The Effect of Extruded Full-Fat Rapeseed on Productivity and Eggs Quality of Isa Brown Laying Hens

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***Abstract*—**The eight-week feeding trial was conducted involving 27-wk-old Isa brown laying hens to study the effect of dry extrusion processing on partial reduction in total glucosinolates content of locally produced rapeseed and on productivity and eggs quality parameters of laying hens. 36 hens were randomly assigned one of three treatments (CONTR, AERS and HERS), each comprising 12, individual caged layers. The main composition of the diets was the same, but extruded soya bean seed were replaced with 2.5% of the extruded rapeseed in the AERS group and 4.5% in the HERS group. Rapeseed was extruded together with faba beans. Due to extrusion process the glucosinolates content was reduced by 7.83 μmol/g of rapeseed. The results of conducted trial shows that during all experimental period, egg production parameters, such as the average feed intake (6529.17 *vs.* 6257 g/hen/14 day; $P<0.05$) and laying intensity (94.35% *vs.* 89.29; $P<0.05$) were statistically different for HERS and CONTR laying hens respectively. Only the feed conversion ratio to produce 1 kg of eggs, kg in AERS group was by 11% lower compared to CONTR group ($P<0.05$). By analysing the effect of extruded rapeseed on egg mass, the statistical differences between treatments were not determined. The dietary treatments did not affect egg weight, albumen height, haugh units, albumen and yolk pH. However, in the HERS group were get eggs with the more intensive yolk color, higher redness (a) and yellowness (b) values. The inclusion of extruded full-fat rapeseed had no effect on egg shell quality parameters, i.e. shell breaking strength, shell weight with and without coat and shell index, but in the experimental groups the eggs were with the thinner shell ($P<0.05$). The internal egg quality analysis showed that with higher content of extruded rapeseed (4.5%) level in the diet, the total cholesterol in the eggs yolk decreased by 1.92 mg/g in comparison with CONTR group ($P <0.05$). Eggs laid by hens fed the diet containing 2.5% and 4.5% extruded full-fat rapeseed had increasing ∑PNRR/∑SRR ratio and decreasing ∑(n-6)/∑(n-3) ratio values of eggs yolk fatty acids than in CONTR group. n-6: n-3 ratio of eggs of the laying hens fed diets with different amount of extruded full-fat rapeseed changed from 5.17 to 4.71. The analysis of the relationship between hypocholesteremia/hypercholesterolemia fatty acids (H/H), which is based on the functional properties of fatty acids, found that the value of this ratio is significantly higher in laying hens fed diet supplemented with 4.5% extruded full-fat rapeseed than the CONTR group, demonstrating the positive effects of extruded rapeseed on egg quality. The results of conducted trial confirmed that 4.5% extruded full fat rapeseed is suitable to replace soyabean in the compound feed of laying hens.

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I. Introduction

RAPESEED or canola is oil crop that is widely grown around the world. The content of nutrient in rapeseed (approximately 40% of oil and 22% of protein) makes it a suitable ingredient for high nutritional value diets [1]. Although content of anti-nutrition factors such as glucosinolates in rapeseed has been markedly reduced through genetic selection, the feeding of higher levels rapeseed can still reduce productivity parameters in animal diets.

For the reduction the glucosinolates content could be used one of the method – extrusion [2], [3]. Reference [2] used dry extrusion at two different temperatures (150 and 130 ºC), and [3] used hydrothermal process at 105 ºC. Full-fat canola, after heat treatment and particle size reduction, is a mainstay protein and energy ingredient in broiler feeds in some countries like Denmark [4]. Also [5] reported about a positive effect of extrusion on the feeding value of rapeseed meal. A previous experiment with extruded rapeseed cake, conducted by [6] has demonstrated that rapeseed expeller cake may be incorporated in laying hens' diets to a level of 6% with no detrimental effect on egg performance and egg quality. The results of the experiment with laying hens, fed diets contained extruded rapeseed, wheat, and peas conducted by [7] did not show a significant effect on the productivity parameters and egg quality of laying hens. Similar results were obtained by [8] in the experiment with commercial layers fed with diets in which 20% of canola seed were replaces of dietary soya bean meal. In both experiments, the reason of insignificant results could be the high level of rapeseed and canola seed in the diets.

In the eggs yolk, content of unsaturated fatty acids, linolenic acid, eicosapentaenoic acid and docosahexaenoic acid can be increased in layer diets enriched with full-fat rapeseed [9]. However, unsaturated fatty acids are the compounds most susceptible to oxidation [10]. Consequently, the inclusion of polyunsaturated fatty acids in laying hens' diets may increase the susceptibility of eggs to lipid oxidation [11]. The egg validity period after lay can be between 4 and 15 days without change of their internal quality by keeping them at room temperature [12]. Consistent with these results, other works [13], [14] found that the shell egg storage time could not influence the content of lipid oxidation. The higher amount concentrations of MDA in eggs (180 ng/g) found may be due to differences in the methods of determining this indicator [15]. According to [16], the eggs enrichment with oil plentiful in omega-3 and omega-6 fatty acids, the MDA levels increased only in the second stage of storage time (60 days).

Nutrition can affect the cholesterol sedimentation in the egg yolk. [17], [18]. The inclusion of vegetable oils rich in unsaturated fatty acids used to change egg lipid profile and to reduce egg cholesterol content. The data presented in literature about the possible effect of the diet on egg cholesterol levels are contrasting. According to [19] and [20], the addition of PUFA-rich oils in the diet reduces blood and egg cholesterol concentrations. Reference [21] evaluated the effects of PUFA supplementation in bird diets and found that linseed oil, canola oil, sunflower oil and fish oil were able to decrease cholesterol levels in hen egg yolks. However, other studies showed that cholesterol content in the yolk couldn't be changed by dietary factors [22].

Therefore, the objective of this study was to investigate the effects of dry extrusion processing of locally produced rapeseed and on productivity and egg quality parameters of laying hens. Rapeseed was extruded together with faba beans because of technical fulfillment.

II. Material and methods

A. Experimental Design and Animals

The trial with laying hens was conducted following the regulations of the Republic of Lithuania and in accordance with EU Directive 2010/63/EEC and the EC recommendation 2007/526 EC for Animal use and storage for experiments and other purposes.

A feeding trial was conducted on 36 Isa Brown laying hens, aged 25 weeks. All laying hens were kept under the same conditions in the individual cages with stationary drinking-bowl and feed box, on a wire-mesh floor.

During the pre-experimental periods (up to 27 weeks of age), a commercial laying hen diet (17.25% crude protein, 11.37 MJ/kg metabolisable energy, 3.44% calcium and 0.40% available phosphorus) was offered at 125 g per day. At 27 weeks of age, the hens were randomly according to weight assigned to one of three treatments (CONTR, AERS and HERS), each comprising 12 individual caged layers. The main diets composition between the groups was the same, but extruded soya bean seeds were replaced with 5% of the mixture, composed of 50% of rapeseed and 50% of faba bean in the AERS group (rapeseed composed 2.5%) and 6.45% in the HERS group (rapeseed composed 4.5%). Rapeseed and faba bean were separately extruded and then ground and mixed together. The extruder was made by Insta-Pro International the model Insta-Pro 2500 (USA). The extruded meals were mixed with the rest of components. Rapeseed with low content of glucosinolates (so called double zeros '00') was used. For the glucosinolate content, samples of non-treated rapeseed and extruded rapeseed were analysed and it was determined that glucosinolates compose 14.7 μmol/g and 6.87 μmol/g, respectively. The total glucosinolates content was analysed in an independent accredited laboratory Labtarna (Lithuania). The birds were fed for 8 weeks, 125 g per day. A corn–wheat experimental diet was formulated to meet the nutrient and energy requirement for laying hens [23]. The content of moisture, crude protein, crude fat, crude fibre, crude ash, starch, calcium and phosphorus was determined with near infrared reflectance spectroscopy (NIRS) analysis. The diets and rapeseed samples reflectance spectra were measured by means of NIRS using a Foss−Tecator equipment DS2500 on milled grain samples. NIRS calibration models for analysed parameters content were developed using software ISIscan Nova (Germany).

B. Determination of Performance Parameters of hens and Internal and External Egg Quality

During the study, all the eggs were calculated and weighed daily, and egg production capacity of laying hens and feed conversion ratio to produce 1 kg of egg mass were determined. Egg weight, albumen high, haugh unit and yolk colour intensity were established by multifunctional automatic egg characteristics analyzer Robotmation (Japan) Egg Multi–Tester EMT–5200, pH of eggs albumen and yolk by Inolab 730 equipment (determination ranges between pH 2 and pH 10±0.5), breaking strength of eggshell by Egg Shell Force Gauge MODEL–II device, and thickness of eggshell by electronic micrometer MITUTOYO Digimatic Micrometer (sharp and, blunt end, and equator).

The colour of egg yolk was determined instrumentally by Minolta Chroma-meter (CR-410, Konica Minolta, Osaka, Japan) in the CIE L* a* b* space. The L* value indicates the lightness, representing dark to light (0–100). The a* (redness) value gives the degree of the red–green colour, with a higher positive a* value indicating more red colour. The b* (yellowness) value indicates the degree of the yellow–blue colour, with a higher positive b* value indicating more yellow colour. White calibration with the specifications of Y=86.2, x=0.3160 and y=0.3231 was used to standardize the chroma-meter.

The eggshell index was calculated according to [24] as (1):

$$SI\ (shell\ index) = (SW/S) \times 100 \tag{1}$$

SW = shell weight;
S = shell surface calculated as (2):

$$S = 4.68 \times egg\ weight\ (EW)^{2/3} \tag{2}$$

C. Determination of Fatty Acids in Yolk of Eggs

The content of fatty acids were determined using a gas chromatograph Shimadzu GC-2010 method [25] methylated according to [26].

The samples were analyzed according to current standards – ISO 5555: 1997 Animal and vegetable fats and oils. The sampling was prepared in accordance with ISO 661: 1997 Animal and vegetable fats and oils. Qualitative and quantitative analysis of fatty acids has been done.

Atherogenic (AI) and trombogenic (TI) indices were calculated according to [27] equations (3) and (4).

$$AI = [C12{:}0 + (4 \times C14{:}0) + C16{:}0] / [n\text{-}6\ PUFA + n\text{-}3\ PUFA + MUFA] \tag{3}$$

$$TI = [C14{:}0 + C16{:}0 + C18{:}0] / [(0.5 \times MUFA) + (0.5 \times n\text{-}6\ PUFA) + (3 \times n\text{-}3\ PUFA) + n\text{-}3/n\text{-}6\ PUFA] \tag{4}$$

MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids (unsaturation ≥2), n-6-omega-6 fatty acids, n-3-omega-3 fatty acids, n-3/n-6-ratio between omega-3 and omega-6 fatty acids

The hypocholesterolemic/hypercholesterolemic (H/H) ratio was calculated according to [28].

D. Determination of Lipid Oxidation and cholesterol content

Lipid oxidation was assessed on the basis of the MDA content in fresh and storage (28 day) egg yolk. MDA is one of the most toxic products of mutagenic and cytotoxic properties [29], [30]. It was determined by high performance liquid chromatography as described by [29].

Total cholesterol content in the eggs yolk was determined according method, described by [31]. Egg yolk was first diluted. Cholesterol was then extracted with ethanol and then conducted saponification with potassium hydroxide. After incubation (1 hour at 50°C temperature) and cholesterol separation with hexane and water, the extracts were centrifuged and dried under the nitrogen steam and the rest of substances were dissolved with mobile phase (acetonytrile : 2-propanole – 55:45). The quantification was performed by HPLC using a 5 μm particle size, 150 mm long and 4.6 mm internal diameter Supelco Ascentis® C18 chromatographic column. The sample injection volume was 10 μl.

E. Statistical Analysis

Statistical significance was established using one–way analysis of variance (ANOVA), and the data were reported as a mean of standard deviation. Mean comparison and separation were done using the Duncan *t* test (P < 0.05). ANOVA was conducted using the statistical package SPSS 22.

III. Results

A. Performance of Laying Hens Fed Diets with Different Amount of Extruded Full-Fat Rapeseed

The effect of 2.5% and 4.5% extruded full-fat rapeseed supplementation on the performance of birds on experimental diets shown in Table I. During all experimental period a higher inclusion level of extruded full-fat rapeseed (4.5 %) increased an average feed intake (g/hen/14 day) and laying intensity (%) of hens (P<0.05), but hadn't no significant effect on the egg-mass (g/hen/day). The lower extruded full-fat rapeseed level (2.5%) in the diet had no effect on the above mentioned productivity parameters only tended to improved feed conversion ratio to produced kg of egg mass (kg/kg) during all trial period (P<0.05).

TABLE I
Effect of 2.5% and 4.5% of full-fat extruded rapeseed on the laying hens' performance (28-34 weeks of age)

Parameter	Groups		
	CONTR	AERS	HERS
Average feed intake (g/hen/14 day)	6257 ± 14.69^{a}	6125 ± 17.66^{ab}	6529 ± 13.17^{b}
Feed conversion ratio to produced kg of egg mass (kg/kg)	2.19 ±0.27^{a}	1.96 ±0.04^{b}	2.08 ±0.05^{ab}
Egg-mass (g/hen/day)	63.45 ±0.65	63.40 ±0.45	61.82 ±0.50
Laying intensity (%)	89.29 ±2.48^{a}	89.44 ±2.83^{ab}	94.35 ±1.31^{b}

ab Means in each row with the different subscripts are significantly different, P<0.05

B. Internal and External Eggs Quality of Laying Hens Fed Diets with Different Amount of Extruded Full-Fat Rapeseed in Diets

Table II indicates the internal egg quality traits. The different extruded full-fat rapeseed in the diets did not affect egg weight, albumen height, albumen weight, pH and yolk pH. However, the haugh units were improved with 4.5% of full-fat extruded rapeseed, the difference between CONTR (78.71%) and HERS groups (83.23%) was significant (P<0.05). Yolk colour intensity of the hens fed higher amount of extruded full-fat rapeseed was higher (P<0.05) than that of the hens fed diets without extruded rapeseed inclusion (CONTR group). The yolk colour evaluation according to lightness (L), redness (a) and yellowness (b) showed that with the higher level of extruded rapeseed in the diets there were higher a and b values.

TABLE II
The internal egg quality of laying hens fed diets with 2.5% and 4.5% of extruded full-fat rapeseed (28-34 weeks of age)

Studied parameter	Group		
	CONTR	AERS	HERS
Egg weight (g)	63.94±0.77	65.01±0.42	61.72±1.03
Albumen height (mm)	7.12±0.44	7.22±0.46	7.29±0.33
Haugh units	78.71±4.51^{a}	80.94±3.63^{ab}	83.23±1.85^{b}
Albumin weight (g)	39.40±0.27	40.32±0.63	37.01±1.27
Albumin pH	8.23±0.04	8.29±0.08	8.20±0.11
Yolk weight (g)	16.48±0.84	16.49±0.51	16.95±0.22
Yolk pH	6.15±0.04	6.26±0.09	6.27±0.04
Yolk colour intensity	3.23±0.10^{a}	3.58±0.26^{ab}	4.10±0.39^{b}
	Yolk colour evaluation		
L*	66.54±1.32	66.67±1.36	66.05±0.51
a*	-6.21±0.44^{a}	-6.70±0.26^{ab}	-5.85±0.31^{b}
b*	46.64±0.56^{a}	47.73±0.58^{ab}	48.20±0.46^{b}

ab Means in each row with the different subscripts are significantly different, P<0.05

*L – lightness, a – redness, and b - yellowness

The dietary treatment had no effect on eggshell quality parameters (Table III) that is shell breaking strength, shell weight with and without coat and shell index. Only the shell thickness was significantly affected by feeding of rapeseed (P<0.05). In this regard, comparisons between treatment means during the all experimental period showed that the CONTR group had the highest values, while hens fed diet containing 2.5% and 4.5% of extruded rapeseed produced eggs with a thinner shell.

TABLE III
Effect of 2.5% and 4.5% of full-fat extruded rapeseed on the laying hens' egg shell quality (28-34 weeks of age)

Item	Group		
	CONTR	AERS	HERS
Shell breaking strength (kg/cm^{2})	3.89±0.25	3.98±0.28	3.86±0.15
Shell weight with coat (g)	8.07±0.09	8.20±0.19	8.17±0.09
Shell weight without coat (g)	5.91±0.10	6.11±0.11	5.88±0.10
Shell thickness (mm)	0.40 ±0.00^{a}	0.38 ±0.00^{b}	0.38 ±0.00^{bc}
Shell index (g/100 cm^{2})	7.91±0.15	8.07±0.14	8.01±0.04

abc Means in each row with the different subscripts are significantly different, P<0.05

C. Fatty Acids of Laying Hens Eggs Yolks

The analysis of fatty acids (FAs) profile of the Isa Brown laying hens' eggs yolk after 0-day storage (Table IV), noticeable, that with the insertion of extruded rapeseed in laying hens' compound feeds, it is possible to change the fatty acids profile and their individual ratios. The statistical significant differences were found between the HERS and CONTR groups. Supplementation of 4.5% extruded rapeseed in the feed decreased saturated FAs (SFA) such as C16:0 and C18:0 and monounsaturated FAs (MUFA) (C16:1) FAs ($P<0.05$). However, content of C18:1 increased by the 5.24 % compared to CONTR group ($P<0.05$). This resulted in a general decline in SFA of HERS group compared with the CONTR group. The increasing of separate polyunsaturated FAs (PUFA) content in feeds increased their deposition in the egg yolks and thus the total amount of n-6 fatty acids. Increasing the amount of extruded rapeseed in laying hens' diets, increasing the deposition of individual n-3 and total n-3 fatty acids in the egg yolk, but no statistically significant difference between treatment and control groups was observed. In this experiment, laying hens, fed diets supplemented with increasing amounts of extruded rapeseed, the appropriate ΣPNRR/ΣSRR ratio increased and Σ(n-6)/Σ (n-3) had tendency to decrease, then in the CONTR group.

The fatty acid profiles also enabled the evaluation of the lipid fractions nutritional quality index. The AI and TI indexes and the H/H ratios could therefore be determined. The AI)and TI indexes in the laying hens' eggs yolks at 1st and 28th lay days were lowered by 0.17 and 0.34 and by 0.20 and 0.41, respectively, for HERS groups, whereas the ratios between hypocholesterolemic and hypercholesterolemic fatty acids (H/H) in HERS group were higher.

By analyzing the amounts of eggs yolk fatty acids in Isa brown laying hens egg yolk after 28 days of storage, there were determine the similar fatty acids change tendencies how in the 1st day of lay: the supplementation of feeds with 4.5 % of full-fat extruded rapeseed, SFA's palmitic (C16: 0) and stearic (C18:0) decreased by 12.6% and 12.3% respectively ($P<0.05$) and MUFA oleic (C18:1) content increased up to 21.4%, compared to the CONTR group ($P<0.05$). The insertion of 2.5% and 4.5% of extruded rapeseed in the diets, the separate PUFA amount increasing, but statistically significant difference was observed only for DHA (C22:6 n-3) fatty acids. This has changed and the total amount of PUFA and SFA content in the HERS group, when the total SFA decreased by 9.85%, and the total PUFA - increased by 3.79 % ($P <0.05$) in comparison to CONTR group. The AI and TI indexes in the laying hens' eggs yolks at 28th lay days were lowered by 0.20 and 0.41, respectively, for HERS groups, whereas the ratios between FAs H/H ratio in HERS group were increased by 1.03 % ($P<0.05$).

D. Lipid Oxidation Extend in Fresh and Storage Eggs

The effect of dietary treatments on lipid oxidation of fresh eggs and those that were refrigerated and stored for 28 days is shown in Table V. The MDA values in fresh eggs of AERS and HERS groups were not affected when compared with CONTR group. The MDA values in stored and enriched eggs by rapeseed oil from extruded full-fat rapeseed was increased, but there were no significant differences among the groups.

TABLE IV
THE EFFECT OF DIFFERENT AMOUNT OF EXTRUDED FULL FAT RAPESEED TO THE DIET ON FATTY ACIDS PROFILES IN EGG YOLK, %

Fatty acids	Groups			Groups		
	CONTR	AERS	HERS	CONTR	AERS	HERS
	After 0-day storage			After 28 days' storage		
C14:0	1.12±0.69	0.84±0.67	0.50±0.77	1.04±0.79	0.68±0.47	0.48±0.48
C15:0	0.06±0.49	0.03±0.24	0.03±0.23	0.04±0.57	0.05±0.14	0.03±0.53
C16:0	26.03±1.17^{a}	23.56±1.79^{ab}	20.70±0.59^{b}	25.97±0.28^{a}	22.18±0.53^{ab}	19.85±0.40^{b}
C16:1 n-7	3.17±0.14^{a}	2.67±0.55^{ab}	1.88±0.34^{b}	2.21±1.14	1.84±2.55	1.67±0.39
C17:0	0.08±0.26	0.03±0.06	0.02±0.17	0.06±0.07	0.03±0.02	0.02±0.08
C17:1	0.05±0.35	0.02±0.09	0.04±0.14	0.05±0.23	0.06±0.22	0.04±0.13
C18:0	12.16±0.19^{a}	11.32±0.12^{ab}	9.63±0.00^{b}	11.23±0.14^{a}	11.04±0.00^{ab}	8.11±0.00^{b}
C18:1 n-9	37.82±1.66^{a}	39.45±0.61^{ab}	43.06±0.95^{b}	35.80±2.69^{a}	37.20±2.09^{ab}	40.01±2.39^{b}
C18:2 n-6	13.73±0.81^{a}	15.61±3.06^{ab}	16.62±0.67^{b}	11.88±2.31	12.57±2.06	13.48±2.60
C18:3 n-3	2.56±0.45	2.79±0.51	3.10±0.75	2.34±3.16	2.53±3.45	2.89±1.73
C20:3 n-6	0.20±0.04^{a}	0.26±0.12^{ab}	0.31±0.17^{b}	0.24±0.04	0.35±0.22	0.38±0.06
C20:4 n-6	2.50±0.26	2.79±0.23	3.15±0.26	1.85±0.40	2.40±0.27	2.87±0.17
C22:6 n-3	0.62±0.29	0.88±0.51	1.07±2.01	0.44±0.57^{a}	0.77±0.30^{a,b}	0.93±0.90^{b}
∑-SFA	39.45±0.87^{a}	35.78±1.12^{ab}	30.88±0.70^{b}	38.34±0.27^{a}	33.98±1.12^{ab}	28.49±0.30^{b}
∑-MUFA	41.04±0.65	42.14±1.23	44.97±0.97	38.06±0.65	39.10±1.23	41.72±0.97
∑-PUFA	19.62±0.33^{a}	22.33±0.80^{ab}	24.24±0.66^{b}	16.76±0.23^{a}	18.62±0.53^{ab}	20.55±0.26^{b}
∑-PUFA/∑-SFA	0.50±0.03	0.62±0.03	0.79±0.06	0.44±0.08	0.55±0.08	0.72±0.15
∑-n-6	16.44±0.52^{a}	18.66±0.92^{ab}	20.07±0.36^{b}	13.98±0.22^{a}	15.32±0.29^{ab}	16.73±0.36^{b}
∑-n-3	3.18±0.05	3.67±0.18	4.17±0.12	2.78±0.09	3.30±0.08	3.82±0.18
∑-n-6/∑n-3	5.17±0.21	5.09±0.15	4.81±0.16	5.03±0.19	4.64±0.25	4.38±0.17
AI	0.50±0.01^{a}	0.42±0.01^{ab}	0.33±0.02^{b}	0.55±0.01^{a}	0.43±0.02^{ab}	0.35±0.02^{b}
TI	1.02±0.05^{a}	0.86±0.09^{ab}	0.68±0.04^{b}	1.11±0.05^{a}	0.91±0.19^{ab}	0.70±0.08^{b}
H/H	2.12±0.13^{a}	2.53±0.26^{ab}	3.17±0.13^{b}	1.95±0.13^{a}	2.44±0.17^{ab}	2.98±0.03^{b}

abc Means in each row with the different subscripts are significantly different, P<0.05, SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids (unsaturation ≥2), n-6-omega-6 fatty acids, n-3-omega-3 fatty acids, PUFA/SFA-ratio between polyunsaturated and saturated fatty acids, n-6/n-3-ratio between omega-6 and omega-3 fatty acids, AI-atherogenicity index, TI-thrombogenicity index, H/H-the ratio of hypocholesterolemic and hypercholesterolemic fatty acids

TABLE V
MALONDYALDEHIDE LEVEL (μMOL/KG) IN FRESH AND STORAGE AT 28 DAYS EGGS YOLK

Parameter	Groups		
	CONTR	AERS	HERS
Fresh egg yolk	0.33±0.02	0.31±0.01	0.30±0.01
Storage at 28 days' egg yolk	0.61±0.06	0.50±0.07	0.53±0.02

E. Total Cholesterol Content in Eggs Yolk

The total cholesterol content in the egg yolk is presented in Table VI. The egg yolk cholesterol in AERS and HERS groups was observed to be from 1.33 till 1.92 mg/g eggs yolk lower than in the CONTR group.

TABLE VI
TOTAL CHOLESTEROL CONTENT OF THE LAYING HENS, FED DIETS SUPPLEMENTED WITH THE 2.5 AND 4.5 % EXTRUDED FULL-FAT RAPESEED, EGGS

Parameter	Groups		
	CONTR	AERS	HERS
Total cholesterol content in eggs yolk (mg/g eggs yolk)	$9.87±0.43^{a}$	$8.54±0.21^{b}$	$7.95±0.57^{bc}$

abc Means in each row with the different subscripts are significantly different, P<0.05

IV. DISCUSSION

A. Performance of Isa Brown Laying Hens Fed Diets with 2.5% and 4.5% of Extruded Full-Fat rapeseed

It was revealed from the results of present experiment that insertion of 4.5% extruded full-fat rapeseed in the laying hens' diets improved almost all performance parameters during all experimental period (28-34 weeks of age). One of the reasons of better performance of laying hens in present study could be the suggestion that in this experiment grinding was used. In general, grinding is used to disrupt the cell wall structure of feed ingredients and oil body structure within oilseeds, thus, increasing the exposure of nutrients to digestive enzymes, which is believed to positively impact the bird performance [32], [33].

In a study with laying hens, [7] reported that the replacement of soya bean meal in diets of layers with extruded rapeseed at up to 13.5% had no effect on laying performance. Another experiment conducted by [34] with 288 Shaver Starcross laying hens from 22 to 42 weeks of age showed no statistical effect on the hens' productivity parameters (feed consumption, egg weight and laying intensity), when 5% of rapeseed cake was incorporated into the diet. In another study where the different levels of locally produced canola seeds were used in the diet of laying hens [35], it was observed that feed intake, egg production and the weight of eggs decreased in parallel with an increase in the level of canola seed. The cause of decrease in egg weight is the lack of linoleic acid in the diet [36], [37]. According to the data presented in scientific literature, the linoleic (LA, 18:2n-6) and alpha-linolenic (LNA, 18:3n-3) acids are necessary by the human body for the maintenance of cell membranes, brain function and the transmission of nervous impulses in normal conditions. These essential FAs also participate in the transmission of atmospheric oxygen to the blood, hemoglobin synthesis and cell division [38].

B. Eggs quality of laying hens fed diets with different amount of extruded full-fat rapeseed

The data obtained from the present study were consistent to those reported by [39], who did not observe a higher weight of eggs, analysed in the term for quality determination, in different groups fed the expeller treated rapeseed.

According to the results of present study, the dietary treatments did not affect internal eggs quality parameters (egg weight, albumen height and weight, pH and yolk pH) however, the haugh units were improved. Other researches, which conducted the experiments with laying hens and analysed the effect of rapeseed and its products on the eggs quality parameters get controversial results. In the conducted experiment with 144 Hy-line (W-36) commercial laying hens [40] determined no significant difference (P>0.05) in haugh unit between experimental diets, but numerically, addition of 10% rapeseed meal, increase haugh unit. References [41] observed significant increase with increasing the proportion of canola seed in diets of layers. References [42] reported that the proportion of rapeseed meal in the diets did not affect the haugh unit, but they suggested addition of iodine had a positive effect on haugh unit. References [43] reported any significant effect of the level of rapeseed meal on haugh unit. The yolk colour in the experimental groups of present trial became more redness and yellowness compared to the control (P<0.05). This difference is thought to be related to the amount ofxanthophylls in the ration [44]. Similarly, [45] found no effect of the dietary level of rapeseed cake (3%, 6% or 9%), except for moderate yolk depigmentation at the highest level of rapeseed cake. The findings of the present study were not in agreement with the findings of [46].

In the present study, the inclusion of 2.5% and 4.5% of extruded full-fat rapeseed had no effect on eggshell quality parameters, except for the shell thickness which was significantly lowered by feeding of rapeseed (P<0.05). Similarly, [6] found no statistically significant effect of the dietary level of rapeseed cake (4, 6, or 8%) on the eggshell quality parameters. In previous studies where the effects of different amounts of rapeseed meal on eggs shell quality in laying hens were investigated by [7], [43] and [40] - it was observed no significant difference (P>0.05) in eggshell thickness among all experimental and control diets. However, in the same investigations [40] found, that laying hens fed diets with 10% rapeseed meal had the highest eggshell weight. References [7] and [47] reported that increasing the level of rapeseed meal had not any effect on eggshell strength. The amount and thickness of the eggshell have been found to be related to eggshell strength [48]. In this study, also the group that fed 10% rapeseed meal, produced eggs with better eggshell thickness and eggshell strength than other groups.

C. Effect of 2.5% and 4.5% of extruded full-fat rapeseed on fatty acids profiles and its categories in egg yolk

In this experiment was expected, that increasing level of extruded full-fat rapeseed (2.5% and 4.5%) in the hens' diets resulted in linear increases in the concentration of MUFA and PUFA and decreases in SFA in egg yolk. For human nutrition, important indicator of n-6/n-3 fatty acid ratio in the eggs yolk after 28 days of storage was also changed and become more favorable, that is from 5: 1 became 4: 1. [49], [50] and [51] also found a linearly decreased ratio of the n-6 to n-3 fatty acids in the yolk fat with increased rapeseed oil in hen feed. In these three studies, the ratio ranged from 4.1 to 5.4: 1 after supplementation of 4–8 % of rapeseed oil.

The values for the three indexes (AI, TI and H/H) showed that extruded full-fat rapeseed insertion into the laying hens' diets had beneficial effects on health, because it is possible get the healthier food. According to [52], nutritional quality indexes can indicate a sample's potential for plaque aggregation. In other words, low AI and TI values indicate high quantities of anti-atherogenic fatty acids in oil or fat. Further, [53] underscored that oil or fat was nutritionally the most adequate based on the relation between hypocholesterolemic and hypercholesterolemic fatty acids. In the current study, H/H ratios of 3.17 and 2.98 were found for the HERS groups in the fresh eggs yolk and in the yolk after 28th days of storage, respectively. These ratios indicate the nutritional adequacy, with an indication of greater quality for the eggs of laying hens, fed diets with adequate level of extruded rapeseed.

D. The effect of 2.5% and 4.5% of extruded full-fat rapeseed on oxidative stability and cholesterol content in eggs yolk

It was revealed from the results that moderate insertion of extruded full-fat rapeseed in the laying hens' diets did not affect the oxidative stability (MDA) of fresh and 28 days storage eggs yolks.

References [54] used different dietary oils to obtain different n- 6/n- 3 ratios and reported that MDA values were higher in fresh eggs with a higher content of n-3 FAs than in those rich in n-6 FAs. References [55] determined, that the major susceptibility to oxidation exhibit in the eggs, enriched with the higher content of total and long chain n-3 PUFA. The results of the present study are in agreement with those published by [56] using different vegetables oils to enrich eggs with different FA and found that MDA (TBA) values from fresh eggs rich in omega-3 FA (fish and flax oils) were higher than those from eggs rich in omega- 6 FA (sunflowers oils or rapeseed oil). In the experiment with fresh and stored eggs enriched with dietary omega-3 and omega-6 polyunsaturated fatty acids conducted by [16], were determined, that extent of MDA values was clearly increased only in the second stage of storage time (60 days).

Studies on layers' diets manipulation for the reducing egg cholesterol content have shown controversial results. Some researchers found, or how in the present study, that the polyunsaturated fatty acids supplemented in the diet in the form of vegetable oils or with the full-fat oilseeds reduce both egg and blood cholesterol levels [57],[58]. However, these results were not obtained by other authors [59],[60]. Yolk cholesterol concentration is very resistant to changes because there is a required yolk cholesterol level to ensure embryo development [61]. However, hens are able to change yolk polyunsaturated fatty acid content in response to dietary lipid source. The reason is that, poultry absorb dietary fat through the portal system as portomicrons, which are directly absorbed into the blood and transported to the main lipogenesis location, *i.e.* liver, thus allowing direct fat absorption by it [62].

A study carried out with commercial white Lohman LSL layers, with 3 weeks of age at the beginning of the experiment, were fed for 112 days with diets supplemented with vegetable oils rich in polyunsaturated fatty acids, particularly in omega-3 and omega-6 fatty acids [18], determined a significant interaction between additives and period for cholesterol content in the yolk of eggs from layers submitted to all experimental treatments. It was observed that egg cholesterol content tended to increase as birds aged. These results are consistent with those reported by [63], who asserted that egg cholesterol level is positively correlated with bird genetics and age, egg weight and yolk weight, and negatively correlated with lay percentage and dietary protein levels.

V. Conclusions

The results of this study showed that 4.5% extruded full-fat rapeseed in the Isa Brown laying hens diet improved laying hens' productivity parameters, such us laying intensity, egg production, feed conversion ratio and egg quality parameters – haugh unit and yolk colour intensity. Besides such level of extruded rapeseed in the diets had beneficial effects on health, because the three indexes (AI, TI and H/H), which report the relationship between fatty acids in food and their contribution to the prevention of coronary diseases were improved. Also, this investigation showed, that the higher polyunsaturated fatty acids supplemented in the diet in the form of full-fat oilseeds reduce egg cholesterol level and the oxidative quality of the eggs were not changed during 28 days of storage.

VI. References

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