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ENRICHING THE DIET IN POLYUNSATURATED FATTY ACIDS FOR LAYING HENS USING FLAXSEED MEAL AND RICE BRAN

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Abstract

Vegetable raw materials rich in polyunsaturated fatty acids, especially in α linolenic (C18:3n3) are represented by flaxseed meal (51.67 ± 0.062% α-linolenic acid) and rice bran (1.79 ± 0.023% α-linolenic acid). A 6-wk study was conducted on 80, Tetra SL layers (38 weeks) assigned to two groups (C and E), to evaluate the effect of using raw materials rich in PUFA in layer diets, on layer performance. Hens were housed in an experimental hall under controlled environmental conditions, in 3-tier batteries (4 layers/cage), and 16h/24 h light regimen. The commercial (C) diet had 2.800 kcal ME, 17.8% CP and 0.885 gα - linolenic acid/ 100 g total FAME. Unlike for diet C, the experimental (E) diet formulations used 2.5% flaxseed meal and 10% rice bran which increased the concentration of α-linolenic acid (4.575 g/100 g total FAME) in this diet. The average daily feed intake, feed conversion ratio, laying percentage and egg weight were monitored throughout the experimental period. Eighteen eggs per group were collected during the final experimental day, and physical measurements were performed on the eggs. Daily feed intake (121.088 g/day/hen) and feed conversion ratio (1.965 kg feed/kg egg) were not influenced by using flaxseed meal (2.5%) and rice bran (10%) in laying hens diet compared with the C group. Also, the laying percentage was higher (P>0.05%) at the E group (95.23%) compared to the C group (94.4%).

Key words*: fatty acids, flaxseed meal, rice bran, laying hens, zootehnical performances.*

INTRODUCTION

The current increase of severity and incidence of degenerative diseases in humans, associated with stress, is largely attributed to the exhaustion of omega-3 fatty acids and antioxidants from the modern diet. Hens eggs are considered a functional food, since they are a source of high-quality proteins, vitamins, minerals and lipids, such as phospholipids and polyunsaturated fatty acids (PUFA) (Nau et al., 2010; Zdrojewicz et al, 2016; Heflin et al., 2018; Marin et al., 2011). The use in feed of laying hens, of plants rich in polyunsaturated fatty acids is a natural way to obtain a qualitative enrichment from the nutritional point of view, by modifying the nutritional components of the egg (Narahari et al., 2005; Benakmoum et al., 2013). In general, oilseeds and their meals are incorporated into the bird ratios both as a source of omega 3 PUFA and for their energy-protein content (Aziza et al., 2010). Of these, flax *(Linumus itatissimum L.)* represents the most widely used raw material included in the diet of laying hens to obtain eggs enriched in omega 3 PUFA (Al-Nasser et al., 2011; Yassein et al., 2015). Generally, flaxseeds are a valuable source of fat contains about 34% oil and has a high content of ALA (>50%), particularly PUFAs (Cherian and Quezada, 2016). The use of flax, in different forms, in animal feed, has led to an increase in the level of PUFA ω-3 acids in food of animal origin (Criste et al., 2009; Panaite et al., 2016). Another source of feed, with a high fat content but with antioxidant properties, is rice bran (Rohrer and Siebenmorgen, 2004; Sumantha et al., 2006). Being rich in lipids, it degrades very easily, which is why enzymatic inactivation of lipase by short-term heat treatments is necessary (Paucar-Menacho et al., 2007; Simone et al., 2012).Unfortunately, feed and foods enriched in PUFAs are exposed to rapid deterioration of nutritional and organoleptic qualities due to the oxidation of double carbon bonds, specific to the molecular structure of PUFAs. Lipid oxidation products have harmful biological effects (Schroepfer, 2002) and

therefore, it is important not only to improve the nutritional value of the feeds but also to minimize the lipid oxidation (rancidity) to provide healthy foods, pleasant to smell and taste (Hayat et al., 2010; King et al., 2012). As an undesirable effect, the enrichment of eggs in PUFA ω 3 leads to the propagation of lipid oxidation processes in egg yolk (Promila et al., 2017; Saracila et al., 2017). This is why PUFA-rich diets have to be supplemented with antioxidants (Galobart et al., 2001). Dietary supplementation with antioxidants is one of the most effective ways to minimize lipid peroxidation in egg yolk, because these compounds are transferred to egg yolk (Sahin et al., 2010; Nour et al., 2018; Panaite et al., 2019).

The purpose of this study was to evaluate the effects of using in laying hens feed, a diet enriched in PUFAs, by using flaxseed meal and rice bran, on the production performance and nutritional quality of the eggs obtained.

MATERIALS AND METHODS

Birds and Housing

The feeding trial was conducted in the experimental halls of The National Research-Development Institute of Animal Biology and Nutrition (IBNA-Balotesti, Romania) according to a protocol approved by the Commission of Ethics of the institute. A 6-wk study was conducted on 80, Tetra SL layers (38 weeks). The hens were weighed individually and assigned to two groups (40 hens/group) depending on their body weight. The layers were housed in an experimental hall under controlled environmental conditions, in 3-tier cages (4 layers/cage) which allow the daily recording of the ingesta and leftovers. The environmental conditions in the hall was according to Tetra SL layers breeder guide (temperature: 23.12±2.03°C and humidity: 44.83±6.20%). The light regimen (16 h daily) was according to the prescriptions for the particular category of poultry. The layers had free access to the feed and water.

Experimental diets

The laying hens received diet formulations according to the particular species, hybrid and feeding requirements. The basal diet formulation (Table 1) was similar for both groups (2800 kcal metabolisable energy and 17.8% crude protein). Unlike for diet C, the experimental (E) diet formulations used 2.5% flaxseed meal and 10% rice bran. The diet formulation for group C was characterized by a content of 0.885 g % total fatty acids. Diet E differed from diet C by increasing the concentration of α-linolenic acid (4.575 g % total fatty acids) in compound feed.

Table 1. Diet formulation and estimated chemical composition of experimental diet

Specification	Experimental diets	
	Control	Flaxseed meal+rice bran
Corn, %	53.74	48.49
Soybean meal, %	23.85	25.48
Flaxseed meal, %	-	2.5
Rapeseed meal, %	-	-
Rice bran, %	-	10
Antioxidant, %	-	0.015
Sunflower oil, %	2.7	1.84
Monocalcium phosphate, %	1.35	1.25
Calcium carbonate, %	8.79	8.81
Salt, %	0.40	0.41
Methionine, %	0.12	0.17
Choline, %	0.05	0.05
Premix*, %	1	1
Total, %	100	100
Analysed		
Dry matter, %	87.24	87.68
Metabolisable energy, kcal/kg	2800	2800
Crude protein, %	17.8	17.8
Calcium, %	3.9	3.9
Phosphorus, %	0.63	0.61
Lysine, %	0.89	0.89
Methionine, %	0.42	0.44
Met+cist, %	0.73	0.73
Threonine, %	0.68	0.65
Tryptophan, %	0.2	0.19

*1 kg premix contained: 1350000 IU/kg vitamin A; 300000 IU/kg vitamin D3; 2700 IU/kg vitamin E; 200 mg/kg vitamin K; 200 mg/kg vitamin B1; 480 mg/kg vitamin B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium

Measurement of laying performance and egg quality analysis

Throughout the trial we monitored layer performance: average daily feed intake (g/layer/dayfeed), conversion ratio (feed intake/egg mass; g/g), laying percentage (%) and egg weight (g) and egg quality parameters. Samples of flaxseed meal and rice bran, compound feeds and eggs were collected and analysed chemically. The basic chemical composition determinations were done according to Regulation (CE) 152/ 2009 (sampling and analytical methods for the official inspection of feeds).The fatty acids content was determined by gas chromatography as described by Panaite et al. (2016). Feed

samples were collected initially and then 14 and 28 days afterward and analyzed for fat acidity index, peroxide value and Kreistest. The fat acidity index (expressed as mg KOH/g fat) was determined in fat extracted with chloroform–methanol by titrating with 0.1 N KOH, and using phenolphthalein as indicator. The peroxide value was determined by iodometric titration according to the method described by the American Oil Chemists' Society (AOCS, 2009) and it was expressed as milli equivalents of peroxide per kilogram of sample. Kreis test was performed on the extracted fat and it was based on the production of red colour as a consequence of the reaction between phloroglucinol and epihydrin aldehyde, a compound present in rancid fats. The absence of a pink colour however indicates no incipient rancidity.

In the end of the trial we collected 18 eggs/group. Measurements were performed on egg weight and its components (albumen, yolk, shell) using an analytical scale (Kerm scales, precision 0.001), eggshell breaking strength, using an Egg Force Reader (Sanovo engineering A/S, Denmark); the pH measurements (albumen and yolk), using a portable pH meter Five Go F2-Food kit with LE 427IP67, Sensor MetlerTolledo. Albumen height, Haugh unit (HU) and yolk colour were considered as the parameters of internal egg quality. Albumen height (mm) was measured by using stage micrometer manually. And based on albumen height, HU was calculated using the equation proposed by Haugh (Stadelman, 1995). Yolk colour (the colour scale from 15, dark orange, to 1, light pale) was determined by comparing yolk colour with the Roche yolk colour fan (Hoffman-La Roche Ltd., Basel, Switzerland), according to the CIE standard colorimetric system. After measurements of the internal and external egg quality, six yolk samples (3 eggs/sample) were formed from the collected eggs (18/group) and assayed for the fatty acids content.

Statistical Analysis

The analytical data were compared using variance analysis (ANOVA) with STATVIEW for Windows (SAS, version 6.0). The experimental results were expressed as mean values with standard errors of means (SEM). The differences are considered statistically significant for $P < 0.05$.

RESULTS AND DISCUSSIONS

Table 2 shows the basic chemical composition of the flaxseed meal, rice bran and of the experimental diets. The flaxseed meal is a feed ingredient rich in crude protein (34.57%). Also, is a good source of fats, 68.40 % of which being polyunsaturated fatty acids (PUFA). Of the PUFA from the flaxseed meal fat, 51.67% are omega-3 fatty acids, and 16.63% are omega-6 fatty acids (Table 2). The chemical composition of the flaxseed meal differs according to the genotype, environmental conditions, oil extraction methods and technologies, etc. (Shim et al., 2014). The literature studies show that the flaxseed meal displays the largest variations of the chemical composition, which depend on the oil extraction process. The crude protein content found was in the range of 29.97-43.30%, ether extractives 1.13-15.69%, crude fibres 8.33-12.94% and 3.87-6.40% ash (Mueller et al., 2010; Olteanu et al., 2017; Vlaicu et al., 2018).

Rice bran is a by-product from milling process of paddy rice to produce polished rice. In general, it contains 12-20 % of total kernel and the rice bran was high in protein (13.77%) and lipid (12.56%). These results, especially the chemical compositions of rice bran, were confirmed by the results obtained by Moongngarm et al. (2012). At the same time, he confirms that the rice bran indicated the strongest antioxidant activity which can conduct at slowing down the peroxidation of linoleic acid. Hence, the ferric thiocyanate formation will be slow (Suja et al., 2005).

On the other hand, the diets (Table 2) were isocaloric and isonitrogenous containing 17.89% CP (diet C) and 17.94% CP (diet E), respectively 2800 kcal ME/kg diet for both diets (Table 1). The α-linolenic acid concentration of the compound feeds given to the experimental group was 4.575%, higher than in C (Table 2). The addition of flaxseedmeal and rice bran to the experimental groups made the omega-3 PUFA concentration to increase about 3.76 times compared to the control formulation (Table 2), therefore also improving omega-6/omega-3 ratio.

Tabelul 2. Chemical composition of the raw materialsand the experimental diets

Specification		Raw materials		Experimental diets	
		Flaxseed meal	Rice bran	Control	Flaxseed meal+rice bran
• *Basic chemical composition**					
Dry matter, %		90.24	89.31	90.30	89.93
Organic matter, %		84.95	82.14	76.64	75.19
Crude protein, %		34.57	13.77	17.89	17.94
Crude fat, %		9.79	12.56	4.09	4.80
Crude fiber, %		8.56	8.86	4.46	4.10
Ash, %		5.29	7.17	13.66	14.74
Nitrogen-free extractives, %		32.02	46.95	50.20	48.35
• *Linolenic acid content and polyunsaturated fatty acids (PUFA) profile*					
Linolenic α C18:3n3, g acid/ 100 g total FAME		51.67	1.79	0.885	4.575
ΣPUFA, g/100g total fatty acids, of which:		68.40	43.34	46.13	50.87
ω-3		51.67	2.27	1.11	4.18
ω-6		16.63	41.07	45.02	46.69
ω-6/ω-3		0.32	18.07	40.41	11.16
• *Lipid peroxidation*					
Peroxide value (ml thyosulphate 0.1 N/g fat)	initial	0.32	0.31	0.468	0.47
	after 14 days	-	-	0.574	0.589
	after 28 days	-	-	0.817	0.917
Fat acidity (mg KOH/g fat)	initial	12.99	12.66	13.86	12.4
	after 14 days	-	-	16.01	16.25
	after 28 days	-	-	17.86	18.47
Kreiss test	initial	negative	negative	negative	negative
	after 14 days	-	-	negative	negative
	after 28 days	-	-	negative	negative

Where: PUFA – polyunsaturated fatty acids; PUFA ω-3-omega 3 polyunsaturated fatty acids; PUFA ω-6- omega 6 polyunsaturated fatty acids; *on dry matter basis. **Analyses performed by the Laboratory of Chemistry and Nutrition Physiology – IBNA Balotești.

The high concentration of unsaturated fatty acids in experimental diet tends toward to lipid peroxidation. Although peroxide value and fat acidity were slightly higher in the diets supplemented with flaxseed meal and rice bran compared to the control diet, their values were below the maximum limits set for compound feeds according the Romanian Standard STAS 12266-84, after both storage periods (14 and 28 days). Kreis test was negative in all samples throughout the period of study.

These results are probably due to the antioxidant capacity coming from rice bran. Numerous earlier studies reported that animal performance is affected by feeding peroxidized lipids (Hung et al. 2017).

Layer performance data (Table 3) show that the flaxseeds meal and rice bran had a positive influence on the production parameters: average daily feed intake (g CF/layer/day), feed conversion ratio (kg CF/kg egg), laying percentage (%), and in the average egg weight, but the results were not statistically supported.

The physical quality parameters of the eggs collected in the end of the trial (Table 3) didn't show any significant differences in egg weight or in the weight of its components.

On the other hand, the albumen pH was significantly ($P \leq 0.05$) higher at the control group than the experimental group, fed with flaxseed meal and rice bran.In the experimental group, the yolk colour intensity increased compared to group C, but not significantly.

After 6 experimental weeks, the colour intensity in egg yolk from E group increased by 1.04% compared to C group.

The results obtained in this study regarding the influence of the dietary flaxseeds meal and rice bran on layer performance and egg quality are in agreement with those obtained by Ianni et al. (2019).

Table 3. Influence of the dietary flaxseeds meal and rice bran on layer performance and egg quality (average values/group)

Specification	Control	Flaxseed meal+rice bran	SEM	P-value
Average daily feed intake (g CF/layer/day)	122.38	121.09	0.442	0.1430
Feed conversion ratio (kg CF/kg egg)	2.004	1.965	0.013	0.1371
Laying intensity (%)	94.405	95.237	0.413	0.3169
Egg weight (g), of which:	65.26	66.65	0.429	0.1142
- albumen	39.75	41.11	0.390	0.0878
- yolk	16.83	16.71	0.165	0.7168
- shell	8.67	8.86	0.100	0.3587
Albumen pH	8.72[a]	8.52[b]	0.026	<0.0001
Yolk pH	6.23	6.12	0.043	0.1837
Yolk colour intensity	5.42	5.61	0.099	0.3311
Eggshell breaking strength (kgF)	4.11	4.08	0.085	0.8578
Haugh units	88.98	87.93	0.154	0.2768

Where: [a-b] Mean values within a row having different superscripts are significantly different by least significant difference test ($P \leq 0.05$). SEM: standard error of the mean. *Analyses performed by the Laboratory of Chemistry and Nutrition Physiology – IBNA Balotești.

Regarding the chemical composition of the egg yolk, from the data presented in Table 4 it can be observed that by using a diet rich in polyunsaturated fatty acids, respectively of a diet that included flaxseed meal and rice bran, the fat content of the egg yolk was not affected.

Table 4. Influence of the dietary flaxseeds meal and rice bran on chemical composition of egg yolkand polyunsaturated fatty acids (PUFA) profile

Specification	Control	Flaxseed meal+rice bran	SEM	P-value
• *Basic chemical composition of egg yolk (%)**				
Dry matter content	52.58	53.45	0.415	0.6151
Crude protein	16.79	16.98	0.545	0.6878
Crude fat	30.71	30.30	0.543	0.7226
Ash	1.92	2.03	0.049	0.2855
• *Linolenic acid content and polyunsaturated fatty acids (PUFA) profile (g acid/ 100 g total FAME)*				
Linolenic α C18:3n3	0.24[a]	0.85[b]	0.100	<0.0001
PUFA of which:	27.89[a]	29.63[b]	0.259	0.0278
ω-3	1.39[a]	4.99[b]	0.282	<0.0001
ω-6	26.49[a]	24.63[b]	0.429	0.0207
ω-6/ ω-3	19.12[a]	4.94[b]	2.141	<0.0001

Where:PUFA–polyunsaturated fatty acids; PUFA ω-3 - omega 3 polyunsaturated fatty acids; PUFA ω-6 - omega 6 polyunsaturated fatty acids; [a-b] Mean values within a row having different superscripts are significantly different by least significant difference test ($P<0.05$). SEM-standard error of the mean;*on dry matter basis; **Analyses performed by the Laboratory of Chemistry and Nutrition Physiology – IBNA Balotești.

A significant increase ($P \leq 0.05$) was recorded both for the amount of α-linolenic acid, representing a significant increase by 3.54 times higher in group E compared to group C, as well as for the content of the PUFA content of the yolks from laying, significantly increase ($P \leq 0.05$) for all omega-3 polyunsaturated fatty acids determined for group E compared to group C. All of the data presented in Table 4 shows that the ratio of omega-6/omega-3 decreased significantly in the experimental group (hens fed with flaxseed meal and rice bran during the experimental period) compared to the hens from group C. Aziza et al. (2013) obtained similar results by using 10% flaxseed meal which had no changes in yolk weight compared with eggs from hens fed the control diet. Also, no significant difference in α-linolenic acid or other n-3 fatty acids in the eggs from hens fed 10% flax meal were reported (Aziza et al., 2013), but feeding flaxseed meal resulted in higher egg production ($P<0.05$) compared with the control. The egg weights and Haugh units increased, and eggshell thickness decreased significantly ($P<0.05$) in diet with 10% flaxseed meal. The egg yolk content of α-linolenic acid and total omega-3 PUFA was higher in flax meal group than in C ($P<0.05$). Regarding the average daily feed intake Vlaicu et al., 2017 found that was lower in groups fed flax meal and rosehip as antioxidant compared with C.

CONCLUSIONS

The results of this study clearly demonstrate that the dietary inclusion of 2.5% of flaxseed meal and 10% rice bran significantly increased the content of PUFA in laying hens' egg yolks; it also notably decreased their the reportomega-6/omega-3. The simultaneous enrichment of hens' feed with flaxseed meal and rice bran like natural antioxidants, did not affect the production performance and the internal and external physical parameters of the eggs, but led to the improvement of their nutritional quality by increasing the content in PUFA fatty acids, especially by increasing the content of alpha-linolenic acid (omega-3).

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