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INFLUENCE OF ORAL FATTY APPLICATIONS ON BIOCHEMICAL INDICATORS OF INFLAMMATION AND DYSBIOSIS IN THE TISSUES OF THE RAT MOUTH

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Abstract

Background. The negative impact on the body of high-fat nutrition largely depends on the fatty acid composition of edible fats and the presence of peroxidation products in them, which are formed during heat treatment. Despite the considerable number of studies on this issue, it remains unclear the nature of the influence of different dietary fats on the condition of the tissues of the oral cavity. The purpose of this study was to clarify this issue

Methods. Ordinary (high-linoleic) sunflower oil, high-oleic sunflower oil and butter in the native state and after heat treatment were used. The experiments were carried out on 35 white rats with an average weight of 252±15 g, who were given oral applications of the above fats at a dose of 0.5 ml per rat for 3 days. After rat killing on the 4th day, the content of malondialdehyde

(MDA), the activity of elastase, urease, lysozyme and catalase were determined in the cheek mucosa homogenate and in the gums. The degree of dysbiosis was calculated by the ratio of the relative activities of urease and lysozyme. The antioxidant-prooxidant API index was calculated from the ratio of catalase activity and MDA content

Results. Oral applications of native and heat-treated oils cause a decrease in the MDA content in both tissues with the exception of heat-treated butter, applications of which do not reduce the MDA level.

Applications of heat-treated oils increase the activity of elastase in the gums, and heat-treated sunflower oil in the cheek mucosa.

Applications of native and heat-treated oils reduce the activity of catalase in the cheek and gums. The API index increases in the gums under the action of native oils and decreases under the action of thermally processed oils.

Applications of butter (native and heat-treated) significantly reduce the activity of urease in the cheek mucosa. Sunflower and high-oleic sunflower oils tend to increase the activity of urease, the least pronounced for the latter.

All oils except high-oleic sunflower, reduce the activity of lysozyme in both tissues and all oils increase the degree of dysbiosis (except butter in the cheek mucosa).

The content of diene conjugates after heat treatment increased the most in butter, and the least in high-oleic sunflower oil.

Conclusion. Oral applications of fats cause a decrease in the formation of active forms of oxygen in the tissues of the oral cavity, which causes the development of dysbiosis and inflammatory processes.

Keywords: fats, oral cavity, dysbiosis, lipid peroxidation

INTRODUCTION

In recent decades, the consumption of edible fats and fat containing products has increased significantly [1-3]. As you know, the excess in the diet of fats has a negative effect on the body, causing under certain conditions the development of obesity [4], liver steatosis [5], type 2 diabetes mellitus [6], and cardiovascular diseases [7].

The negative effect on the body of high-fat diet is to a large extent dependent on the fatty acid composition of edible fats [8] and the presence of peroxidation products that are formed

during prolonged storage [9] and, especially, in the heat treatment of fats in the processes of fatty cooking [10, 11].

Unfortunately, the issue of the influence of edible fats on the condition of the tissues of the oral cavity, which first comes in contact with them, remains unclear.

Therefore, the purpose of this study was to determine the effect of oral applications of various edible fats on the condition of the tissues of the oral cavity, which was assessed by indicators of inflammation, antioxidant defense and dysbiosis.

As the edible fats, sunflower oil, butter and the new promising form of edible fats - the high oleic sunflower oil "Olivka" - were used in Ukraine [12].

MATERIAL AND RESEARCH METHODS

In the work, the usual (high-linoleic) sunflower oil was refined (Fig. 1), high oleic sunflower oil "Olivka", production of NVA "Odessa biotechnology" (Fig. 2) and butter (fig. 3). Fatty acid composition of these fats is presented in Table 1.

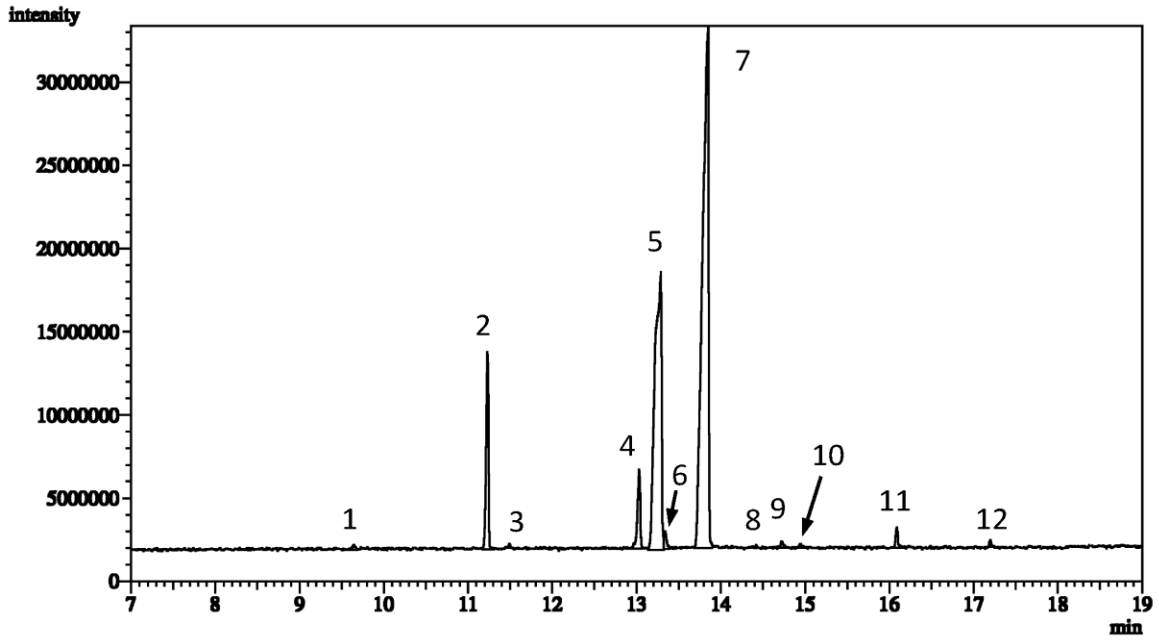

Fig. 1. Chromatogram of hexane solution of sunflower oil after methylation
(Acids: 1 - myristic, 2 - palmitic, 3 - palmitoleic, 4 - stearic, 5 - oleinic,
6 - vacenic, 7 - linoleic, 8 - linolenic, 9 - arachinic, 10 - eicosenic, 11 - behenic,
12 - lignocerin)

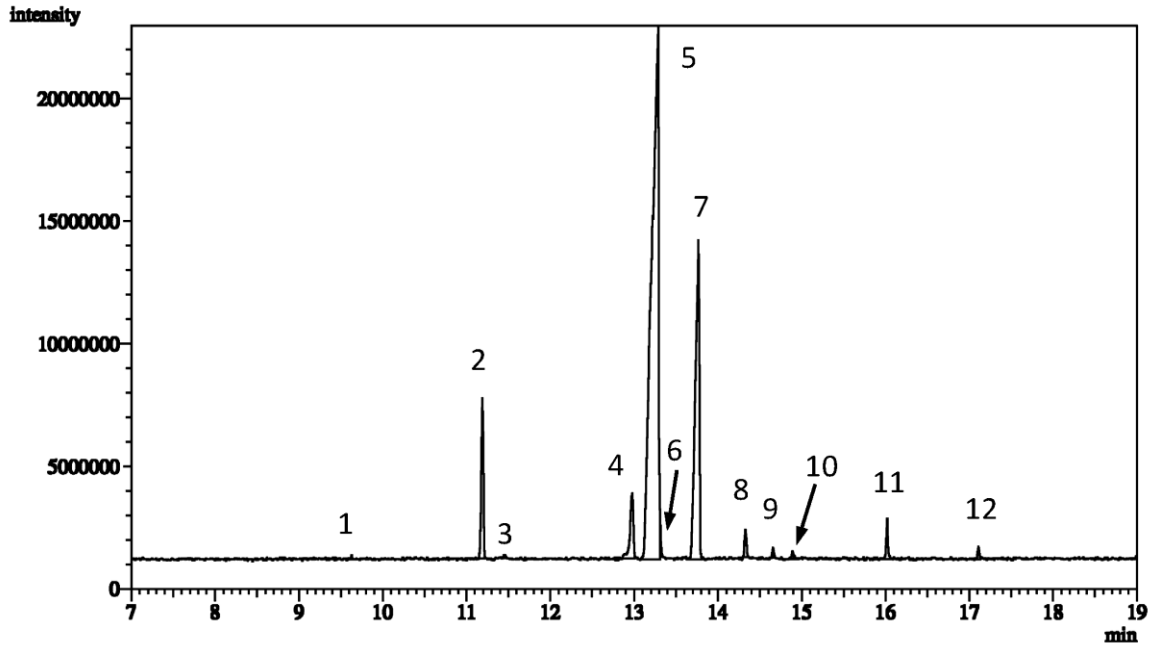

Fig. 2. Chromatogram of hexane oil "Olivka" after methylation (1-12 – see fig. 1)

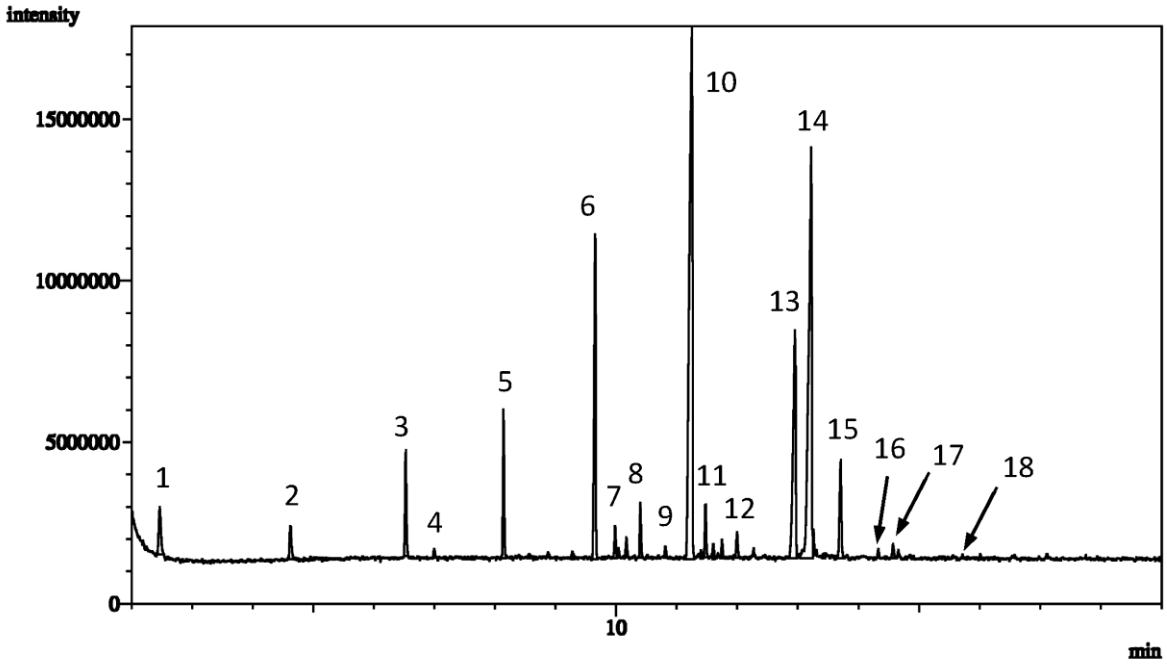

Fig. 3. Chromatogram of hexane solution of butter after methylation
(Acids: 1 - kapron, 2 - capryl, 3 - kaprinov, 4 - undecyl, 5 - laurinov, 6 - myristic, 7 - myristoleic, 8-pentadecyl, 9-pentadecanoic, 10-palmitic, 11-palmitoleic, 12-margarine, 13-stearic, 14-oleic, 15-linoleic,16 - linolenic, 17 - arachidic, 18 - arachidone)

Table 1. Fatty acid composition of fats

Fatty acid	Fat		
	Sunflowers	Sunflowers	Sunflowers
kapron			1,96
capryl			1,19
kaprinov			2,78
undecyl			0,22
laurinov			3,39
myristic	0,28	0,08	10,61
myristoleic			0,82
pentadecyl			1,32
pentadecanone			0,31
palmitic	8,02	5,68	31,00
palmitoleic n-9			0,22
palmitoleic n-7	0,09	0,13	1,43
margarine			0,71
stearic acid	3,72	3,71	11,18
oleic	32,40	64,50	25,28
vaccine	0,91	0,82	0,58
linoleic	53,09	22,20	3,43
linolenic	0,07	0,87	0,20
peanut	0,23	0,26	0,15
eukosenic	0,18	0,28	
arachidic			0,08
behenic	0,80	1,12	0,13
lignocerin	0,21	0,36	0,13

To study the effect on the organism of heat-treated fats, we added 1.5% of 30% hydrogen peroxide (H_2O_2) to the oil, heated to 125 °C in a glycerin bath and kept at that temperature for 60 minutes.

Biological studies were performed on 35 white rats of the Vistar line (males, 13 months, mean live weight 252 ± 15 g), distributed in 7 equal groups: 1-a - control; 2nd - received oral applications to the oral mucosa for 0.5 ml of sunflower oil; 3rd group - 0.5 ml of heat-treated (TO) sunflower oil; 4th group received applications for 0.5 ml of high oleic sunflower oil

"Olivka"; 5th group - 0.5 ml of high-oleic sunflower oil "Olivka"; 6th group - 0.5 ml of butter and 7th group of 0.5 ml of butter.

Fat applications were made for three days, and on the 4th day of the rats they were subjected to euthanasia under thiopental anesthesia (20 mg / kg) by total bleeding from the heart. The mucous membranes of the cheeks were clear and the levels of biochemical markers of inflammation were determined in the homogenates of these tissues: the activity of elastase and the content of MDA [13], the activity of the antioxidant enzymes of catalase [13], and the antioxidant-prooxidant index API [13], based on the ratio of catalase activity and MDA content. . The degree of bacterial insemination was calculated by the activity of urease [14], the state of non-specific immunity - by the activity of lysozyme [15] and the ratio of relative activity of urease and lysozyme calculated the degree of dysbiosis [14].

The evaluation of the thermal damage of fats was done by the content of diene conjugates [16]. Fatty acid composition of fats was investigated on a gas chromatograph [17].

The results of experiments were subjected to standard stamping.

RESULTS AND DISCUSSION

In fig. 4 shows the results of determining the content of diene conjugates in fats before and after heat treatment.

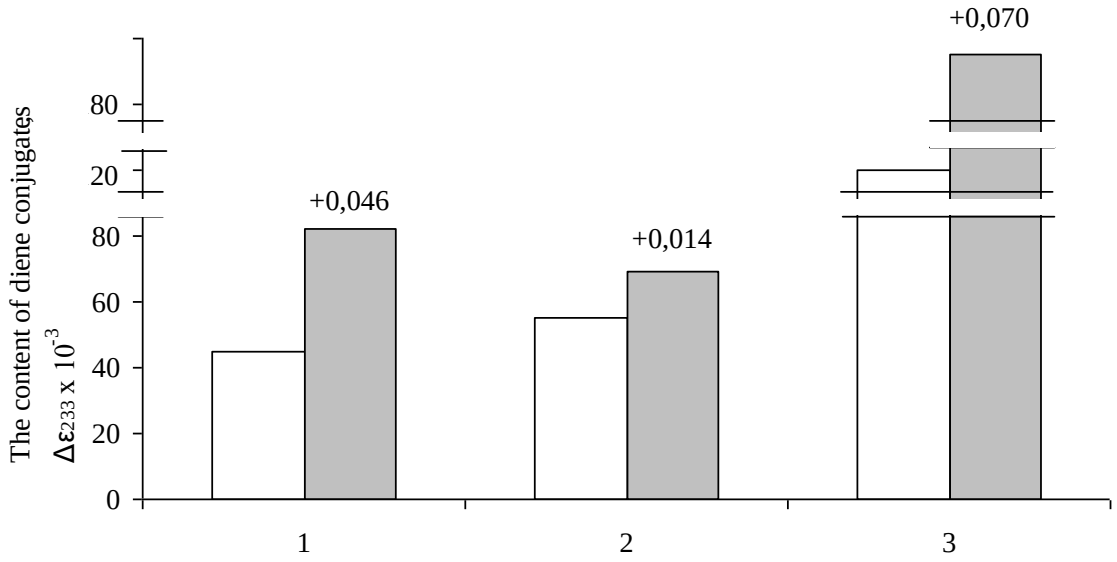

Fig. 4. The content of diene conjugates in fats after heat treatment (+ 125 ° C, 1 hour)

From these data, it can be seen that diene conjugates, as the primary products of peroxidation of unsaturated fatty acids, are present in a significant amount in butter (0.120 units of extinction at 233 nm). In sunflower oil they are only 0,045 units. After heat treatment, the growth of diene conjugates in butter was 0,070 units, in sunflower oil 0,096 units and in high-oleic sunflower oil only 0,014 units. These data confirm the higher thermal resistance of "Olivka" [12].

Table 2 presents the results of determination in the tissues of the oral cavity of the biochemical marker of inflammation - elastase. From these data it is clear that oral applications "Olivka" lead to an obvious tendency to increase activity, and the activity of elastase increases reliably after the application of butter. The heat treatment of sunflower oil greatly increases its ability to induce elastase activation in the mucous membrane of the cheek and in the gums. The heat treatment of butter significantly increases the activity of elastase in the gums.

Table 2. Elastase activity (mcat/kg) in the mucous membrane of the cheek and in the gums of rats after oral applications of different fats (M ± m)

№№	Fat applications	Cheek	Gums
1	Control	70,3±6,5	64,9±3,8
2	Sunflower oil	71,7±6,9 $p>0,6$	69,3±3,3 $p>0,3$
3	HT sunflower oil	84,1±3,1 $p<0,05$; $p_1>0,05$	81,2±10,6 $p>0,05$; $p_1>0,05$
4	High Oleic Sunflower Oil (HOSO)	83,2±8,0 $p>0,05$	72,8±3,1 $p>0,05$
5	HT HOSO	83,1±7,1 $p>0,05$; $p_1>0,05$	78,4±2,7 $p<0,05$; $p_1<0,05$
6	Butter	82,1±8,1 $p>0,05$	75,7±2,0 $p<0,05$
7	HT Butter	77,5±3,5 $p>0,1$; $p_1>0,3$	98,8±9,6 $p<0,01$; $p_1<0,01$

Notes: p – compared with gr. 1; p_1 - in comparison with gr. 2, 4, 6, respectively.

Table 3 presents the results of determining the content of MDA in the tissues of the oral cavity of rats. It is seen that oral application of fats significantly reduces the level of MDA in the tissues of the oral cavity. After the heat treatment of butter and "Olivka", the MDA content in the gums is significantly increased.

Table 3. The content of MDA (mmol/kg) in the mucous membrane of the cheek and in the gums of rats after oral applications of different fats (M ± m)

№№	Fat applications	Cheek	Gums
1	Control	16,9±1,3	16,0±0,8
2	Sunflower oil	10,6±1,4 $p<0,01$	9,6±1,1 $p<0,01$
3	HT sunflower oil	12,7±1,0 $p<0,05$; $p_1>0,05$	10,3±1,0 $p<0,01$; $p_1>0,3$
4	High Oleic Sunflower Oil (HOSO)	11,7±1,1 $p>0,01$	9,1±0,6 $p<0,001$
5	HT HOSO	11,8±0,9 $p<0,01$; $p_1>0,8$	12,9±0,9 $p<0,05$; $p_1<0,05$
6	Butter	11,3±0,6 $p<0,05$	8,3±0,5 $p<0,001$
7	HT Butter	13,3±1,4 $p>0,05$; $p_1>0,05$	16,0±0,8 $p=1$; $p_1<0,001$

Notes: see tab. 2.

Table 4 presents the results of determining the activity of catalase in the tissues of the oral cavity of rats after application of different fats. It is seen that fatty applications reduce the activity of catalase in the mucous membrane of the cheek, and to a greater extent after the application of sunflower oil. In gums, fatty applications also reduce the activity of catalase, in particular, heat-treated fats.

Table 4. Catalase activity (mcat/kg) in the mucous membrane of the cheek and in the gums of rats after oral applications of different fats (M ± m)

№№	Fat applications	Cheek	Gums
1	Control	7,91±0,21	9,54±0,33
2	Sunflower oil	4,49±0,47 $p<0,01$	8,70±0,58 $p>0,05$
3	HT sunflower oil	4,32±0,35 $p<0,01$; $p_1>0,5$	6,34±0,20 $p<0,01$; $p_1<0,05$
4	High Oleic Sunflower Oil (HOSO)	6,68±0,53 $p<0,05$	8,64±0,25 $p<0,05$
5	HT HOSO	6,53±0,71 $p<0,05$; $p_1>0,5$	8,27±0,09 $p<0,05$; $p_1>0,1$
6	Butter	6,97±0,50 $p<0,05$	8,24±0,09 $p<0,01$
7	HT Butter	6,25±0,53 $p<0,05$; $p_1>0,3$	7,78±0,34 $p<0,05$; $p_1>0,05$

Notes: see tab. 2.

Table 5 presents the results of the definition of the API index. Fat applications greatly increase the AII index in the gums. In the mucous membrane, the cheeks significantly increase the API index for applications of high-oleic sunflower oil. Heat treated sunflower oil reduces the API index. As for heat-treated fats, they all reduce the API index in gums, and HT sunflower oil is also in the cheek mucus.

Table 5. Index API in the mucous membrane of the cheek and in the gums of rats after oral applications of different fats (M ± m)

№№	Fat applications	Cheek	Gums
1	Control	4,68±0,20	5,96±0,28
2	Sunflower oil	4,24±0,27 $p>0,05$	9,06±0,61 $p<0,01$
3	HT sunflower oil	3,40±0,22 $p<0,01$; $p_1<0,05$	8,10±0,58 $p<0,05$; $p_1>0,2$
4	High Oleic Sunflower Oil (HOSO)	5,71±0,41 $p<0,05$	9,49±0,61 $p<0,01$
5	HT HOSO	5,53±0,48 $p>0,05$; $p_1>0,5$	6,41±0,37 $p>0,05$; $p_1<0,05$
6	Butter	4,30±0,38 $p>0,2$	9,93±0,98 $p<0,05$
7	HT Butter	4,70±0,37 $p>0,8$; $p_1>0,3$	4,86±0,28 $p<0,05$; $p_1<0,01$

Notes: see tab. 2.

Table 6 shows the results of determining the urease activity. These data show that in the mucosa of the cheek applications sunflower oil (groups 2 and 3) significantly (in 1,6-1,9 times) increase the activity of urease, which indicates the growth of microbial insemination of the tissue. At the same time, applications of butter, on the contrary, reduce the activity of urease in 2 times. As for the gum, the application of all fats (native and heat-treated) significantly increase the activity of urease (in 1,4-1,5 times). Such an increase in the level of bacterial insemination of the gums can indicate the ability of fats to promote the translocation of bacteria from the oral cavity.

Table 7 shows that fatty applications (especially butter) reduce the activity of lysozyme in the tissues of the oral cavity, and applications of heat treated fats cause even more reduction in the mucous membrane of the cheek, whereas in the gums, heat-treated fats show a clear tendency to increase the activity of lysozyme.

Table 6. Urease activity (mc-cat/kg) in the mucous membrane of the cheek and in the gums of rats after oral applications of different fats (M ± m)

№№	Fat applications	Cheek	Gums
1	Control	0,21=0,05	0,30±0,13
2	Sunflower oil	0,39±0,05 p<0,05	0,53±0,07 p>0,05
3	HT sunflower oil	0,35±0,07 p>0,05; p_1>0,3	0,55±0,08 p>0,05; p_1>0,8
4	High Oleic Sunflower Oil (HOSO)	0,30±0,05 p>0,05	0,41±0,06 p>0,3
5	HT HOSO	0,22±0,07 p>0,7; p_1>0,05	0,49±0,06 p>0,05; p_1>0,3
6	Butter	0,10±0,02 p<0,05	0,46±0,07 p>0,05
7	HT Butter	0,12±0,02 p<0,05; p_1<0,01	0,53±0,10 p>0,05; p_1>0,3

Notes: see tab. 2.

Table 7. Lysozyme activity (units/kg) in the mucous membrane of the cheek and in the gums of rats after oral applications of different fats (M ± m)

№№	Fat applications	Cheek	Gums
1	Control	93±4	254±14
2	Sunflower oil	73±12 p<0,05	207±25 p>0,05
3	HT sunflower oil	55±12 p<0,05; p_1>0,3	254±34 p=1; p_1>0,1
4	High Oleic Sunflower Oil (HOSO)	83±9 p>0,05	191±22 p<0,05
5	HT HOSO	54±13 p<0,05; p_1>0,1	231±24 p>0,3; p_1>0,1
6	Butter	41±11 p<0,05	172±30 p<0,01
7	HT Butter	39±7 p<0,05; p_1>0,5	183±28 p<0,05; p_1>0,5

Notes: see tab. 2.

Table 8 shows the estimated data on the degree of dysbiosis in the oral tissues of rats receiving oral applications of different fats. From these data it is clear that the mucous membrane of the cheek and gums are characterized by an increase in bacterial insemination and an increase in the degree of dysbiosis in 1.6-2.8 times after applications of sunflower, high oleic sunflower oil and butter. Unlike gums, the application of butter in the mucous membrane reduces bacterial

insemination and almost to the same extent and the activity of lysozyme, which ultimately results in indicators of the degree of dysbiosis, as in the control group rats.

Table 8. Dysbiosis degree into in the mucous membrane of the cheek and in the gums of rats after oral applications of different fats (M ± m)

№№	Fat applications	Cheek	Gums
1	Control	1,00±0,13	1,00±0,15
2	Sunflower oil	2,38±0,25 $p<0,01$	2,19±0,22 $p<0,05$
3	HT sunflower oil	2,83±0,31 $p<0,01$; $p_1>0,1$	1,83±0,19 $p<0,05$; $p_1>0,3$
4	High Oleic Sunflower Oil (HOSO)	1,61±0,18 $p<0,05$	1,83±0,20 $p<0,05$
5	HT HOSO	1,81±0,19 $p<0,05$; $p_1>0,3$	1,79±0,18 $p<0,05$; $p_1>0,8$
6	Butter	1,09±0,17 $p>0,5$	2,25±0,25 $p<0,01$
7	HT Butter	1,36±0,16 $p>0,05$; $p_1>0,2$	2,45±0,25 $p<0,01$; $p_1>0,4$

Notes: see tab. 2.

Thus, our study showed that oral tissues differentiatedly react to edible fats, reducing the level of peroxide oxidation of unsaturated fatty acids, as evidenced by a decrease in the content of MDA and an increase in the level of antioxidant defense in the gums.

Taking into account that active forms of oxygen (AFO), which are formed during the process of peroxidation of lipids, have antimicrobial activity [18], then the reduction of their level should contribute to the increase in the number of bacteria in the tissues of the oral cavity (as evidenced by the increase in urease activity) and, as consequence, an increase in the degree of dysbiosis.

In turn, AFO can activate inflammatory and dystrophic processes in the tissues of the oral cavity, as evidenced by increased elastase activity after applications of thermally oxidized fats (Table 2).

CONCLUSIONS

1. Oral applications of fats reduce the intensity of processes of peroxidation of unsaturated fatty acids and the formation of AFO.

2. The result of reducing the level of AFO is an increase in bacterial insemination and the growth of the degree of dysbiosis.

3. In turn, an increase in the level of AFO contributes to the development of inflammatory-dystrophic processes in the tissues of the oral cavity.

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