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COMPARATIVE ASSESSMENT OF THE EFFECT OF CONSUMPTION OF VEGETABLE OILS WITH DIFFERENT FATTY ACID COMPOSITION ON LIPOPEROXIDATION AND THE DEVELOPMENT OF LIVER STEATOSIS

Actuality. *Liver steatosis is an extremely common pathological condition caused by the excessive accumulation of fat in this organ, which leads to the activation of lipoperoxidation and, as a result, to the development of steatohepatitis, which often ends in liver cirrhosis.*

Aim of work. *To investigate the effect of consumption of vegetable oils with different fatty acid composition on lipoperoxidation processes and the development of steatosis in rats.*

Research and methods. *They used ordinary (high-linoleic) sunflower oil (HLSO), high-oleic sunflower oil "Olyvka" (HOSO), palm oil and coconut oil. Rats were fed a fat-free diet (FFD) or a high-fat diet (HFD) containing 15% fat. Lipoperoxidation in the liver was evaluated by the content of malondialdehyde (MDA) and by the level of the antioxidant-prooxidant index API (the ratio of catalase activity and MDA content). Liver steatosis was determined by the amount of triglycerides and cholesterol esters. Dysbiosis was caused by the antibiotic lincomycin. As an anti-dysbiotic agent, the drug "Kvertulin" (quercetin + inulin + calcium citrate) was used.*

Results. *It was established that the consumption of HLSO or palm oil increases the increase in live weight of rats, in contrast to the consumption of coconut oil and HOSO. The consumption of HOSO does not increase the level of lipoperoxidation in the liver, in contrast to HLSO and palm oil, which significantly increase it. A high-fat diet increases the content of fat in the liver, especially when consumed against the background of dysbiosis. The use of the drug "Kvertulin" prevents the development of steatosis. Consumption of FFD after long-term consumption of palm oil normalizes the content of fat in the liver.*

Conclusions. *High-fat diets with the use of ordinary sunflower or palm oil cause steatosis of the liver and activation of lipoperoxidation, especially against the background of dysbiosis. The use of an anti-dysbiotic agent or a fat-free diet prevent the development of steatosis and lipoperoxidation in the liver.*

Key words: *fatty diet, disease, steatosis, lipoperoxidation, antidysbiotic agents.*

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ПОРІВНЯЛЬНА ОЦІНКА ДІЇ НА ЛІПОПЕРОКСИДАЦІЮ ТА РОЗВИТОК СТЕАТОЗУ ПЕЧІНКИ СПОЖИВАННЯ РОСЛИННИХ ОЛІЙ ІЗ РІЗНИМ ЖИРНОКИСЛОТНИМ СКЛАДОМ

Актуальність. *Стеатоз печінки – надзвичайно поширений патологічний стан, зумовлений надмірним накопиченням жиру у цьому органі, що призводить до активації ліпопероксидації і, як наслідок, до розвитку стеатогепатиту, який часто завершується цирозом печінки.*

Мета дослідження. *Дослідити вплив споживання рослинних олій із різним жирнокислотним складом на процеси ліпопероксидації і розвиток стеатозу печінки у щурів.*

Матеріали та методи. *Використовували звичайну (високолінолеву) соняшникову олію (ВЛСО), високоолеїнову соняшникову олію «Оливка» (ВОСО), пальмову олію і кокосову олію. Щурі отримували безжировий раціон (БЖР) або високожирові раціони (ВЖР) з умістом 15% жиру. Ліпопероксидацію у печінці оцінювали за вмістом малонового діальдегіду (МДА) та за рівнем антиоксидантно-прооксидантного індексу АПІ (співвідношення активності каталази і вмісту МДА). Стеатоз печінки визначали за кількістю тригліцеридів та ефірів холестерину. Дисбіоз викликали за допомогою антибіотика лінкоміцина. Як антидисбіотичний засіб використовували препарат «Квертулін» (кверцетин + інулін + цитрат кальцію).*

Результати дослідження. *Установлено, що споживання ВЛСО або пальмової олії збільшує приріст живої маси щурів на відміну від споживання кокосової олії і ВОСО. Споживання ВОСО не збільшує рівень ліпопероксидації у печінці на відміну від ВЛСО і пальмової олії, які його значно збільшують. Високожирове харчування збільшує вміст жиру в печінці, особливо під час споживання на тлі дисбіозу. Застосування препарату «Квертулін» попереджає розвиток стеатозу. Споживання БЖР після тривалого споживання пальмової олії нормалізує вміст жиру в печінці.*

Висновок. *Високожирові раціони з використанням звичайної соняшникової або пальмової олій викликають стеатоз печінки та активізацію ліпопероксидації, особливо на тлі дисбіозу. Застосування антидисбіотичного засобу або безжирове харчування попереджають розвиток стеатозу і ліпопероксидації у печінці.*

Ключові слова: *жирове харчування, печінка, стеатоз, ліпопероксидація, антидисбіотичні засоби.*

Introduction. The basis of the fat diet of the population of Ukraine is the consumption of vegetable oils, in particular ordinary (high-linoleic) sunflower oil (HLSO).

Recently, the consumption of palm oil, which contains more than 40 % palmitic acid ($C_{16:0}$) is increasing.

The consumption of new high-oleic sunflower oil is increasing every year, which differs from HLSO in its high content of oleic acid ($C_{18:1}$, ω-9), the amount of which is more than 75 % (Khodakov, 2019). High-oleic sunflower oil (HOSO) is similar to olive oil, but differs in that it contains significantly less palmitic acid.

There are data on the negative impact of linoleic (Pokotylo, 2007) and palmitic (Yuzefovych, Solodushko, Wilson, Rachek, 2012) acids on the body.

We have shown the negative effect of linoleic acid on the endogenous biosynthesis of ω-3 polyunsaturated fatty acids (PUFA) (Levitsky, Khodakov, Levchenko, 2015), while oleic acid significantly activates it (Levitsky, Velichko, Selivanska et al., 2022).

It has been established that excessive consumption of fats causes the activation of free radical oxidation of unsaturated fatty acids with the formation of toxic peroxidation products (Levitsky, Egorov, Lapinskaya et al., 2020).

The aim of this work was the study of the influence of the consumption of various vegetable oils on the state of lipoperoxidation and the development of steatosis in the liver of rats receiving high-fat diets. The state of lipoperoxidation was assessed by the content of the final product of peroxidation of fatty acids - malondialdehyde (MDA), and the level of antioxidant protection was determined by the activity of the enzyme catalase and the API index (antioxidant-prooxidant index).

Materials and research methods. The following vegetable edible fats were used in the work:

− unrefined, frozen, pressed sunflower oil (producer "Smak sun oil " "V.V. Marchenko", Ukraine ;

− high-oleic sunflower oil "Olyvka", TS 10.4-37420386-007:2023 (producer LLC "Biohimtech", Ukraine

− palm oil producer «Dukees RBD» (Malaysia);

− coconut oil brand «Bees» (producer PGFO Edible Oils SDN BHD, Malaysia).

The fatty acid composition of these fats was determined by the gas chromatography method (Khodakov, 2019). The results of the determination of fatty acids are presented in Table 1. From these data, it can be seen that the main fatty acid of ordinary sunflower oil is linoleic acid ($C_{18:2}$), high-oleic sunflower oil "Olyvka" contains 88 % of oleic acid ($C_{18:1}$), in palm oil approximately equally contains palmitic ($C_{16:0}$) and oleic acids, while coconut oil contains the most lauric acid ($C_{12:0}$) and almost no unsaturated fatty acids.

Experimental studies of the influence of the consumption of various fats on the state of the body and, in particular, the liver, were conducted in 2 series. In the first series, experiments with nutrition were carried out on white rats of the Wistar line (males, 8-9 months, live weight 240-260 g), which were divided into 5 equal groups of 6 heads in each. The 1st group received a standard balanced diet with a content of 5 % feed fat (almost 90 % consists of linoleic, oleic and palmitic acids). Groups 2-a-5-a received high-fat diets (HFD), in which 15 % of the grain component was replaced by the corresponding amount of the studied fat: the 2nd group - sunflower oil, the 3rd - high-oleic sunflower oil, the 4th - palm oil and 5th – coconut oil. The duration of feeding was 64 days.

Before euthanasia, rats under thiopental anesthesia (20 mg/kg) received blood from *v. porta* and *v. cava*

Table 1

Fatty acid composition of used vegetable food fats (% of total fatty acids)

Fatty acid	Short formula	Sunflower oil	High oleic sunflower oil	Palm oil	Coconut oil
Caprylic acid	$C_{8:0}$	0	0	0	2,00
Capric acid	$C_{10:0}$	0	0	0	3,02
Lauric acid	$C_{12:0}$	0	0	0,19	46,57
Myristic acid	$C_{14:0}$	0,15	0,03	1,16	22,70
Palmitic acid	$C_{16:0}$	9,74	4,44	42,02	11,67
Stearic acid	$C_{18:0}$	3,90	3,07	4,87	13,60
Oleic acid	$C_{18:1}$	30,60	88,06	40,93	0,30
Linoleic acid	$C_{18:2}$	53,46	1,21	9,49	0,02
Linolenic acid	$C_{18:3}$	0,03	0,11	0,17	0
Arachinic acid	$C_{20:3}$	0,20	0,27	0,47	0,12
Eicosenoic acid	$C_{20:1}$	0,22	0,16	0,16	0
Arachidonic acid	$C_{20:4}$	0	0	0	0
Behenic acid	$C_{22:0}$	0,72	1,07	0,13	0
Lignoceric acid	$C_{24:0}$	0,25	0,81	0,10	0

inferior, and then total bleeding from the heart was carried out. The liver was isolated and blood serum was obtained.

In blood serum and liver homogenate, MDA content was determined by the thiobarbiturate method (Levitsky, Makarenko, Demyanenko, 2018) and catalase activity (Levitsky, Makarenko, Demyanenko, 2018). Based on the ratio of catalase activity and MDA content, the antioxidant-prooxidant index of API was calculated according to the formula (Levitsky, Makarenko, Demyanenko, 2018):

$$API = \frac{A_{cat} \cdot 10}{C_{MDA}}$$

In the second series of experiments on white rats of the Wistar line, the effect of consumption of high-oleic sunflower oil "Olyvka" and palm oil on the content of fat in the liver of rats was determined. There were a total of 6 groups of rats (males, 8 months old) with 6 heads in each. For 35 days, the 1st group received a fat-free diet (FFD), the composition of which is presented in Table 2. The 2nd group received a diet with "Olyvka" (15 % instead of 15 % starch), the 3rd group received a diet with 15 % palm oil, the 4th group received a diet with 15 % palm oil against the background of experimental dysbiosis, which was induced in rats using lincomycin (60 mg/kg with drinking water during the first 5 days of the experiment (Levitsky, 2019), the 5th group received 15 % palm oil oil on the background of dysbiosis, but for 25 days, and from day 26 received FFD. The 6th group received a diet with 15 % palm oil on the background of dysbiosis for 35 days and from the first day of the experiment with feed received the anti-dysbiotic agent "Kvertulin » in a dose of 300 mg/kg (Levitsky, Makarenko, Selivanskaya et al., 2012).

Table 2

The composition of fat-free (FFD) and fat rations of rats (%)

Component	FFD	Fat diet
Corn starch	65	50
Defatted soybean meal	20	20
Ovalbumin	6	6
Sugar	4	4
Mineral mixture	4	4
Vitamin mixture	1	1
Oil	0	15

After euthanasia of the animals, neutral lipids (triglycerides + cholesterol esters) were extracted from the liver (Khodakov, Tkachuk, Velichko, Levitsky, 2017) and their content was determined.

The results of the experiments were subjected to standard statistical processing (Truhacheva, 2012).

Results and discussion. In fig. 1 presents the results of determining the increase in the live weight of rats for 64 days of feeding HFD. From these data, it can be seen that the consumption of ordinary sunflower oil or palm oil caused an increase in live weight gain by 2 and 2.5 times, respectively. Consumption of coconut oil had little effect on body weight gain and consumption of high-oleic sunflower oil had no effect at all.

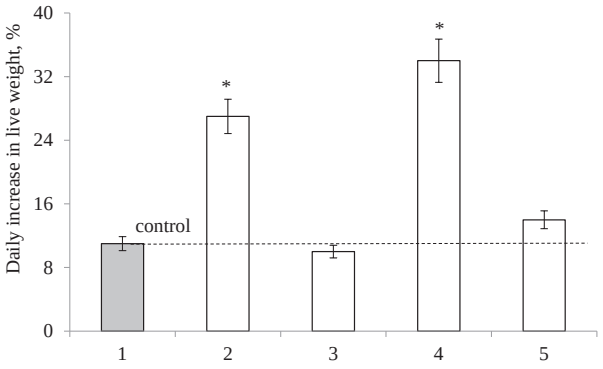

Fig. 1. The effect of a HFD on the relative increase in live weight of rats in 64 days: 1 – control, 2 – sunflower oil, 3 – high-oleic sunflower oil, 4 – palm oil, 5 – coconut oil

Table 3 presents the results of determining the organ index of the liver in rats treated with HFD. Consumption of ordinary sunflower oil and palm oil significantly increases the organ index of the liver. However, coconut oil and high-oleic sunflower oil did not affect the organ index of the liver.

Table 3

The effect of HFD on the organ index of the liver of rats

№№	Dietary fat	Organ index of the liver, g/kg
1	Control	31,7±2,9
2	Sunflower oil	39,3±2,3 p<0,05
3	High oleic sunflower oil	35,9±4,1 p>0,3
4	Palm oil	42,8±3,1 p<0,05
5	Coconut oil	32,7±2,8 p>0,5

In fig. 2 presents the results of determination of the influence of HFD on the content of MDA in the liver of rats. The content of MDA in the liver of rats that received HFD, especially with palm oil, increased to the greatest

extent. Only HFD with high-oleic sunflower oil does not increase MDA content in the liver.

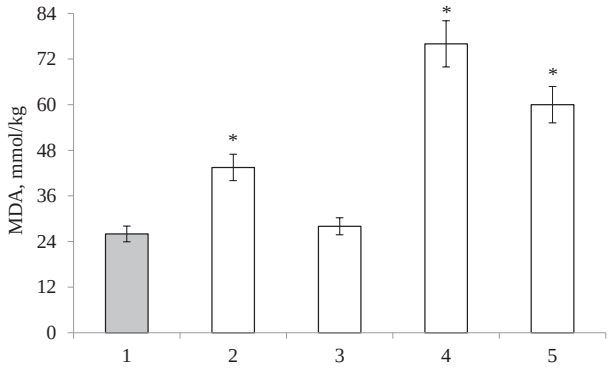

Fig. 2. The effect of HFD on the level of MDA in the liver of rats: 1 – control, 2 – sunflower oil, 3 – high oleic sunflower oil, 4 –palm oil, 5 – coconut oil

Table 4 presents the results of determining the activity of the antioxidant enzyme catalase. The highest activity of this enzyme is observed in the liver, under the conditions of HFD, this indicator shows only a tendency to decrease its level. It can be assumed that the level of catalase does not have a significant effect on the level of MDA.

Table 4

Effect of HFD on catalase activity in rat liver

№№	Dietary fat	Catalase activity, mcat/kg
1	Control	6,3±0,1
2	Sunflower oil	6,1±0,1 p>0,05
3	High oleic sunflower oil	6,1±0,1 p>0,05
4	Palm oil	5,9±0,1 p<0,05
5	Coconut oil	6,1±0,1 p>0,05

A more sensitive indicator of the state of antioxidant systems is the API index, the results of which are presented in fig. 3. As can be seen from these data, all dietary fats (with the exception of high-oleic sunflower oil) reduce the level of the API index.

Thus, our research (the first series) showed that HFD activates the processes of lipid peroxidation (LPO) in the liver, which is evidenced by a significant increase in the content of the final product of LPO − malondialdehyde.

An increase in the content of MDA during HFD leads to a significant decrease in the API index, although the activity of the antioxidant enzyme catalase is slightly reduced after HFD. It is possible that other antioxidant systems of the body play a decisive role in counteracting

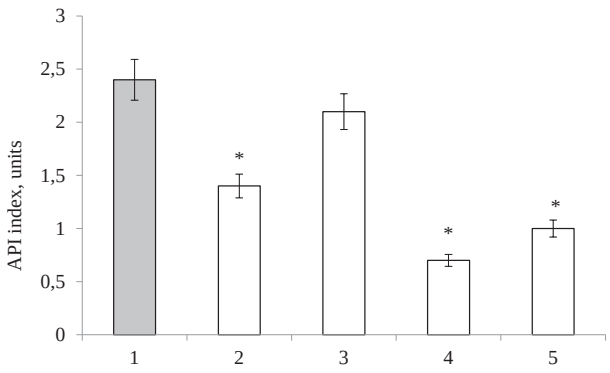

Fig. 3. The influence of HFD on the level of the API index in the liver of rats: 1 – control, 2 – sunflower oil, 3 – high-oleic sunflower oil,4 – palm oil, 5 – coconut oil

LPO. It can be glutathione system, selenium system, tocopherol and many other antioxidant systems.

In any case, on the basis of the obtained data, it can be stated that in order to prevent LPO activation, it is necessary to use high-oleic sunflower oil in food (Levitsky, Makarenko, Selivanskaya et al., 2016), since oleic acid performs an antioxidant function in the body (Titov, Lisitsyn, 2005).

In the first series of experiments, we determined the content of MDA in the blood serum of rats. The content of MDA was significantly higher in blood with *v. cava* than with *v. porta,* which indicates the possible incretion of this substance from the liver, which has the highest concentration of MDA, compared to other organs.

As can be seen from the data in Table 5, all fats, with the exception of high-oleic sunflower and coconut oils, increase the concentration of MDA, which may indicate the activation of lipid peroxidation in the liver.

Table 5

Incretion of MDA by the liver of rats receiving different dietary fats

№ №	Group	MDA, mmol/l		Δ MDA +
		v. porta	v. cava	
1	Control	0,75±0,08	1,84	1,09±0,06
2	Sunflower oil	0,78±0,04 p>0,3	2,15±0,07 p<0,01	1,37±0,05 p<0,05
3	High oleic sunflower oil	0,92±0,04 p>0,05	1,82±0,05 p>0,3	0,85±0,04 p<0,01
4	Palm oil	0,88±0,04 p>0,05	2,51±0,10 p<0,01	1,63±0,09 p<0,01
5	Coconut oil	0,78±0,06 p>0,3	1,55±0,07 p<0,05	0,77±0,06 p<0,01

In the second series of experiments, we determined the effect on the fat content in the liver of two oils: high-

oleic sunflower oil and palm oil. The relevant data are presented in fig. 4. From these data, it can be seen that the fat content in the liver is only 3 % in rats that received FFD. Consumption of high-oleic sunflower oil increases it by 2.5 times, and consumption of palm oil increases the fat content by 6 times. The content of fat in the liver of rats that consumed palm oil against the background of dysbiosis increases 7 times and amounts to 22.5 %.

In rats fed a diet with 15 % palm oil against the background of dysbiosis for 25 days, and then treated with FFD for 10 days, the content of fat in the liver decreased significantly (less than 4 %). We obtained a similar result in a group of rats that consumed palm oil against the background of dysbiosis, but from the first day of the experiment received the anti-dysbiotic agent "Kvertulin".

Conclusions. The consumption of dietary fats causes the development of steatosis and the activation of lipid peroxidation in the liver.

High-oleic sunflower oil accumulates in the liver to a lesser extent and does not cause activation of peroxidation in the liver.

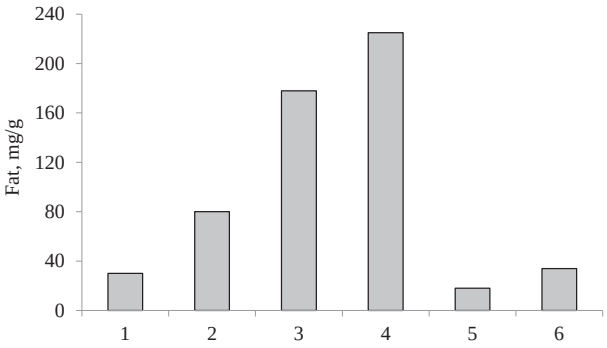

Fig. 4. The effect of HFD on the fat content in the liver of rats: 1 – FFD, 2 – 15% "Olivka", 3 – 15% palm oil, 4 – 15% palm oil+dysbiosis, 5-15% palm oil+dysbiosis+FFD (last 10 days), 6 – 15% palm oil+dysbiosis+anti-dysbiotic agent "Kvertulin"

The presence of dysbiosis under the conditions of consumption of HFD increases steatosis of the liver.

Eating a fat-free diet eliminates steatosis of the liver.

The use of the antioxidant Kvertulin prevents the development of hepatosteatosis.

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