented in Table 2 show that during the 10 weeks of the trial there was no statistically significant difference in egg weight between the control group and the observed treatments, while all groups reported an increase in weight. *Baucells et al. (2000); Novak and Scheideler (2001)* and *Ebeid (2011)* did not report the impact of PUFA sources on egg production and weight. *Sari et al. (2001)* reported that the addition of 15% flax seed reduced significantly egg weight ($p < 0.01$). *Scheideler and Froning (1996)* reported significantly lower egg weight of the group fed with 5% and 15% of added flaxseed concerning the control group and the group fed with fish oil. *Zotte et al. (2015)* found no effect of nutritional treatment on average egg weight.

Table 3 presents the results of the measured yolk weight during the experiment.

Table 3. Egg yolk weight in experimental groups ((g) ± standard deviation)

Week of the experiment	Treatment				p-value
	Control group	Flax cake+oil	Flax 5%	Flax 10%	
1.	14.59 ± 0.58	14.29 ± 0.29	14.13 ± 0.25	14.74 ± 0.60	0.05
2.	14.45 ± 0.70	14.50 ± 0.30	14.68 ± 0.42	14.50 ± 0.42	0.80
3.	14.60 ± 0.34	14.85 ± 0.39	14.86 ± 0.30	14.73 ± 0.65	0.61
4.	15.03 ± 0.40	14.80 ± 0.52	14.96 ± 0.32	14.85 ± 0.59	0.77
5.	15.18 ± 0.35	14.98 ± 0.28	15.14 ± 0.33	15.01 ± 0.38	0.59
6.	15.23 ± 0.45	15.04 ± 0.41	15.21 ± 0.33	15.18 ± 0.50	0.81
7.	15.30 ± 0.36	15.14 ± 0.48	15.23 ± 0.35	15.35 ± 0.47	0.76
8.	15.55 ± 0.47	15.19 ± 0.65	15.45 ± 0.44	15.38 ± 0.71	0.65
9.	15.68 ± 0.51	15.61 ± 0.82	15.49 ± 0.36	15.45 ± 0.72	0.88
10.	15.70 ± 0.46	15.69 ± 0.46	15.64 ± 0.49	15.61 ± 0.46	0.98

The results presented in Table 3 show that there was no statistically significant difference between the weight of the control group yolk and the observed treatments in week 1, and this trend persisted until the end of week 10 of the experiment. Also, the results show that in all treatments the weight of the yolk increased during the experiment. *Hayat et al. (2009)* reported that there was no effect on egg yolk weight when hens were fed flaxseed. *Sari et al. (2001)* report a decrease in yolk weight with an increase in flaxseed in feed. This decrease in yolk weight was not significant between the control group and the groups with added 5% and 10% of flaxseed, but was significant ($p < 0.01$) in the group with the added 15% of flaxseed. These results show that they are in accordance with the results of this paper where there was also no statistically significant deviation of the yolk weight between the control group and the groups with the added 5 and 10% of ground flax seed. *Scheideler and Froning (1996)*; *Novak and Scheideler (2001)* report a decrease in the proportion of yolk when fish oil or flax is present in feed. *Ebeid (2011)* used fish and flaxseed oil as a source of omega-3 PUFAs where he

did not find significant treatment effects on feed consumption, egg production, egg weight, or egg yolk height. In this paper, the weight of yolk in the treatment of flax cake + oil did not differ significantly concerning the weight of the yolk of the control group, as well as the other treatments included in the experiment. The yolk weight was not influenced by the level (0, 10 or 20%) of ground flax in feed *(Caston et al., 1994)*.
Table 4 presents the results of the analysis of certain chemical indicators of egg quality.

Table 4. Egg quality parameters in experimental groups (mean ± standard deviation)

Parameters	Week of the experiment	Treatment				p-value
		Control group	Flax cake + flax oil	Flax 5%	Flax 10%	
pH of white egg	5.	8.35±0.15[a]	8.46±0.15[ab]	8.61±0.08[b]	8.49±0.06[ab]	0.00
	10.	8.62±0.14	8.68±0.09	8.62±0.06	8.63±0.14	0.65
pH of yolk	5.	5.90±0.08	6.07±0.24	6.07±0.06	6.01±0.07	0.06
	10.	6.02±0.05	6.30±0.51	6.00±0.07	6.03±0.13	0.11
PUFA/SFA	5.	0.86±0.12[a]	1.08±0.07[bc]	0.94±0.12[ab]	1.11±0.11[c]	0.00
	10.	0.95±0.09[a]	1.19±0.11c	0.97±0.07[ab]	1.10±0.12[bc]	0.00
Ratio ∑omega-6/∑omega-3 fatty acids	5.	9.73±0.75[c]	1.48±0.17[a]	2.89±0.26[b]	1.90±0.27[a]	0.00
	10.	9.28±0.82[c]	1.57±0.17[a]	3.34±0.62[b]	1.63±0.19[a]	0.00

* a-c Average values with different letters in one line differ significantly at the level of 1%

The effect of the treatment on the pH value of the egg white at the end of week 5 of the experiment was significant (P <0.01). The results presented in Table 4 show that at the end of week 5, the lowest pH of the egg white was found in the control group, but it did not differ significantly with respect to flaxseed + oil and flax treatments of 10%, while compared to 5% with flax treatment was significantly lower. Flax treatment of 5% had the highest pH but it did not differ significantly from the treatments of flax cake + oil and flax 10%. At the end of the 10th week of the experiment, the effect of the treatment was not significant (p> 0.01) on the pH of the egg white and therefore there were no statistically significant differences between the groups. The effect of treatment on the yolk pH registered at the end of week 5 and 10 was not significant (p> 0.01), so there was no statistically significant difference between the observed groups.
The effect of treatment was significant (p <0.01) on the ratio of polyunsaturated (PUFA) and saturated fatty acids (SFA), showing an increase in agreement with the amount of PUFAs added through feed supported by other works *(Ferrier et al., 1995; Scheideler and Froning, 1996; Zotte et al., 2015).* Also, the results presented in Table 4 show that the effect of the treatment is significant (p <0.01) on the ratio of total omega-6 and omega-3 fatty acids, which decreases in line with the increase

in PUFA in feed, as reported by other authors *(Jiang et al., 1991; Scheideler and Froning, 1996; Sari et al., 2001; Zotte et al., 2015).*

Conclusion

From the results presented in this experiment it can be seen that by adding natural ground flax at a concentration of 5 and 10% as well as adding flax cake at a concentration of 10% together with flax oil at a concentration of 2% had no negative effects on the production characteristics of the laying hens in the experiment (egg production, egg weight, egg yolk weight). Also, the results obtained in this experiment show that the pH of the egg white showed a significant effect of PUFA enriched treatments at the end of week 5 of the trial, while at the end of week 10 there was no significant effect of treatments. However, the pH of the egg yolk did not show a significant effect of PUFA enriched treatments on its value at either the end of the 5th or the end of the 10th week of the experiment. Flax treatments of 10% and flax cake + oil had a significantly higher polyunsaturated / saturated fatty acid ratio at the end of week 5 and 10 compared to the control group.

The ratio of ∑omega-6 / ∑omega-3 fatty acids in both analyzes was significant statistically lower in the treatment of flax cake + oil and flax 10%, while in the treatment flax 5% was significantly higher concerning the previous two treatments but also significantly lower concerning the control group that had the statistically significant highest ratio.

From the above it can be concluded that PUFA enriched treatments in the experiment confirmed the possibility of enrichment of table eggs omega-3 with fatty acids with the specified nutrients, at the same time without undermining the production characteristics of consumable laying hens.

Uticaj korišćenja različitih izvora omega-3 masnih kiselina u hrani kokoši nosilja na proizvodne parametre i odnos omega-6 i omega-3 masnih kiselina

Jovo Perić, Milanka Drinić, Nenad Mićić

Rezime

Obogaćivanje konzumnih jaja određenim hranljivim materijama kao što su omega-3 masne kiseline je tema koja je stalno aktuelna. Sprovedeno je istraživanje

sa ciljem ispitavanja mogućnosti ishrane nosilja sa dodatkom mlevenog lanenog zrna, lanene pogače i lanenog ulja i njihovog uticaja na odnos omega-6 i omega-3 masnih kiselina u konzumnim jajima, kao i na određene proizvodne parametre. Eksperiment je postavljen u proizvodnom objektu sa 192 nosilje starosti 27 nedelja. Nosilje su bile podeljene u 4 grupe: kontrolna grupa; grupa hranjena sa lanenom pogačom (10%) + lanenim uljem (2%); grupa hranjena sa 5% mlevenog lanenog zrna i grupa hranjena sa 10% mlevenog zrna lana. Grupe su imale svaka po 8 kaveza u kojima je smešteno po 6 nosilja, ukupno 48 nosilja po grupi. Proizvodni parametri koji su prikazani u ovom radu su sledeći: broj jaja po useljenoj nosilji, težina jaja i težina žumanca. Pokazatelji kvaliteta jaja su određivani na kraju 5. i 10. nedelje ogleda i obuhvatili su: pH belanca i žumanca, odnos polinezasićenih i zasićenih masnih kiselina, kao i odnos omega-6 i omega-3 masnih kiselina. Rezultati istraživanja su pokazali da uključivanje lanene pogače, lanenog ulja i mlevenog lanenog zrna nije imalo negativnih efekata na prikazane proizvodne parametre, kao i da postoji pozitivan efekat navedenih hraniva na odnos omega-6 i omega-3 masnih kiselina u konzumnim jajima.

Ključne reči: kokoši nosilje, proizvodni parametri, masne kiseline

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