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The Effect of Evening Primrose (*Oenothera biennis* L.) Oil on Digestive Tract and the Activity of Pancreatic Enzymes of Wistar Rats

Wpływ oleju z nasion wiesiołka dwuletniego (*Oenothera biennis* L.) na przewód pokarmowy i aktywność enzymów trzustkowych szczurów rasy Wistar

The therapeutic qualities of evening primrose (*Oenothera biennis* L.) seeds, proved in recent years, are conditioned by biologically active components incorporated in them, the most significant of which are essential unasturated fatty acids (EFA) (4, 5, 6). The analysis of fatty acids composition of evening primrose revealed high content of gammalinoleic and di-homo-gammalinoleic acid (GLA and DGLA) (4, 5, 9). Under regular physiological conditions, the acids are synthesized from linoleic acid, yet, with higher organisms, the process is limited by specific metabolic mechanism. The cases of deficient supply, as well as increased demand of an organism for essential unsaturated fatty acids (EFA) may lead to growth and developmental handicaps and also those of the functions conditioned by the synthesis of biologically active derivative compounds.

The aim of the assumed studies was to determine the effects of feeding rats with fodder supplemented by primrose oil and to compare them with the results of applying fodder with the addition of soybean oil, which is widely used with human nutrition and contains polyunsaturated fatty acids in its composition.

MATERIAL AND METHODS

The studies were conducted on 18 female Wistar rats with body wieght from 210 to 245 g, divided at random into 3 groups and kept in cages, 6 specimens in each. Four weeks the animals were fed with mixtures prepared on the basis of typical fodder of laboratory rats LSM (Table 1) which differed in the 5% supplement of evening primrose oil (W group) or soybean oil (S group). Not greased standard mixture was used with the control (K group). During the experiment the rats were ensured constant and free access to fodder and water. The consumption of fodder and water was checked daily and the body weight weekly. After having finished the feeding stage of the research, the rats were etherized and decapitated and then liver, stomach, intestines, pancreas and spare fat were immediately sampled in succession. The length of intestines was measured and then weighted, after having removed their content and having applied additional perfusion with physiological saline. Before having been

Tab. 1. Composition of mixture
Skład mieszanek

Compound	Content %
Wheat barn	12.5
Corn ground grain	18.5
Wheat ground grain	26.0
Barley ground grain	25.0
Fish flour	4.2
Powder defated milk	6.2
Fodder yeats	4.2
Fodder chalk	1.3
Fodder phosphate	1.2
Polfamiks LSM	1.0
Protein content % 15.5 EM (MJ/kg) 11.1	

5% evening primrose oil (group W) or 5% soybean oil (group S) was added to standard mixtures applied to experimental rats.

5% olej z nasion wiesiołka (grupa W) lub 5% olej sojowy (grupa S) dodano do mieszanek standardowych podawanych szczurom doświadczalnym.

prepared, liver was perfused with 10 ml of physiological saline through a portal vein, then, after weighing it was homogenized in glass Potter homogenizers, in the medium of 0,05M phosphate buffer of pH = 10.2 for the determination of alkaline phosphatase and superoxidase dismutase. Pancreases were weighed individually and then cumulative tests were performed with respect to every group, which was homogenized also in phosphate buffer, yet that of 0.06 M and pH = 5,6. Homogenates were centrifuged at 13000 × g for 20 min. Liver and pancreas samples were prepared in ice and in cooled centrifuges.

Proteolytic (7), amylolytic (12) and lipolytic (3) activities were determined in pancreas supernatant, obtained after centrifugation of homogenates. The activity of asparagine (GOT – E.C.2.6.1.1.) and alanine aminotrasferases (GTP – E.C.2.6.1.2.) (12), isocitrate dehydrogenase (ICDH – E.C.1.1.1.42) (2), alkaline phosphatase (AP – E.C.3.3.2.1.) (2) as well as superoxide dismutase (SOD – E.C.1.15.1.1.) (10) were determined in liver supernatant as well as protein by Folin method and the content of glycogen with anthrone method (8).

The results were subject to the statistic analysis of variance.

RESULTS AND DISCUSSION

The most significant increase in body weight was observed with the rats fed on fodder supplementd by primrose oil (Table 2). The control group revealed it to be 2.5 times lower. The rats fed with fodder enriched by soybean oil showed the increase higher by 7.5 g than the control group, yet it was lower by 13g as compared with the increases in the group of rats obtaining fodder with the addition of primrose oil.

Fodder consumption treaded differently in conversion to 1g of body weight. Control rats and the ones fed with fodder with soybean oil consumed more fodder by 52 and 27% than the rats obtaining primrose oil. The quantities of

Tab. 2. Parameters of rats and digestive tract features in groups fed on diet with the addition of 5% of evening primrose oil (W), 5% of soybean oil (S) and the control without fats (K) (x, ±s, n=6)
Parametry szczurów i cechy przewodu pokarmowego w grupach szczurów żywionych mieszankami z dodatkiem 5% oleju wiesiołka (W), 5% oleju sojowego (S) oraz szczurów grupy kontrolnej karmionych bez dodatków olejowych (K) (x, ±s, n=6)

Group	Tested characteristic and parametres of digestive tract					
	K		W		S	
Body weight (g)	256.60[a]	±11.69	273.30[a]	±23.59	229.17[b]	±16.56
Body weight increase (g)	13.33[a]	±4.08	33.33[b]	±12.11	20.83[a]	±7.36
Fodder consumption (g/g of increase)	38.91[a]	±10.70	18.75[b]	±6.59	25.88[a]	±7.02
Fat weight (g)	17.70[a]	±4.00	5.42[a]	±4.21	9.00[b]	±1.58
Stomach weight (g)	2.77	±0.48	3.30	±1.16	2.08	±0.53
Relative stomach weight (% of body weight)	1.07	±0.19	1.21	±0.39	0.90	±0.21
Intestine length (cm)	114.17	±4.49	118.33	±7.71	104.00	±8.21
Relative intestine length (cm/g body weight)	0.45	±0.03	0.43	±0.03	0.45	±0.03
Relative intestine weight % of body weight)	1.62[b]	±0.23	2.99[a]	±0.29	3.13[a]	±0.40
Liver weight (g)	7.80[a]	±0.64	9.12[b]	±1.03	7.10[a]	±0.95
Relative liver weight (% of body weight)	3.04	±0.25	3.33	±0.21	3.09	±0.26
Pancreas weight (g)	0.57	±0.13	0.48	±0.15	0.50	±0.08
Relative pancreas (% of body weight)	0.22	±0.06	0.17	±0.04	0.22	±0.03

a, b – statistically significant differences with $p < 0.05$.
a, b – różnice statystycznie istotne, $p < 0,05$.

consumed fodder mixtures in the control group and the one supplemented by primrose oil differed significantly.

The highest amount of spare fats was observed with the rats of control group and the lowest with the ones fed on fodder supplemented by soybean oil. The difference was about 50% and was statistically significant (Table 2). The rats obtaining primrose oil accumulated 13% of fat less than the control group.

What seems to be interesting while comparing the results of feeding is their significant differentiation both in the kind of oil added and to control diet. Despite approximate energetic quality of mixtures significantly better results of rearing were obtained with the rats given primrose oil. It betokens stimulating influence of this oil on the growth of rats.

The highest stomach weight, higher by 17% than the one observed with the control group of rats and by 38% higher with the rats fed with the addition of soybean oil, was noted in the group fed with the addition of primrose oil. Concerning the intergroup dispersion of results, the differences proved to be statistically irrelevant. Similar dependence was shown for relative stomach

Tab. 3. Enzymes activity (U/mg of protein) with three groups of rats fed on experimental mixtures with 5% addition of primrose oil (W), 5% addition of soybean oil (S) and the control without fatty additions (K) (x, ±s, n=6)

Aktywność enzymów (U/mg białka) w trzech grupach szczurów żywionych mieszankami doświadczalnymi z 5% dodatkiem oleju z wiesiołka (W), 5% dodatkiem oleju sojowego (S) i szczurów z grupy kontrolnej, żywionych bez dodatków olejowych (K) (x, ±s, n=6)

Enzyme	Group		
	K	W	S
	Liver		
Protein (mg/g)	159.77[a]	130.25[b]	154.66[a]
AspAT (E.C.2.6.1.1.)	274.50	290.33	293.66
AlAT (E.C.2.6.1.2.)	247.50	218.83	214.17
AP (E.C.3.3.2.1.)	3.80[a]	4.82[b]	4.51[b]
ICDH (E.C.1.1.1.42)	0.58	0.64	0.48
SOD (E.C.1.15.1.1.)	61.68	55.09	51.01
Glicogen (mg/g)	1.45[a]	2.13[a]	0.68[b]
	Pancreas		
Protein (mg/g)	29.33	21.81	23.51
Proteolytic activity	4.61	1.17	0.34
Lipolytic activity	27.20	25.50	19.00
Amylolytic activity	414.00	2339.00	1726.00

weight and the differences were 12 and 15%, respectively. Rats fed on diet supplemented by primrose oil had longer intestines (Table 2) than the ones in other groups, the differences, however, between the means were slight and statistically irrelevant for the compared groups. The highest relative intestine mass, higher by only 6% than the one noted with the rats receiving primrose oil and by as much as 49% than with the control, was recorded with the rats fed with fodder supplemented by soybean oil. The values of this intestine characteristic of rats fed on supplemented diet differed significantly from the ones observed in the control group. Therefore, one can presume that intestine weight depended mainly on the presence of supplemented fats in a diet and not on the kind of added fat.

The livers of the highest weight were recorded with the rats fed on fodder enriched with primrose oil. They were statistically considerably larger by 18% as compared with the rats of the remaining groups.

The rats from the cotrol group were characterized by the highest weight of pancreas and the ones receiving primrose oil revealed the lowest. These weights varied by 15%. Similar dependence was noted for relative pancreas weight although the difference reaching a value above 22% did not occur to be statistically significant.

The rats of the control group had by 22% more protein in pancreas than the

ones fed with the addition of oils. The sort of oil differentiated this value only to a slight degree, that is by about 7%. Lower content of protein in the pancreas of the rats fed with greased mixtures proves directly the depressive influence of fat on the ability to synthesize and collect digestive enzymes, mainly in the nature of proteases and lipases.

Three times higher proteolytic activity of pancreas (Table 3) was noted with the rats of the control group, as compared with the ones fed with diet completed by the addition of soybean oil. The rats receiving primrose oil had this activity only 4 times lower than in the control group. The effect of repressing the activity of pancreatic proteases was pronounced in this model of feeding not only in relation to fat added to fodder but also to its kind.

The higher lipolytic activity (Table 3) was recorded with the control group. As compared with the rats fed with fodder with primrose oil, it was higher by as much as 30%. This activity stands in simple relation to the amount of spare fats accumulated by rats, irrespective of both the supplementing the diet by the addition of fat and the differentiation of the kind. One can suppose that the higher activity of pancreatic lipase results in greater lipogenesis. However, it should be explained which fodder ingredients of applied oil additions, or its binding with the substrates of digestion or the products of enzymatic decomposition or digestive juices constituents is responsible for the inhibition of synthesis and the activity of pancreas lipolytic enzymes. However, the rats receiving diet with primrose oil were characterized by the highest amylolytic activity (Table 3). It was 1.5 times higher than with the ones fed on fodder with soybean oil and 7 times higher than in the controls.

On the average, less by 16.5% protein in liver was observed in rats fed with the mixtures supplemented with primrose oil than with the remaining rats and the arisen differences were statistically significant. It may be connected with the observed highest amount of liver glycogen in this group. The opposite relation between the content of protein and glycogen is not to be explained with the simple mechanism of superseding of this components in liver tissue and one should suppose that it is inherent in other casual mechanisms. The highest amount of glycogen in the liver of rats fed on primrose oil diet, differing significantly from the ones found in other grups, is probably connected not only with much enlarged amylolytic activity of the pancreas of tested rats but also with more efficient pathway for glycogen synthesis and storing capacities of hepatocytes. Connection of these dependences seems to be obvious as the heightened possibilities of freeing monosaccharides from a nutritive dose lead to their heightened absorption, and what follows, also their transformation into glycogen (11).

The conducted studies did not reveal any basic changes of liver transaminases activity (Table 3). It was proved that oil addition to fodder did not exert any destructive influence on the walls of both hepatocytes and mitochondria (2).

The superoxide dismutase activity remaining on appropriate level in comparable groups indicates the heightening amount of superoxides and also free radicals in the liver of rats fed on fodder supplemented by oil, with high content of polyunsaturated fatty acids (10). Slightly higher activity of isocitrate dehydrogenase in rats fed on diet enriched with primrose oil betokens increased transformation of citrate acid cycle. This fact may be connected with faster transformation of sugars into glycogen in the hepatocytes. Only the activity of alcaline phosphatase in the control rats differed significantly from the one recorded with remaining groups and was lower by 18.5%, on the average. Therefore, the increase in the activity of this enzyme with rats fed on greased mixtures may be connected with the deficiency of calcium ions both in the serum and the organs of tested rats (3). The calcium deficiency often occurs under the conditions of feeding diets with the addition of fat as this component of fodder decreases the absorption of Ca from the digestive tract as a result of creating undissolvable soaps (1).

The obtained results of the studies prove the existence of stimulating influence of evening primrose oil addition to diet on the growth of rats, the activity mechanism of which, revealing positively in the results of rearing, seems to be complex and requires further studies with particular explanation of the metabolism of EFA.

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STRESZCZENIE

W doświadczeniach na szczurach rasy Wistar badano wpływ 5% dodatku oleju z nasion wiesiołka lub oleju sojowego do paszy na przyjmowanie pokarmu, odkładanie tłuszczu, długość i masę przewodu pokarmowego oraz aktywność proteolityczną, lipolityczną i amylolityczną enzymów trzustkowych, a także aktywność wybranych enzymów wątrobowych. U szczurów żywionych paszą z dodatkiem oleju wiesiołkowego stwierdzono większe pobieranie pokarmu, większe przyrosty masy ciała i większą masę wątroby, która zawierała mniej białka, a więcej glikogenu w porównaniu do szczurów żywionych paszą z dodatkiem oleju sojowego i szczurów grupy kontrolnej (które otrzymywały paszę bez dodatków olejowych).

Szczury żywione mieszankami z dodatkiem oleju wiesiołkowego i sojowego wykazywały większą aktywność fosfatazy alkalicznej w wątrobie. Najwyższą aktywność alfa-amylazy trzustkowej stwierdzono w grupie szczurów karmionych paszą z dodatkiem oleju wiesiołkowego i sojowego, zaś najwyższą aktywność proteolityczną – w grupie szczurów kontrolnych karmionych paszą standardową, mniejszą – w grupie otrzymującej paszę z dodatkiem oleju wiesiołkowego i najniższą – u zwierząt karmionych paszą z dodatkiem oleju sojowego. Zastosowanie w żywieniu szczurów oleju wiesiołkowego (jako 5% dodatku do paszy) stymuluje pobieranie pokarmu i zwiększa przyrosty masy ciała oraz aktywność alfa-amylazy trzustkowej, zaś zmniejsza aktywność proteaz trzustkowych, nie zmieniając aktywności enzymów wątrobowych.

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