

LIPID COMPOSITION OF LIVER IN RATS FED DIETS SUPPLEMENTED WITH EGG YOLKS OF MODIFIED COMPOSITION

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The aim of this study was to examine the effects of diets supplemented with egg yolks of modified composition on the fatty-acid composition and lipid content in rat's liver. During four weeks of the experiment 64 Wistar rats were divided into four groups of 16 individuals each (eight individuals of both sexes) and fed a commercial feed mixture for rats (group C) or diet containing 70% commercial mixture for rats and 30% freshly cooked egg yolks from laying hens fed diets with 3% fish oil (group F), 3% palm olein (group P) or 3% lard (group L).

Dietary supplementation with egg yolks significantly increased the hepatic cholesterol pool in rats, regardless of the type of fat in the diet of laying hens from which the eggs originated. The content of α -linolenic acid in the liver of male rats in group P was 4-6 times higher compared to males in the other groups. Liver lipids and their fatty-acid composition differ by both, sex and dietary modified egg yolk composition in rats.

Key words: cholesterol, dietary egg yolk, fatty-acid composition, liver, rat, total lipids

INTRODUCTION

Fats are the major and most efficient source of energy in the body and represent almost half of the total daily substrate for oxidation. The main component of the dietary and body fats are triglycerides. The second one is cholesterol, which plays an important role in a variety of specific functions in cell membranes and is the precursor of bile acids and steroid hormones. Body cholesterol originates from either dietary sources of animal origin or *de novo* synthesis in most cells in the body.

The liver has a central role in lipid metabolism, and a particularly important role in cholesterol transport. Cholesterol is mainly synthesized *de novo* by hepatocytes, but it is also partially taken from the blood plasma lipoproteins. In some species such as rat, mouse and *Chrysothrix* monkey, cholesterol synthesis in the liver takes place rapidly and makes 40-50% of the total synthesis of sterols in

the organism. In most other species, however, including the rabbit, guinea pig, *Cynomolgus* monkey, hamster and humans, liver is responsible for only about 5-20% of total sterol synthesis. Furthermore, when any of these animals is switched to a diet that contains cholesterol amounts typical for human food (100-300 mg/1000 kcal) cholesterol synthesis in the liver is suppressed, but not in extrahepatic tissues (Spady *et al.*, 1993).

Because of the capability for suppression of hepatic synthesis, rat and *Chrysothrix* monkey, unlike hamster and *Cynomolgus* monkey, can be adapted to high loads of dietary cholesterol before cholesterol-esters begin to accumulate in the liver, and before any changes in the metabolism of low density lipoprotein (LDL) cholesterol. When the amount of dietary sterol is small the net entry of cholesterol into the liver from the intestine and extrahepatic tissues is probably about 90% of the total circulating cholesterol, so it is with most species that contribution of liver to total synthesis of cholesterol is of minor importance (Spady *et al.*, 1993).

Cholesterol content in egg yolk is one of the main reasons for permanent reduction of egg consumption *per capita*, although the relationship between egg consumption and cholesterol metabolism in humans has not yet been completely clarified. It should also be kept in mind that egg yolk contains about 60% lipids (on dry matter basis), mainly triglycerides and phospholipids, because the metabolism of cholesterol in humans may be influenced by the quantity and fatty-acid composition of dietary lipids (Hodzic, 2003).

In the experiment of Jiang and Sim (1991) isonitrogenous feed mixtures, one contained 18.7% egg yolk powder, and second balanced with casein, pork fat and cholesterol, resulted in similar values of the total and high density lipoprotein (HDL) cholesterol in the plasma of rats fed these mixtures. In addition, the contents of free and total cholesterol in the liver, but not in the heart, were significantly lower in rats fed mixtures with egg yolk powder. The same authors (Jiang and Sim, 1992) later found that feeding the yolks enriched with n-3 polyunsaturated fatty acids (PUFA) in rat's diet significantly reduced cholesterol in plasma and liver compared with the yolks of normal composition. Different fish oils and meals (Hargis *et al.*, 1991), flax seed and oil (Jiang and Sim, 1991), or linolenic acid (Ahn *et al.*, 1995) and rapeseed oil (Lewis *et al.*, 2000) in the diet for laying hens increased the content of n-3 PUFA in egg yolks.

Assuming that diet supplemented with egg yolks represents a diet rich in fat, the aim of this study was to examine the impact of diet supplemented with egg yolks of modified composition on the fatty-acid composition and lipid content in rat's liver, depending on the type of added fat in the diet for laying hens from which the eggs originate.

MATERIAL AND METHODS

A preliminary study, which lasted six weeks, was carried out to produce the yolks of certain quality under defined conditions of laying hens feeding regimen. Ninety Lohman Brown laying hens in the 34th week of production and in the 56th

week of age were used in the study. The hens were kept under standard conditions of intensive egg production.

The animals were randomly divided into three groups - FO, PO and LA. Hens in group FO were fed a feed mixture with 3% fish oil ("Henry Lamotte" GmbH, Bremen, Germany), group PO was fed with 3% palm olein ("Alami Corporation SDN, BHD", Selangor, Malaysia), and group LA with 3% lard ("Meat Industry Gradiska" Gradiska, B&H). During the fifth and sixth week of preliminary study eggs were collected (complete production) for the preparation of food for rats.

Concentration and content of total lipids, triglycerides and cholesterol, as well as fatty-acid composition of total lipids were determined in the egg yolks. The results of these analyses were shown in an earlier article (Hodzic *et al.*, 2008).

The experimental part of the study, for a total period of four weeks, was conducted on 64 Wistar rats, 32 females and 32 males, four months old at the beginning of the experiment.

Diets for rats

The rats were fed *ad libitum* and had free access to water during the experiment. Three days before the start of the experiment all rats were fed a commercial pelleted feed mixture ("MB-MIX", Banja Luka, B&H). After three days of adaptation to experimental housing conditions, rats were weighed and randomly allocated to four groups (C, F, P and L) with 16 individuals in each (eight of each sex).

Three experimental diets for rats were prepared to contain 70% of the commercial feed mixture for rats and 30% freshly cooked yolk from eggs of laying hens from groups FO, PO and LA. The eggs were cooked for 15 minutes, cooled, and the yolks were separated and homogenized. Samples of the commercial feed mixture were ground and soaked in the same amount of water and mixed with egg yolk. The obtained mixture was manually homogenized and then cakes were made and dried during 24 hours at room temperature, and then dried at 50°C to a constant weight. The prepared cakes were kept in paper bags in a dry and dark place.

The control rats (group C), females and males, were fed a commercial pelleted feed mixture. The diet for rats in group F was made of cakes containing 30% cooked egg yolk from hens in group FO. Group P was fed cakes containing 30% cooked yolk from eggs of laying hens in group PO, and rats in group L were offered the diet with 30% of cooked egg yolk from hens in group LA. Fresh diets, commercial and experimental, were offered every two days and feed consumption and weight gain were measured weekly.

Diets were analyzed for dry matter, crude protein, fiber, and ether extract, as well as Ca, P, and cholesterol content. Absolute dry matter content was determined by drying at 103-105°C for 24 h. Dried samples were ground through a 1-mm screen and ashed at 550°C overnight. Total nitrogen (crude protein) content was determined using a Kjeltac automatic analyzer (Model 1030, Tecator AB, Hoganas, Sweden). Crude fiber analysis and fat extraction were performed according to standard ISO procedures. Calcium content was determined via atomic absorption spectrophotometry (AAAnalyst 300, Perkin Elmer Corp.,

Norwalk, CT, USA) in ashed samples dissolved in a 3N HCl solution and diluted 1/50 with strontium chloride. Phosphorus was measured colorimetrically using the ammonium molybdate procedure. Dietary cholesterol concentration was determined spectrophotometrically by Lieberman-Burchard method using commercial tests (Semikem, Sarajevo, B&H) and standard of 50 mg/dL in the extract obtained by Folch method (Folch *et al.*, 1957). The procedure for the determination of fatty-acid composition of rat's diets was the same as described in a previous article for samples of feeds for laying hens (Hodzic *et al.*, 2005).

Table 1. Chemical composition, Ca, P, and cholesterol content of diets for rats

Ingredients (%)	Group [†]			
	C	F	P	L
Dry matter	92.71	94.41	93.79	94.33
Crude protein	21.97	24.09	23.98	24.01
Crude fat (ether extract)	5.87	15.59	15.74	15.88
Crude fiber	9.16	6.68	7.66	8.00
Ash	7.54	6.68	6.67	6.68
Nitrogen free extract	48.17	40.10	39.74	39.76
Ca	1.21	1.03	1.07	1.07
P	0.95	0.94	0.86	0.88
Cholesterol (mg/g)	0.70	7.03	5.62	6.74

[†] C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed the diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L).

Table 2. Fatty acid composition of total lipids in the diets for rats

Fatty acid (%)	Group [†]			
	C	F	P	L
C14:0	0.96	-	0.60	-
C16:0	16.45	17.74	21.30	20.30
C16:1	0.83	1.41	1.64	1.50
C18:0	7.68	9.13	9.92	10.43
C18:1	30.05	37.17	42.72	42.37
C18:2n-6	36.20	18.15	7.11	17.92

[†] C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L).

The chemical composition of diets for rats and its fatty-acid composition is given in Tables 1 and 2. Experimental diets (groups F, P and L) differed in

comparison to the control (group C) primarily in the higher total fat content as a result of adding cooked egg yolks in a quantity of 30%, and in lower content of fiber (Table 1). Experimental diets also contained more saturated fatty acids – stearic (C18:0) and palmitic (C16:0), as well as monounsaturated oleic (C18:1) and palmitoleic (C16:1) acids, but the content of linoleic acid (C18:2n-6) was much lower in relation to the diet of control rats (Table 2).

Preparation and analyses of samples

After 28 days of experimental feeding, the rats were subjected to 12-hour fasting. The next day the animals were weighed, and their blood was taken from the abdominal aorta under mild anesthesia with diethyl-ether. Blood samples were collected in the vacutainers of 3 mL with EDTA as an anticoagulant. The rats were sacrificed and then exenteration was performed. Liver samples for histological examination were fixed with 10% buffered formalin. Histological cuts of 3-5 μm thickness were made from prepared paraffin tissue blocks on sliding Leitz microtome. Histological slices of liver were stained with two staining methods, Hematoxylin-eosin and Sudan III.

Individual liver samples were homogenized, and 1 g of each was taken and added 19 mL of chloroform/methanol mixture in the ratio 2:1 as extraction solvent (Folch *et al.*, 1957). Prepared liver samples in test tubes were covered with double tin foil and kept at -18°C until later processing. The remains of homogenizate were also stored at -18°C as pooled samples (all animals of one group) for determination of fatty-acid composition of total liver lipids, which was performed with the procedure previously described for samples of feeds for laying hens (Hodzic *et al.*, 2005).

After removal from the freezer lipid extracts of liver were kept at room temperature for 24 hours to complete fat extraction. Subsequently, the extracts were filtered and the filtrate was directly used as the sample for the determination of total lipids, triglycerides and total cholesterol. Methods and procedures for the determination of these three parameters have been described previously for egg yolks (Hodzic *et al.*, 2005), except that a standard of 50 mg/dL was used for total cholesterol. Concentration and content of total lipids, triglycerides and total cholesterol have been expressed in mg/g or mg/total liver weight.

Statistical analysis

The data were processed by two-way ANOVA. The differences between the treatment means were further analyzed by Duncan's multiple range test at a significance level of $p < 0.05$, but only if ANOVA showed a significant effect of the treatments.

RESULTS

The animals of the same sex in different groups did not significantly differ in body weight at the beginning of the experiment, but there were evident and expected differences between individuals of opposite sexes in the same groups. However, the difference in behavior of females and males was also evident as

expressed in difference in weight gain (Table 3). Significantly different feed consumption between the groups did not produce significant differences in weight gain in females. The highest consumption of feed was recorded in group C, while the highest body weight gain was recorded in group P, and this was the case for both sexes.

Table 3. Initial body weight, weight gain and feed consumption in rats (mean \pm SE)

	Group [†]	Initial body weight (g)	Body weight gain (g/rat/4 wk)	Feed consumption (g/rat/4 wk)
Females	C	171.25 \pm 5.49 ^a	19.38 \pm 4.77 ^a	442.99 \pm 11.46 ^c
	F	168.75 \pm 7.18 ^a	25.63 \pm 6.23 ^a	333.75 \pm 6.75 ^a
	P	183.75 \pm 7.30 ^a	35.00 \pm 4.72 ^a	365.62 \pm 18.93 ^{ab}
	L	184.38 \pm 4.17 ^a	19.38 \pm 4.38 ^a	377.26 \pm 7.55 ^b
Males	C	234.38 \pm 6.30 ^{a*}	91.25 \pm 6.93 ^{b*}	598.54 \pm 14.39 ^{c*}
	F	244.38 \pm 3.83 ^{a*}	72.50 \pm 3.13 ^{a*}	501.22 \pm 7.82 ^{b*}
	P	246.25 \pm 5.96 ^{a*}	108.75 \pm 2.80 ^{c*}	467.06 \pm 8.70 ^{ab*}
	L	247.50 \pm 5.35 ^{a*}	78.75 \pm 7.95 ^{ab*}	492.83 \pm 10.22 ^{b*}

[†] C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L). ^{a, b, c} = the values for the same sex in the same column with different superscript are significantly different ($p < 0.05$). * = significant difference ($p < 0.05$) between the opposite sex in the same group. SE = standard error of the mean.

Liver lipids

Concentrations (mg/g) and contents (mg/whole liver) of total lipids, triglycerides and total cholesterol were determined in the rat's liver. The significance of differences between the sexes for the same feeding treatment is marked in the figures for males. The lowest concentrations and total content of lipids, triglycerides and total cholesterol in the liver of males and females were found in group C. The highest values, however, females showed in group L, except total cholesterol. Males showed highest values in group P for all determined parameters. Concerning the differences between feeding treatments, they were not always identical in females and males, which is probably due to the influence of the treatment on liver weight (Hodzic, 2003).

Table 4 shows the fatty-acid composition of total liver lipids in groups of male and female rats determined in a pooled sample of all 8 animals of one group. Females of all groups had more saturated fatty acids in the liver compared to males. Females in experimental groups had less stearic and linoleic acid in the liver and more oleic acid compared to control. For males this finding was confirmed only for oleic acid.

Figure 1. Concentration and content (mean \pm SE) of total lipids in the liver of female rats

C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L). ^{a, b, c} = the values of the same series with different superscript are significantly different ($p < 0.05$). SE = standard error of the mean

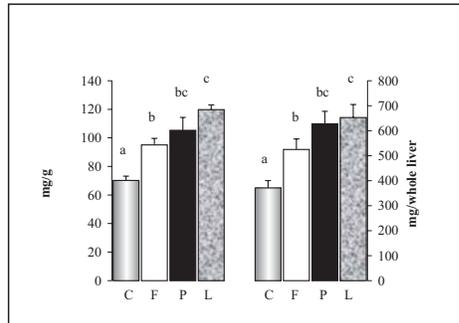


Figure 2. Concentration and content (mean \pm SE) of total lipids in the liver of male rats

C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L). ^{a, b, c} = the values of the same series with different superscript are significantly different ($p < 0.05$). * = significant difference ($p < 0.05$) compared to females of the same group (Figure 1). SE = standard error of the mean

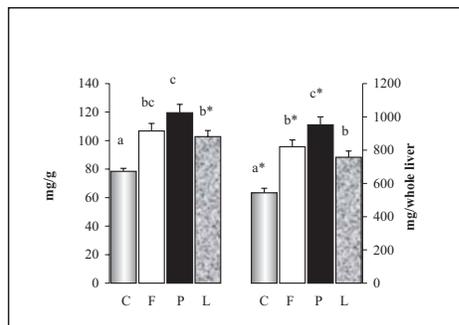


Figure 3. Concentration and content (mean \pm SE) of triglycerides in the liver of female rats

C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L). ^{a, b, c} = the values of the same series with different superscript are significantly different ($p < 0.05$). SE = standard error of the mean

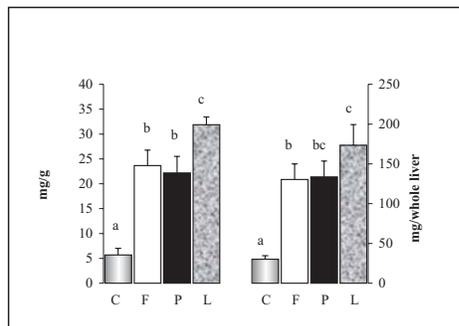
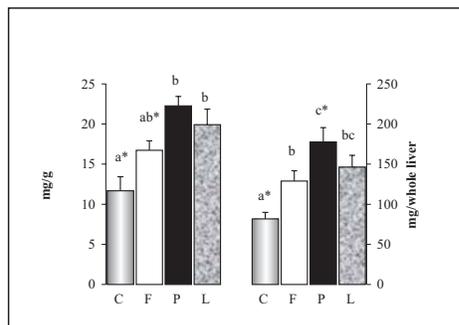


Figure 4. Concentration and content (mean \pm SE) of triglycerides in the liver of male rats

C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L). ^{a, b, c} = the values of the same series with different superscript are significantly different ($p < 0.05$). * = significant difference ($p < 0.05$) compared to females of the same group (Figure 3). SE = standard error of the mean



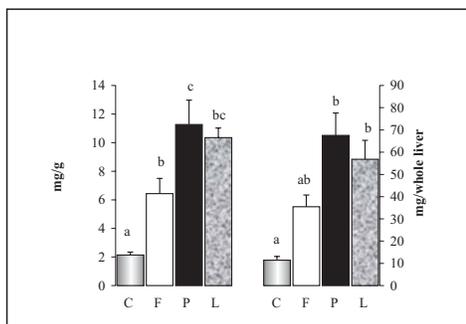


Figure 5. Concentration and content (mean \pm SE) of total cholesterol in the liver of female rats

C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L). ^a, ^b, ^c = the values of the same series with different superscript are significantly different ($p < 0.05$). SE = standard error of the mean

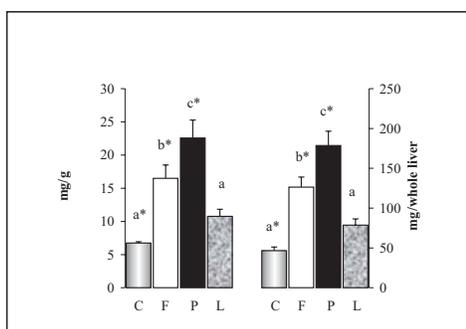


Figure 6. Concentration and content (mean \pm SE) of total cholesterol in the liver of male rats

C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L). ^a, ^b, ^c = the values of the same series with different superscript are significantly different ($p < 0.05$). * = significant difference ($p < 0.05$) compared to females of the same group (Figure 5). SE = standard error of the mean.

Table 4. Fatty acid composition of total lipids in the liver of rats

Fatty acid (%)	Females				Males			
	C †	F	P	L	C	F	P	L
C16:0	20.91	19.48	20.84	22.32	16.10	17.01	16.41	19.74
C16:1	1.59	1.95	2.38	2.12	1.45	1.42	1.53	1.57
C18:0	27.13	16.95	15.82	16.35	19.60	15.00	13.80	20.06
C18:1	12.28	20.75	26.44	26.62	8.60	21.75	25.91	22.18
C18:2n-6	24.90	21.19	23.16	25.81	24.0	24.60	21.92	27.21
C18:3n-3	2.61	1.61	1.82	1.14	2.24	3.0	13.66	2.08

† C, F, P and L represent the group of rats according to feeding treatment: control group (C) fed a commercial feed mixture for rats and the experimental groups fed diets containing 30% egg yolk from laying hens fed mixtures with fish oil (F), palm olein (P), or lard (L)

Pathomorphology of the liver

Macroscopic findings – The livers of animals of both sexes in group P were observed and changes were found in terms of something lighter yellowish parenchyma with easily friable consistency. The livers of animals in group F had a more intensive bright-yellow color and ruinous consistency, while the livers of groups C and L were reddish-yellow.

Microscopic findings – Hepatocytes stained with Haematoxylin and eosin had lost their polyedric shape and become more rounded. Nuclei of some individual cells were pushed to the periphery, in the part of the cell where a part of cytoplasm remained. In most of the hepatocytes many different bright vacuoles of various sizes were found. These hepatocytes were distributed more peripherally in the hepatic lobules.

Hepatocytes stained with Sudan III had numerous small red droplets diffusely present in the cytoplasm, which represented fat droplets stained with Sudan III. Nuclei of some individual cells were shifted marginally without necrobiotic changes on them.

DISCUSSION

In comparison to the control group, accumulation of cholesterol in the liver was significantly increased in all experimental groups of rats except in males in group L (Figure 5 and 6). Thus, addition of egg yolks in rat's diet, regardless of the type of fat in the diet of laying hens from which the eggs originate, increased the hepatic cholesterol pool. The main difference in chemical composition between commercial feed mixture and experimental diets for rats with added egg yolks was in the contents of total fat and cholesterol (Table 1) which were higher in the experimental diets. Taking into consideration these differences, as well as differences in plasma lipid levels in experimental groups influenced by egg yolks of different composition in their diet (Hodzic, 2003), it is obvious that achieving a balance of dietary cholesterol with plasma cholesterol takes only a few days, but it takes a couple of weeks for a balance with tissue cholesterol (Srilatha *al.*, 1997).

The differences in the fatty-acid composition of rat's diets influenced the differences in concentrations and contents of cholesterol in the liver between experimental groups (Figures 5 and 6). Animals in group P, both males and females, had the highest accumulated cholesterol. High hepatic pool of cholesterol in this group may be partially related to high levels of total and LDL-cholesterol in the plasma of these rats (Hodzic, 2003), considering that the liver is the primary organ of its metabolism (Kutchai, 1996). Obvious difference between males and females was found in group L. In the males of this group there were no significant differences in concentration and content of total cholesterol in the liver comparing to control group (Figure 6), but these differences were very clear in females (Figure 5). Consumption of egg yolks enriched with n-3 polyunsaturated fatty acids not only reduced the pool of cholesterol in the body, but, more importantly, enriched total lipids and phospholipids in the liver with linoleic, eicosapentaenoic (C20:5n-3), docosapentaenoic (C22:5n-3) and docosaheptaenoic (C22:6n-3) acid (Jiang and Sim, 1992). The greatest enrichment of yolks with n-3 polyunsaturated fatty acids was achieved after supplementation of diet for laying hens with flax seeds and oil (Jiang and Sim, 1992) and with fish oils (Hargis *et al.*, 1991).

If eggs of group FO laying hens are considered as n-3 enriched eggs, then our findings regarding the content of linoleic acid in the total liver lipids of rats differ somewhat from the findings of Jiang and Sim (1992). The liver of males in

group F had a higher content of linoleic acid compared to groups C and L, but not in relation to group P which had the highest content of linoleic acid - even 13.66% (Table 4). Diet of group P had the lowest content of linoleic acid (Table 2). According to Farrell (1993), linoleic acid can interfere with the conversion α -linolenic acid (C18:3n-3) to eicosapentaenoic and docosahexaenoic acid and with the efficiency of incorporation of n-3 polyunsaturated fatty acids into the tissues. Differences in expression of conversion enzyme explain why the liver capacity to synthesize docosahexaenoic acid from circulating α -linolenic acid is increased by dietary deprivation of n-3 PUFA (Igarashi *et al.*, 2007). Furthermore, female rats replete their docosahexaenoic acid status more readily than males, probably due to a higher expression of liver desaturases (Extier *et al.*, 2010). Sex is an important determinant of the effect of variations in fat and fatty acid intake on long chain polyunsaturated fatty acids status (Childs *et al.*, 2010). Is it possible that a low content of linoleic acid in the diet was the main reason for the most efficient incorporation of n-3 polyunsaturated α -linolenic acid into the liver lipids in males of the group P?

In the analysis of fatty-acid composition of the total liver lipids it is also interesting to comment the content of saturated acids - palmitic and stearic, and unsaturated oleic acid. The highest total content of measured saturated fatty acids and lowest content of oleic acid in the liver was recorded in group C, with the exception of males in group L (Table 4). This finding can be correlated with the same trend, but of opposite direction, found for total lipids (Figures 1 and 2), triglycerides (Figures 3 and 4) and total cholesterol content (Figure 5 and 6) of the liver. This should mean that quantity and quality of fat in rats' diet influenced the lipogenic activity of the liver (Van Elswyk *et al.*, 1994) and fat metabolism. Greater amounts of total fat in the experimental diets and its higher saturation (Table 2) increased the deposition of less saturated fat in the liver (Table 4) in experimental groups. Part of saturated fatty acids in the liver are converted to oleic. The conversion of palmitic to oleic acid involves two stages - elongation and desaturation. Elongation can be relatively slow, while desaturation is a fast process. Stearic acid does not require elongation, and could be quickly converted into oleic, while palmitic acid can be accumulated in the tissues (Grundy and Denke, 1990).

Macroscopic examination of livers immediately after exenteration showed that they were enlarged in most rats, with rounded edges, lighter in color and with a yellow colored pearly infiltrate. These changes were observed in all rats in the experimental groups and in two females in the control group. Unlike males, in three females of each experimental group lesions on the skin were also observed.

Microscopic findings confirmed fatty infiltration of the liver in all experimental groups, with no visible degenerative changes, as a probable consequence of diets with high fat content (Table 1) derived from egg yolks. Long term feeding of rats with a diet supplemented with canola oil, lard and egg yolks increased the accumulation of fat in the liver, while reducing its sinusoids (Aguila *et al.*, 2003). Diets rich in fat increased accumulation of the total lipids in rat's liver (Figures 1 and 2), which includes increased accumulation of triglycerides (Figures 3 and 4) and cholesterol (Figures 5 and 6), similar to findings of Xie *et al.* (2010). However,

in the case of increased influx of nonesterified fatty acids and the metabolic blockage lipid components can accumulate in the liver causing its fatty infiltration (Beitz and Allen, 1984).

CONCLUSION

Supplementation of diets for rats with egg yolks significantly increased the hepatic pool of cholesterol, regardless of the type of fat in the diets for laying hens from which the eggs originate. It seems that the most responsible factors for this finding were a high total fat and high cholesterol content in the diets.

The diet for rats supplemented with egg yolks from laying hens fed diet with 3% palm olein had a low content of linoleic acid. It could be one of the reasons for the highly efficient incorporation of α -linolenic acid in the liver of these rats, concerning that dietary linoleic acid can interfere with the efficiency of incorporation of polyunsaturated fatty acids in tissues.

Liver lipids and their fatty-acid composition differ by both, sex and dietary fat intake in rats.

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REFERENCES

1. Aguila MB, Pinheiro AR, Parente LB, Mandarim-de-Lacerda CA, 2003, Dietary effect of different high-fat diet on rat liver stereology, *Liver Int*, 23, 363-70.
2. Ahn DU, Sunwoo HH, Wolfe FH, Sim JS, 1995, Effects of dietary α -linolenic acid and strain of hen on the fatty acid composition, storage stability, and flavor characteristics of chicken eggs, *Poult Sci*, 74, 1540-7.
3. Beitz DC, Allen RS, 1984, Lipid Metabolism, In: Swenson MJ, editor, *Dukes' Physiology of Domestic Animals*, London: Cornell University Press Ltd, 386-97.
4. Childs CE, Romeu-Nadal M, Burdge GC, Calder PC, 2010, The polyunsaturated fatty acid composition of hepatic and plasma lipids differ by both sex and dietary fat intake in rats, *J Nutr*, 140, 245-50.
5. Extier A, Langelier B, Perruchot MH, Guesnet P, Van Veldhoven PP, Lavielle M *et al.*, 2010, Gender affects liver desaturase expression in a rat model of n-3 fatty acid repletion, *J Nutr Biochem*, 21, 180-7.
6. Farrell DJ, 1993, Une's designer egg: recent advances, In: Farrell DJ, editor, *Recent advances in animal nutrition*, Armidale: University of New England, 291-302.
7. Folch J, Lees M, Stanley GHS, 1957, A simple method for the isolation and purification of total lipids from animal tissues, *J Biol Chem*, 226, 497-509.
8. Grundy SM, Denke MA, 1990, Dietary influences on serum lipids and lipoproteins, *J Lipid Res*, 31, 1149-72.
9. Hargis PS, Van Elswyk ME, Hargis BM, 1991, Dietary modification of yolk lipid with menhaden oil, *Poult Sci*, 70, 874-83.
10. Hodzic A, Hamamdžić M, Gagić A, Mihaljević M, Krnić J, Vegara M *et al.*, 2005, Egg yolk lipid modifications by fat supplemented diets of laying hens, *Acta Vet Beograd*, 55, 41-51.

11. Hodzic A, Hamamdžić M, Gagić A, Mihaljević M, Vegara M, Krnić J *et al.*, 2008, The influence of dietary palm olein, fish oil and lard on the egg yolk and plasma lipid composition, and performances of laying hens, *Pol J Vet Sci*, 11, 1-7.
12. Hodzic A, 2003, Plasma and parenchyma organs lipids in rats fed a diet supplemented with egg yolk, PhD thesis, Veterinary Faculty, University of Sarajevo, B&H.
13. Igarashi M, Ma K, Chang L, Bell JM, Rapoport SI, 2007, Dietary n-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not brain, *J Lipid Res*, 48, 2463-70.
14. Jiang Z, Sim JS, 1991, Research note: effect of feeding egg yolk powder on the plasma and tissue cholesterol levels in rats, *Poult Sci*, 70, 401-3.
15. Jiang Z, Sim JS, 1992, Effect of dietary n-3 fatty acid-enriched chicken eggs on plasma and tissue cholesterol and fatty acid composition of rats, *Lipids*, 27, 279-84.
16. Kutchai HC, 1996, Gastrointestinal System, In: Berne RM, Levy MN, editors, Principles of Physiology, St. Louis: Mosby-Year Book Inc, 437-500.
17. Lewis NM, Seburg S, Flanagan NL, 2000, Enriched eggs as a source of n-3 polyunsaturated fatty acids for humans, *Poult Sci*, 79, 971-4.
18. Spady DK, Woollett LA, Dietschy JM, 1993, Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids, *Annu Rev Nutr*, 13, 355-81.
19. Sripatha B, Adayan PG, Ng SC, Aulkumarar S, 1997, Effects of feeding egg yolk on the serum lipid levels in rabbits, *Meth Find Exp Clin Pharmacol*, 19, 489-92.
20. Van Elswyk ME, Hargis BM, Williams JD, Hargis PS, 1994, Dietary menhaden oil contributes to hepatic lipidosis in laying hens, *Poult Sci*, 73, 653-62.
21. Xie Z, Li H, Wang K, Lin J, Wang Q, Zhao G, *et al.*, 2010, Analysis of transcriptome and metabolome profiles alterations in fatty liver induced by high-fat diet in rat, *Metabolism*, 59, 554-60.

LIPIDI JETRE PACOVA U USLOVIMA ISHRANE SA DODATKOM KOKOŠIJEG ŽUMANJKA MODIFIKOVANOG SASTAVA

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SADRŽAJ

Cilj ovog rada je bio ispitati kakav uticaj na sadržaj lipida i njihovu masno-kiselinsku kompoziciju u jetri pacova ima ishrana sa dodatkom kokošijeg žumanjka modifikovanog sastava.

Tokom četiri nedjelje eksperimenta 64 Wistar pacova, podijeljenih u četiri grupe od po 16 jedinki (po osam jedinki oba spola) hranjeno je komercijalnom hranom za pacove (grupa C) ili hranom koja je sadržavala 70% komercijalne smješe za pacove i 30% svježeg kuhanog žumanjka poreklom od jaja koka nosilja hranjenih sa 3% ribljeg ulja (grupa F), 3% palminog oleina (grupa P) ili 3% svinjske masti (grupa L).

Suplementacija hrane za pacove kokošijim žumanjcima značajno je povećala hepatični „pool“ holesterola, neovisno o vrsti masti u hrani koka nosilja od kojih žumanjak potiče. Sadržaj α -linolenske kiseline u jetri mužjaka pacova grupe P bio je 4-6 puta veći u usporedbi sa mužjacima drugih grupa. Lipidi jetre kod pacova i njihova masno-kiselinska kompozicija zavise kako od spola, tako i od kokošijeg žumanjka modifikovanog sastava dodanog u hranu.