EFFECT OF DIETARY FATTY ACID COMPOSITION ON WEIGHT OF MODEL ANIMALS

PETRA PESKOVA[1], TOMAS KOMPRDA[1], JANA NEUWIRTHOVA[2], BRETISLAV GAL[2], VERONIKA ROZIKOVA[1]

[1]Department of Food Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
[2]Faculty of Medicine
Masaryk University
Kamenice 5, 625 00 Brno
CZECH REPUBLIC

petra.peskova@mendelu.cz

Abstract: The aim of the present study was to compare the effect of diet enriched with 6% fish oil (source of polyunsaturated fatty acids), 6% of safflower oil, 6% of oil from *Schyzichytrium* microalga oil and the effect of diet enriched with 6% of palm oil (source of saturated fatty acids; control) on weight of model animals. Like model animals were used 48 adult male *Rattus norvegicus* Wistar Albino. They were divided into 4 groups with 12 animals each and they were fed for 8.5 weeks. They were weighed every week. There were found no significant differences among all diet in feed intake and final live weight at the day of sacrifice. The only significant variety was observed in total weight gain. It was lower in SF-group in comparison with A-group, which is in contrast with our assumption. The duration of experiment was probably too short to show differences among diet interventions.

Key Words: obesity, safflower oil, fish oil, DHA, palm oil, *Rattus norvegicus*

INTRODUCTION

Obesity is one of the main risk factors contributing on development of many serious diseases. It goes together with higher risc of cardiovascular diseases. This study is focused on important differences among types of fats because of their fatty acid composition. It was compared the influence of safflower oil (source of long chained polyunsaturated fatty acids n-6 – LC-PUFA n-6, SF-diet), palm oil (source of LC-PUFA n-6 which was used as a control group, P-diet), fish oil (source of LC-PUFA n-3, respectively of eicosapentoenoic fatty acid, EPA, 20 : 5n-3) and oil from *Schizochytrium* microalga oil (source of LC-PUFA n-3, mainly of dokosahexaenoic fatty acid, DHA, 22 : 6n-3). EPA and DHA are important parts of cell membranes, they influence their fluidity and behavior of the integral membrane proteins (Schitz and Ecker 2008). If it comes to our eating habits there is high difference in intake of PUFA n-6 and PUFA n-3. It is recommended to consump them in ratio 1 : 1–1 : 2, but the reality is on the other side and we eat them in ratio 1 : 15–1 : 20, it is called western type of diet. Mainly founded fatty acid in this diet are PUFA n-6, the key metabolite is arachidonic acid (AA, 20 : 4n-6). This ratio can contribute to higher risc of chronical degenerative diseases. EPA and DHA are metabolite by the same pathway but it leads to different products. Eicosanoids which are produced from AA have pro-inflammatory effect, they increase cluttering of blood plates and they have vasoconstrictor effect. The products from EPA have in many cases opposite effects (Das, 2006). Current studies also suggest that the right ratio of PUFA n-6 and n-3 improves obesity-linked inflammation and insulin resistance (Liu et al. 2013, Glass and Olefsky 2012).

MATERIAL AND METHODS

Animals, dietary interventions, analysed tissues

A total of 48 adult male rats of the laboratory strain Wistar Albino (Bio Test Ltd., Konárovice, Czech Republic) at the age of 10 weeks (the mean live weght of 230 ± 12 g) were used. Rats were

housed in plastic boxes (53.5 cm × 32.5 cm × 30.5 cm) of 4 animals each in a room temperature at 23 ± 1 °C, humidity 60%, and 12/12 h light/dark cycle (maximum intensity of 200 lx). The experiment was performed in compliance with Czech National Council Act No. 246/1992 Coll. to protect animals against cruelty, the amended Act No. 162/1993 Coll., and was approved by the „Commission to protect animals against cruelty" of the Mendel University in Brno (Experiment Project Number 2017/4).

Basic feed mixture, pelletized complete chow for mice and rats (Biokron, Blučina, Czech Republic), composed of wheat, oat, wheat sprouts, soybean meal, extruded soybean, maize, dried milk, dried whey, dried yeast, grounded limestone, monocalcium phosphate, salt, L-lysine hydrochloride, and a premix of vitamins and minerals, was fed to all animals for the whole experiment with addition of different types of oils.

The rats were randomly divided into 4 groups (12 animals per group) and following dietary interventions were applied for 8.5 weeks: basic feed mixture with fish oil (commercial *oleum jecoris aselli*, 60 g/kg, 12 animals), basic feed mixture with palm oil (60 g/kg, 12 animals), basic feed mixture with oil extracted from *Schizochytrium* microalga (60 g/kg, 12 animals) and basic feed mixture with safflower oil), all of the diets also included extra vitamins/minerals premix (1 g/kg, 48 animals). The mixtures were prepared at the laboratory this way: pelletized chow was ground, homogenized with an appropriate amount of required oil and premix. It was weighted 150 g, 200 g, respectively 50 g of the mixture (dependent on the part of our project), mixed with small amount of water and feeding dose was prepared. The animals were fed daily *ad libitum* and had free access to tap water. Feed consumption was measured daily and animals were weighed each week.

After 8.5 week of feeding all animals were sacrificed (after 12 h fasting) under anesthesia with isoflurane; blood samples were collected from the heart and samples of different tissues were taken.

Fat determination

The sample of feeding mixture was homogenized in Moulinex blender (model D56, Moulinex, France) and subsequently transferred to 150 ml Erlenmeyer flask. The mixture of hexane/2-propanol 3 : 2 (*v/v*) – HIP 1 was added and the sample was sonicated for 15 min using PS10000 apparatus (Notus-Powersonic, Vráble, Slovakia). The extract was filtered using Büchner funnel, then 24 ml of NA_2SO_4 was added. After shaking and separation of the layers in the separation funnel, n-hexane layer was filtered through anhydrous NA_2SO_4 to 50 ml volumetric flask. The water layer was re-extracted with 10 ml of HIP 2 (7 : 2, *v/v*). Layer of n-hexan was filtered through anhydrous NA_2SO_4 after re-extraction. Then it was transferred to 50 ml volumetric flask. Combined extracts were taken to 100 ml round-bottom flask and the content was evaporated on a rotary vacuum evaporator (RV 05-ST 1P-B model; IKA Labortechnik, Staufen, Germany) at 40 °C. Evaporation was finished under nitrogen and total lipids were gravimetrically determined.

Statistical evaluation

One-way analysis of the variance ratio test, including *post-hoc* Tukey's test, was used for evaluation of the differences among the dietary intervations. For all evaluations STATISTICA 12 package (StatSoft, Tulsa, OK, USA) and MATLAB were used.

RESULTS AND DISCUSSION

The composition of the diets is shown in Table 1. As it is shown all of diets were isocaloric.

Feed intake, daily weight gain and final live weight of rats are presented in Table 2. No significant differences in feed intake between groups were found. Daily weight gain was significantly decreased in rats with SF-diet in comparison with other diets, among P-, F- and A-diet were not found any significant differences.

Table 1 The composition of the diets

		Diet			
		P	**SF**	**F**	**A**
Components in the diet (g/kg)	**Basic feed mixture**	939	939	939	939
	Maize starch	-	-	-	-
	Palm oil	60	-	-	-
	Oil extracted from *Schizochytrium microalga*	-	-	-	60
	Fish oil	-	-	60	-
	Safflower oil	-	60	-	-
	Extra premix of vitamins/minerals	1	1	1	1
Nutrients (g/kg)	**Crude Protein**	228	228	228	228
	Fat	56	56	56	56
	Crude fibre	49	49	49	49
	Nitrogen-free extractives	667	667	667	667
Metabolizable energy (MJ/kg)		15.2	15.2	15.2	15.2

Legend: P – basic feed mixture with 6% of palm oil; SF – basic feed mixture with 6% of safflower oil; F – basic feed mixture with 6% of fish oil (commercial oleum jecoris asseli); A – basic feed mixture with 6% of oil extracted from the Schizochytrium microalga.
Basic feed mixture – pelletized complete chow for mice and rats; composition [g/kg]: wheat 475; extruded soybean 180; oat 50; maize 50; wheat sprouts 50; maize sprouts 40; potato protein 22.5; dried whey 53; flax meal 20; dried yeast 12; grounded limestone ($CaCO_3$) 17; monocalcium phosphate 15; common salt (NaCl) 2.5; L-lysine hydrochloride 3; premix of vitamins + minerals 10 (premix composition [mg/kg]: vitamin A 900; vitamin D3 8; vitamin E 10 000; vitamin K3 250; vitamin B1 800; vitamin B2 1000; vitamin B6 500; $FeSO_4$ H_2O 8300; $Mn(x)_{1-3}$ nH_2O + MnO 6500; $Zn(x)_{1-3}$ nH_2O + ZnO 8500; Cu(x) nH2O + $CuSO_4$ 5H2O 1200; KI 100; Na_2SeO_3 16.5; $CoSO_4$ 7 H_2O 51.5; butylhydroxytoluene 3400; propyl gallate 1400; carrier: wheat flour + $CaCO_3$ added to 1 kg).
Crude was protein determined using KD 310-A-1015 Kjel ROC Analyser (Furulund, Sweden).
Fat was determined gravimetrically as hexane/2-propanol extract (see fat determination).
Crude fiber was determined using ANCOM^{220} Fiber Analyser (Ancom Technology, Macedon, NY, USA).
Nitrogen-free extractives were calculated as a remainder to 100% after subtracting the content of crude protein, fat, crude fibre and ash (determined gravimetrically after incinerating an aliyuot at 550 °C.
Metabolizable energy was calculated from nutrient contents.

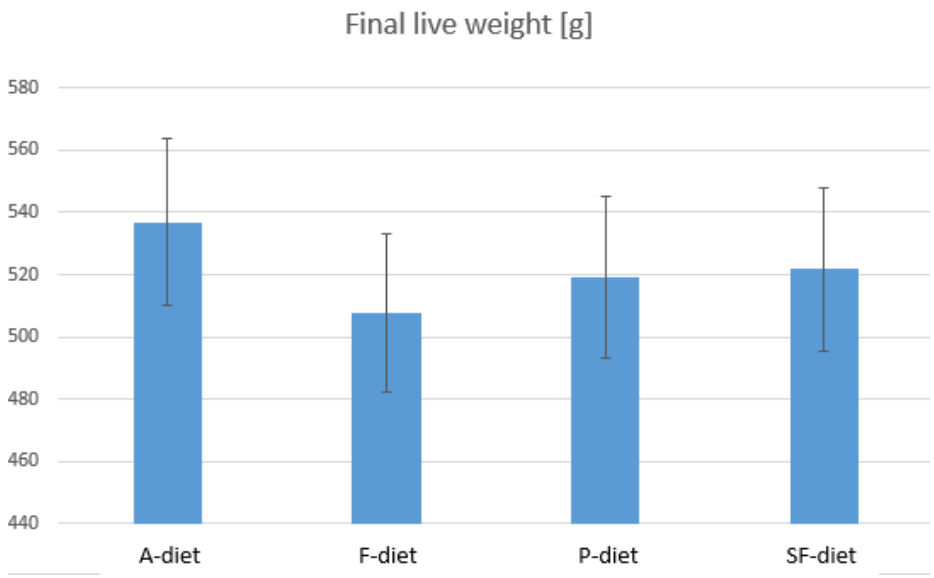
Table 2 Feed intake, daily weight gain and final live weight of rats

Diet	Feed intake[1] [g/day]	Daily weight gain[2] [g] (mean ± SEM)	Final live weight[3] [g] (mean ± SEM)
P	45.6 ± 0.05	5.3 ± 0.5	536.8 ± 30.1
SF	45.1 ± 0.05	4.6 ± 0.3	507.5 ± 16.9
F	46.1 ± 0.05	4.9 ± 0.3	519.1 ± 21.5
A	46 ± 0.05	5.1 ± 0.2	521.7 ± 13.6

Legend: [1]The mean of whole feeding period; daily feed intake of individual rats was calculated as ¼ of the total daily intake per a given box (four animals). [2]The mean of whole experiment. [3]At the day of sacrifice.

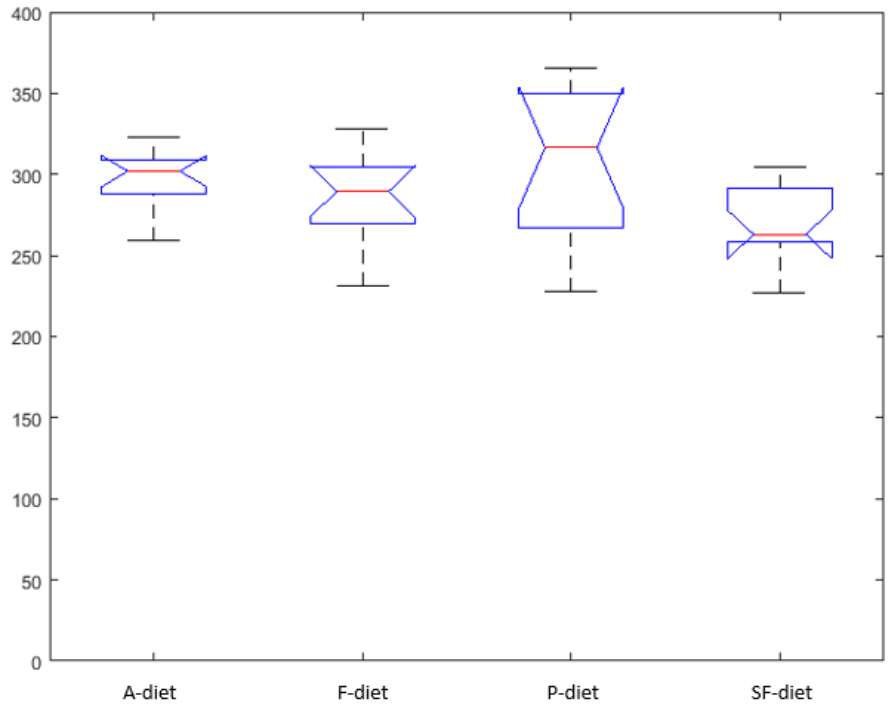
When final live weights are compared, there were found no significant differences (Figure 1). But if it comes to total weight gain it was observed significant difference between A- and SF-diet. Animals which were fed with SF-diet had lower weight gain during whole experiment. The highest weight gain was observed in A-diet. It was probably caused by lower live weight at the beginning of our experiment. There were not found any differences among F-diet and P-diet compared with other groups (Figure 2).

Figure 1 Final live weight [g] ± SEM at the date of sacrifice



Legend: P – basic feed mixture with 6% of palm oil; SF – basic feed mixture with 6% of safflower oil; F – basic feed mixture with 6% of fish oil (commercial oleum jecoris asseli); A – basic feed mixture with 6% of oil extracted from the Schizochytrium microalga. Tested by one-way ANOVA with post-hoc Tukey´s test ($p < 0.05$). There were not observed any significant differences among diets.

Figure 2 Final weight gain [g] during whole experiment



Legend: P – basic feed mixture with 6% of palm oil; SF – basic feed mixture with 6% of safflower oil; F – basic feed mixture with 6% of fish oil (commercial oleum jecoris asseli); A – basic feed mixture with 6% of oil extracted from the Schizochytrium microalga. Tested by one-way ANOVA with post-hoc Tukey´s test ($p < 0.05$).

CONCLUSION

The purpose of the present study was to determine the effect of the diet enriched with 6% of fish, palm, alga and of safflower respectively on weight, gain of weigt and feed intake of animal models (*Rattus norvegicus*). It was analysed composition of feed mixtures, all of them were isocaloric.

There were found no significant differences among all diet in feed intake and final live weight at the day of sacrifice. The only significant variety was observed in total weight gain. It was lower in SF-group in comparison with A-group, which is in contrast with our assumption The duration of experiment was probably too short to show differences among diet interventions.

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