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EFFECT OF AN ANTIDISBIOTIC AGENT ON THE BIOSYNTHESIS OF FATTY ACIDS OF LIVER LIPIDS OF RATS WHICH RECEIVED PALM OIL ON THE BACKGROUND OF DYSBIOSIS

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Abstract

Background. To determine the effect of antidysbiotic agent on the biosynthesis of fatty acids of lipids in the liver of rats fed a high-fat diet (HFD) with palm oil against the background of dysbiosis.

Methods. The HFD contained 15% palm oil. In a biological experiment, white rats were used, divided into 4 groups: the 1st group received a fat-free diet (FFD), the 2nd, 3rd, and 4th received HFD. In rats of the 3rd and 4th groups, dysbiosis was reproduced using lincomycin. Rats of the 4th group from the first day of the experiment received an antidysbiotic agent (inulin + quercetin, ADA) with food. The duration of feeding is 39 days. Liver lipids were divided into 3 fractions: neutral lipids (NL), phospholipids (PL), and free fatty acids (FFA). The fatty acid composition of the fractions was determined by gas

chromatography. The “activities” of fatty acid synthase, palmitic acid elongase, and stearyl-CoA- desaturase (SCD18 and SCD16) were determined.

Results. The presence of all classes of fatty acids (FA) in the liver lipids of rats treated with FFD was established. Consumption of HFD with palm oil increased the content of NL in the liver by 6 times (gr. 2), and in group 3 by 8 times. The introduction of ADA reduces the content of NL almost to the level of the 1st group. The content of ω-3 polyunsaturated fatty acids (PUFA) decreases in the PL fraction in rats of the 3rd group and is restored in rats of the 4th group. In rats treated with HFD, the "activity" of synthase, SCD18 and very strongly SCD16 are reduced.

Conclusion: The negative effect of palm oil on the background of dysbiosis on the biosynthesis of fatty acids in the liver, leading to hepatic steatosis and deficiency of ω-3 PUFA, can be prevented by the use of an antidysbiotic agent.

Keywords: palm oil; fatty acids; liver lipids; dysbiosis; antidysbiotic agent.

Introduction

In our previous work [1] it was shown that the consumption of high-fat diet rats (HFD) with a content of 15% of palm oil increases the fraction of liver phospholipids in the fraction of ω-6 PUFA (polyunsaturated fatty acids) by 15% and reduces the content of ω-3 PUFA at 16.4%.

In rats with experimental lincomycin dysbiosis, the consumption of HFD with palm oil increased the content of ω-6 PUFA by only 3.7%, but significantly reduced the content of ω-3 PUFA by 42.6%. If rats with experimental dysbiosis and receiving HFD with palm oil, were fed with antidisbiotic agent "Kvertulin" (containing the prebiotic inulin and bioflavonoid quercetin), the content of ω-6 PUFA in liver phospholipids increased by 79.5% compared to who received the antibiotic lincomycin and 23% compared with the group that received a diet of palm oil.

It is known that the fraction of phospholipids is only 20-25% of the total lipid of the liver, and the main share of lipids are neutral lipids, represented by triglycerides and cholesterol esters [2].

Therefore, the aim of this study was to determine the effect of antidisbiotic agent on the biosynthesis of fatty acids of all fractions of liver lipids in rats that received HFD with palm oil on the background of dysbiosis.

Materials and research methods

Palm oil produced by PGFO Edible Oils SDN (Malaysia) was used, the fatty acid composition of which is presented in table 1.

Table 1. Fatty acid composition of palm oil

Fat acid	Short formula	Content, %
Lauric acid	$C_{12:0}$	0,31
Myristic acid	$C_{14:0}$	1,09
Palmitic acid	$C_{16:0}$	43,81
Stearic acid	$C_{18:0}$	5,05
Palmitoleic acid	$C_{16:1}$, ω-7	0,13
Oleic acid	$C_{18:1}$, ω-9	40,05
Linoleic acid	$C_{18:2}$, ω-6	9,90
α-linolenic	$C_{18:3}$, ω-3	0,08
Arachidonic acid	$C_{20:4}$, ω-6	0
Eicosapentaenoic acid	$C_{20:5}$, ω-3	0
Docosapentaenoic acid	$C_{22:5}$, ω-3	0
Docosahexaenoic acid	$C_{22:6}$, ω-3	0

As an antidisbiotic agent was used the drug "Kvertulin", which contains the prebiotic inulin, bioflavonoid quercetin and calcium citrate [3], produced by the SPA Odesa Biotechnology (Ukraine).

The experiments were performed on white Wistar rats (28 males, 8-9 months, mean live weight 255±12 g), divided into 4 levels of the group: 1st received a fat-free semi-synthetic diet (FFD), the composition of which is shown in table 2; 2nd received HFD with 15% palm oil (instead of 15% starch), 3rd received HFD and from 1st to 5th day – antibiotic lincomycin with drinking water at a dose of 60 mg / kg to reproduce dysbiosis [4]; 4th received HFD, lincomycin and from the first to the 39th day - the drug "Kvertulin" at a dose of 300 mg/kg.

Euthanasia of animals was performed on the 40th day of the experiment under thiopental anesthesia by total bleeding from the heart. Lipids were isolated in the liver by the method of Dole [5] and were divided into three fractions: neutral lipids (NL), phospholipids (PL) and free fatty acids (FFA) [6].

The fatty acid composition of the lipid fractions combined for each group was determined by gas chromatographic method on a Shimadzu chromato-mass spectrometer [7].

The content of energy fatty acids (EFA) was determined by the sum $C_{16:0}+C_{16:1}+C_{18:0}+C_{18:1}$.

The «activity» of fatty acid synthase was determined by the content of $C_{16:0}+C_{16:1}$.

Palmitic acid elongase «activity» was determined by the formula $A=(C_{18:0}+C_{18:1})/(C_{16:0}–C_{16:1})$.

Stearyl-CoA-desaturase (SCD) activity was determined by the formulas $SCD18=C_{18:1}/C_{18:0}$ і $SCD16=C_{16:1}/C_{16:0}$ [8].

Table 2. Content diet for rats [4]

Components	Experimental groups			
	1 FFD	2 PO	3 PO+D	4 PO+D+ADA
Corn starch	65	50	50	50
Soybean meal defatted	20	20	20	20
Ovalbumin	6	6	6	6
Sucrose	4	4	4	4
Mineral mixture	4	4	4	4
Vitamin mixture	1	1	1	1
Palm oil (PO)	0	15	15	15
Lincomycin	0	0	+	+
Antidisbiotic agent (ADA) «Kvertulin»	0	0	0	+

Notes: D – disbiosis (lincomycin).

Results and discussion

In fig. 1 shows how the content of NL and HFD in the liver of rats receiving FFA with palm oil changes. It can be seen that in rats receiving HFD, the NL content was 2.7% and the FFA content was 0.3%. Consumption of HFD with palm oil increases the content of NL to 16.8% (i. e., 6 times), and the content of FFA – up to 0.46% (i. e., 1.6 times).

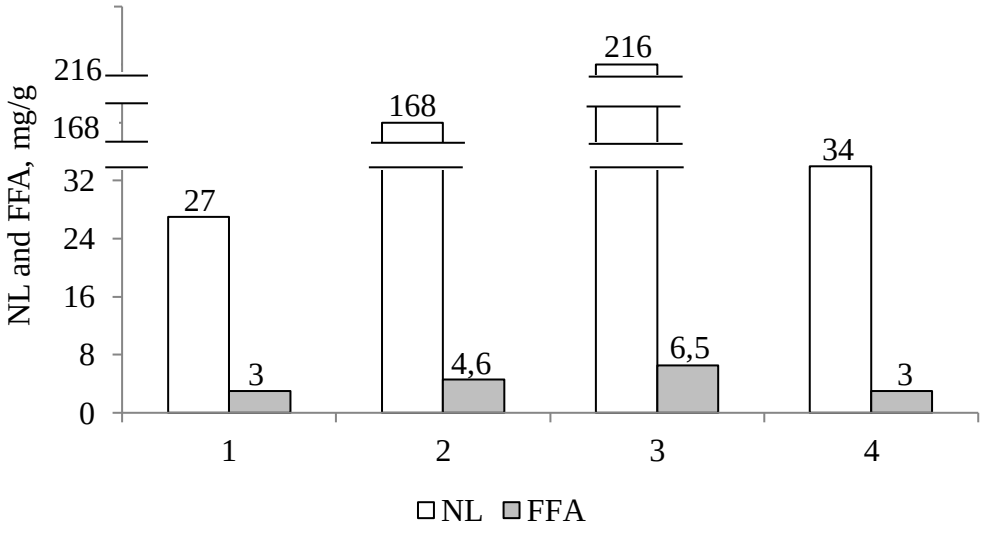

Fig. 1. The content of NL and FFA in the liver of rats fed a diet of palm oil (gr. 2, 3, 4) on the background of dysbiosis (gr. 3, 4) and diet with ADA (gr. 4), gr. 1 – FFD

Consumption of HFD on the background of dysbiosis increases the content of NL to 21.6%, and the content of FFD – to 0.68%. The use of antidisbiotic agent reduces the content of NL in 6 times and 2 times reduces the content of FFD.

Table 3 presents the results of determining the fatty acid composition of the fraction of neutral lipids, which is the main lipid in the liver. The highest content in this fraction is EFA (74-75%). The content of PUFA is 10-16%, and other FA account for 8-15%. It should be noted that the consumption of HFD with palm oil has little effect on the content of EFA, but significantly increases the content of PUFA (by 26-54%) and significantly reduces the content of other FA (by 40-50%).

Table 3. Fatty acid composition (%) of neutral lipids of liver of rats receiving fat-free diet (FFD) and fatty diets on the background of dysbiosis and antidisbiotic agent (ADA).

Fatty acids(FA)	Groups of rats			
	1 FFD	2 Palm oil (PO)	3 PO+disbiosis (D)	4 PO+D+ADA
A. EFA				
Palmitic, $C_{16:0}$	27,56	27,28	27,34	28,00
Palmitoleic, $C_{16:1}$	7,89	2,43	2,66	2,33
Stearic, $C_{18:0}$	2,71	3,53	4,42	3,84
Oleic, $C_{18:1}$	36,05	42,38	40,27	44,95
Total	74,21	75,62	74,69	79,12
B. PUFA	10,48	15,89	16,19	13,23
C. Other FA	15,31	8,49	9,12	7,65

Notes: FA – fatty acids; EFA – energy fatty acids; PUFA – polyunsaturated fatty acids; ADA – antidisbiotic agent; D – dysbiosis.

Table 4 shows the fatty acid composition of liver phospholipids, which indicates the predominant content of EFA (70-73%). PUFA account for 17-21.5% of all FA of the phospholipid fraction, and their content is the lowest in rats that received HFD on the background of dysbiosis. The use of antidisbiotic agent increases the content of PUFA by 22%.

Table 5 presents the results of determining the fatty acid composition of the fractions. These data show that HFD it palm oil increases the content of EFA, especially when consuming HFD on the background of dysbiosis – by 15.5%. The content of PUFA in the fraction of HFD decreases when consuming palm oil, especially when consuming on the background of dysbiosis – by 20.3%. However, the use of antidisbiotics increases the content of PUFA by 29.3%.

Table 4. Fatty acid composition (%) of phospholipids of liver of rats receiving fat-free (FFD) and fatty diets on the background of dysbiosis and antidisbiotic agent

Fat acid (FA)	Groups of rats			
	1 FFD	2 Palm oil (PO)	3 PO+disbiosis (D)	4 PO+D+ADA
A. EFA				
Palmitic, $C_{16:0}$	27,03	26,68	27,70	24,43
Palmitoleic, $C_{16:1}$	4,27	1,67	2,35	1,04
Stearic, $C_{18:0}$	25,85	16,37	7,24	24,66
Oleic, $C_{18:1}$	13,65	27,31	35,80	21,91
Total	70,80	72,03	73,09	72,04
B. PUFA	17,86	19,95	17,62	21,50
C. Other FA	11,34	8,02	9,29	6,46

Notes: see tabl. 3.

Table 5. Fatty acid composition (%) of the fraction of free fatty acids in the liver of rats treated with free -fat (FFD) and fatty diets on the background of dysbiosis and antidisbiotic agent (ADA)

Fatty acids (FA)	Groups of rats			
	1 FFD	2 Palm oil (PO)	3 PO+disbiosis (D)	4 PO+D+ADA
A. EFA				
Palmitic, $C_{16:0}$	23,07	22,77	25,33	23,90
Palmitoleic, $C_{16:1}$	5,74	2,00	2,17	1,53
Stearic, $C_{18:0}$	13,28	11,65	8,67	13,68
Oleic, $C_{18:1}$	15,75	27,42	30,66	23,65
Total	57,84	63,84	66,83	62,76
B. PUFA	26,75	25,76	21,33	27,59
C. Other FA	15,41	10,40	11,84	9,65

Notes: see tabl. 3.

In fig. 2 presents the results of determining the content of ω-6 PUFA ($C_{18:2}$+$C_{20:4}$) in three fractions of liver lipids of rats that consumed HFD with palm oil. It is seen that the content of these PUFA increases in rats that consumed palm oil in the fractions of NL and PL. The content of ω-6 PUFA in the fraction of FFA decreases in rats treated with palm oil (groups 2 and 3), but even slightly increases with the use of antidisbiotic agent.

In fig. 3 shows the content of ω-3 PUFA in liver lipids. It is seen that the content of these acids is significantly reduced in the fraction of FFA of liver lipids in rats treated with HFD with palm oil (groups 2, 3 and 4). In the PL fraction, the content of ω-3 PUFA decreased the most in rats of the 3rd group and was completely normalized in rats of the 4th group. As for the NL fraction, it has the lowest level of ω-3 PUFA, which is reduced in rats of the 2nd and 4th groups.

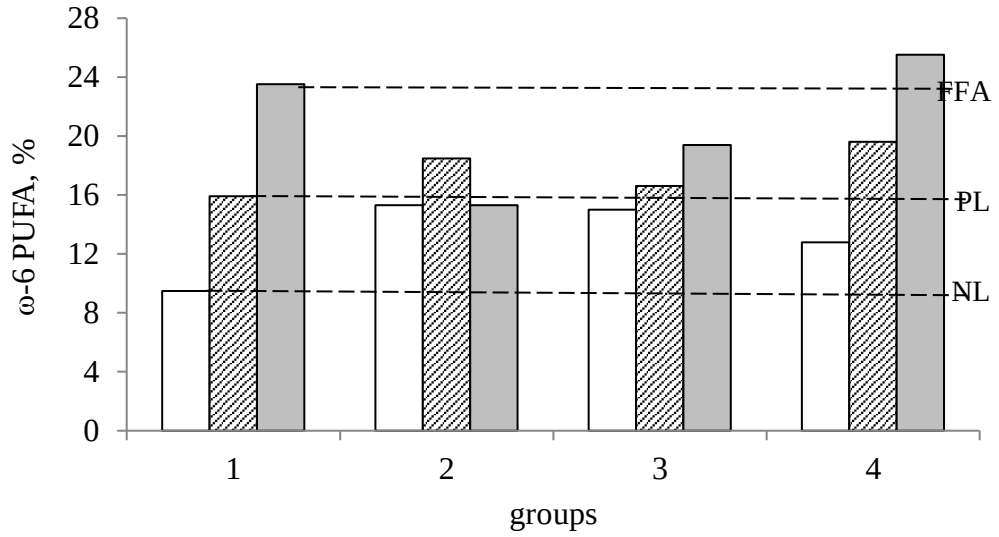

Fig. 2. The effect of rations with palm oil (gr. 2, 3, 4) on the content of ω-6 PUFA in liver lipids of rats with dysbiosis (gr. 3) and who received ADA (gr. 4), gr. 1 – FFD

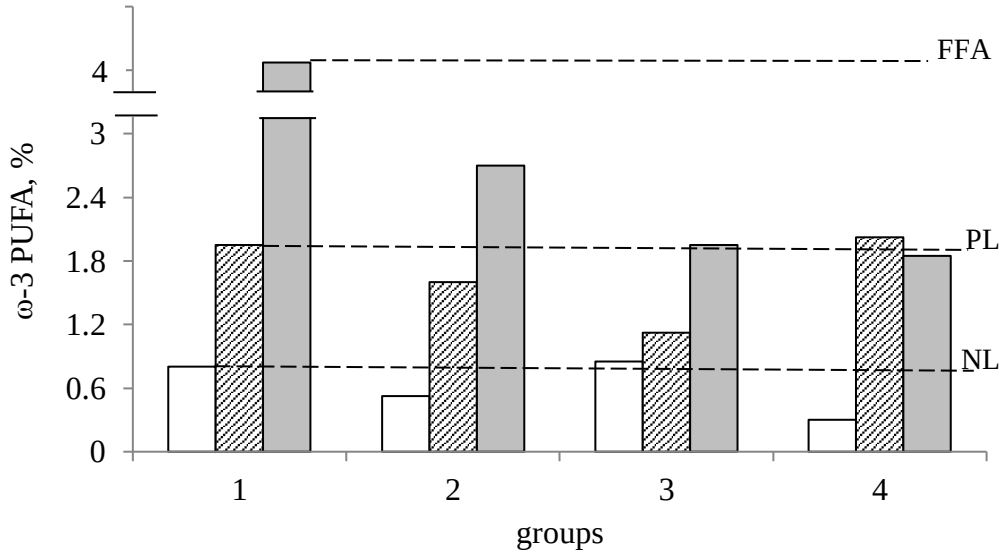

Fig. 3. The effect of rations with palm oil (gr. 2, 3, 4) on the content of ω-3 PUFA in liver lipids of rats with dysbiosis (gr. 3) and who received ADA (gr. 4), gr. 1 – FFD

Based on the data of fig. 2 and 3, the ratio ω-6/ω-3 PUFA was calculated, presented in fig. 4. It is seen that this ratio increases in all fractions of liver lipids of rats that received HFD with palm oil, especially in group 4 in the fraction of NL. In the PL fraction, the ratio of ω-6/ω-3 PUFA increases the most in rats that received HFD on the background of dysbiosis.

In fig. 5 shows the results of determining the "activity" of fatty acid synthase. It can be seen that the consumption of palm oil slightly reduces this figure in all lipid fractions, but only slightly: in the NL fraction by 14-16%, in the PL fraction by 5-18%, in the FFA fraction by 4.5-14%.

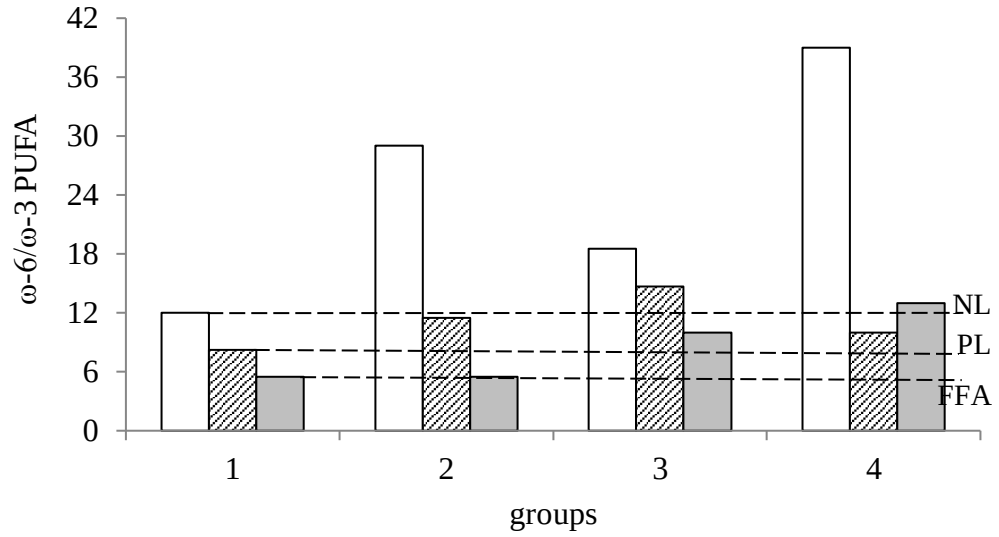

Fig. 4. The effect of rations with palm oil (gr. 2, 3, 4) on the ratio of ω-6/ω-3 PUFA in liver lipids of rats with dysbiosis (gr. 3) and who received ADA (gr. 4), gr. 1 – FFD

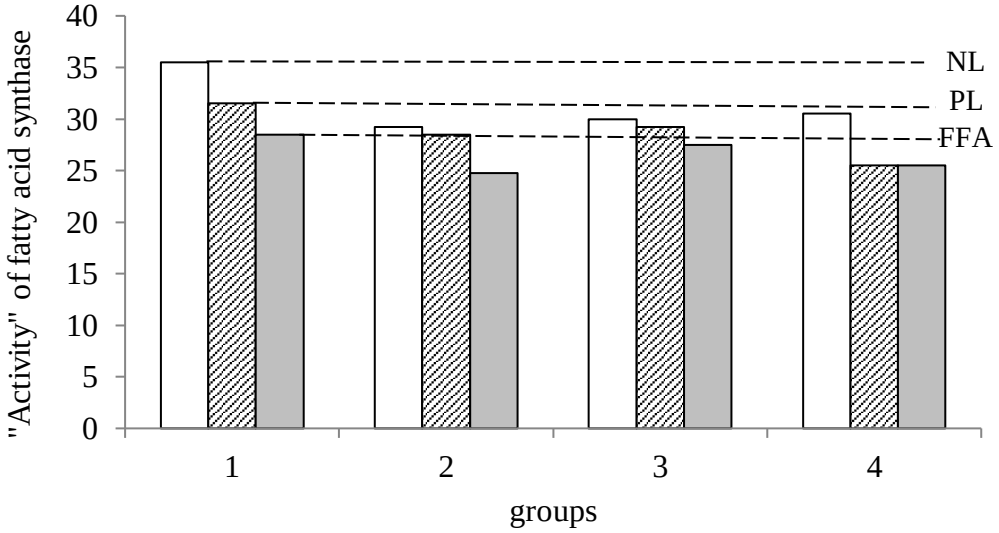

Fig. 5. The effect of rations with palm oil (gr. 2, 3, 4) on the "activity" of fatty acid synthase in the liver of rats with dysbiosis (gr. 3) and who received ADA (gr. 4), gr. 1 – FFD

In fig. 6 presents the results of determining the "activity" of palmitic acid elongase. It is seen that the "activity" of this enzyme does not change with the consumption of palm oil in all groups.

In fig. 7 shows the "activity" of stearic acid desaturase (SCD18). It is seen that the consumption of palm oil reduces the "activity" of SCD18 in the NL fraction, especially in group 3. In the fractions of PL and FFA, on the contrary, the consumption of palm oil increased the "activity" of SCD18 and also most in rats of group 3, which reproduced

dysbiosis. The use of antidisbiotic agent significantly reduced the "activity" of SCD18: in the PL fraction 5.5 times and in the FFA fraction 2.5 times.

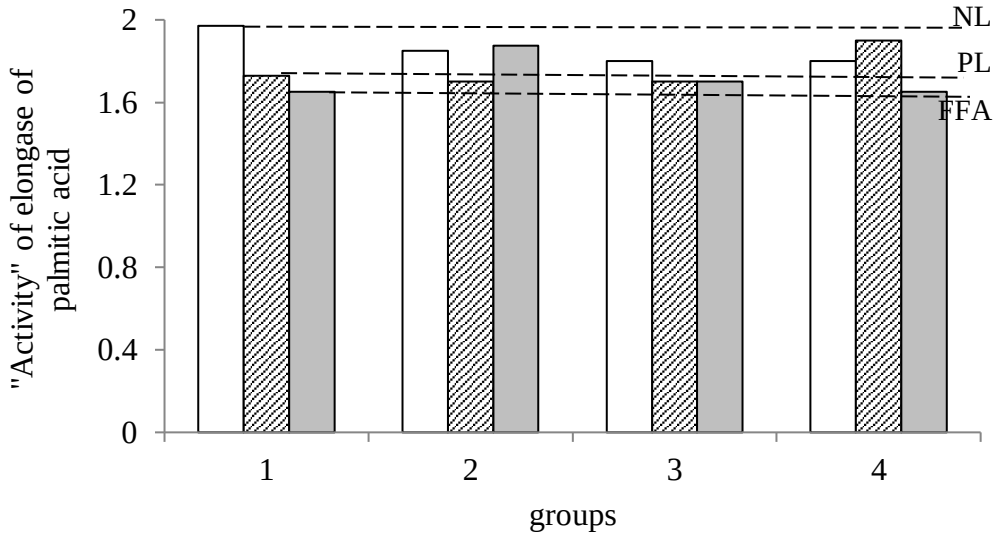

Fig. 6. The effect of rations with palm oil (gr. 2, 3, 4) on the "activity" of elongase of palmitic acid in the liver of rats with dysbiosis (gr. 3) and who received ADA (gr. 4), gr. 1 – FFD

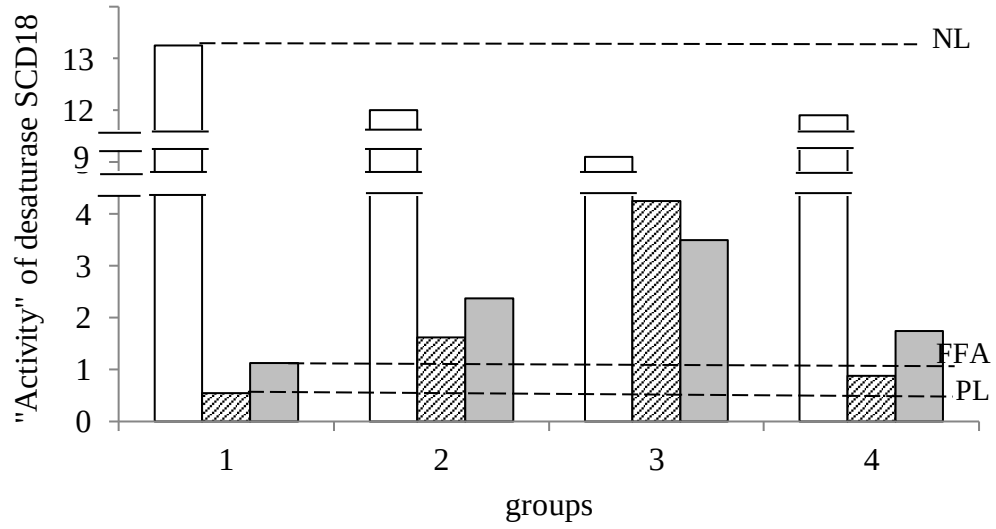

Fig. 7. The effect of rations with palm oil (gr. 2, 3, 4) on the "activity" of stearic acid desaturase (SCD18) in the liver of rats with dysbiosis (gr. 3) and who received ADA (gr. 4), gr. 1 – FFD

In fig. 8 shows the results of determining the "activity" of palmitic acid desaturase (SCD16). It is seen that in all fractions of liver lipids of rats treated with HFD with palm oil, significantly reduces the "activity" of SCD16: in the fraction of NL in 2.9-3.4 times, in the fraction of PL in 1.9-6.6 times and in the fraction of FFA in 2.8-3.9 times. To the greatest extent, the "activity" of SCD16 is reduced with the use of antidisbiotic agent.

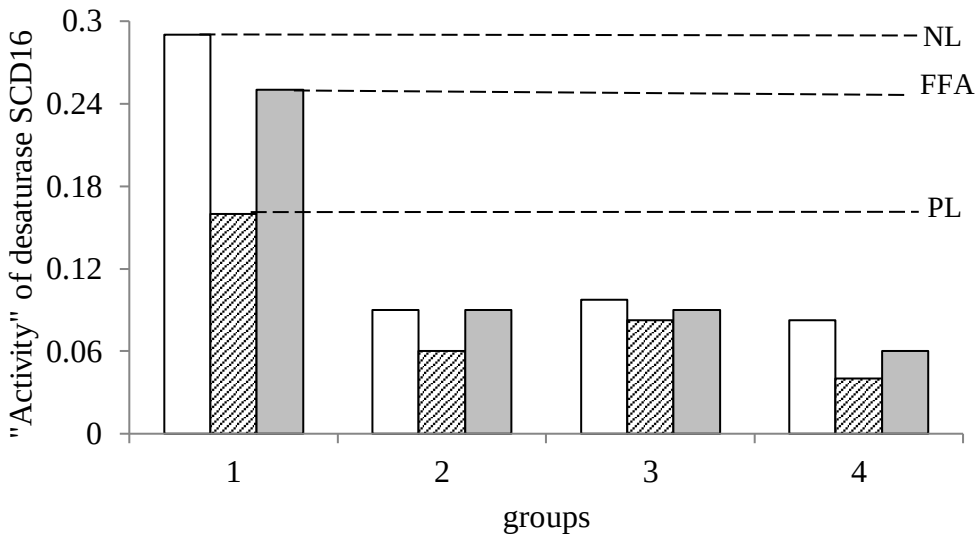

Fig. 8. The effect of rations with palm oil (gr. 2, 3, 4) on the "activity" of desaturase SCD16 in the liver of rats with dysbiosis (gr. 3) and who received ADA (gr. 4) , gr. 1 – FFD

The palm oil used in our experiments contains not only a lot of palmitic acid (43.8%), but also a significant amount of oleic (40%). The latter is the main energy acid of the animal organism [2]. At the same time, palm oil contains very few fatty acids that inhibit the growth of a number of endogenous bacteria, namely palmitoleic (0.13%) and linoleic (9.9%) [9-11].

Consumption of HFD with palm oil has little effect on the content of palmitic acid in all lipid fractions of rat liver, but significantly increases the content of oleic acid: 21% (NL), 103% (PL) and 74% (FFA).

Consumption of a diet with palm oil increases the content of NL in the liver (6 times) and FFA (1.6 times), which can be assessed as hepatosteatosis. However, the consumption of palm oil increases the content of ω-6 PUFA in NL by 60%, and reduces the content of ω-3 PUFA by 38%. As a result, the ratio of ω-6/ω-3 PUFA increases from 12 to 29.

Consumption of palm oil slightly reduces the "activity" of fatty acid synthase and the "activity" of stearic acid desaturase (SCD18), possibly due to the inhibitory effect of oleic acid from palm oil.

It is clearly established that the consumption of HFD with palm oil significantly reduces the content of palmitoleic acid: 3.2 times (NL), 2.6 times (PL) and 2.9 times (FFA). At the same time, the "activity" of palmitic acid desaturase (SCD16) is greatly reduced: 3.2 times (NL), 2.6 times (PL) and 2.8 times (FFA). A possible inhibitor of this enzyme may be linoleic acid [9].

The indicators of the fatty acid composition of NL in the conditions of experimental dysbiosis when consuming a diet with palm oil differ little from the group of rats that received

palm oil without dysbiosis. However, in the fractions of PL and FFA decreases the content of PUFA, mainly due to ω-3 PUFA. It is important to emphasize that dysbiosis contributes to the further development of hepatosteatosis.

The use of the antidisbiotic agent "Kvertulin" showed an antistetogenic effect and reduced the content of NL in the liver by 6.4 times. Moreover, the antidisbiotic agent completely restored the level of ω-3 PUFA in the PL fraction.

Conclusions

The results of determining the fatty acid composition of liver lipids in rats under conditions of dysbiosis and under the conditions of antidisbiotic use indicate an important role of microbial factor in endogenous biosynthesis ω-3 PUFA, which is inhibited by consumption of HFD with palm oil.

The use of antidisbiotic agent can prevent the negative effects of palm oil on the development of hepatosteatosis and deficiency of ω-3 PUFA.

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