

### EGG YOLK LIPID MODIFICATIONS BY FAT SUPPLEMENTED DIETS OF LAYING HENS

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*The aim of this study was to investigate the possibility to modify the total lipid and cholesterol level, as well as fatty acid composition of egg yolks, by supplementing diets of laying hens with different fats.*

*The trial was conducted in two six week experiments. Experiment I was conducted on 180 Isa Brown hens assigned to two age categories: 36 months - old (O), and 27 weeks of age - young (Y) hens. Both age categories were divided into three groups: control groups fed a diet I with no supplemented fat (OC and YC); experimental groups fed a diet II supplemented with 3.2% of palm oil (OP and YP) and experimental groups fed a diet III supplemented with 2.5% of lard (OL and YL). In Experiment II 45 Lohman Brown hens of 56 weeks of age were randomly assigned into three groups of 15 birds each and were fed with three experimental diets supplemented with either 3% fish oil (group FO), 3% palm olein (group PO) or with 3% lard (group L).*

*The results of our trial support the thesis of constant cholesterol content in egg yolk, that was accepted by the majority of researchers, although it was possible to affect the levels only in some conditions, as for example by the age of hens in Experiment I, or by feeding Lohman Brown hens with 3% of supplemented lard in Experiment II. However, the experiment proved the possibility of altering egg yolk fatty acid composition, this being a trend in actual investigations of egg yolk cholesterogenic modification.*

*Key words: cholesterol, diet, fat, hen, lipid, yolk.*

### INTRODUCTION

Hyperlipidemia, particularly hypercholesterolemia, is commonly accepted as a major risk factor for atherosclerosis and coronary heart disease, which have been at epidemic levels for a long time. The growing role of diet in both progression and prevention of these diseases has led to the convergence of consumer and government attention on the health quality of food (Van Elswyk, 1997).

More than 60% of total lipid, 70% saturated fat and 100% of cholesterol in the Western diet are originate from animal products (Jiang and Sim, 1993). Consumer preferences for animal products probably will be continued. Thus, it is of essential interest to designe and/or modify animal products in the way to minimise a dietary risk for cardiovascular and other (hypertension, autoimmune, allergic and neurological) diseases.

Egg yolk is one of the richest sources of dietary cholesterol in human nutrition. Due, at least in part, to consumer concern on dietary cholesterol a significant decline in *per capita* whole-egg consumption has been observed during the past several decades. Many attempts to improve the health quality of eggs through reduction in yolk cholesterol have met with only marginal success (Noble, 1987). It has been hypothesized that the inability to reduce egg cholesterol levels is due to a physiological control mechanism that ultimately causes cessation of egg production when yolk cholesterol deposition is inadequate for embryo survival (Marks and Washburn, 1977). Fortunately, the tide of public and medical opinion concerning the role of dietary cholesterol in the initiation and progression of heart disease has begun to change (Van Elswyk, 1997).

An alternative way to change of cholesterogenic features of egg yolk is the alteration of its fatty acid composition. Meat from animals and fish in the wild, chicken eggs produced under natural conditions, and wild plants contain higher amounts of n-3 polyunsaturated fatty acids (PUFA) in comparison with domesticated or cultivated ones (Simopoulos, 1999). The composition of meats, fish, and eggs is dependent on animal feed. Fish oil and meal (Hargis *et al.*, 1991), flax seed and oil (Jiang and Sim, 1991), canola oil (Lewis *et al.*, 2000),  $\alpha$ -linolenic acid (Ahn *et al.*, 1995), and n-3 PUFA from algae (Abril and Barclay, 1998) in hen feeds increase the n-3 fatty acid content of egg yolks and lead to the availability of n-3 PUFA enriched eggs on the market. Three n-3 PUFA enriched eggs provide approximately the same amount of n-3 PUFA as one meal containing fish (Lewis *et al.*, 2000). Research is ongoing for the production of n-3 fatty acid enriched products from poultry beef, lambs, pork, milk, bakerey products, etc. (Simopoulos, 1999). In the case of n-3 fatty acid enriched eggs, the egg from hens kept under natural conditions can serve as reference for proper composition.

A major advantage of the n-3 enriched egg, compared for example to fish, is not only because it is a cheap and complete nutritional package for human (Farrell, 1997), but the hen acts as a «biological sieve» to remove or reduce any undesirable contaminants, (Farell, 1994). In addition, the concentration and mix of n-3 PUFA in the yolk can be controlled by dietary changes. Moreover, the hen egg is a widely accepted animal product, taking part in human daily nutrition, and with no limitations for its use. n-3 enriched egg yolk may be an acceptable alternative supplement in infant formula as well (Heird, 2001).

According to the above, the aim of this trial was to investigate the possibility of egg yolk total lipid, total cholesterol and fatty acid composition modifications by different fat supplemented diets of laying hens.

## MATERIAL AND METHODS

The trial was conducted in two experiments, each lasting for six weeks.

### Experiment I

Experiment I was conducted on 180 Isa Brown laying hens divided between two age categories: 36 months (two times moulted) - old (O), and 27 weeks of age - young (Y) hens. According to the experimental design, three types of diet were prepared, marked as the diet I, II, and III: diet I with no supplemented fat, diet II supplemented with 3.2% of palm oil ("Felda Refinery Corporation", Pandamaran Oil Products, Malaysia), and diet III supplemented with 2.5% of lard (Mesna industrija "PIK Vrbovec", Croatia). Both age categories of hens were divided into three groups: OC (n=16) and YC (n=20), control groups fed diet I; OP (n=32) and YP (n=40), experimental groups fed diet II; OL (n=32) and YL (n=40), experimental groups fed a diet III. Feed intake was 115 g/hen/day for all groups, and access to water was *ad libitum*. The birds were housed according to the "California" system of four (O) and five (Y) birds in a cage, and with a lighting cycle of 16 h light/8 h dark.

The major carbohydrate component in the diets was maize; maize contents were 63%, 59.8%, and 60.5% in diets I, II, and III. Dietary fat components, in quantities noted above, were added as a substitution for the carbohydrate component. There was no difference among diets in their content of other feedstuffs: soybean meal 13%, fish meal 4.5%, sunflower meal 4%, alfalfa meal 4%, ground wheat 2%, limestone 7.4%, dicalcium phosphate 1.4%, salt 0.2%, and vitamin-mineral premix 0.5%. Chemical composition of the diet I, II, and III was determined using the standard Wende procedure and was shown in Table 1.

Table 1. Chemical analysis of diets for Isa Brown laying hens

Chemical component (%)	Diet		
	I*	II	III
Dry matter	89.16	89.60	89.80
Crude protein	15.94	16.19	16.37
Crude fat	4.00	4.66	4.51
Crude fiber	4.33	4.11	4.20
Crude ash	10.27	11.42	9.65
Nitrogen free extract	54.62	53.22	55.07
Calcium	3.812	4.340	4.143
Phosphor	0.819	0.812	0.808

\* I, II, and III are diets for Isa Brown laying hens: with no supplemented fat, with 3.2% of supplemented palm oil, and 2.5% of supplemented lard, respectively.

Samples of 30 eggs per group were collected at the end of the experiment to determine total lipid and total cholesterol levels in yolks. Preparation of eggs and

hard-boiled yolks for laboratory analysis, as well as total lipid extraction were done as described by Berrio and Hebert (1990), with the only difference being that we have done lipid extraction from 5-gram pooled samples of five yolks. Total lipids in hard-boiled yolks were determined gravimetrically, by drying 5 ml of lipid extract and weighting the residual fat, and were expressed as mg/g (concentration) and g/egg (content). Total cholesterol concentration (mg/g) in the egg yolks was determined colorimetrically by the Liebermann-Burchard analytical methodology specific for cholesterol and its derivatives (Chung *et al.*, 1965). Total cholesterol content (mg/egg) was calculated from cholesterol concentration and yolk weight values.

Data were statistically analysed using the two-tailed unpaired *t*-test.

### Experiment II

Forty-five Lohman Brown hens at 56 weeks of age were randomly assigned into three groups of 15 birds each and were fed with three experimental diets during a 6-week experimental period. To obtain the experimental diets, the basal diet was supplemented with either 3% of fish oil ("Henry Lamotte GMBH", Bremen, Germany) - group FO, 3% of palm olein ("Alami Corporation SDN, BHD", Selangor, Malaysia) - group PO or with 3% of lard ("Mesna industrija Bosanska Gradiška", Bosanska Gradiška, Bosnia & Herzegovina) - Group L. Housing, lighting cycle, daily feed intake, and access to water were as described for Experiment I. The nutritional composition of the three experimental diets was as follows: maize 54.7%, ground wheat 5.5%, alfalfa meal 4%, sunflower seed 3%, soybean meal 13.5%, maize gluten 4.5%, fish meal 2%, fat component (fish oil, palm olein, or lard) 3%, limestone 8.3%, dicalcium phosphate 0.8%, salt 0.2%, and vitamin-mineral premix 0.5%.

Chemical analysis of diets was done in the same manner as in Experiment I and is shown in Table 2. Dietary total cholesterol concentration was determined as the egg yolk total cholesterol concentration in Experiment I.

Table 2. Chemical analysis of diets for Lohman Brown laying hens

Chemical component (%)	Group		
	FO*	PO	L
Dry matter	89.84	90.30	90.20
Crude protein	17.73	17.71	17.07
Crude fat	6.09	6.63	5.57
Crude fiber	5.61	5.44	5.80
Crude ash	9.30	9.02	11.26
Nitrogen free extract	51.11	51.50	50.50
Calcium	3.230	3.181	3.223
Phosphor	0.658	0.656	0.655
Cholesterol (mg/g)	1.686	0.281	1.545

\*FO, PO, and L are groups of Lohman Brown laying hens fed diets supplemented with 3% fish oil, palm olein, and lard, respectively.

Fatty acid composition of total lipids was determined in Lohman Brown hen diets as well ("Galenika", Zemun, Serbia & Montenegro). Total lipids of the experimental diets were extracted by the method of Folch *et al.* (1957). Hydrolysis and extraction of lipids was done with 1M potassium hydroxide in methanol at room temperature according to the method of Christie (1982). To obtain methyl esters of fatty acids the lipid samples were mixed with 14% boron trifluoride-methanol. Finally, to determine fatty acid composition, the fatty acid methyl esters were analyzed by gas-liquid chromatography (GLC) (United States Pharmacopoeia Convention, 1999). A GLC instrument (Hewlett Packard 6890) equipped with flame ionization detector (FID) and Supelcowax column (30 mm x 0.32 mm ID x 0.5 mm) was used. Operating temperatures for the column and detector were 240 °C and 300 °C, respectively. The operating temperature for injection port was 250 °C. Helium gas was used as a carrier at a flow rate of 1 ml/min. Fatty acid composition in the experimental diets was obtained after addition of 3% fish oil, palm olein, or lard is shown in Table 3. Owing a limitation in fatty acid standards we determined only the following fatty acids: palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), and linoleic (C18:2n-6).

Table 3. Fatty acid composition of dietary total lipid of Lohman Brown laying hens

Fatty acid (%)	Group		
	FO*	PO	L
C16:0	17.62	18.60	16.98
C16:1	2.89	—	1.40
C18:0	11.00	—	11.83
C18:1	36.20	58.66	36.68
C18:2n-6	25.83	11.61	22.07

\*FO, PO, and L are groups of Lohman Brown laying hens fed diets supplemented with 3% fish oil, palm olein, and lard, respectively.

At the end of the experiment, 10 eggs per group were randomly sampled from each of the experimental groups, for assessment of the concentration (mg/g) and content (mg/egg) of total lipid and total cholesterol in the egg yolks. Preparation of eggs and hard-boiled yolks for laboratory analysis was done as in Experiment I. Total lipids of hard-boiled yolks were extracted by the method of Folch *et al.* (1957) and lipid extract was used as a sample for the determination of egg yolk total lipid and total cholesterol in the manner described in Experiment I. The fatty acid composition in the yolk total lipid was determined by gas liquid chromatography in the same manner as in the experimental diets using pooled samples of 10 hard-boiled yolks per each experimental group.

Data were analyzed using ANOVA for a one-way randomized test. Multiple range comparisons were made according to Duncan's multiple range test by comparing means of different treatment groups to determine significant differences among treatments ( $P < 0.05$ ).

## RESULTS

Egg yolk total lipid concentration and content were not significantly affected by different dietary treatments, regardless of the provenience of hens (Table 4 and Table 5). However, the age of hens fed an identical diet did affect total lipid content (Table 4). Dietary treatments also had no effect on total cholesterol concentrations and content in egg yolks of Isa Brown laying hens, however the age of the hens significantly affected both of them.

Table 4. Egg yolk total lipid and total cholesterol concentrations and contents of Isa Brown laying hens

Group	Egg yolk total lipid		Egg yolk total cholesterol	
	mg/g <sup>▲</sup>	g/egg <sup>◆</sup>	mg/g	mg/egg
OC*	267.63±5.47	4.48±0.22	14.04±0.14	234.69±9.60
OP	281.94±6.70	5.01±0.10	13.20±0.44	228.20±4.71
OL	279.16±1.99	4.75±0.08	13.41±0.30	228.17±6.67
YC	275.57±5.29	3.98±0.08	12.88±0.26	186.12±5.17
YP	269.99±6.02	3.84±0.07	12.92±0.45	183.40±5.48
YL	266.03±5.92	3.76±0.10	12.47±0.27	176.36±4.44
Differences among same dietary treatments, but different age				
OC/YC	N/S	N/S	P<0.005	P<0.004
OP/YP	N/S	N/A	N/S	P<0.0002
OL/YL	N/S	N/A	P<0.05	P<0.0002

All values are presented as Mean±SEM (n=6). \*Groups of old (O) and yang (Y) Isa Brown laying hens: OC and YC - control groups fed a diet with no supplemented fat; OP and YP - experimental groups fed a diet supplemented with 3.2% palm oil; OL and YL - experimental groups fed a diet supplemented with 2.5% lard. <sup>▲</sup> and <sup>◆</sup> = concentration and content of determined parameters. N/S = non significant; N/A = non analytical significance.

Table 5. Egg yolk total lipid and total cholesterol concentrations and contents of Lohman Brown laying hens

Group	Total lipid		Total cholesterol	
	mg/g <sup>▲</sup>	g/egg <sup>◆</sup>	mg/g	mg/egg
FO*	360.82±7.21 <sup>a</sup>	5.79±0.20 <sup>a</sup>	12.36±0.35 <sup>a</sup>	197.53±5.49 <sup>a</sup>
PO	360.82±3.85 <sup>a</sup>	6.01±0.19 <sup>a</sup>	12.29±0.57 <sup>a</sup>	203.75±9.08 <sup>a</sup>
L	369.76±3.93 <sup>a</sup>	6.16±0.16 <sup>a</sup>	14.23±0.41 <sup>b</sup>	236.29±7.03 <sup>b</sup>

All values are presented as Mean±SEM (n=10). \*FO, PO, and L are groups of Lohman Brown laying hens fed diets with supplemented 3% fish oil, palm olein, and lard, respectively. <sup>▲</sup> and <sup>◆</sup> = concentration and content of determined parameters. a,b = values within column with a different letter in superscript differ significantly (P<0.05).

Over a six week period, feeding Lohman Brown laying hens with 3% of lard in Experiment II significantly increased egg yolk total cholesterol, in comparison with the ones fed with 3% fish oil or 3% palm olein (Table 5).

Dietary fish oil, palm olein and lard at level of 3% altered the fatty acid composition of egg yolk total lipid in Lohman Brown laying hens (Table 6).

Table 6. Fatty acid composition of egg yolk total lipid of Lohman Brown laying hens

Fatty acid (%)	Group		
	FO*	PO	L
C16:0	25.62	19.96	21.22
C16:1	5.05	8.40	6.24
C18:0	11.01	8.30	10.31
C18:1	28.82	29.10	27.66
C18:2n-6	12.24	10.61	12.72

\*FO, PO, and L are groups of Lohman Brown laying hens fed diets supplemented with 3% fish oil, palm olein, and lard, respectively.

## DISCUSSION

### *Yolk total lipid*

According to the results of numerous authors (Pankey and Stadelman, 1969; Jiang *et al.*, 1991; Hargis *et al.*, 1991) dietary treatments had no effects on total lipid concentration in egg yolks, independent of the kind and quantity of the added fat, in laying hens of both proveniences - Isa (Table 4) and Lohman Brown (Table 5). Even the age of the hens, fed with the experimental diets (OP/YP and OS/YS) had no effects on the yolk total lipid concentrations. However, the differences in the yolk total lipid content are a consequence of the effects of palm oil (3.2%) and lard (2.5%) from the diets of the laying hens on the weight of their yolk, which is reported in earlier studies (Hodzic, 1999; Hodzic *et al.*, 2003). In the case when the laying hens are housed adequately, when they are in high production phase supplied by adequate quantities of the necessary nutrients, the hatched eggs show a relatively uniform increase of weight and constant lipid contents (Hargis, 1988). Marked domination the hepatic synthesis, in comparison to the dietary sources, makes supplying lipids for the yolk formation more stable, and less susceptible to the occasional dietary and husbandry disbalances (Kuksis, 1992).

### *Yolk total cholesterol*

Yolk total cholesterol in constant, and can be influenced only by some conditions, as for example by feeding the Lohman Brown laying hens with 3% lard (Table 5). Egg yolk total cholesterol content has also shown no differences among laying hens fed diets containing soybean or coconut oil, lard or tallow (Hirata *et*

*al.*, 1986), as well as laying hens fed with the mixture of sunflower and palm oil, in comparison to the three dietary regimens - tallow, crude and refined sunflower phospholipids (An *et al.*, 1997). Also, in the investigations performed by Hodžić *et al.* (2000) statistically significant differences were found in the concentration and content of yolk total cholesterol in experimental groups, in comparison to the controls.

The age of laying hens, however, has a significant influence on the concentration and the content of the yolk total cholesterol (Table 4). The old, two times moulted, hens had higher values and this is the finding in all groups. In Tetra-SL hens the values of the yolk weight, and the concentration and content of yolk total cholesterol were also slightly higher after moulting (at the age of 108 weeks) compared to the first production period (in the age of 48 weeks), but the differences were not statistically significant (Kovacs *et al.*, 1998). In the study of Nielsen (1998), however, the contents of total lipids, total cholesterol and phospholipids, expressed as g/yolk, were the same in the eggs of the laying hens aged 21 and 57 weeks. In that study the age difference was not so marked as in Experiment I.

The fact that there were no significant differences in the concentration and the content of the yolk total cholesterol when the hens were fed with fish oil or palm olein in Experiment II, may represent a very significant finding from the practical and economical point of view. On the other hand, it appears that the very high contents of oleic acid in the PO diet (Table 3) could give the same effect as PUFA from the FO diet. Unfortunately, we were not able to determine the polyunsaturated fatty acids except linoleic acid, but it is well known that the fish oil represents one of the richest sources of the PUFA, especially those of the n-3 series, with a remark that these oils greatly differ in the contents of the PUFA (Childs *et al.*, 1990; Farrell, 1998). Dietary cholesterol (Table 2) did not affect the concentration and the content of the yolk cholesterol.

The thesis of constant cholesterol concentration in the egg yolk is supported by numerous authors (Naber, 1983; Hargis *et al.*, 1991; Kuksis, 1992), so the recent studies of improving the health quality of egg are directed more to modification of the fatty acid composition of yolk lipids than to the yolk cholesterol content. All these findings indicate that the physiological mechanisms of egg yolk formation are flexible enough to overcome the dietary intervention and keep the cholesterol homeostasis in the egg (Beyer and Jensen, 1993).

#### *Fatty acid composition of the yolk total lipid*

In the study of Cherian *et al.* (1996), the supplementation of palm oil to the feed of laying hens did not result in any changes in the fatty acid content of the yolk. In our experiments, however, 3% palm olein in the feed for Lohman Brown laying hens resulted in the lowest content of saturated fatty acids - palmitic and stearic acids, as well as linoleic acid from the group of n-6 PUFA (Table 5).

At the same time, the PO group showed the highest content of the monounsaturated fatty acids - palmitoleic and oleic acids. Furthermore, the group PO had also the lowest total score of determined fatty acids (76.37% in comparison to the 82.74% for the group FO and 78.15% for the group L). It



represents also the highest difference for the group PO, which can be composed of middle chain saturated or long chain fatty acids, in the first place PUFA. However, in regard to the reduced (due to objective reasons) profile of the determined fatty acids, it is not rewarding to make a definitive conclusion of the possible beneficial fatty acid composition of yolk total lipid in the laying hens fed with the palm olein in comparison to the other two groups.

The mentioned differences in fatty acid composition of yolk total lipids are probably the consequence, in the first place, of the differences in fatty acid composition of the diets used for feeding the Lohman Brown laying hens. The PO diet had the lowest total score of unsaturated fatty acids determined (palmitic and stearic) - 18.60%, in comparison to 30.58% for the FO diet, and 29.56% for the L diet. In addition, the PO diet contained most oleic, and least linoleic acid (Table 3), which we consider an interesting result. Gibson found in 1989 (cited by Farrell, 1994) that the dietary linoleic acid competes with all n-3 PUFA for the incorporation into the egg lipids. He succeeded in the enriching the yolk with 8% dokosahexaenoic acid, but linoleic acid was represented only with 5% from the total fatty acids in the feed for laying hens. In animals and humans, namely,  $\alpha$ -linolenic acid converts to the long chain PUFA, like eicosapentaenoic and docosahexaenoic acid, by the processes of desaturation and elongation. The efficiency of that conversion depends, among other, on the contents of dietary n-6 PUFA, like linoleic acid which competes for the same enzymatic systems as  $\alpha$ -linolenic acid, which results in the lower production of n-3 PUFA (Crawford *et al.*, 2000). Olive oil, with high contents in oleic acid, however, increases the incorporation of n-3 fatty acids into cell membranes (Simopoulos, 2002).

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#### **MODIFIKACIJA LIPIDA ŽUMANJKA DODAVANJEM MASTI U HRANU KOKA NOSILJA**

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#### **SADRŽAJ**

Cilj ovog rada je bio da se ispita mogućnost izmene nivoa ukupnih lipida i ukupnog holesterola kokošijeg žumanjka, kao i njegove masno-kiselinske kompozicije kod koka nosilja hranjenih smešama sa dodatkom različitih vrsta masti.

Istraživanje je provedeno u dva eksperimenta u trajanju od po šest nedelja. Eksperiment I je proveden sa dve dobne kategorije koka nosilja provenijence Isa Brown: 36 mjeseci - stare (O) i 27 nedjelja - mlade (Y). Obe dobne kategorije koka nosilja bile su podeljene u po tri grupe: OK i YK, kontrolne grupe hranjene smešom I bez dodate masti; OP i YP, eksperimentalne grupe hranjene smešom II sa 3.2% palminog ulja; OL i YL, eksperimentalne grupe hranjene smešom III sa 2.5% svinjske masti. U eksperimentu II 45 koka nosilja provenijence Lohman Brown u dobi od 56 nedjelja metodom slučajnog uzorka bile su podeljene u tri grupe od po 15 životinja i hranjene eksperimentalnim smešama sa dodatkom 3% ribljeg ulja (grupa FO), 3% palminog oleina (grupa PO) ili 3% svinjske masti (grupa L).

Rezultati naših istraživanja podupiru tezu o konstantnosti sadržaja ukupnog holesterola žumanjka koju zastupaju brojni istraživači, premda je moguća i njegova izmena, ali samo u određenim uslovima, kao što je, na primer, dob koka nosilja u eksperimentu I, ili ishrana Lohman Brown koka nosilja sa 3% svinjske masti u našem Eksperimentu II. Dokazana je, pak, mogućnost promene masno-kiselinske kompozicije ukupnih lipida žumanjka, što i jeste trend u savremenim istraživanjima mogućnosti menjanja holesterogenih svojstava kokošijeg žumanjka.