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Research article

EFFECTS OF DIETARY SUPPLEMENTATION WITH A MIXTURE OF BUCKWHEAT LEAF AND FLOWER ON FATTY ACID COMPOSITION OF RAT BRAIN PHOSPHOLIPIDS

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The aim of our study was to stablish the possible alternations in fatty acid composition of brain phospholipids in rats on a standard and high-fat diet supplemented with buckwheat leaf and flower mixture (BLF) and subsequent possible beneficial effects of BLF. Four months old male Wistar rats were randomly divided into five experimental groups fed a standard diet, standard diet supplemented with 5% BLF, high-fat diet, high-fat diet with full-period (13 weeks) of 5% BLF supplementation and high-fat diet with partial-period (7 weeks) of 5% BLF supplementation. Gas-liquid chromatography was performed to analyze the fatty acids in hexane lipid extracts of whole rat brains.

Supplementation with BLF did not induce significant changes in fatty acid composition of whole brain phospholipids in rats fed the standard diet. In rats on high-fat diet concomitant (full-period) BLF supplementation increased eicosapentaenoic acid (20:5n-3, EPA), total n-6 and n-6/n-3 ratio, and decreased the percentage of oleic acid (18:1n-9) and estimated activity of Δ-9 desaturase. When BLF application was postponed (partial-period) in the case of developed hyperlipidemia, a decrease of stearic acid (18:0) accompanied with an increased estimated Δ-9 desaturase activity was observed. Regardless of BLF supplementation all high-fat diet-fed groups showed an elevated percentage of linoleic acid (18:2n-6, LA) and a reduced estimated Δ-6 desaturase activity.

BLF contributes to the maintenance of stable fatty acid composition of brain phospholipids and supports normal brain function in high-fat diet rats, with more positive effects when BLF was applied before hyperlipidemia developed. This could be the mode of buckwheat health beneficial effects on the brain.

Key words: buckwheat leaf and flower mixture, high-fat diet, brain phospholipids, fatty acids

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INTRODUCTION

Phospholipids are complex, polar lipids essential for the lipid bilayer of cell membranes. Above all other tissues, phospholipids are especially abundant in the central nervous system (CNS) of mammals, representing about a half of total lipids in the adult brain. The fatty acids (FAs) composition, the chain length, the degree of saturation and the ratio between saturated and unsaturated FAs in phospholipids are of great importance for the maintenance of neural membrane integrity and fluidity, as well as for its function [1,2]. Evidence indicates that the brain phospholipids contain a very high amount of polyunsaturated FAs (PUFA), one FA of three is PUFA, predominantly arachidonic acid (20:4 n–6, AA) and docosahexaenoic acid (22:6 n–3, DHA) [3,4]. Although, this composition seems to be quite constant it could be modulated by environmental influences such as diet or dietary supplementation.

Buckwheat (*Fagopyrum esculentum* Moench) is a herbaceous plant, which belongs to the P*olygonaceae* family. Despite popular belief, it is actually a fruit seed not a grain, related to rhubarb and sorrel. Due to containing nutritional ingredients with high biological value it is an important food source for animal and human consumption. Moreover, diets that contain buckwheat have been linked to many health-beneficial effects such as: lowered risk of developing high cholesterol and blood pressure, less progression in stenosis and atherosclerosis, protection against breast and other hormone-dependent cancers as well as heart disease and diabetes [5-8]. The buckwheat's lipid-lowering activity is largely due to its rich supply of flavonoids, particularly rutin. Among others, rutin acts as a powerful antioxidant inhibiting lipid peroxidation and reactive oxygen species (ROS) induced damages. The statistically significant relationship between buckwheat's antioxidant activity and its total phenolic, as well as rutin content, has been confirmed. About 2-10% of rutin per dry weight can be found in buckwheat flowers and leaves, and their total phenolic content is higher than that of seeds (flower > leaves > seed > stem > root) [9]. However, as buckwheat is mainly used as seed products flour and groat, there is still an open area of research of health-beneficial effects of other parts of the buckwheat plant (e.g. flowers and leaves) on tissues, including the nervous tissue.

A previous study, using buckwheat leaf and flower mixture (BLF) has shown the hypolipidemic, antiatherogenic and antioxidative effects in rats fed a high-fat diet. These effects were accompanied with improvement of FAs profiles of plasma phospholipids where supplementation with BLF significantly decreased the percentage of palmitic acid and total saturated fatty acids (SFA) and increased the percentage of stearic acid and total PUFA [10]. To our knowledge, no reports are available assessing buckwheat effects on fatty acids in the brain. Since phospholipids in various tissues, including the brain tissue, basically mirror the FAs state in the plasma, the aim of this study was to establish the alternations in fatty acid composition of brain phospholipids in rats on standard and high-fat diet supplemented with BLF. Possible beneficial effect of buckwheat on brain fatty acid profiles and consequently brain function were examined.

MATERIALS AND METHODS

Experimental animals and diets

The experiments were performed on four months aged male Wistar rats (b.w. 310-440 g; n=33). The animals were housed in groups of two or three per standard cage, in a room with controlled conditions (12 h light-dark cycle, ambient temperature of 24°C), on a pelletized commercial diet (Veterinary Institute, Subotica) for 2 weeks after arrival. After this pre-experimental period the animals were randomly divided into five groups each having a specific dietary regime for the next 13 weeks. The first group of rats (group I, n=7) was fed the standard diet. The second group of rats (group II, n=6) was fed the standard diet with 5% BLF (Institute for Medicinal Plants Research "Dr Josif Pančić", Belgrade, Serbia). The animals of group III and IV were on a lipogenic diet consisting of 2.5% cholesterol, 20% sunflower oil and 0.5% sodium cholate added to standard chow without (group III, n=7) or with 5% BLF (group IV, n=6). The animals of group V (n=7) were maintained on the same food regime as those in group III during the first 7 weeks. After this period, they doubled plasma cholesterol concentration and switched to the same feed intake as the animals in group IV for the next 6 weeks.

In our previous study, standard methods of analysis (AOAC, 1984) were used to determine the proximate composition of each type of experimental diets. The content of protein (g kg^{-1}) in standard diet, standard diet +BLF, high-fat diet and high-fat diet +BLF is given in Table 1 [11]. The fatty acid composition of sunflower oil is described in Table 2. During the entire experimental period food and tap water were given ad libitum. Feed intake and weight gain were measured daily and weekly, respectively. The animals were decapitated with Harvard Guillotine under ether anesthesia. They were fasted overnight prior to sacrifice. The brains were removed, rinsed with saline and stored at -80°C until analysis. All the experiments used in the study were reviewed and approved by the Institutional Animal Care and Use Committee [No.III-2011-01]

Table 1. Feed composition of each type of experimental diet

	Standard diet	Standard diet+BLF	High-fat diet	High-fat diet+BLF
Proteins (g/kg)	217.4	206.4	171.1	169.2
Moisture (g/kg)	72.2	85.0	68.4	68.7
Cellulose (g/kg)	48.6	63.4	21.9	42.6
Fat (g/kg)	36.3	29.1	210.8	201.9
Carbohydrate (g/kg)	436.5	405.8	344.1	352.2
Ash (g/kg)	69.3	69.0	56.5	56.5
Energy value (kJ/kg)	15710	15470	19270	19250

Table 2. Fatty acids composition of sunflower oil

Palmitic acid (16:0) (g/kg)	62.3
Stearic acid (18:0) (g/kg)	40.7
Oleic acid (18:1,n-9) (g/kg)	266.3
18:1, n-9 trans	6.1
Linoleic acid (18:2, n-6)	624.8
SFA	103.0
MUFA	272.1
PUFA	624.8

Lipid extraction and fatty acids analysis

Whole rat brains (~2 g) were homogenized in 6 ml of chlorophorm/methanol (1:2, by volume). Aliquots of 3 ml of the brain homogenate were used for total lipid extraction, according to the procedure [12]. After centrifugation and removal of the supernatant, the remaining tissue was resuspended in 3 ml of solvent (chlorophorm/methanol, 1:2, by volume) and centrifugation was repeated. The combined supernatants were evaporated to dryness and the residue was dissolved in chlorophorm/methanol/water (60:30:4.5, by volume). Low molecular weight contaminants were eliminated by partition with chlorophorm/methanol/KCl (4:2:1, by volume). After centrifugation the upper layer was discarded and the lower lipid layer was used for further analysis.

Direct transesterification of fatty acids was carried out according to a modified method [13]. The hexane extract containing fatty acids methyl esters was evaporated under a stream of nitrogen to complete dryness. The sample was dissolved in 200 μl of hexane and 1 μl was manually injected into the GC.

Fatty acids methyl esters were analyzed by gas-liquid chromatography on a Shimadzu chromatograph GC 2014 equipped with a flame ionization detector on Rtx 2330 column (60 m x 0.25 mm ID, film thickness 0.2 μm, Restek, Bellefonte, PA). Separation was obtained over a 51 min period with an initial temperature of 140 °C held for 5 minutes. The temperature was then increased to 220 °C at a rate of 3 °C/min and held on final temperature for 20 minutes. The injection was performed with a split ratio 50:1 and constant flow operating mode at 11 ml/min, with helium as carrier gas. The injector temperature was 220 °C and detector temperature was 240°C. The identification of fatty acid methyl esters was done comparing retention times with standard mixtures (PUFA-2 and/or 37 FAMEs mix, Supelco, Bellefonte, PA). Individual fatty acids were expressed as a percentage of total fatty acids identified.

Desaturase and elongase activities were estimated from the primary data by using specific FA indices. The ratio of 20:4n-6/20:3n-6 was used to estimate Δ-5 desaturase activity. The activities of Δ-6 desaturase and elongase were estimated from the ratio

of 20:3n-6/18:2n-6, while the ratios of 18:1n-9/18:0 and 18:0/16:0 represented estimated Δ-9 desaturase and elongase activities, respectively.

Statistical analysis

The results are expressed as means ± SD. Normality was tested using the Shapiro-Wilk test. When variables showed normal distribution, statistical analysis was performed using one-way ANOVA, followed by Tukey's *post hoc* test to identify inter-group differences. For non-normally distributed variables (22:4n-6 and MUFA), Kruskal-Wallis and Mann-Whitney tests were applied. A value of $p < 0.05$ was considered statistically significant.

Table 3. Values are expressed as mean ± SD. Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between groups

Duration 13 weeks	Standard diet (13w)	Standard diet + BLF (13w)	High-fat diet (13w)	High-fat diet + BLF (13w)	High-fat diet (7w) BLF (6w)
	group I (n=7)	group II (n=6)	group III (n=7)	group IV (n=6)	group V (n=7)
Food intake (g/day)	26.16±1.14 a	24.37±1.58 b	19.19±0.29 c	18.51±1.04 d	18.93±0.75 cd
Weight gain (g/day)	8.96±0.72 a	7.22±0.82 a	13.31±1.15 b	9.75±0.73 a	10.67±1.35 a

RESULTS

As presented in Table 1, the food intake in all groups supplemented with BLF (group II) and/or fed high-fat diet (group III, IV and V) was significantly lower in comparison to the control group fed the standard diet (group I), the differences were $p<0.05$, $p<0.001$, $p<0.001$ and $p<0.001$, respectively. However, high-fat diet-fed animals (group III, IV and V) showed significantly higher weight gains compared to both groups of standard diet-fed animals (group I and II), the differences were $p<0.001$, $p<0.05$ and $p<0.05$ in comparison to group I; and $p<0.001$, $p<0.05$ and $p<0.001$ in comparison to group II, respectively.

Alternations in percentage of individual fatty acids observed in whole brains phospholipids of rats on standard and high-fat diet no-supplemented or supplemented with BLF are presented in Table 4, 5 and 6. When compared to all other groups, the lowest level of SFA stearic acid (18:0) was obtained in partial-period BLF supplemented high-fat diet rats (group V) (25.14±0.5%), where statistically significant difference in comparison to full-period supplemented high-fat diet rats (group IV) (26.96±1.26%) was detected ($p<0.01$) (Table 4). At the same time, the highest level of MUFA vaccenic acid (18:1n-7, VV) was found in full-period BLF supplemented high-fat diet-fed rats (group IV) (7.99±1.03%), being significantly different compared to BLF supplemented standard diet rats (groups II) (5.33±0.91%) and partial-period BLF supplemented high-fat diet rats (group V) (5.48±0.61%) ($p<0.01$) (Table 5).

Contrary, when considering a percentage of the other MUFA oleic acid (18:1n-9), full-period BLF supplemented high-fat diet rats (group IV) showed the lowest level (20.75±1.31%), significantly lower in comparison to BLF supplemented standard diet rats (group II) (23.73±0.90%) ($p<0.05$) (Table 5). In addition, all high-fat diet rats (group III, IV and V) showed a significant increase in PUFA linoleic acid (18:2n-6) compared to BLF supplemented standard diet rats (group II) ($p<0.05$, $p<0.01$, $p<0.05$, respectively), as well as in total n-6 PUFA as compared with the same group ($p<0.05$, $p<0.001$, $p<0.01$, respectively) (Table 6). The percentage of PUFA eicosapentaenoic acid (20:5n-3, EPA) and n-6/n-3 ratio, also increased in full-period BLF supplemented high-fat diet-fed rats (group IV), making again a significant difference between groups II and IV ($p<0.01$) (Table 6). All other fatty acids of brain phospholipids remained unchanged in standard as well as high-fat diet-fed animals regardless of the BLF supplementation.

Table 4. Values are presented as mean ± SD.SFA, saturated fatty acids; Significantly different from group IV: b $p<0.01$

FA (%)	Standard diet	Standard diet +BLF	High-fat diet	High-fat diet +BLF	High-fat diet (7) BLF(6)
	group I (n=7)	group II (n=6)	group III (n=7)	group IV (n=6)	group V (n=7)
16:0	20.92±2.12	21.41±0.46	20.60±0.96	20.17±1.36	21.90±0.97
18:0	26.32±0.74	26.15±0.31	26.45±0.75	26.96±1.26	25.14±0.57 b
SFA	47.24±2.16	47.56±0.34	47.05±0.83	47.13±2.10	47.04±1.15

Table 5. Values are presented as mean ± SD. saturated fatty acids; MUFA, monounsaturated fatty acids. Significantly different from group II: a_1 $p<0.05$, a_2 $p<0.01$. Significantly different from group IV: b $p<0.01$

FA (%)	Standard diet	Standard diet +BLF	High-fat diet	High-fat diet +BLF	High-fat diet (7) BLF(6)
	group I (n=7)	group II (n=6)	group III (n=7)	group IV (n=6)	group V (n=7)
16:1n-7	0.31±0.06	0.30±0.02	0.27±0.02	0.31±0.04	0.29±0.02
18:1n-9	21.85±1.57	23.73±0.90	21.41±1.57	20.75±1.31 a_1	22.82±1.20
18:1n-7	6.70±1.16	5.33±0.91	6.76±1.67	7.99±1.03 a_2	5.48±0.61 b
MUFA	28.85±2.45	29.36±0.80	28.44±1.36	29.05±2.00	28.58±1.71

Table 7 presents the estimated activities of desaturases and elongase systems in rat brain phospholipids. The highest estimated activity of Δ-9 desaturase was found in BLF supplemented standard diet-fed rats (group II) and partial-period BLF supplemented high-fat die-fed rats (group V), being significantly different compared to full-period BLF supplemented high-fat diet-fed rats (group IV) ($p<0.05$). All high-

fat diet fed groups (group III, IV and V) showed reduced estimated Δ-6 desaturase activity, significantly different in comparison to BLF supplemented standard diet-fed rats (group II) ($p<0.001$, $p<0.001$ $p<0.01$, respectively). There was no differences in the estimated activities of Δ-5 desaturase, while elongase activity appeared to be significantly different between full-period and partial-period BLF supplemented high-fat diet-fed rats (group IV and V) ($p<0.05$).

Table 6.Values are presented as mean ± SD. PUFA, polyunsaturated fatty acids. Significantly different from control (group I): * $p<0.05$. Significantly different from group II: a_1 $p<0.05$, a_2 $p<0.01$, a_3 $p<0.001$

FA (%)	Standard diet	Standard diet +BLF	High-fat diet	High-fat diet +BLF	High-fat diet(7) BLF(6)
	group I (n=7)	group II (n=6)	group III (n=7)	group IV (n=6)	group V (n=7)
18:2n-6	1.11±0.30	0.69±0.07	1.34±0.15 a_1	1.58±0.68 a_2	1.46±0.08 a_1
20:3n-6	1.10±0.32	1.07±0.09	1.06±0.08	1.18±0.27	1.26±0.26
20:4n-6	7.95±0.48	7.67±0.36	8.32±0.40	8.03±0.69	7.88±0.53
22:4n-6	3.28±0.24	3.15±0.21	3.26±0.27	3.66±0.84	3.38±0.40
n-6	13.44±0.35	12.58±0.46	13.98±0.38 a_1	14.45±0.98 a_3	13.98±0.58 a_2
20:5n-3	0.05±0.02	0.04±0.01	0.05±0.02	0.06±0.02 a_1	0.05±0.02
22:5n-3	0.39±0.09	0.38±0.02	0.32±0.06	0.35±0.06	0.36±0.03
22:6n-3	10.02±0.91	10.08±0.51	10.15±0.82	8.93±0.95	9.96±0.58
n-3	10.47±0.90	10.50±0.52	10.53±0.80	9.37±0.90	10.39±0.54
PUFA	23.91±1.16	23.08±0.72	24.51±1.10	23.82±0.69	24.37±0.78
n-6/n-3	1.29±0.09	1.20±0.07	1.33±0.08	1.56±0.25 *, a_2	1.35±0.09

Table 7. Values are presented as mean ± SD. Significantly different from control (group I): * $p<0.05$. Significantly different from group II: a_1 $p<0.05$, a_2 $p<0.01$, a_3 $p<0.001$. Significantly different from group IV: b $p<0.05$

	Standard diet	Standard diet+BLF	High-fat diet	High fat diet+BLF	High fat diet(7) BLF(6)
	group I (n=7)	group II (n=6)	group III (n=7)	group IV (n=6)	group V (n=7)
18:1n-9/18:0 (Δ-9 desaturase)	0.83±0.08	0.91±0.04	0.81±0.06	0.77±0.08 a_1	0.91±0.06 b
20:3n-6/18:2n-6 (Δ-6 desaturase and elongase)	1.06±0.43	1.57±0.14 *	0.81±0.15 a_3	0.79±0.14 a_3	0.87±0.21 a_2
20:4n-6/20:3n-6 (Δ-5 desaturase)	7.83±2.14	7.23±0.83	7.87±0.88	7.04±1.22	6.58±1.61
18:0/16:0 (elongase)	1.27±0.14	1.22±0.04	1.29±0.08	1.34±0.10	1.15±0.06 b_1

DISCUSSION

The difference between feed intakes in rats maintained on high-fat diet and those on standard diets could be explained by an increased feeling of satiety in high-fat diet animals [14]. Due to enrichment in fats, high-fat diet has a higher calorific value, likely being required in somewhat smaller amounts to fulfill rat's feeding needs. Additionally, BLF supplementation probably affects the distinctive taste of a standard rat chow, resulting in reduced daily feed intake, at least during the first days of supplementation [15]. However, despite the lower feed intake rats fed a diet rich in fat exhibited a significantly greater weight gain, what can be again attributed to the high-energy diet they consumed [16].

Despite the well evidenced association between dietary fat and the fatty acid content of the brain, to the author's knowledge, this is the first study dealing with alternations in brain fatty acids in the case of BLF supplementation. In our previous supplementation trials on BLF by decreasing the percentage of palmitic acid and total SFA and increasing stearic acid and total PUFA improved FAs profiles in rat plasma, demonstrating an overall hypolipidemic effect in hyperlipidemic rats [10].

In this study, BLF supplementation did not induce any significant changes in fatty acid composition of whole brain phospholipids in rats fed standard pelletized diet. At the same time, our results clearly demonstrate the differences in fatty acid composition of brain phospholipids between rats fed the standard diet with BLF supplementation (group II) and those fed high-fat diet with full-period (13 weeks) or partial-period (6 weeks) BLF supplementation (group IV and V, respectively). The main reason to distinguish between the high-fat diet fed rats with full-period and partial-period BLF supplementation is that in the first group BLF supplementation was provided concomitantly with the lipogenic diet therefore the possible preventive effect of BLF mixture on rat brains during the development of hyperlipidemia can be followed, while the second group with postponed BLF supplementation may reflect BLF supplementation effects in rats after the onset of hyperlipidemia.

The significant decrease of SFA stearic acid (18:0) in animals fed a lipogenic diet with partial-period BLF supplementation as observed in our study, could probably be related to the estimated elevated activity of Δ-9 desturase i.e. an enzyme that uses stearic acid as a precursor in biosynthesis of oleic acid (18:1-n9). Taking into account that increased Δ-9 desturase activity is a characteristic of adverse condition such as metabolic syndrome, obesity, hypertriglyceridemia, atherosclerosis or cancer [17,18], it can be said that BLF supplementation failed to have health beneficial effects mediated by this enzyme in rats with developed hyperlipidemia. In contrast, in full-period BLF supplemented rats the reduction of estimated Δ-9 desaturase activity was detected. Decreased Δ-9 desaturase activity is associated with many beneficial effects on human health, such as prevention of fatty liver and insulin resistance, reduced risk of obesity and cancer, and reduced inflammation [19-22], speaking in favor of positive effects of supplementation exerted when BLF mixture is applied concomitantly with the

lipogenic diet, but not in the case of delayed supplementation as in partial-period BLF supplemented rats. The exogenous control of Δ-9 desturase activity is already well documented, being inhibited by a high intake of n-3 PUFA, n-6 PUFA and conjugated linoleic acid, and stimulated by carbohydrates, alcohol, cholesterol and liposoluble vitamins A and D [23].

Full-period BLF supplementation increased the level of MUFA vaccenic acid (18:1n-7, VV) in high-fat diet fed rats when compared to rats on lipogenic diet with partial-period BLF supplementation. *Cis*-vaccenic acid which is followed in this paper, is long been known to improve cell membrane fluidity [24], while more recent findings report on the association between the content of *cis*-vaccenic acid in red blood cells and lower risk of total coronary heart disease [25]. Also, in mouse mesencephalic cell line positive effects of *cis*-vaccenic acid on dopaminergic function in primary neurons were found [26]. Therefore, in view of the possible beneficial effects in the brain, the increase of vaccenic acid as detected herein, speaks in favor of full-period rather than partial-period BLF supplementation.

Full-period BLF supplementation decreased the level of MUFA oleic acid (18:1-n9) in high-fat diet rats compared to animals on standard diet with BLF supplementation. This result is in line with the recent study, reporting the lower level of oleic acid in plasma phospholipids of children fed a diet rich in fiber [27]. In the brain tissue oleic acid is synthesized from its unsaturated precursor stearic acid (18:0), which is also synthesized by the brain or can be exogenously imported by passing the blood-brain barrier [28]. Since the lower level of oleic acid in full-period supplemented high-fat diet rats was not accompanied with corresponding decrease in stearic acid, it was reasonable to assume that BLF mixture exerts its effect by inhibiting oleic acid biosynthesis. This assumption is supported with the previously discussed inhibition of Δ-9 desturase in the brains of full-period supplemented high-fat diet rats. Oleic acid is known to be the major component of CNS myelin as well as a modulator of either activity or expression of key enzymes in brain cell signaling (protein kinase C, synaptosomal Na^+/K^+-ATPase), lipogenesis (acetyl-coenzyme A carboxylase) and cholesterolgenesis (3- hydroxy-3-methylglutaryl coenzyme A reductase) [29,30]. Thus, fluctuation in brain oleic acid concentration, as observed in this study, could have an impact on many aspects of neural activity. In addition, alternations in FA such as oleic, linoleic (18:2n-6, LA) and alpha-linolenic acid (18:3n-3) may affect the stability of the erythrocyte membrane having an effect on blood-brain barrier permeability [31]. Result of Lee *et al* [32] suggests an effect of oleic acid on infarct volume in ischemic rat brain exerted through the activation of peroxisome proliferator activated receptors.

The significant increase in the level of LA in high-fat diet rats as observed in our study, appears as a consequence of the high intake of sunflower oil having an appreciable amount (624.8 g kg^{-1}) of LA. This result is in line with the more recent studies that reported that supplementation of various dietary oils modified the fatty acid composition of cell membranes in the brain [33-37]. High levels of LA in sunflower oil down-regulated n-3 PUFA desaturation, resulting in the loss of membrane n-3 PUFA.

More specifically, a recent study has shown that rats fed a sunflower oil diet through two generations have significantly lower levels of DHA in the brain phospholipids. Although the deficiency of n-3 PUFA in the brain does not affect the motor and reflex abilities in rats, those animals exhibit weaker exploratory behavior and poor performing maze-learning tasks [38]. On the other hand, lowering of dietary intake of LA can reduce the synthesis and/or accumulation of oxidized LA derivatives that have been implicated in a variety of pathological conditions such as Alzheimer disease and dementia [39,40].

Small fractions of LA and alpha-linolenic acid in the brain are converted to arachidonic acid (20:4n-6, AA) and docosahexaenoic acid (22:6n-3, DHA), respectively [41,42]. Besides, the alternations in the level of LA, the levels of dihomo-gamma-linolenic acid (20:3n-6) remained unchanged among all experimental groups. Results from literature also show that consumption of LA-rich diets could have an impact on the alpha-linolenic acid conversion to DHA, in a manner that increased the level of docosapentaenoic acid (22:5n-3, DPA) and reduced concentrations of DHA in the developing brain [43]. In our study, elevated LA content increased the level of total n-6 PUFA in the brains of rats fed high-fed diet + BLF, but no effect on DHA or total n-3 PUFA level was detected. However, despite an elevated level of total n-6 PUFA in rats fed high-fat diet, the estimated ratio of n-6/n-3 remained below the value of 2 in all experimental groups, which is in line with optimal ratios 1/1 or 2/1 in the brain, providing normal brain function [44]. In addition, the level of AA remained constant what could be important as a stable content of AA and DHA is crucial in neurodevelopment, while deficiency of these two fatty acids may hinder the development of brain function and lead to behavioral disorders. Speaking in favor of positive effects in the brain, our findings also revealed that full-period BLF supplementation increased the level of eicosapentaenoic acid (20:5n-3, EPA) in high-fat diet rats. Along with DHA, EPA by protecting the structural integrity, balancing the immune function and decreasing neuronal apoptosis in the brain tissue is the most important PUFA in relation to normal neuronal activity and prevention of mental health problems and mood dysregulation [45,46].

In this study, the ratios between specific FA products and their precursors were used to estimate the enzymatic activity of brain desturase and elongase systems, which is a common approach in some other tissues, as liver or plasma [47]. Brain FA composition is a complex mixture of de novo synthesized FA in the brain tissue and FA derived from the diet or synthesized in the liver. At the same time the activities of enzymes, elongases 2 and 5 and Δ-5 and Δ-6 desaturases, are generally much lower in the brain than in the liver [48]. Therefore, some limitations occur when interpreting the results on the estimated activity of these enzymes as reported herein. Although our results indicated a significant increase of estimated Δ-6 desaturases activity in BLF supplemented standard diet rats when compared to those without BLF supplementation, and particularly those on the lipogenic diet, additional experiments are needed to evaluate the possible impact of buckwheat on Δ-6 desaturases activity

in the brain. It may be important in view of the major role Δ-6 desaturases play in the cellular supply of long-chain PUFA, as the changes in the activity of this enzyme is associated with various pathological processes as shown in the liver [49]. The positive effect of full-period BLF supplementation on the prevention of decrease in elongase activity, as detected in the partial-period supplemented hyperlipidemic rats, also remains to be further investigated.

In summary, our study revealed that buckwheat given in a form of BLF supplementation leads to moderate changes in fatty acid profiles and estimated desaturase and elongase activity in the brain, supporting the maintenance of stable levels of fatty acids in brain phospholipids and normal brain function in high-fat diet rats. More positive effects have been achieved when BLF was applied before hyperlipidemia developed. These findings encourage further research on the buckwheat beneficial effects in the brain.

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EFEKAT DIJETARNE SUPLEMENTACIJE SA MEŠAVINOM LISTA I CVETA HELJDE NA MASNOKISELINSKI PROFIL FOSFOLIPIDA MOZGA PACOVA

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Cilj naše studije koja je trajala 13 nedelja bila je određivanje masnokiselinskog profila fosfolipida mozga kod pacova na standardnoj hrani i masnoj dijeti saplementiranoj sa listom heljde i mešavinom cveta heljde (BLF) kao i ispitivanje potencijalnih benefit efekata BLF. Četiri meseca stari pacovi (muškog pola) su nasumično podeljeni na 5 eksperimentalnih grupa. Prva (I) hranjena standardnom hranom, druga (II) hranjena standardnom hranom +BLF (5%), treća (III) grupa na masnoj dijeti, četvrta (IV) na masnoj dijeti +BLF (13 nedelja), peta (V) grupa na masnoj dijeti (7 nedelja) + BLFsaplementacija (6nedelja). Saplementcija BLF nije indukovala značajne promene u masnokiselinskom profilu fosfolipida mozga kod pacova hranjenih standardnom hranom. Kod pacova (grupa IV) na masnoj dijeti i BLF saplementaciji povećao se procenat EPA (20:5, n3), ukupnih n-6 i n-6/n-3 odnosa, a smanjio se procenat oleinske kiseline (18:1 n9) i procenjene aktivnosti delta-9 desaturaze. U grupi (V) u kojoj se već razvila hiperlipidemija a BLF saplementiran posle 7 nedelja smanjio se procenat stearinske kiseline (18:0) udružen sa povećanom procenjenom vrednosti delta 9 desaturazne aktivnosti. Generalno, BLF saplementacija u svim grupama sa masnom hranom dovela je do povećanja linolne kiseline (18:2, n-6, LA) i smanjenja procenjene vrednosti delta-6 desaturazne aktivnosti. BLF doprinosi očuvanju masnokiselinskog sastava fosfolipida mozga i pomaže normalnom funkcionisanju mozga kod pacova hranjenih masnom dijetom ali sa mnogo više efekta pre razvijene hiperlipidemije.

Na ovome bi se mogao zasnivati pozitivan efekat heljde na mozak.