



TITLE: Buckwheat – enriched instant porridge improves lipid profile and reduces inflammation in participants with mild to moderate hypercholesterolemia

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Abstract

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Buckwheat (BW) food is a good source of beneficial substances. Based on increased interest in product development approach to formulate new types of BW food products with health claims, three different instant porridges were developed: BW – enriched high-protein (A); corn-based high-protein (B); corn-based non-protein (C) and their effects were investigated on selected clinical markers. A randomized, crossover intervention study was performed among 34 participants with mild to modest hypercholesterolemia. Intake of porridge A significantly reduced serum levels of total cholesterol, LDL cholesterol, triacylglycerol, uric acid and significantly increased serum adiponectin levels, HDL cholesterol, and fat free mass. In conclusion, intake of porridge A as a functional food may improve lipid profile and may reduce inflammation. These findings may support that BW as a part of porridge consumption is an effective and convenient nutritional therapy for the improvement of metabolic parameters in participants with metabolic syndrome components.

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Keywords: buckwheat, porridge, hypercholesterolemia, metabolic syndrome, inflammation, nutrition therapy

1. Introduction

In recent decade, buckwheat (BW) has gained increased attention of food processors and consumers due to its high nutritional value and numerous other beneficial health effects its consumption may provide. A large number of studies have inferred to remarkable nutritional quality of BW, high biological value of its components and their potential beneficial effects as reviewed in (Gimenez-Bastida & Zielinski, 2015; Zhu, 2016). Additionally, BW is suitable for celiac sufferers as it is free from toxic prolamins (De Francischi, Salgado, & da Costa, 1994).

BW has superior protein quality in comparison to cereals due to well-balanced amino acid composition in spite of their low digestibility (Krkošková & Mrazova, 2005; Christa & Soral-Smietana, 2008). BW contains soluble carbohydrates, fagopyritols, which are a source of *D-chiro*-inositol (Janet, Horbowicz, & Obendorf, 2005), a component appearing to be beneficial in the treatment of polycystic ovarian syndrome and diabetes (Larner, Brautigan, & Thorner, 2010; Unfer & Porcaro, 2014). Due to presence of resistant starch, BW is suggested as an ingredient in design of low glycemic index (GI) food (Alvarez-Jubete, Arendt, & Gallagher, 2010). Fats in BW are mostly unsaturated with the predominance of oleic and essential linoleic acids (Christa & Soral-Smietana, 2008). Besides high-quality proteins, fat and favourable carbohydrate composition, BW is an abundant source of numerous other components with healing benefits: dietary fibres, flavonoids and flavones (rutin, quercetin, isovitexin), phytosterols (β -sitosterol), macro and microelements, B vitamins (B2, B6), etc. (Krkošková & Mrazova, 2005; Christa & Soral-Smietana, 2008; Alvarez-Jubete, Arendt, & Gallagher, 2010).

Health effects and pharmacological activities of BW were mostly reported in animal studies and only scarce human data exist (Gimenez-Bastida & Zielinski, 2015; Zhu, 2016). In

humans, BW leaf tea was confirmed to have pharmacological action in the treatment of leg oedema (Ihme et al., 1996). Furthermore, it has been reported successful application of tartary BW in the treatment of gastritis and diabetic patients (Lin, Jia, & Ren, 1998). He (1998) reviewed the medical use of tartary BW in China and reported its use as a bactericidal agent in the treatment of wounds and ulcers, to control hyperlipidemia, diabetic hypertension and diabetic coronary disease (He, 1998). In addition, daily administration of BW supplement was proven to reduce triacylglycerols (TAG), total cholesterol, low density lipoprotein (LDL) cholesterol, blood pressure, body weight and increase high density lipoprotein (HDL) cholesterol in senile hyperlipemia patients (Xiping & Xianqiong, 1995). Moreover, a study involving human subjects reported the positive effect of consuming tartary BW cookies on the reduction of myeloperoxidase which is a biomarker of neutrophilic inflammation caused by lower airway inflammation (Wieslander et al., 2011). The same study inferred that consumption of cookies made from common and tartary BW may lower cholesterol levels and cardiovascular risks as well as improve lung vital capacity. These effects were ascribed to the presence of rutin and other flavonoids in BW (Wieslander et al., 2011). Recently, Nishimura et al. (2016) have shown that rutin-rich tartary BW was effective in lowering body weight, body mass index and body fat percentage of human subjects. The ability of tartary BW to change body composition was associated to its high antioxidant activity. Other beneficial health effects associated with regular daily intake of BW-enriched food were evidenced in recent human studies such as a fatigue reduction in healthy subjects after regular consumption of BW cookies providing an intake of 359.7 mg rutin equivalents per day (Wieslander et al., 2012) and an alleviation of renal dysfunction in type-2 diabetic patients (Qiu, Li, Qin, Yue, & Liu, 2016).

Considering all these powerful functional properties and beneficial health effects provided by BW, it is not odd that regular BW consumption has been associated with

preventive nutrition (Krkoškova & Mrazova, 2005; Zhang et al., 2012). So far, the preventive health impact of BW has been related to radical scavenging effect of BW peptides as well as high concentration of numerous other components with healing effects such as flavones, flavonoids, phytosterols, D-chiro-inositol and myo-inositol (Zhang et al., 2012). Consequently, BW has been regarded as an ingredient particularly suitable for use in a novel class of food preparations known as FOSHU (Food for Specified Health Use) (Brunori et al., 2009). BW is used for food in many different forms: as (un)roasted decorticated grains for traditional dishes referred to as “kasha”, served as an alternative to rice or porridge or as an addition to soups/stews or in the form of flour, combined with other cereal flours in bread, noodles, cakes, pancakes, muffins, etc. Apart from health benefit, an important feature of food is its convenience. Processing may offer the convenience of consumption to food product. Following this trend, BW has been lately used as an ingredient in processed ready-to-eat food such as puffed snacks (Wojtowicz, Kolasa, & Moscicki, 2013) and ginger-nut biscuits (Filipčev, Šimurina, & Bodroža-Solarov, 2015). Porridge is a popular healthy meal consumed mostly as a breakfast and consists of various cereals or legumes cooked in water or milk to a mushy consistency. A variant of porridge is instant porridge which preparation is simple as it does not require cooking but pouring over with warm water. BW can be used as an ingredient for instant porridges.

The objective of the current randomized, placebo-controlled, crossover intervention study was to evaluate the effects of a porridge A on metabolic health parameters.

2. Methods

2.1 Study participants

39 Caucasian healthy male and female volunteers were recruited (age 46.6 ± 8.2 y; BMI 25.7 ± 4.2 kg/m²) according to the following inclusion criteria: non-smoking men and women, with a BMI between 20 and 35 kg/m², and serum concentration of total cholesterol and/or triacylglycerol above normal (total cholesterol >5.2 mmol/L or/and LDL cholesterol >3.3 mmol/L or/and TAG >1.7 mmol/L). Exclusion criteria included the presence of diabetes mellitus; cardiovascular, gastrointestinal, or liver disease; usage of antihypertensive or cholesterol-lowering medication; adherence to a prescribed diet; unstable weight in the past 3 months; vigorous physical activity; pregnancy or lactation. A physician confirmed the health conditions of the participants through a complete medical history and physical examination. The study was approved by the National Ethics Committee of Slovenia. All the participants signed an informed consent. The metabolic studies were performed at the Faculty of Health Sciences of the Primorska University, Isola, Slovenia.

2.2 Intervention

The randomized, placebo-controlled, crossover intervention study was conducted between October 2015 and March 2016 (Fig. 1). After enrolment, subjects were randomly allocated into one of the 3 intervention groups by a staff member not involved in the study. All products were in bags that were identical in appearance and coded A, B, and C. Encoding was performed by a staff member not involved in the study. Encoding was broken after all data analysis had been performed. The study was organized in 3 subsequent phases to evaluate

metabolic effects of A, B and C. Each phase lasted five weeks. Phase 1 included a washout period (7-days) during which the participants abstained from eating any BW products, followed by an intervention period (35-days), during which subjects ate 1 bag of product A, B, or C each day (instead of breakfast). Then, the phase 2 and phase 3 included a washout period followed by A, B, or C intervention period as in phase 1. Flowchart of participants through the study is presented in Fig. 2.

All A, B and C porridges used in the study came from the same batch. In order to standardize the eating process, all participants received the same instructions. Before the study, an expert dietitian assessed eating and physical activity habits of each participant. To minimize potential confounding variables, derived from individual lifestyle, subjects followed some simple rules throughout the study phases. These included exclusion of any food containing BW (with the exception of the test products taken during the intervention periods), maintenance of habitual levels of physical activity, maintenance of the usual eating pattern, avoidance of foods particularly rich in polyphenols, vitamins, other nutrient supplements, herbal products and medications. Food intake and physical activity were monitored weekly throughout the study, by 3-day diet records (two weekdays and one weekend). An expert dietitian carefully explained how to record everything they were eating and drinking and how to record their physical activity. Dietary data was analysed using the Open Platform for Clinical Nutrition (OPEN) that is accessible through the website <http://opkp.si/>. At the end of each washout and intervention period body weight and composition were measured in the fasting state, while food and exercise diaries were checked by a dietitian and discussed with the subjects. At the same time-points, body composition indices were obtained using bioelectrical impedance (Tanita MC-980MA (Maeno-cho, Japan) and dedicated software (GMON Pro-Tanita)).

>> INSERT FIG. 1

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2.3 Products characteristics

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The products included three types of porridges (A based on extruded BW, B and C based on extruded maize). Porridge was formulated to contain min 57% cereal-based ingredients (on as is basis). Porridge B contained min 47% cereal-based ingredients and was fortified with proteins (soy protein isolate + casein). Porridge A was defined as containing min 39% (pseudo)cereal-based ingredients and proteins (soy protein isolate + casein). All the ingredients (dried apples, oat flakes, cinnamon) except maize and BW extruded were present in equal amounts in the porridge formulations.

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Proximate composition of porridges is displayed in Table 1. Composition of porridges was analysed using the methods of AOAC (2000), which included protein (Official Method No. 950.36), fat (Official Method No. 935.38), reducing sugar (Official Method No. 975.14), total dietary fibre (Official Method No. 958.29), ash (Official Method No. 930.22), starch (14.031), and moisture (Official Method No.926.5) determinations.

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>> INSERT TABLE 1

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The descriptive profiles of porridges A, B and C were evaluated by a panel of 7 trained panellist aged between 30-50 years. During training, porridges were described with attributes by reaching a panel consensus. The panel used 22 descriptive terms grouped under odour, appearance, colour, oral consistency, taste, flavour and aftertaste (Table 2). Attributes were scored according to their intensities on a 9-point scale in which 0 denoted a not

perceived attribute and 9 denoted a strongly perceived attribute. The sensory evaluation was carried out in a sensory laboratory with booths for each panellist, equipped according to ISO 8589:2007.

Porridges were prepared by vigorous mixing of dry porridge blend with warm water (50°C) at 1:2.5 proportion (w/w), after which it was allowed to swell for 10 minutes. The porridges were served in randomized order, in portions of approximately 100 ml placed in glass containers on a white plate. The samples were evaluated using stainless steel teaspoons. The sensory evaluation was carried out in duplicate.

Back-extrusion method was used to instrumentally determine porridge firmness and cohesiveness on a Texture Analyser (TA-XTplus, SMS, Godalming, UK). The test settings were the following: test speed 1.0 mm/s, trigger force 10 g and travel distance 30 mm. The maximum force registered during penetration of the probe into the sample was used as an indicator of firmness. On probe return, sample resisted the flow off the disc and the registered peak force was taken as an indicator of cohesiveness.

General Atwater conversion factors were used to calculate the energy content of all three porridges. The contribution of soluble fibres to the energy content was also taken into account (2 kcal/g) as well as the contribution of sweetener. When calculating the energy value of porridges, carbohydrate content was determined by calculation: available carbohydrates = 100 – (protein + fat + water + ash + alcohol + total fibre) according to FAO/WHO guidelines that are accessible through the website <http://www.fao.org/docrep/006/y5022e/y5022e03.htm>.

>> INSERT TABLE 2

2.4 Metabolic assessment

Venous blood samples were collected in vacuum test tubes between 7 A.M. and 9 A.M to obtain serum which was treated according to the analytical protocols and stored at -80°C, until measurements. Serum concentrations of glucose, TAG, total cholesterol, LDL cholesterol and HDL cholesterol were measured using Olympus reagents and performed on an AU 680 analyser (Beckman Coulter). In addition, serum levels of uric acid, bilirubin and adiponectin were also measured. Total bilirubin and uric acid were measured with the cobas c111 analyser (Roche). Serum adiponectin concentrations were measured using enzyme-linked immunosorbent assay by means of commercially available kits. Assay sensitivity was 10 pg/mL. Assays interassay and intraassay CVs were typically <10%.

2.5 Statistics

Data are expressed as mean $\pm$ SEM or mean $\pm$ SD. In order to evaluate protein effects and BW $\times$ protein interactions (A and B), we have used repeated measured ANOVA or ANCOVA (washout values as covariates), where appropriate. When the results of BW $\times$ protein interactions were significant ($p \leq 0.05$), the changes induced by the products, evaluated as the difference between the values obtained at the end of the intervention and the washout periods, were assessed through paired *T*-test. Values were logarithmically transformed when appropriate. P-values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS software (version 21; SPSS, Inc., Chicago, IL).

3. RESULTS

3.1 Study population

39 apparently healthy subjects were randomly allocated at the start of the study. The mean age, BMI, SBP, and DBP were 46.6 ± 8.2 years old, $25.7 \pm 4.2 \text{ kg/m}^2$, $136 \pm 28 \text{ mmHg}$, $72 \pm 19 \text{ mmHg}$, respectively. The mean glucose was $5.16 \pm 0.51 \text{ mmol/L}$, most of the subjects were hyperlipidemic, and the mean total, LDL and HDL cholesterol, and TAG were, $6.79 \pm 1.02 \text{ mmol/L}$, $4.54 \pm 0.82 \text{ mmol/L}$, $1.35 \pm 0.35 \text{ mmol/L}$, and $1.86 \pm 0.84 \text{ mmol/L}$, respectively. The mean energy intake was $8744.6 \pm 2278.1 \text{ kJ/day}$ ($2092 \pm 545 \text{ kcal/day}$).

This randomized, placebo-control, crossover intervention study was organized in 3 subsequent phases (each phase lasted five weeks with a washout period during 7-days) to evaluate the effects of porridges A, B and C on lipid profile (Fig. 1). Of the 39 subjects (17 men, 21 women) who started the study, 34 completed all interventions (Fig. 2). Three subjects dropped out because of illness and two subjects did not withdraw the consent.

>> INSERT FIG. 2

3.2 Descriptive profile of porridges, including firmness and cohesiveness

The tested porridges exhibited significant ($p < 0.05$) differences regarding the majority of descriptive attributes and textural properties (Table 3). The porridge A was firmer and more viscous in relation to the others. It had similar cohesiveness to the non-protein maize porridge C. There was similar trend between instrumentally measured texture and orally

perceived viscosity. Increase in texture and consistency for C and A porridge appears to be related to higher fibre content. It is likely that fibre swelling contributed to the formation of stronger extensive networks. Although gelatinized starch is a main contributor to viscosity of porridges, fibres (especially insoluble ones) have an ability to form stronger rigid structures. The surface of porridge A was significantly less glossy but smoother than C and B, with marked greyish nuance. It had intensive odour, flavour and aftertaste on BW and slightly bitter taste which partially masked the cinnamon/apple aroma. Intensive aroma and perceived bitterness might be attributed to the presence of phenolic compounds in BW. Beany and milky flavour was lightly perceived in B porridge due to presence of soy protein isolate and casein. Although A porridge also contained the same protein sources in the same amounts, beany and milky flavour as well as beany aftertaste was masked by strong BW aroma. At the same time, the highest sweetness could be attributed to this porridge formulation A.

>> INSERT TABLE 3

3.3 Daily intake of bioactive compounds

Analysis of phenolic compounds was performed according to the method of Mišan et al. (2011). As expected, the porridge A contributed to significantly higher intake of rutin and quercetin due to being based on BW. BW is known as a good source of rutin, quercetin, and flavonoids with healing effect. Porridge A was also high in gallic acid and catechin whereas the porridges B and C were dominated by protocatehuic acid (Table 4).

>>INSERT TABLE 4

3.4 Food intake

Food intake and physical activity were monitored weekly throughout the study, by 3-day diet records. The calculation of energy values of porridges was performed and is as follows: for A 365.92 kcal/100g, for C 366.28 kcal/100g, and for B 374.87 kcal/100g. During intervention, instead of breakfast, participants consumed 80 g of porridge, approximately 300 kcal (Table 5; data for participant A01). Overall, there were no statistically significant differences in daily energy intakes between three groups at baseline (1985 kcal; 2019 kcal; 1997 kcal; $p > 0.05$) and after the 5-week intervention period of all three phases of the study ($p > 0.05$) (data not shown).

>> INSERT TABLE 5

3.5 Effects of porridges A, B and C on body composition and metabolic profile

The effects of porridges A, B and C on body composition and anthropometric data were also evaluated (Table 6). Weight, fat mass (FM), fat free mass (FFM), metabolic age, and total body water (TBW) of all participants before and in the end of each phase of the study were measured. Table 8 shows that the interaction of BW and protein did not give significantly differences when studying body weight, FM, metabolic age and TBW. However, FFM significantly increased at week 5 in the group who consumed porridge A, confirming the significant BW x protein effect on FFM (0.70 ± 2.1 kg, $p=0.037$).

The effects of porridges A, B and C on serum glucose levels, lipid profile, inflammatory marker CRP, anti-inflammatory adiponectin, and on serum bilirubin and uric acid levels were also analysed (Table 6). Serum glucose level did not significantly differ

between the groups, nor did inflammatory marker CRP and serum bilirubin levels. However, BW x protein interaction gave marginally significant difference to serum bilirubin levels, a potent endogenous antioxidant ($p=0.051$). In addition, there was a significant BW x protein effect on serum total cholesterol, LDL cholesterol, HDL cholesterol, and TAG levels. The ingestion of porridge A significantly decreased total cholesterol (-0.69 ± 0.19 mmol/L, $p=0.002$), LDL cholesterol (-0.63 ± 0.16 mmol/L, $p=0.001$), TAG (-0.27 ± 0.15 mmol/L, $p=0.028$); on the other hand, significant increase in HDL cholesterol was observed (0.14 ± 0.07 mmol/L, $p=0.003$). Moreover, there was also a significant protein effects on total cholesterol (-0.25 ± 0.18 mmol/L, $p=0.034$). Furthermore, there was also a significant BW x protein effect on serum adiponectin and uric acid levels. Anti-inflammatory adiponectin significantly increased in the group which consumed porridge A compared to the group which consumed porridge B and C (4.6 ± 2.8 $\mu\text{g/mL}$, $p=0.021$); whereas, endogenous antioxidant uric acid decreased from baseline to week 5 in the group which consumed porridge A (-17.6 ± 15.9 $\mu\text{mol/L}$, $p=0.024$). In addition, significant correlation between reduction in LDL cholesterol and production of anti-inflammatory adiponectin was observed at week 5 in the group which consumed porridge A ($R^2=0.764$; $p < 0.01$).

>> INSERT TABLE 6

4. DISCUSSION

In recent years, there is renewed interest in the utilization of BW due to the “re-discovered” nutritional benefits (Izydorczyk et al., 2014; Zhu, 2016). Based on increased potential to produce a new generation of food products that can deliver specific health benefits, the porridge A was developed and a randomized controlled study was performed.

Porridge A was observed to show lipid lowering and anti-inflammatory effects in healthy participants with mild to modest hypercholesterolemia during a randomized controlled trial. A number of animal studies have shown that bioactive compounds of BW could positively impact on the metabolic outcomes (Tomotake et al., 2007; Yao et al., 2008; Merendino et al., 2014), whereas proteins, dietary fibres, and flavonoids were the effective components of BW (Izydorczyk et al., 2014). These compounds have been shown to improve hypercholesterolemia (Tomotake et al., 2007; Yang et al., 2014), hyperlipidemia (Wang, Liu, Gao, Parry, & Wei, 2009), hyperglycemia and oxidative stress (Yao et al., 2008; Gong, Li, Zhang, Li, & Zhang, 2012) in rodent models. Furthermore, numerous studies on BW confirmed that this plant has beneficial metabolic effect and plays an antioxidant, anti-inflammatory and anti-hypertensive role also in humans (Weislander et al., 2011; Weislander et al., 2012; Nishimura et al., 2016; Qiu, Li, Qin, Yue, & Liu, 2016).

The foods used in this study were based on commercially available ingredients and recipes and were well accepted by the participants. BW is not quite common in industrially produced food; therefore, the daily intake of nutrients and phytochemicals from these products is limited (Guo et al., 2012; Qin, Wu, Yao, & Ren, 2013). The porridge A from the present study retained most of the beneficial nutrients, while keeping acceptable sensory characteristics for consumption. To mask the bitterness of BW, dried apples, sweetener and cinnamon were used. Compared with C and B, porridge A contains more insoluble fibres,

more rutin, quercetin, gallic acid, and less sugar. The choice has been determined by the aim of evaluating the metabolic effects of porridge A on lipid profile, inflammation, and oxidative stress, while at the same time preserving the most appreciated porridge properties, such as taste, odour, appearance, oral consistency, flavour, and minimizing undesirable consequences. Indeed, during the whole intervention periods neither side effects, allergic reactions, gastrointestinal discomforts nor any other manifestation were noticed in any of the subjects.

Anthropometrical and biochemical analysis were performed before and at the end of each intervention periods. The combined nutrient and phytochemical composition of porridge A likely exerted synergistic effects beneficial on human health. Although the number of studies investigating the cholesterol-lowering activity of BW in humans is small (Weislander et al., 2011; Gimenez-Bastida & Zielinski, 2015), our results indicated strong association between porridge A intake and improved lipid profile, as shown by lower total cholesterol, LDL-cholesterol, and TAG. Indeed, TAG, total cholesterol and LDL-cholesterol were significantly reduced by the porridge A dietary intervention, as significant differences were observed in the changes from baseline to week 5. The dietary intake showed that after 5-weeks of intervention, the energy intake was not affected. Therefore, porridge A intervention showed a significant decrease in lipids with a relatively stable energy intake and stable weight during the test period. Proteins extracted from BW have low digestibility and are known to suppress hypercholesterolemia by increasing faecal excretion of sterol in mice (Tomotake et al., 2006) and rats (Tomotake et al., 2007) and down-regulation of intestinal NPC1L1 and ACAT2 (Yang et al., 2014). In addition to BW proteins, previous studies showed that also dietary fibre, phytosterols and polyphenols can lower the cholesterol level in animal models (Park et al., 2002; Yang et al., 2014). However, in the present study it is difficult to dissect whether these effects are due to the beneficial effects of one bioactive compound or because of the synergistic effects of different chemical compounds, including added proteins in porridge A.

Indeed, significant reduction in total cholesterol has been observed due to dietary intervention with both, BW and protein – enriched porridges. Recently, by performing meta-analysis, it has been shown that soy protein supplementation reduces clinical indices in type 2 diabetes and metabolic syndrome (Zhang, Zhang, & Chi, 2016).

Moreover, the ingestion of the porridge A, significantly increased FFM at week 5, probably, due to synergistic effects of different chemical compounds and added proteins in the porridge A. Recently, Nishimura et al. (2016) revealed that rutin-rich Tartary BW showed potential effects on decreasing body weight and body fat percentage. Furthermore, Egawa et al. (2012) reported that the ingestion of 110 mg quercetin for 12 weeks decreased visceral fat area in subjects with BMI between 25 kg/m² and 30 kg/m². However, little is known about the beneficial effects of BW on FFM until now. It is well known that high-protein diets exert beneficial effects on appetite, anthropometry, and body composition; indeed, in the present study casein and soy protein isolate have influence on FFM, which is in agreement with previous report (Tahavorgar, Vafa, Shidfar, Gohari, & Heydari, 2014), although, the influence was not statistically significant.

There are inconsistent data about antidiabetic effect of the BW in *in vitro* and *in vivo* studies (Zeng, Pu, Du, Yang, & Jia, 2012; Su-Que et al., 2013; Qiu, Li, Qin, Yue, & Liu, 2016). BW, for example, is used as a dietary use in China in order to prevent diabetes (Zeng, Pu, Du, Yang, & Jia, 2012). In addition, consumption of BW bread or BW groats compared to white wheat bread showed a decrease in the postprandial plasma glucose in both type 2 diabetic subjects and healthy subjects (Su-Que et al., 2013). It has been shown, that quercetin, isoquercetin, rutin, and D-chiro-inositol, extracted from BW are effective in lowering glucose level (Yao et al., 2008). This is consistent with the *in vitro* and *in vivo* studies that BW extract and polyphenols showed inhibitory activities against starch digestive enzymes, thereby lowering blood glucose level (Qin, Wu, Yao, & Ren, 2013; Zhu, 2015). Moreover, Lee et al.

demonstrated that rutin and quercetin extracted from BW improved hyperglycemia and hyperinsulinemia in C57BL6 mice (Lee, Hsu, Shen, Cheng, & Wu, 2012). However, in the present study intervention with A did not show an improvement in serum glucose level. But, it has to be stress out, that participants in the present study were normoglycemic.

Adiponectin, a 244-amino acid peptide secreted from adipocytes, protects against metabolic and cardiovascular diseases (Turer & Scherer, 2016); while low serum levels of adiponectin is common in obesity (Petelin et al., 2014). In the present study, serum adiponectin levels were higher when the diet was enriched with BW, but they declined when BW was absent, which was all the more remarkable because weight and FM was similar between interventional periods in all three dietary interventions. Previously, it has been shown that rutin has the potential to inhibit the expression of leptin and up-regulate the expression of adiponectin at the protein level in 3T3-L1 adipocytes (Hsu, Wu, Huang, & Yen, 2009). BW, especially rutin has therefore the potential to stimulate adiponectin secretion from adipose tissue and improve adipokine imbalance. In addition, it has been recently shown that tartary BW extract and rutin inhibited the nuclear factor kappa β activation in LPS-stimulated macrophages (Karki, Park, & Kim, 2013), confirming the positive effects of BW on reducing the inflammation.

Increasing appreciation of functional properties of BW has also encouraged some researchers about its beneficial effects through its antioxidant properties. Zhou et al. have recently reported that BW honey showed protective effects on hydroxyl radical-induced DNA damage (Zhou et al., 2012). The high antioxidant capacity of BW is mainly due to its polyphenol content, especially rutin (Kreft, Fabjan, & Yasumoto, 2006). Indeed, it has been suggested that rutin may be involved in activation and upregulation of endogenous anti-oxidative enzymes (Yeh, Yang, Yang, Li, & Kuan, 2014); one proposed mechanism was through the activation of heme oxygenase (HO-1). In the present study, increase in serum

447 bilirubin levels, the potent endogenous antioxidant, was detected, confirming the expression
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 2 448 of HO-1. In addition to increasing serum bilirubin levels, heme oxygenase-1 system is
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 4 449 involved also in enhancing adiponectin synthesis and release (Nicolai et al., 2009).
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 7 450 Overexpression of HO-1 resulted in a marked increase in adiponectin with a corresponding
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 9 451 decrease of inflammatory cytokines in animal models of obesity (Li et al., 2008). Therefore,
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 11 452 BW, especially rutin, may be linked to improved metabolic factors through an anti-
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 13 453 inflammatory and anti-oxidative related mechanism. However, further studies investigating
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 15 454 the anti-inflammatory and anti-oxidative effects of the BW are required to draw conclusions
 16
 17 455 due to the low number of studies performing so far.
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21
 22 456 Uric acid is another potent water-soluble antioxidant and an increase in its serum
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 24 457 levels could be associated with the lipid disorders in subjects with metabolic syndrome.
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 26 458 However, several observational studies indicate that high levels of uric acid increase the risk
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 28 459 of cardiovascular events by metabolism-related inflammation (Kushiyama et al., 2016). In
 29
 30 460 agreement with previous studies on high fat-fed rats (Amin, Kamel, & Eltawab, 2011; Joanna,
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 32 461 Henryk, Jadwiga, & Renata, 2013), enrichment of the diet with BW resulted in decrease of
 33
 34 462 serum uric acid levels. The elevated serum uric acid levels in the present study may
 35
 36 463 compensate the oxidative stress related to metabolic syndrome related disorders. It is therefore
 37
 38 464 possible that the 5-weeks of dietary intervention with A resulted in lower oxidative stress and
 39
 40 465 consequently, decreased serum uric acid levels were detected.
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46 466 We have to stress out that the present study has several limitations. First of all, the
 47
 48 467 duration of the present study was only 5 weeks, which might be insufficient to detect
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 50 468 considerable changes in glucose levels. Second, the present study allowed participants to
 51
 52 469 remain in “free-living” environments; therefore, the results are based on participant’s
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 54 470 motivation and compliance. Because of the length of the study, we had concerns about
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 56 471 compliance; participants in “free-living” environments might add instead of substituting foods
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472 to their usual diet, leading to higher overall energy intake. Third, we did not use any
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2 473 biomarkers related to BW.
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4 474 However, overall, these findings may support that BW as a part of porridge
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7 475 consumption is an effective and convenient nutritional therapy for the improvement of lipid
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10 476 profile, inflammation, and oxidative stress in participants with metabolic syndrome
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12 477 components.
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64
65

494 REFERENCES

1
2 495

3
4 496 Alvarez-Jubete, L., Arendt, E.K., & Gallagher, E. (2010). Nutritive value of pseudocereals
5
6
7 497 and
8
9 498 their increasing use as functional gluten-free ingredients. *Trends in Food Science &*
10
11
12 499 *Technology*, 21, 106-113.

13
14 500

15
16 501 Amin, K.A., Kamel, H.H., & Eltawab, M.A.Abd. (2011). The relation of high fat diet,
17
18
19 502 metabolic
20
21 503 disturbances and brain oxidative dysfunction: modulation by hydroxy citric acid. *Lipids in*
22
23
24 504 *Health and Disease*, 10, 74.

25
26 505

27
28 506 AOAC. (2000). Official methods of analysis (17th ed.). Maryland, USA: Association of
29
30
31 507 Official
32
33 508 Analytical Chemists 2000.

34
35
36 509

37
38 510 Brunori, A., Baviello, G., Zannettino, C., Corsini, G., Sándor, G., & Végvári, G. (2009). The
39
40
41 511 use of *Fagopyrum tataricum* Gaertn. whole flour to confer preventive contents of rutin to
42
43
44 512 some traditional Tuscany biscuits. *AUDJG Food Technology*, 34, 38-41.

45
46 513

47
48 514 Christa, K., & Soral-Šmietana, M. (2008). Buckwheat grains and buckwheat products-
49
50
51 515 Nutritional and prophylactic value of their components- a Review. *Czech Journal of Food*
52
53 516 *Sciences*, 26, 153-162.

54
55
56 517

57
58
59
60
61
62
63
64
65

- 518 De Francischi, M.L., Salgado, J.M., & da Costa, C.P. (1994). Immunological analysis of
 519 serum
 520 for buckwheat fed celiac patients. *Plant Foods for Human Nutrition*, 46, 323-329.
 521
- 522 Egawa, K., Yoshimura, M., Kanzaki, N., & Fukuhara, I. (2012). Body fat reducing effect and
 523 safety evaluation of long-term consumption of green tea containing quercetin glucoside in
 524 obese subjects. *Japan Pharmacology & Therapeutics*, 40, 495-503.
 525
- 526 Filipčev, B., Šimurina, O., & Bodroža-Solarov, M. (2015). Impact of buckwheat flour
 527 granulation and supplementation level on the quality of composite wheat/buckwheat ginger-
 528 nut-type biscuits. *Italian Journal of Food Science*, 27, 495-504.
 529
- 530 Giménez-Bastida, J.A., & Zieliński, H. (2015). Buckwheat as a Functional Food and Its
 531 Effects
 532 on Health. *Journal of Agricultural and Food Chemistry*, 63, 7896-7913.
 533
- 534 Gong, F., Li, F., Zhang, W., Li, J., & Zhang, Z. (2012). Effects of crude flavonoids from
 535 tartary
 536 buckwheat on alloxan induced oxidative stress in mice. *Bangladesh Journal of*
 537 *Pharmacology*, 7, 124–130.
 538
- 539 Guo, X.D., Wu, C.S., Ma, Y.J., Parry, J., Xu, Y.Y., Liu, H., & Wang, M. (2012). Comparison
 540 of milling fractions of tartary buckwheat for their phenolics and antioxidant properties. *Food*
 541 *Research International*, 49, 53-59.
 542

- 543 He, L. (1998). Review on the research status of tartary buckwheat. Proceedings of the 1st
 544 International Conference Asian product Development, 6-12. Taiyuan, China (Eds. H. Corke,
 545 R. Lin), Science Press Beijing, 366-372.
 546
 547 Hsu, C.L., Wu, C.H., Huang, S.L., & Yen, G.C. (2009). Phenolic compounds rutin and o-
 548 coumaric acid ameliorate obesity induced by high-fat diet in rats. *Journal of Agriculture and*
 549 *Food Chemistry*, 57, 425-431.
 550
 551 Ihme, N., Kieseewetter, F., Jung, F., Hoffmann, K.H., Birk, A., Muller, A., & Grutzner, K.I.
 552 (1996). Leg oedema protection from a buckwheat herb tea in patients with cronic venous
 553 insufficiency: a single, randomized, double blind, placebo controlled clinical trial. *European*
 554 *Journal of Clinical Pharmacology*, 50, 443-447.
 555
 556 Izydorczyk, M.S., McMillan, T., Bazin, S., Kletke, J., Dushnicky, L., & Dexter, J. (2014).
 557 Canadian buckwheat: A unique, useful and under-utilized crop. *Canadian Journal of Plant*
 558 *Science*, 94, 509-524.
 559
 560 Janet, M., Horbowicz, M., & Obendorf, R.L. (2005). Cyclitol galactosides in embryos of
 561 buckwheat steam-leaf-seed explants fed D-chiro-inositol, myo-inositol or D-pinitol. *Seed*
 562 *Science Research*, 25, 329-338.
 563
 564 Joanna, C., Henryk, B., Jadwiga, K., & Renata, F. (2013). The Effect of Buckwheat
 565 (Fagopyrum

566 esculentum Moench) Groats Addition to the Lard Diet on Antioxidant Parameters of Plasma
 567 and Selected Tissues in Wistar Rats. *International Journal of Biological, Biomolecular,*
 568 *Agricultural, Food and Biotechnological Engineering*, 7, 2013.

569
 570 Karki, R., Park, C.H., & Kim, D.W. (2013). Extract of buckwheat sprouts scavenges
 571 oxidation
 572 and inhibits pro-inflammatory mediators in lipopolysaccharide-stimulated macrophages
 573 (RAW264.7). *Journal of Integrative Medicine*, 11, 246-252.

574 Kreft, I., Fabjan, N., & Yasumoto, K. (2006). Rutin Content in Buckwheat (*Fagopyrum*
 575 *esculentum* Moench) Food Materials and Products. *Food Chemistry*, 98, 508-512.

576
 577 Krkošková, B., & Mrázová, Z. (2005). Prophylactic components of buckwheat. *Food*
 578 *Research*
 579 *International*, 38, 561-568.

580
 581 Kushiyama, A., Nakatsu, Y., Matsunaga, Y., Yamamotoya, T., Mori, K., Ueda, K., ... Asano,
 582 T. (2016). Role of Uric Acid Metabolism-Related Inflammation in the Pathogenesis of
 583 Metabolic Syndrome Components Such as Atherosclerosis and Nonalcoholic Steatohepatitis.
 584 *Mediators of Inflammation*, 2016, 8603164.

585
 586 Larner, J., Brautigan, D.L., & Thorner, M.O. (2010). D-*chiro*-inositol glycans in insulin
 587 signaling and insulin resistance. *Molecular Medicine*, 16, 543-552.

588

- 589 Lee, C.C., Hsu, W.H., Shen, S.R., Cheng, Y.H., & Wu, S.C. (2012). Fagopyrum tataricum
 590 (buckwheat) improved high-glucose-induced insulin resistance in mouse hepatocytes and
 591 diabetes in fructose-rich diet-induced mice. *Experimental Diabetes Research*, 1, 83–88.
- 592
- 593 Li, M., Kim, D.H., Tsenovoy, P.L., Peterson, S.J., Rezzani, R., Rodella, L.F, ... Abraham,
 594 N.G.
 (2008). Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and
 595 subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and
 596 glucose tolerance. *Diabetes*, 57, 1526-1535.
- 597
- 598
- 599 Lin, R., Jia, W., & Ren, J. (1998). Research and utilization of tartary buckwheat. Proceedings
 600 of the 1st International Conference Asian product Development, 6-12, Taiyuan, China (Eds. H.
 601 Corke, R. Lin), Science Press Beijing, 1998:187-97.
- 602
- 603 Mišan, A., Mimica-Dukić, N., Mandić, A., Sakač, M., Milovanović, I., & Sedej, I. (2011).
 604 Development of a rapid resolution HPLC method for the separation and determination of 17
 605 phenolic compounds in crude plant extracts. *Central European Journal of Chemistry*, 9, 133-
 606 142.
- 607
- 608 Merendino, N., Molinari, R., Costantini, L., Mazzucato, A., Pucci, A., Bonafaccia F,...
 609 Bonafaccia, G. (2014). A new "functional" pasta containing tartary buckwheat sprouts as an
 610 ingredient improves the oxidative status and normalizes some blood pressure parameters in
 611 spontaneously hypertensive rats. *Food & Function*, 5, 1017-1026.

- 613 Nicolai, A., Li, M., Kim, D.H., Peterson, S.J., Vanella, L., Positano, V., ... Abraham, N.G.
 614 (2009). Heme oxygenase-1 induction remodels adipose tissue and improves insulin sensitivity
 615 in obesity-induced diabetic rats. *Hypertension*, 53, 508-515.
- 616
- 617 Nishimura, M., Ohkawara, T., Sato, Y., Satoh, H., Suzuki, T., Ishiguro, K., ... Nishihira, J.
 618 (2016). Effectiveness of rutin et al. Effectiveness of rutin-rich Tartary buckwheat (*Fagopyrum*
 619 *tataricum* Gaertn.) 'Manten-Kirari' in body weight reduction related to its antioxidant
 620 properties: A randomised, double-blind, placebo-controlled study. *Journal of Functional*
 621 *Foods*, 26, 460–469.
- 622
- 623 Park, S.Y., Bok, S.H., Geon, S.M., Park, Y.B., Lee, S.J., Jeong, T.S., & Choi, M.S. (2002).
 624 Effects of rutin and tannic acid supplements on cholesterol metabolism in rats. *Nutrition*
 625 *Research*, 22, 283-295.
- 626
- 627 Petelin, A., Bizjak, M., Černelič-Bizjak, M., Jurdana, M., Jakus, T., & Jenko-Pražnikar, Z.
 628 (2014). Low-grade inflammation in overweight and obese adults is affected by weight loss
 629 program. *Journal of Endocrinological Investigation*, 37, 745-755.
- 630
- 631 Qin, P., Wu, L., Yao, Y., & Ren, G. (2013). Changes in phytochemical compositions,
 632 antioxidant and α -glucosidase inhibitory activities during the processing of tartary buckwheat
 633 tea. *Food Research International*, 50, 562-567.
- 634
- 635 Qiu, J., Li, Z., Qin, Y., Yue, Y., & Liu, Y. (2016). Protective effect of tartary buckwheat on
 636 renal function in type 2 diabetics: a randomized controlled trial. *Therapeutics and Clinical*
 637 *Risk Management*, 12, 1721-1727.

638

639 Su-Que, L., Ya-Ning, M., Xing-Pu, L., Ye-Lun, Z., Guang-Yao, S., & Hui-Juan, M. (2013).
 640 Effect of consumption of micronutrient enriched wheat steamed bread on postprandial plasma
 641 glucose in healthy and type 2 diabetic subjects. *Nutrition Journal*, 12, 64.

642

643 Tahavorgar, A., Vafa, M., Shidfar, F., Gohari, M., & Heydari, I. (2014). Whey protein
 644 preloads are more beneficial than soy protein preloads in regulating appetite, calorie intake,
 645 anthropometry, and body composition of overweight and obese men. *Nutrition Research*, 34,
 646 856-861.

647 Tomotake, H., Yamamoto, N., Yanaka, N., Ohinata, H., Yamazaki, R., Kayashita, J., & Kato,
 648 N. (2006). High protein buckwheat flour suppresses hypercholesterolemia in rats and
 649 gallstone formation in mice by hypercholesterolemic diet and body fat in rats because of its
 650 low protein digestibility. *Nutrition*, 22, 166–173.

651

652 Tomotake, H., Yamanoto, N., Kitabayashi, H., Kawakami, A., Kayashita, J., Ohinata, H., ...
 653 Kato, N. (2007). Preparation of tartary buckwheat protein product and its improving effect on
 654 cholesterol metabolism in rats and mice fed cholesterol-enriched diet. *Journal of Food*
 655 *Science*, 72, S528–S533.

656

657 Turer, A.T., & Scherer, P.E. (2016). Adiponectin: Just Along for the Ride? *Circulation*
 658 *Research*, 119, 407-408.

659

660 Unfer, V., & Porcaro, G. (2014). Updates on the myo-inositol plus D-*chiro*-inositol combined
 661 therapy in polycystic ovary syndrome. *Expert Review of Clinical Pharmacology*, 7, 623-631.

662

663 Zhang, Z.L., Zhou, M.L., Tang, Y., Li, F.L., Tang, Y.X., Shao, J.R., ... Wu, J.M. (2012).

664 Bioactive compounds in functional buckwheat food. *Food Research International*, 49, 389–
665 395.

666

667 Zhang, X.M., Zhang, Y.B., & Chi, M.H. (2016). Soy Protein Supplementation Reduces
668 Clinical

669 Indices in Type 2 Diabetes and Metabolic Syndrome. *Yonsei Medical Journal*, 57, 681-899.

670

671 Zeng, Y., Pu, X., Du, J., Yang, S., Yang, T., & Jia, P. (2012). Use of functional foods for
672 diabetes prevention in China. *African Journal of Pharmacy and Pharmacology*, 6, 2570-2579.

673

674 Zhou, J., Li, P., Cheng, N., Gao, H., Wang, B., Wei, Y., & Cao, W. (2012). Protective effects
675 of buckwheat honey on DNA damage induced by hydroxyl radicals. *Food and Chemical*
676 *Toxicology*, 50, 2766-2773.

677

678 Zhu, F. (2015). Interactions between starch and phenolic compound. *Trends in Food Science*
679 *&*
680 *Technology*, 34, 129-143.

681

682 Zhu, F. (2016). Chemical composition and health effects of tartary buckwheat. *Food*
683 *Chemistry*,
684 203, 231-245.

685

686 Wang, M., Liu, J.R., Gao, J.M., Parry, J.W., & Wei, Y.M. (2009). Antioxidant activity of
687 tartary

- 688 buckwheat bran extract and its effect on the lipid profile of hyperlipidemic rats. *Journal of*
 689 *Agricultural and Food Chemistry*, 57, 5106–5112.
- 690
- 691 Wieslander, G., Fabjan, N., Vogrincic, M., Kreft, I., Janson, C., Spetz-Nyström, U., ...
 692 Norbäck, D. (2011). Eating buckwheat cookies is associated with the reduction in serum
 693 levels of myeloperoxidase and cholesterol: a double blind crossover study in day-care centre
 694 staffs. *The Tohoku Journal of Experimental Medicine*, 225, 123-130.
- 695
- 696 Wieslander, G., Fabjan, N., Vogrincic, M., Kreft, I., Vombergar, B., & Norback, D. (2012).
 697 Effects of common and Tartary buckwheat consumption on mucosal symptoms, headache
 698 and tiredness: A double-blind crossover intervention study. *Journal of Food, Agriculture and*
 699 *Environment*, 10, 107–110.
- 700
- 701 Wójtowicz, A., Kolasa, A., & Mościcki, L. (2013). Influence of buckwheat addition on
 702 physical
 703 properties, texture and sensory characteristics of extruded corn snacks. *Polish Journal of*
 704 *Food and Nutrition Sciences*, 63, 239–244.
- 705
- 706 Xiping, L., & Xianqiong, F. (1995). Clinical effects of tartary buckwheat on senile
 707 hyperlipemia. In *Current Advances in Buckwheat Research*, vol. II, Eds. T. Matano, A.
 708 Ujihara, Shinshu University Press, Matsomoto, 947-950.
- 709
- 710 Yang, N., Li, Y.M., Zhang, K., Jiao, R., Ma, K.Y., Zhang, R., ... Chen, Z.Y. (2014).
 711 Hypocholesterolemic activity of buckwheat flour is mediated by increasing sterol excretion

712 and down-regulation of intestinal NPC1L1 and ACAT2. *Journal of Functional Foods*, 6,
713 311–318.

714

715 Yao, Y., Shan, F., Bian, J.S., Chen, F., Wang, M., & Ren, G. (2008). D-chiro-inositol-enriched
716 tartary buckwheat bran extract lowers the blood glucose level in KK-Ay mice. *Journal of*
717 *Agriculture and Food Chemistry*, 56, 10027–10031.

718

719 Yeh, C.H., Yang, J.J., Yang, M.L., Li, Y.C., & Kuan, Y.H. (2014). Rutin decreases
720 lipopolysaccharide-induced acute lung injury via inhibition of oxidative stress and the
721 MAPK-NF- κ B pathway. *Free Radical Biology and Medicine*, 69, 249-257.

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725 **TABLES**

726

727 **Table 1**

728 Proximate composition of the tested porridges A, B and C.

Parameter (g/100 g)	Porridge		
	A	C	B
Starch content	26.79±0.32	41.08±0.54	36.18±0.46
Total sugar content	9.77±0.13	17.17±0.27	14.77±0.21
saccharose	2.29±0.07	1.98±0.01	1.69±0.02
fructose	5.26±0.08	12.70±0.14	11.07±0.10
glucose	2.22±0.01	2.49±0.02	2.02±0.01
Protein content	17.33±0.12	7.66±0.10	17.50±0.15
Total fibre content	8.74±0.90	7.60±0.18	6.71±0.50
insoluble	7.19±0.62	4.80±0.06	3.23±0.08
soluble	1.65±0.10	3.08±0.08	3.38±0.18
Moisture content	8.11±0.21	7.51±0.10	7.37±0.17
Fat content	4.68±0.15	5.48±0.11	6.57±0.17
Ash content	2.55±0.06	1.71±0.08	2.11±0.01
Available carbohydrates (by calculation) ¹ (mg/100 g)	58.61	70.94	58.85
Na	299.10±0.10	10.83±0.03	239.27±0.06
K	407.93±4.93	319.77±5.95	256.5±1.82
Ca	39.44±0.04	18.56±0.04	16.48±0.02
Mg	10.08±0.10	53.35±0.12	34.53±0.05
Fe	10.68±0.06	8.33±0.04	7.44±0.08

Zn	5.58±0.05	3.10±0.03	3.20±0.02
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Values are presented as means ± standard deviation.

¹calculated according to FAO/WHO Guidelines retrieved from

(<http://www.fao.org/docrep/006/y5022e/y5022e03.htm>), available carbs=100-

(prot+fat+water+ash+alcohol+total fibre)

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753 **Table 2**

754 Terminology used by panel to describe sensory attributes of porridges A, B and C.

Property	Descriptive profile	Definition
Odour	Odour intensity	The overall odour intensity of the porridge
		Intensity of odour associated with cooked maize porridge
	Buckwheat odour	Intensity of odour associated with cooked buckwheat
	Cinnamon/apple odour	Intensity of odour associated with cinnamon and apple
Appearance	Glossiness	The amount of shine or gloss perceived on the surface when the cup is bent in the direction of light
		Evenness of porridge associated with presence of lumps or clumps
Colour	Yellow	-
	Ochre	-
	Grey	-
Oral consistency	Coarseness	The degree to which grittiness of porridge due to presence of small particles could be perceived
		Amount of force required to draw a paste from spoon over the tongue
	Viscosity	

	Creaminess	Intensity of mouthfeel associated with fatty product such as dairy cream
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	Taste	The overall taste intensity of the porridge
	Taste intensity	
	Sweet	Basic sweet taste associated with saccharose
	Bitter	Basic bitter taste associated with quinine
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	Flavour	Intensity of flavour associated with combination of cinnamon and apple
	Cinnamon/apple	
	Maize porridge	Intensity of flavour associated with cooked maize porridge
	Buckwheat	Intensity of flavour associated with cooked buckwheat
	Beany	Intensity of flavour associated with undercooked soy
	Milky	Intensity of flavour associated with dairy products, butter
	<hr/>	
	Aftertaste	Intensity of flavour associated with undercooked soy perceived after swallowing the porridge
	Beany	
	Buckwheat	Intensity of flavour associated with cooked buckwheat perceived after swallowing the porridge

756 **Table 3**

757 Descriptive profile of porridges A, B and C, including firmness and cohesiveness measured
 758 instrumentally on TA-XTplus Texture Analyser.

Property	Descriptive profile	Porridge			p value *
		A	B	C	
Odour	Odour intensity	7.71±0.45	4.57±0.51	7.43±0.58	0.0005
	Maize porridge odour	0.43±0.38	2.67±0.50	1.57±0.56	0.0040
	Buckwheat odour	8.83±0.44	0.00±0.00	0.00±0.00	0.00000
	Cinnamon/apple odour	2.00±1.18	4.57±0.96	7.29±0.74	0.0017
Appearance	Glossiness	1.29±0.52	8.43±0.73	7.00±0.85	0.00004
	Smoothness	4.14±0.63	3.71±0.76	2.17±0.75	0.03376
Colour	Yellow	0.71±0.71	4.29±0.75	5.43±0.82	0.00066
	Ochre	0.86±0.64	7.17±0.87	8.83±0.50	0.00002
	Grey	8.29±0.79	1.57±0.74	2.21±0.84	0.00008
Oral consistency	Coarseness	7.13±0.87	5.71±0.72	7.71±0.93	0.06523
	Viscosity	7.86±0.67	3.14±0.70	5.51±0.26	0.00018
	Creaminess	4.14±0.91	3.29±0.79	4.08±0.87	0.44933
Taste	Taste intensity	8.71±0.49	5.43±0.53	8.36±0.80	0.00121
	Sweet	5.62±0.82	4.29±0.49	3.14±0.69	0.01233
	Bitter	3.86±0.74	0.14±0.38	0.29±0.46	0.00026
Flavour	Cinnamon/apple	2.13±0.61	3.86±0.72	5.71±0.78	0.00245
	Maize porridge	0.14±0.38	6.86±0.83	4.36±0.65	0.00004
	Buckwheat	8.86±0.58	0.00±0.00	0.00±0.00	0.00000
	Beany	0.00±0.00	1.43±0.85	0.00±0.00	0.01836

	Milky	0.00±0.00	1.86±1.07	0.14±0.12	0.01882
Aftertaste	Buckwheat	5.86±0.69	0.00±0.00	0.00±0.00	0.00000
	Beany	0.57±0.53	2.14±0.66	0.00±0.00	0.00440
Instrumental	Firmness (g)	126.28±3.01	40.77±2.37	96.76±4.41	0.00000
texture	Cohesiveness (g)	117.47±18.86	37.91±4.10	101.72±4.97	0.00033

*p-values (ANOVA). Attributes highlighted in bold have significant effect in the ANOVA

model. P value greater than 0.05 is considered non-significant

761 **Table 4**

762 Daily intake of bioactive (polyphenolic) compounds of porridges A, B and C (if 80 g of
 763 porridges A, B and C was consumed daily).

Bioactive compounds (mg/day)	Porridges		
	A	B	C
Gallic acid	3.184±0.262	1.511±0.105	0.749±0.073
Protocatehuic acid	2.165±0.019	6.114±0.074	8.862±0.093
<i>p</i> -Hydroxybenzoic acid	0.000±0.000	1.235±0.107	1.127±0.383
Catechin	2.355±0.126	1.813±0.103	2.125±0.111
Caffeic acid	1.735±0.017	1.572±0.073	1.583±0.077
Epicatechin	0.720±0.009	0.987±0.029	0.877±0.078
Ferulic acid	0.527±0.058	0.587±0.094	0.575±0.078
Rutin	2.139±0.106	0.074±0.002	0.067±0.011
Cinnamic acid	0.310±0.009	0.141±0.018	0.157±0.012
Quercetin	3.124±0.169	0.209±0.017	0.269±0.026

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766 **Table 5**

767 Daily menu, before and during the intervention, for participant A01.

Daily Menu, before	Food (g)	Energy (kcal)	Daily Menu, during	Food (g)	Energy (kcal)
Breakfast		383.96	Breakfast		366.73
Coffee with milk	200	73.99	Coffee with milk	200	73.99
Cornflakes	40	151.82	Porridge A	80	292.74
Buttermilk	220	66.43			
White Grapes	120	91.72			
Lunch		800.40	Lunch		783.22
Bolognese sauce	300	284.06	Gorgonzola sauce	150	268.28
Cooked Pasta	200	262.00	Cooked Pasta	250	327.50
Parmesan	25	97.35	Cabbage	100	25.05
Red wine	100	83.00	Vegetable oil	10	88.40
Coffee with milk	200	73.99	Coffee with milk	200	73.99
Afternoon snack		292.47	Afternoon snack		398.44
Ice cream	126	292.47	Cheesecake	100	286.00
			Milk 1.4%	244	112.44
Dinner		378.31	Dinner		349.11
Cabbage with	100	25.05	Chicory with	79	23.92
Beans and	50	47.99	Beans and	144	138.20
Tuna	50	199.15	Sweet corn	78	63.18
Olive oil	12	106.12	Olive oil	14	123.81
		1855.14			1904.76

768

Table 6
Effect of porridges A, B and C on body composition, anthropometric data, and metabolic profile.

		Study Phase 1		Study Phase 2		Study Phase 3		p-value	
		Washout	A	Washout	B	Washout	C	protein effect	BW ×protein effect
Fasting state									
Weight (kg)		75.7±2.8	76.2±2.9	76.1±2.7	76.5±2.9	76.3±2.8	75.9±2.7	0.061	0.057
FFM (kg)		55.2±2.1	55.9±2.2	55.7±2.1	56.1±2.2	55.8±2.2	55.5±2.1	0.058	0.037
FM (kg)		20.5±1.2	20.3±1.4	20.4±1.3	20.4±1.2	20.5±1.2	20.4±1.2	0.876	0.644
Metabolic age		44.5±2.0	44.0±1.8	44.2±2.0	43.9±1.9	44.2±2.0	44.3±1.9	0.121	0.075
TBW (%)		51.9±0.9	52.2±0.9	52.0±1.0	52.1±0.9	51.9±1.1	52.0±0.9	0.920	0.756
Glucose (mmol/L)		5.25±0.07	5.21±0.09	5.12±0.12	5.11±0.10	5.09±0.07	5.07±0.08	0.921	0.979
Lipid metabolism									
Total	cholesterol	6.82±0.19	6.13±0.17	6.72±0.17	6.47±0.18	6.81±0.17	6.67±0.18	0.034	0.002
(mmol/L)									
LDL	cholesterol	4.55±0.16	3.92±0.15	4.49±0.16	4.28±0.15	4.57±0.17	4.45±0.15	0.051	0.001
(mmol/L)									
HDL	cholesterol	1.37±0.06	1.51±0.07	1.32±0.07	1.39±0.08	1.35±0.07	1.30±0.08	0.053	0.003

(mmol/L)								
TAG (mmol/L)	1.89±0.14	1.62±0.15	1.90±0.14	1.76±0.16	1.82±0.15	1.71±0.17	0.345	0.028
Inflammatory/anti-inflammatory mediators								
CRP (mg/L)	2.9±0.8	2.0±0.6	2.4±0.7	2.3±0.7	2.5±0.8	2.3±0.6	0.351	0.245
Adiponectin (µg/mL)	15.9±2.2	20.5±2.8	14.8±2.1	15.3±2.3	16.7±2.5	14.9±2.1	0.291	0.021
Antioxidants								
Bilirubin (µmol/L)	9.37±1.21	11.25±1.61	9.10±1.11	9.34±1.31	8.67±1.43	9.02±1.27	0.765	0.051
Uric acid (µmol/L)	332.6±15.9	315.0±15.0	335.4±15.7	333.9±16.0	339.4±14.7	341.9±15.5	0.621	0.024

771 Data are expressed as mean ± SEM. Repeated measurements ANOVA or ANCOVA (washout values as covariates) were used where appropriate.

772 P value greater than 0.05 is considered non-significant. BW, buckwheat; CRP, C-reactive protein; FFM, fat free mass; FM, fat mass; HDL, high
773 density lipoprotein; LDL, low density lipoprotein; TAG, triacylglycerols; TBW, total body water.

774

775 Highlights:

- 776 • A new type of buckwheat food product with health benefits was developed
- 777 • A new buckwheat-enriched porridge is effective in improving lipid profile
- 778 • A new buckwheat-enriched porridge is effective in reducing inflammation
- 779 • Buckwheat-enriched porridge is a convenient food product for nutritional therapy

780

781

782 FIGURE CAPTIONS

783

784 **Figure 1.** Design of the experimental study.

785

786 **Figure 2.** Participant flow diagram. The diagram depicts number of recruited volunteer and
787 actual number of participant included in data analysis.

788

Figure 1
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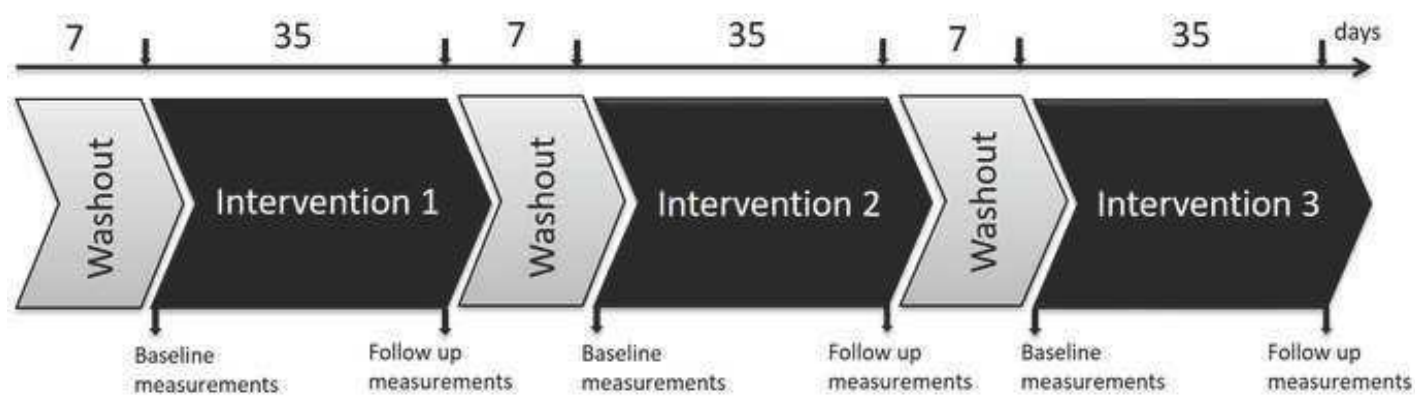


Figure 2
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