Acta Veterinaria (Beograd), Vol. 62, No. 5-6, 641-652, 2012.

DOI: 10.2298/AVB1206641H UDK 577.115:612.82:599.1.3/8.4.:637.424

BRAIN LIPIDS IN RATS FED A DIET SUPPLEMENTED WITH HEN EGGS OF MODIFIED LIPID CONTENT

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(Received 22nd August 2012)

The aim of this study was to research the impact of a diet supplemented with egg yolks of modified content, having in mind the type of fat added to the laying hens diet, on the brain lipids and their fatty acid composition in rats.

During four weeks of the experiment, 64 Wistar rats, divided into four groups of 16 animals each (eight animals of both sexes), were fed the commercial rat feed (group C), or the feed that contained 70% of the commercial rat feed and 30% of freshly boiled yolks from the eggs originating from laying hens fed with 3% fish oil (group F), 3% palm olein (group P) or 3% lard (group L). Concentration and content of total lipids and total cholesterol, as well as the fatty-acid composition of the total brain lipids were determined in the lipid extracts of the rats brains.

Under unfavourable conditions, which in our case could be high dietary intake of the total fat due to egg yolk addition, the amount of total fat in the brain tissue or the mass of the organ itself can be changed. Applied dietary treatments could also influence the level of de novo synthesis of total cholesterol in the rat brain. High dietary fat intake, as well as the fat quality regarding its fatty acid composition, appear to be able to significantly influence the fatty acid profile of the total brain lipids in adult rats, whereas the level and quality of the changes also depend on sex.

Key words: brain lipids, diet, egg yolk, fatty-acid composition, rat

INTRODUCTION

The main structural brain material are lipids (60%) which have a unique profile of the long-chain polyunsaturated fatty acids of the essential fatty acids group (Crawford, 1993). This profile remains stable in all species regardless of the testing conditions, so that size rather than composition is influenced under unfavourable conditions (Crawford *et al*., 1976). Such findings point to the conclusion that providing these fatty acids is a limiting factor for the brain development and evolution (Crawford and Marsh, 1989).

Brain lipids are mostly complex polar phospholipids, sphingolipids, gangliosides and cholesterol which are involved in the structure and functioning of the brain cell membranes (Sinclair *et al*., 2007). Phosphoglycerides are rich in long-chain fatty acids, primarily arachidonic and docosahexaenoic ones, but not in the essential fatty acids from which they originate. For example, in rats long-chain fatty acids with 20-22 C atoms are incorporated in the developing brain 10 times more efficiently than the basic essential fatty acids (Sinclair, 1975). Most experts agree that both n-6 and n-3 polyunsaturated fatty acids are necessary in the diet. It is also clear that dietary provision of the essential fatty acids is limiting for the brain development (Crawford, 1992).

Normal brain and retina development requires α-linolenic acid and its metabolite with the longer chain docosahexaenoic acid (Crawford, 1992; Connor *et al*., 1992; Heird, 2001). Research has confirmed the link between dietary docosahexaenoic acid and the optimal brain and retina development, especially in prematurely born low birth weight babies (Carlson and Salem, 1991; Wilbert *et al*., 1997).

Brain requires arachidonic and docosahexaenoic acids for growth, functional activity and integrity maintenance (Neuringer *et al*., 1988; Bazan, 1989). Both of these acids are constituents of the mother's milk. Adding the long-chained polyunsaturated fatty acids to milk replacements is useful only if the use of supplements is safe since all the sources of long-chain polyunsaturated fatty acids do not have the same biological value (Amate *et al*., 2001; Childs *et al*., 2011). Some experiments offered the reasons for consensus on the safety of oils originating from single-cell oils, low eicosapentaenoic acid fish oils and phospholipids and triglyceride fractions of the egg yolk as the source of the long-chain polyunsaturated fatty acids in milk replacements (Heird, 2001).

Experimental studies of animals showed that the lack of n-3 polyunsaturated docosahexaenoic acid in the brain was linked to memory loss and diminished cognitive function (Petursdottir *et al*., 2008). The diet lacking n-3 polyunsaturated fatty acids leads to disorders, the most of which can be remedied by the introduction of n-3 polyunsaturated fatty acids in the diet (Sinclair *et al*., 2007).

Egg yolk and cerebral phospholipids were the efficient source of n-3 polyunsaturated fatty acids in reversible behavioural changes (Carrie *et al*., 2000a) and changed fatty acid composition caused by the diet deficient in n-3 polyunsaturated fatty acids in mice (Carrie *et al*., 2000b). For the reasons listed above, the last 15 years are marked with a heightened interest in treating neurophysiologic disorders (depression and schizophrenia) with n-3 polyunsaturated fatty acids (Sinclair *et al*., 2007). In this sense, aim of this study was to examine how is the lipid content in the rat brain influenced by the diet with added yolk of modified composition, due to the type of fat added to the laying hens' diet.

MATERIAL AND METHODS

A preliminary study, which lasted six weeks, was carried out to produce egg yolks of certain quality under defined conditions of laying hens feeding regimen.

Ninety Lohman Brown laying hens in the 34th week of production and at 56 weeks of age were used in the study. The hens were kept under standard conditions for commercial 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, Bremmen, Germany), group PO 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 the 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 a previously published 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 start of the experiment.

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 the experimental housing conditions, rats were weighted and randomly allocated to four groups (C, F, P and L) with 16 individuals in each (eight of each sex).

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

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

The composition of diets for rats and its fatty-acid compositions are given in Tables 1 and 2. Experimental diets differed in comparison to the control primarily in the higher total fat content as a result of adding cooked egg yolks in a quantity of 30%. This increase was mostly on behalf of nitrogen free extract (NFE) and fibres. Regarding the fatty-acid composition of total lipids, the experimental diets also contained more saturated fatty acids – stearic and palmitic, but the content of linoleic acid (C18:2n-6) was much lower in relation to the diet of control rats (Table 2).

Table 1. Chemical composition 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
Fat	5.87	15.59	15.74	15.88
Fiber	9.16	6.68	7.66	8.00
Ash	7.54	6.68	6.67	6.68
NFE	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 groups of rats according to feeding treatments: 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 the total lipids in 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 groups of rats according to feeding treatments: 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)

After 28 days of experimental feeding, the rats were subjected to 12-hour fasting. The next day the animals were weighed, and then their blood was taken from the abdominal aorta under mild anesthesia with diethyl-ether. Blood samples were collected in vacutainers of 3 mL with EDTA as an anticoagulant, and then exenteration was performed.

Individual brain samples were homogenized, and then a 1 g sample was added to 19 mL of chloroform/methanol mixture in a ratio 2:1 (Folch *et al*., 1957) and the samples were covered with double tin foil and kept at -18^{o}C until later processing. The homogenizate remains were also stored at -18^{o}C as pooled samples (all animals of one group) for the determination of fatty-acid composition

of total brain lipids, which was performed with the procedure previously described for feed samples feeds for laying hens (Hodzic *et al*., 2005).

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

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

RESULTS

Table 3 contains the results of the initial body mass, its increase and food consumption in rats.

Table 3. Initial body weight, weight gain and feed consumption in rats

Sex	Group	Initial body weight (g)	Body weight gain (g/rat/4 wk)	Feed consumption (g/rat/4 wk)
Females	C*	171.25 ± 5.49^{a}	19.38 ± 4.77^{a}	442.99 ± 11.46^{c}
	F	168.75 ± 7.18^{a}	25.63 ± 6.23^{a}	333.75 ± 6.75^{a}
	P	183.75 ± 7.30^{a}	35.00 ± 4.72^{a}	365.62 ± 18.93^{ab}
	L	184.38 ± 4.17^{a}	19.38 ± 4.38^{a}	377.26 ± 7.55^{b}
Males	C	$234.38\pm6.30^{a*}$	$91.25\pm6.93^{b*}$	$598.54\pm14.39^{c*}$
	F	$244.38\pm3.83^{a*}$	$72.50\pm3.13^{a*}$	$501.22\pm7.82^{b*}$
	P	$246.25\pm5.96^{a*}$	$108.75\pm2.80^{c*}$	$467.06\pm8.70^{ab*}$
	L	$247.50\pm5.35^{a*}$	$78.75\pm7.95^{ab*}$	$492.83\pm10.22^{b*}$

All values represent mean±SE (n=8). C, F, P and L represent groups of rats, females and males, according to the dietary treatments: control group, groups with 30% added egg yolk from the laying hens fed by fish oil, palm olein and lard respectively. a,b,c = values of the same sex and in the same column with the different letter in the superscript are significantly different ($p<0.05$). * = significant difference ($p<0.05$) between the same groups of the opposite sexes. SE = standard error of the mean.

Animals of the same sex in different groups showed no significant difference in body mass in the beginning of the experiment. However, there was an evident and understandable difference between the individuals in the same group but of the opposite gender. Also, there was a difference in female and male behaviour regarding body mass increase expressed in g/rat/4 weeks (Table 3). Significantly different feed consumption between the groups did not result in significant differences in body mass increase in females. The highest food consumption was in individuals in the control group, and the highest increase of body mass was found in group P. This finding was valid for both sexes.

Table 4. Rats organs mass after experimental feeding

Sex	Group	Liver	Brain	Ovaries/ testicles (g/100 g)	Adrenal body mass	Heart	Kidneys	Lungs
Females	C*	2.915±0.127^{a}	0.975±0.011^{bc}	0.071±0.003^{a}	0.044±0.002^{a}	0.311±0.010^{a}	0.600±0.016^{a}	0.598±0.015^{ab}
	F	2.994±0.082^{a}	1.018±0.020^{c}	0.076±0.003^{a}	0.044±0.002^{a}	0.301±0.009^{a}	0.591±0.024^{a}	0.635±0.018^{b}
	P	2.854±0.042^{a}	0.898±0.031^{a}	0.068±0.002^{a}	0.041±0.001^{a}	0.290±0.006^{a}	0.573±0.008^{a}	0.593±0.014^{a}
	L	2.825±0.058^{a}	0.935±0.013^{ab}	0.071±0.002^{a}	0.044±0.002^{a}	0.295±0.005^{a}	0.594±0.008^{a}	0.591±0.009^{a}
Males	C	2.345±0.030^{a*}	0.654±0.019^{b*}	1.373±0.031^{b}	0.023±0.002^{a*}	0.279±0.009^{b*}	0.579±0.012^{c}	0.474±0.016^{a*}
	F	2.498±0.048^{ab*}	0.581±0.006^{a*}	1.206±0.029^{a}	0.020±0.000^{a*}	0.256±0.005^{a*}	0.506±0.006^{a*}	0.476±0.011^{a*}
	P	2.620±0.045^{b*}	0.646±0.015^{b*}	1.381±0.042^{b}	0.020±0.000^{a*}	0.266±0.003^{ab*}	0.559±0.014^{bc}	0.469±0.010^{a*}
	L	2.415±0.029^{a*}	0.633±0.016^{b*}	1.261±0.026^{a}	0.020±0.000^{a*}	0.265±0.006^{ab*}	0.528±0.010^{ab*}	0.461±0.012^{a*}

Allvalues represent mean±SE (n=8). ¨C, F, P and L represent groups of rats, females and males, according to the dietary treatments: control group, groups with 30% added egg yolk from the laying hens fed by fish oil, palm olein and lard respectively. a,b,c = values of the same sex and in the same column with the different letter in the superscript are significantly different ($p<0.05$). * = significant difference ($p<0.05$) between the same groups of the opposite sexes. SE = standard error of the mean.

Dietary treatments significantly influenced only the mass of the brain and lungs in females, while in males they influenced the mass of all organs, except the adrenal gland and lungs (Table 4).

Dietary treatments did not significantly influence the concentration and content of total lipids in the brain of females (Figure 1); while in males, they influenced only the content of total lipids (Figure 2).

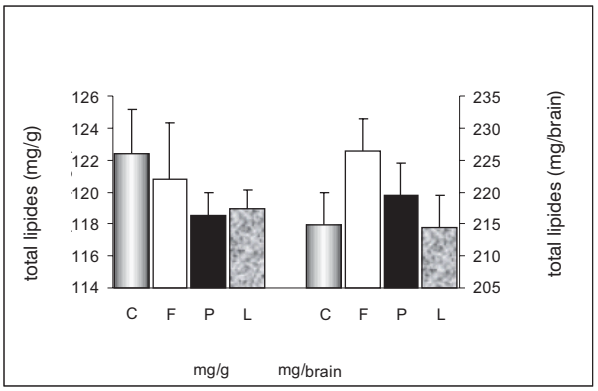

Figure 1. Concentration and content (mean±SE) of total lipids in the brain of female rats. C, F, P and L represent groups of rats, according to dietary treatments: control group, groups with 30% added egg yolk from laying hens fed by fish oil, palm olein and lard respectively. SE = standard error of the mean

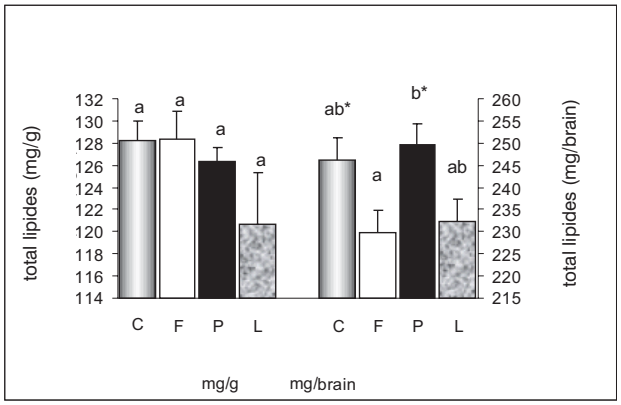

Figure 2. Concentration and content (mean±SE) of total lipids in the brain of male rats. C, F, P and L represent groups of rats, according to dietary treatments: control group, groups with 30% added egg yolk from laying hens fed fish oil, palm olein and lard respectively. a,b = values of the same series with a different letter are significantly different ($p<0.05$). * = significant difference ($p<0.05$) compared to the females of the same group (Figure 1). SE = standard error of the mean

Regarding total cholesterol, different responses of females (Figure 3) and males (Figure 4) under the influence of the same dietary treatments were

recorded. Namely, the highest concentration of total brain cholesterol was found in the control group females, while it was not the case with the males. Moreover, cross-relations of various dietary treatments differed for males as well, regarding the content of total brain cholesterol.

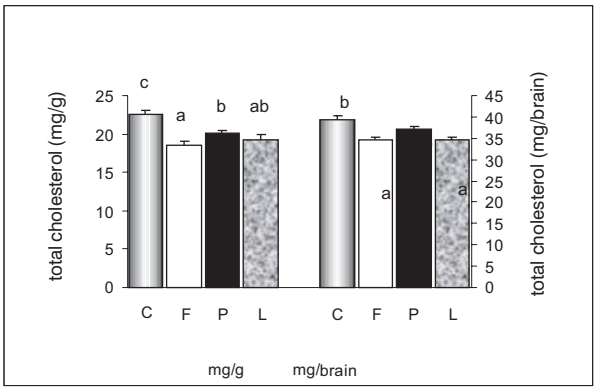

Figure 3. Concentration and content (mean±SE) of total cholesterol in the brain of female rats (n=8). C, F, P and L represent groups of rats, according to dietary treatments: control group, groups with 30% added egg yolk from laying hens fed fish oil, palm olein and lard respectively. a,b,c = values of the same series with a different letter are significantly different ($p<0.05$). SE = standard error of the mean

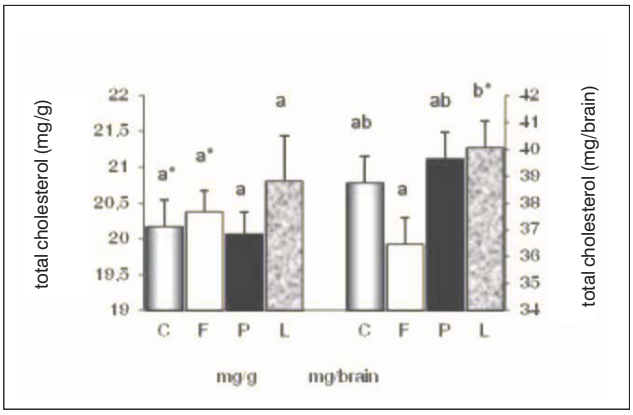

Figure 4. Concentration and content (mean±SE) of the total cholesterol in the brain of the male rats (n=8). C, F, P and L represent groups of rats, according to dietary treatments: control group, groups with 30% added egg yolk from the laying hens fed fish oil, palm olein and lard respectively. a,b = values of the same series with a different letter 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

Table 5 shows the fatty acid composition of brain lipids by groups and gender, determined in pooled samples from all individuals of the same group. In females of the experimental groups, a higher presence of palmitic, stearic and oleic acid was found in the brain lipids compared to the control group. Group P showed the highest values. In males, there was neither a consistent distinction between the experimental groups on one side and the control group on the other nor between individual experimental groups.

Table 5. Fatty acid composition of the total brain lipids in rats

Fatty acid (%)	Females				Males			
	C	F	P	L	C	F	P	L
C16:0	6.00	15.73	20.96	13.27	18.79	9.57	15.51	19.31
C16:1	1.61	10.78	1.41	5.34	4.04	0.64	4.86	3.41
C18:0	11.03	18.97	24.76	16.87	21.96	8.78	16.11	21.02
C18:1	11.35	21.47	27.76	21.88	22.06	9.26	21.19	28.53
C18:2n-6	5.64	4.13	4.12	4.47	4.58	4.26	4.72	7.59
Σ determining of f.a.	35.63	71.08	79.01	61.83	71.43	32.51	62.39	79.86

*C, F, P and L represent groups of rats, females and males, according to the dietary treatments: control group, groups with 30% added egg yolk from the laying hens fed by fish oil, palm olein and lard respectively

DISCUSSION

In our research, dietary treatments had no significant influence on the level of total lipids as the main structural brain material (Figures 1 and 2), although quite high individual variations were noticed, especially in females (Figure 1). In males, important differences were recorded only in content (mg/brain) of the total lipids between F and P group (Figure 2), as a possible confirmation of the thesis that in unfavourable conditions, which in our case could be high dietary intake of total fat due to egg yolk addition, the amount of total fat in the brain tissue (Crawford *et al*., 1976) or the mass of the organ itself can be changed. Namely, in females, statistically significant differences in the brain mass were established, expressed in g/100 g of body mass, between all the groups, and in males, statistically significant lower brain mass, compared to all three remaining groups, were in individuals in group F (Table 4). Moreover, the brain was the only exentered organ in females the mass of which was significantly influenced by the dietary treatment, except the lung mass but just in group F. However, the situation was different for male rats as dietary treatment had no significant influence only on the mass of the adrenal glands and of the lungs (Table 4). Statistical significance of differences between organ mass between the same groups, and of different sex is a consequence of statistically significant differences in their body mass (Table 3).

Cholesterol metabolism in the developed brain is extremely slow (Lütjohann *et al*., 1996). Practically the entire cholesterol, present in the developing or developed brain, comes from *de novo* synthesis, so that the animal brain does not use LDL-cholesterol (Turley *et al*., 1996). According to our results and ascertained differences between the groups (Figures 3 and 4), dietary sources could influence the level of its synthesis after 28 days of consumption, but differently in males and females.

Brain lipids are cholesterol and phosphoglicerides rich in long-chain fatty acids, primarily arachidonic and docosahexaenoic ones (Crawford, 1992). Docosahexaenoic acid is one of the most represented components of the structural brain lipids. Like eicosapentaenoic acid it can come directly only from food or by synthesis from eicosapentaenoic and linolenic acids also from food (Simopoulos, 1999). Having in mind the above statements, as well as the importance of docosahexaenoic acid for the nervous tissue and retina, it is clear that any meaningful discussion on the brain lipids implies analysis of changes in the content of arachidonic and especially docosahexaenoic acid which are incorporated in the phospholipids of the brain in adult animals (Petursdottir *et al*., 2008). Due to the impossibility of determining the content of polyunsaturated fatty acids, there will be no discussion on the dietary influence of egg yolks of different quality on the content of essential long-chain polyunsaturated fatty acids of brain lipids. In the framework of measured fatty acids, more visible differences were noted, compared to the other groups, i.e. in the control group females and F group males (Table 5). Primarily, it pertains to the total percentage of the measured fatty acids which was almost half in the said groups, compared to the other groups, determined inside the same sex. This, on one hand, raises the question which fatty acids represent the remaining part of the 100%, and, on the other hand, differences between females and males are evident in the incorporation of certain fatty acids in the brain tissue lipids, which is, also, influenced by the diet (Rapoport, 2008; Childs *et al*., 2010).

CONCLUSION

Under unfavourable conditions, which in our case could be a high dietary intake of total fat due to egg yolk addition, the amount of total fat in the brain tissue or the mass of the organ itself can be changed. The applied dietary treatments could also influence the level of *de novo* synthesis of total cholesterol in the rat brain. High dietary fat intake, as well as the fat quality regarding its fatty-acid composition, appear to be able to significantly influence the fatty-acid profile of total brain lipids in adult rats, whereas the level and quality of the changes also depend on the sex. However, for a more purposeful analysis of the possibility of dietary modification of total brain lipids composition, it is necessary to determine the long-chain polyunsaturated fatty acids, especially arachidonic and docosahexaenoic ones.

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LIPIDI MOZGA PACOVA U USLOVIMA ISHRANE SA DODATKOM KOKOŠIJEG ŽUMANJKA MODIFIKOVANOG LIPIDNOG SASTAVA

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SADRŽAJ

Cilj ovog rada je bio ispitati kakav uticaj na sadržaj lipida u mozgu pacova kao sisara i njihovu masno-kiselinsku kompoziciju ima ishrana sa dodatkom žumanjka modifikovanog sastava, obzirom na vrstu dodane masti u hranu koka nosilja od kojih isti potiče.

Tokom četiri nedjelje eksperimenta 64 Wistar pacova, podeljeni u četiri grupe od po 16 jedinki (po osam jedinki oba spola), hranjeni su komercijalnom hranom za pacove (grupa C) ili hranom koja je sadržavala 70% komercijalne smješe za pacove i 30% svježe kuhanog žumanjka porijeklom od jaja koka nosilja hranjenih sa 3% ribljeg ulja (grupa F), 3% palminog oleina (grupa P) ili 3% svinjske masti (grupa L). U lipidnim ekstraktima mozga pacova određivani su koncentracija i sadržaj ukupnih lipida i ukupnog holesterola, te masno-kiselinska kompozicija ukupnih lipida mozga.

U nepovoljnim uslovima, u našem slučaju bi to mogao biti visok dietarni unos ukupne masti dodavanjem kokošijeg žumanjka, može da se menja količina ukupne masti u moždanom tkivu ili pak masa samog organa. Korišteni dietarni tretmani bi mogli uticati i na nivo *de novo* sinteze ukupnog holesterola u mozgu pacova. Visok unos dietarne masti, kao i njen kvalitet u pogledu masno-kiselinske kompozicije, čini se mogu značajno uticati na masno-kiselinski profil ukupnih lipida mozga kod odraslih pacova, pri čemu nivo i kvalitet tih promjena zavise i od spola.