Original Article

Symptomatic efficacy of buckwheat products in Non-Celiac Gluten Sensitivity (NCGS)

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Background and Objectives: Buckwheat (*Fagopyrum esculentum*) is a gluten-free grain with acclaimed beneficial effects on human health. Our aim was to assess the effect of buckwheat products on intestinal/extra-intestinal symptoms and biochemical parameters in patients with Non-Celiac Gluten Sensitivity (NCGS). **Methods and Study Design:** A randomized, crossover trial with two intervention phases was conducted on 19 NCGS patients over a 12 week-period. The participants were assigned to consume products made from buckwheat or to maintain their normal gluten-free diet for 6 weeks in a random order. Symptoms due to NCGS were evaluated using two questionnaires. **Results:** During the intervention period with buckwheat products, patients experienced a significant decrease in the severity of abdominal pain and bloating (p=0.03). In contrast, the control group showed a significant worsening trend for the majority of NCGS symptoms such as nausea, headache, joint/muscle pain, and attention disorders. The replacement diet with buckwheat products also resulted in a significant increase of serum magnesium (+4.7%) and a significant reduction in the circulating levels of some pro-inflammatory cytokines such as interferon gamma (-33.3%) and monocyte chemotactic protein-1 (-46.5%). **Conclusion:** The study supports the positive effects of buckwheat for NCGS patients, showing that this alternative cereal can contribute to the reduction of both negative gastro-intestinal and related symptoms, and nutritional deficiencies, and lead to an improvement in inflammatory profile.

Key Words: NCGS, buckwheat, diet, symptoms severity, inflammatory profile

INTRODUCTION

Non-celiac gluten sensitivity (NCGS) is an emerging syndrome, affecting up to 6% of the general population in Western countries, although the exact prevalence is as yet unknown.[1] NCGS consists of a combination of intestinal and/or systemic symptoms, similar to those of celiac disease (CD) and irritable bowel syndrome (IBS).[2] The most frequent intestinal symptoms are bloating and abdominal pain, alternating bowel habits, and nausea. Additional extra-intestinal manifestations include exhaustion, lack of wellbeing, headaches, anxiety, "foggy mind", arm/leg numbness, muscle or joint pain and depression.[3]

Given that there are no serologic and histological markers available, an objective approach to NCGS diagnosis is unavailable to date. According to the London criteria, a diagnosis of NCGS is made in patients with negative immune allergy tests to wheat, negative celiac disease serology, normal duodenal histopathology and the reduction of symptoms under a gluten-free diet (GFD).[1] However, gluten is only one protein contained within wheat. Recent studies have been suggested that wheat proteins other than gluten, such as amylase trypsin inhibitors (ATIs), can elicit immunomediate reactions (innate immunity).[4]

Nevertheless, in NCGS gastro-intestinal (GI) and extra-GI symptoms occur after gluten ingestion, and rapidly improve after its withdrawal from the diet. Therefore, a GFD seems to be the only effective therapy.[2] On the other hand, people who adopt a GFD commonly experience deficiencies in essential nutrients and vitamins.[5] In fact, gluten-free products tend to be low in a wide range of important nutrients, including vitamin B, calcium, iron,

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zinc, magnesium, and fiber.[5] Moreover, sugar or fat are added to gluten-free substitute flours to make the products more palatable, thereby leading to a high calorie content. Hence, the possibility of replacing wheat flour with grains that are naturally gluten-free may represent a healthier option.[6]

The technological and nutritional properties of alternative cereals, as a replacement for wheat, have been investigated and it has been suggested that the use of these pseudo cereals could improve the intake of protein, iron, calcium and fibre.[7-8] Among these gluten-free grains, buckwheat seems to be one of the most valuable in terms of nutritional composition.[9]

There is no research available so far on the use of this alternative cereal for patients with NCGS. Therefore, the present dietary intervention trial was conducted with the aim of evaluating the impact of buckwheat on intestinal/extra-intestinal symptoms and biochemical parameters in NCGS patients.

METHODS

Study population

Nineteen subjects with a diagnosis of NCGS were recruited among the patients visiting the Unit of Allergology and Clinical Immunology of the S. Giovanni di Dio Hospital, Florence. Inclusion criteria were as follows: (1) age >18 years, (2) presence of symptoms of NCGS, (3) absence of celiac disease, (4) negative allergy tests to wheat (according to guidelines for IgE mediate food reactions)[10] (5) negative for HLA DQ 2 and DQ8 (6) positive in the double-blinded, placebo-controlled crossover challenge (DBPC) clinical trials with gluten muffin.[4,11]

Exclusion criteria included other significant gastrointestinal diseases (such as cirrhosis or inflammatory bowel disease); other clinically significant co-morbidities; BMI >35 or <18 kg/m^2 (suggesting an abnormal diet or health status); excessive alcohol intake; pregnancy or breast-feeding; inability to give written informed consent.

Buckwheat products

The buckwheat products employed in the study were pasta, hard tacks, biscuits and buckwheat-flakes. All of them were gluten free according to the Codex Alimentarius Standard and were provided by PROBIOS srl (*Calenzano, Florence, Italy*). With the exception of pasta, which was 100% buckwheat, the other items also contained whole rice flour, corn flour, almonds flour, quinoa flour and flaxseeds. The macronutrient composition of these products is presented in Table 1. Overall, participants received 80 g of pasta per day, 60 g of hard tacks per day, 40 g of biscuits per day and 50 g of flakes per day. They were instructed not to consume any other cereal products throughout the experimental period of the study.

Study design

The present study was randomised, cross-over trial, with two intervention phases spanning a total of 12 weeks. Baseline data were collected during a 2-week run-in period. After the run-in period, eligible participants were randomly assigned with a 1:1 ratio either to the experimental group or the control group. During the experimental period, patients were asked to substitute all gluten-free products, usually consumed, with a range of suitable commercially-available buckwheat products that were provided for the study. On the contrary, during the control period, patients were asked to maintain their normal GFD. At the end of the first 6-week intervention phase, patients crossed over to the other treatment condition for the remaining 6 weeks of the study. Symptoms due to NCGS were evaluated using two questionnaires, which were compiled both at baseline and on a weekly basis for the duration of the study. Patients were instructed to maintain their normal dietary and lifestyle habits throughout the study, and not to change or take any medication without consulting the investigator.

The study was performed in accordance with the Helsinki II declaration. Written informed consent was obtained from each participant before the initial screening visit and before randomization. The institutional review board at the University of Florence, aimed at protecting human participants, approved the study protocol (SPE 13.115)

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Data collection and measurements

At baseline and at the end of the two intervention phases, respectively, all patients were examined between 7:00 a.m. and 9:30 a.m. after an overnight fasting period. Moreover, each patient underwent an interview, according to standardized methods, to obtain information about personal medical history, demographics, medication, and lifestyle habits. Physicians, using standardized protocols, conducted a physical examination and laboratory tests. Patients were asked not to engage in strenuous physical activity during the day before the examination. Anthropometric measurements and body composition were made on entry to the study and at the end of the two phases. Weight and height were measured through use of a stadiometer. Body mass index (BMI) was calculated as weight (kg)/height (m)^2. The body composition was determined with a bioelectrical impedance analyser device (Tanita, model SC 330 P).

Because of the absence of reliable biomarkers for NCGS, a symptomatic trend was measured using scores validated for functional diseases. In particular, NCGS

Table 1. Macro-nutritional composition in 100 g buckwheat products used in the intervention study

Product	Energy (kJ)	Energy (kcal)	Fat (g)	Carbohydrate (g)	Protein (g)	Dietary fibre (g)	Salt (g)	Saturatedfat (g)	Total sugars (g)
Pasta	1456	344	3.4	61.1	13.3	6	0.8	0.7	0.6
Cakes	1543	369	4.0	68.4	11.1	7.4	0.5	0.8	0.2
Flakes	1682	397	1.2	86	7.5	6	1.2	0.1	8.7
Biscuits	1870	445	16	68	6.1	2.3	0.8	2.6	23

symptom frequency and severity were assessed using a modified version of two IBS-specific questionnaires: the Global Assessment of Improvement Scale (GAI)[12] and the Symptom Severity Scale (SSS).[13] Assessments were made at the start of each week for the entire duration of the respective intervention phases. The modified GAI Scale assessed NCGS symptoms using a 7-pointscale. The severity of abdominal pain, severity of abdominal distention, satisfaction with bowel habits, severity of headache, severity of exhaustion, severity of nausea, attention disorder, muscle/joint pain, and quality of life was investigated in response to the following question: "Compared to the way you felt before you entered the study, have your NCGS symptoms over the past 7 days been: 1) "Substantially Worse", 2) "Moderately Worse, 3) "Slightly Worse", 4) "No Change", 5) "Slightly Improved", 6) "Moderately Improved" or 7) "Substantially Improved".

The modified IBS-SSS is a multidimensional rating scale assessing overall NCGS severity on a Visual Analogue Scale (VAS). An overall NCGS score was calculated from six items: pain severity, pain frequency, abdominal bloating, bowel habit dissatisfaction, abdominal heaviness, and life interference. The modified IBS-SSS ranges from 0 to 600, with higher scores meaning more severe symptoms. SSS can be used to classify NCGS severity as mild (<200), moderate (200–400), and severe (>400). SSS has been validated and found to be responsive to changes in symptom severity; a change in 50 is considered to be adequate to detect a clinical improvement.[13]

A participant was defined as a subject who (1) answered that, compared with prior to the intervention, his symptoms were either 'moderately improved' or 'substantially improved' to the GAI questions and (2) who reported a change of ≥50 points on the SSS.

Laboratory analyses

Blood sampling, after overnight fasting, was obtained from each participant. Lipid variables, blood glucose, serum electrolytes, serum minerals and liver enzymes were assessed by conventional methods. Venous blood samples were taken from the subjects by the study physician and collected into evacuated plastic tubes (Vacutainer). Samples obtained by centrifuging at 3,000 g for 15 min at 4°C were stored in aliquots at - 80°C until analysis.

Statistical analysis

Statistical analysis was performed using the statistical package PASW 20.0 for Macintosh (SPSS, Inc.). All variables were checked for a normal distribution before data analysis. Data were expressed as arithmetic means and standard deviations for normally distributed variables and as medians and ranges for non-normally distributed data. The Mann–Whitney U test was used for testing the differences between the groups. All data were treated as paired samples from a crossover study. The two interventions were analyzed by taking into account both periods in the two groups of subjects at different stages. A general linear model for repeated measurements, with adjustments for age and sex, was used to compare the effect of the two different treatments. A p value <0.05 was considered to indicate statistical significance.

RESULTS

The median age of the study population (18 women; 1 man) was 44 (range 28-65) years and the mean body mass index was 23.7±5. Three patients were defined as smokers, and one was hypertensive under an optimal therapeutic control. Baseline questionnaires for evaluating the severity of symptoms related to NCGS showed a moderate severity in all the participants (total score for SSS: 261±153, with a score ranging from 0 to 600). In particular, 37% had mild, 37% moderate, and 26% severe NCGS symptoms.

With regard to the gastrointestinal symptoms, the most frequent were bloating (53%) and abdominal pain (47%). About one-third of the patients reported abdominal heaviness, while 37% had alternating bowel habits. The most frequent extra-intestinal manifestations were joint/muscle pain and exhaustion, reported by 68% and 63%, respectively. No statistically significant differences between the two randomized groups of intervention with regard to age, demographic characteristics, and pain scores were observed (data not reported).

Modifications of symptoms

The changes in gastrointestinal and extra-intestinal symptoms were compared from baseline to the end of the intervention periods using a general linear model adjusted for age and sex. The SSS-score showed that patients consuming buckwheat products reported a significantly improved score at the end of the intervention period, compared with those who maintained their normal eating habits. In particular, during the buckwheat period, the severity score of abdominal pain significantly decreased from 41.3±30.8 at baseline to 31.1±25.3 at week 6 (p<0.05) (Figure 1), and a significant trend of reduction in the severity of bloating was observed (p<0.05) (Figure 2).

Eleven (58%) patients reported an improvement of more than 50 points according to the SSS-score, after consumption of the buckwheat products, compared with only 4 (21%) patients after the control period. In addition,

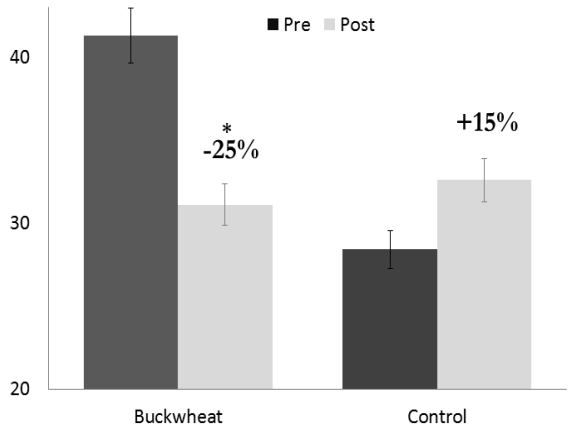

Figure 1. Change in the SSS-score for severity of abdominal pain in the buckwheat and control groups over a 6-week period. *The reduction in the severity of symptoms was significant (p<0.05)

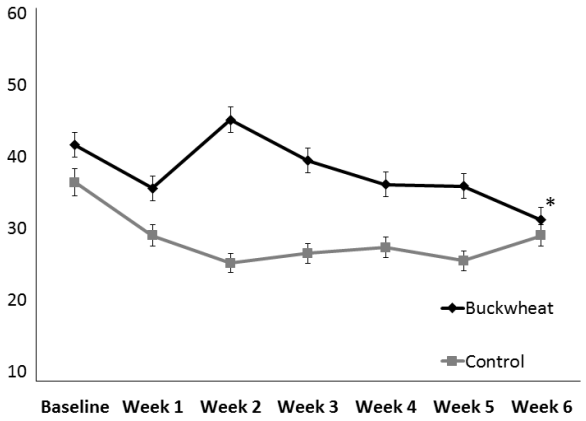

Figure 2. Change in the SSS-score for severity of bloating in the buckwheat and control diet-treated groups over a 6-week period. *The trend of reduction in the severity of symptom was significant (p<0.05).

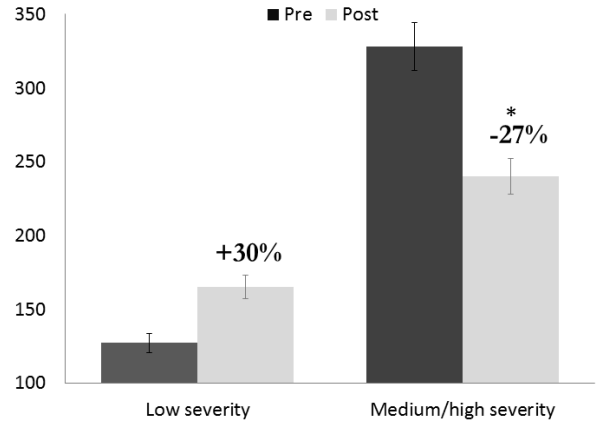

Figure 3. Change in total score for SSS in patients with mild NCGS symptoms at baseline compared to patients with moderate and severe NCGS manifestations at baseline. *The reduction in the severity of symptoms was significant (p<0.05).

we observed that during the buckwheat intervention period patients with moderate and severe NCGS manifestations at baseline were more likely to indicate significant improvements in NCGS symptoms (change in SSS-score: -88 (CI -167; -8) p<0.05), compared with patients with mild NCGS symptoms at baseline (change in SSS-score: 38 (-29; 105) p=0.2) (Figure 3).

The GAI questionnaire also showed a trend for an improvement in NCGS symptoms in patients who consumed the buckwheat products but this change did not reach statistical significance. In contrast, the control group showed a significant trend of worsening for the majority of the NCGS symptoms: nausea (5.4±1.5 at baseline vs 4.1±0.9 at week 6; p<0.05), headache (5.1±1.4 vs 3.8±1.0; p<0.05), joint/muscle pain (4.6±1.7 at baseline vs 6 3.3±1.3 at week 6; p<0.05), attention disorder (4.8±1.5 at baseline vs 3.9±0.7 at week 6; p<0.05), and satisfaction with stool consistency (4.8±1.4 at baseline vs 3.9±1.6 at week 6; p<0.05).

Four (21%) patients responded positively for the symptom of joint/muscle pain after the buckwheat-product intervention period, compared with only 1 (5%) subject after the control period. Similarly, 3 (16%) patients reported to have reduced nausea and lack concentration at the end of the buckwheat-product intervention period, whereas only 1 (5%) subject reported a significant amelioration after the control period.

Changes in other measurements

At the end of the intervention period, anthropometrical measurements did not change significantly with respect to baseline in both groups (data not reported). With regard to the biochemical parameters, no significant difference was observed for the vast majority of the investigated parameters, after both dietary intervention periods. However, significant improvements in the circulating levels of magnesium (+0.08 mg/dL (+4.7%); p<0.05) were reported after the intervention period with only the buckwheat products (Table 2).

In addition, the circulating levels of some pro-inflammatory cytokines such as interferon gamma and monocyte chemotactic protein-1 decreased significantly (-9.2 pg/mL (-33.3%); -20.6 pg/mL (-46.5%), respectively; p<0.05) after the intervention phase with the buckwheat products (Table 3). This effect was not evident after the consumption of control GFD phase.

DISCUSSION

The aim of the current study was to assess the effect of buckwheat products on gastro-intestinal manifestations, related extra-gastro-intestinal symptoms, as well as biochemical parameters in patients with NCGS. After the 6-week buckwheat intervention period, the majority of patients reported an overall significant reduction in the severity of the two most frequent symptoms such as bloating and abdominal pain. Conversely, significant worsening was shown during the control period, when patients returned to their normal GFD. With regard to the biochemical parameters, there were no diet-specific changes in any biomarker, except for circulating levels of magnesium, that increased after the buckwheat intervention period.

Because of their suspected involvement in NCGS pathogenesis,[14] inflammatory cytokine levels were also evaluated. Patients experienced a significant decrease in both interferon gamma and monocyte chemotactic protein-1 levels after the intervention period, but further research is needed to clarify this issue.

This is the first dietary intervention trial that assessed the efficacy of buckwheat products on gastrointestinal symptoms, biochemical parameters and the inflammatory profile among NCGS patients. Over the last few years, there has been a resurgence in research interest regarding NCGS, as demonstrated by several scientific contributions on this topic.[15-18] Nevertheless, our knowledge is still limited, and there are many unresolved points warranting clarification. On the one hand, NCGS is a symptom-based condition, in which diagnosis remains highly presumptive, being exclusively based on clinical criteria and on exclusion of celiac disease and wheat allergy. On the other hand, despite the general agreement that gluten exclusion improves NCGS symptoms,[19] growing evidence indicates that gluten is not the only emerging trigger. Other components are likely to play a relevant role in the pathogenesis of this syndrome.[20] In addition, several double-blind trials underlined the possibility that many

Table 2. Modifications of biochemical parameters

Variables	Buckwheat			p^{**}	Control			p^{**}
	Pre	Post	Change		Pre	Post	Change	
Total cholesterol (mg/dL)	205.9±7.1	196.4±6.6	-9.5 (-26.7; 7.7)	0.26	196.1±6.3	196.5±7.1	0.4 (-12.9; 13.8)	0.95
LDL-cholesterol (mg/dL)	111.7±6.0	112.2±6.3	-3.6 (-10.5; 6.6)	0.28	108.8±5.0	109.3±6.1	1.3 (-5.8; 8.5)	0.70
HDL-cholesterol (mg/dL)	72.0±4.3	68.4±4.1	0.5 (-12.9; 13.9)	0.98	69.9±3.5	71.3±3.2	0.4 (-8.1; 8.9)	0.91
Triglycerides (mg/dL)	84.2±10.8	84.0±0.9	-0.2 (-27.0; 26.7)	0.99	92.5±12.4	84.5±10.0	-8.0 (-34.0; 18.0)	0.52
Blood glucose (g/L)	0.79±0.02	0.82±0.02	0.03 (-0.01; 0.07)	0.16	0.82±0.02	0.79±0.02	-0.03 (-0.07; 0.01	0.14
Magnesium (mg/dL)	1.93±0.03	2.02±0.02*	0.08 (0.02; 0.15)	0.01	1.99±0.03	2.02±0.04	0.02 (-0.09; 0.14)	0.69

Data are reported as mean and standard deviation.
*p<0.05 for paired t-test.
**p for general linear model adjusted for age and gender.

Table 3. Modifications of inflammatory profile

Variables	Buckwheat			p^{**}	Control			p^{**}
	Pre	Post	Change		Pre	Post	Change	
IL-1ra (pg/mL)	32.8±25.3	36.2±27.0	3.40 (-6.88; 13.7)	0.49	29.4±21.4	35.3±26.4	5.87 (-5.06; 16.8)	0.27
IL-4 (pg/mL)	0.34±0.25	0.27±0.22	-0.07 (-0.19; 0.04)	0.18	0.32±0.30	0.28±0.26	-0.04 (-0.12; 0.04)	0.26
IL-6 (pg/mL)	3.6±3.1	2.4±1.6 *	-1.18 (-2.42; 0.07)	0.06	3.6±4.0	3.1±2.8	0.52 (-1.85; 0.80)	0.42
IL-8 (pg/mL)	23.0±26.6	21.2±35.9	-1.82 (-12.3; 8.7)	0.72	37.5±43.1	28.2±57.5	-9.29 (-33.7; 15.1)	0.43
IL-10 (pg/mL)	9.9±5.0	11.4±8.4	1.44 (-2.29; 5.18)	0.43	11.1±8.1	10.2±5.1	10.2 (7.6; 12.8)	0.45
IL-12 (pg/mL)	15.0±7.5	12.7±6.6	-2.24 (-5.08; 0.60)	0.11	14.0±6.7	13.2±5.9	-0.84 (-2.69; 1.00)	0.35
VEGF (pg/mL)	150.4±147.7	112.5±82.1	-38.0 (-106.8; 30.8)	0.26	191.1±164.3	153.9±155.2 *	-37.2 (-70.5; -3.8)	0.03
IFN-gamma (pg/mL)	27.6±21.5	18.4±7.2 *	-9.16 (-18.5; 0.14)	0.05	24.0±17.6	19.6±12.2	-4.35 (-11.0; 2.26)	0.18
MCP-1 (pg/mL)	44.3±27.8	23.7±12.1 *	-20.6 (-33.6; -7.58)	0.01	36.3±25.5	26.8±16.9	-9.48 (-24.0; 5.0)	0.19
TNF-alpha (pg/mL)	7.7±4.7	7.1±4.1	-0.62 (-3.72; 2.49)	0.68	7.2±7.1	6.5±7.2	-0.69 (-5.05; 3.66)	0.74

IL-1ra: interleukin-1ra; IL-4: interleukin-4; IL-8: interleukin-8; IL-10: interleukin-10; IL-12: interleukin-12; VEGF: Vascular Endothelial Growth Factor; INF-gamma: interferon-gamma; MCP-1: Monocyte Chemotactic Protein-1; TNF-alpha: Tumor Necrosis Factor-alpha.
Data are reported as mean and standard deviation.
*p<0.05 for paired t-test.
**p for general linear model adjusted for age and gender.

patients with a self-diagnosis of food hypersensitivity display an imaginary syndrome that is caused by the nocebo effect of gluten ingestion.[21,22]

To date, five placebo-controlled dietary interventions in patients with presumptive NCGS have been published with contrasting results in terms of the culprit agent.[21-27] In the study by Biesiekierski et al[23] patients who received the gluten challenge reported worsening of the abdominal symptoms than those who received the placebo. Similarly, in a second trial, conducted by the same research group, no differences among high-gluten, low-gluten or placebo challenge were demonstrated.[24] However, in the latter study, patients showed a significant clinical improvement during the run-in period of the study, i.e. when they were under a low FODMAPs-diet (Fermentable Oligo-Di-Monosaccharides and Polyols). Therefore, the possibility that NCGS patients could be sensitive to FODMAPs has been hypothesized, and it has been later supported by the results of other two most recent studies[25,27] that reported an improvement in patients with gluten-free flour sensitivity when a low FODMAPs-diet was recommended.[28]

Actually, dietary management of patients with NCGS still presents unanswered questions concerning elimination diets, supplements, and foods that may help symptoms of the disorder. Gluten-free diet is the first recommended dietary change for these patients, but an increasing number of patients shift to gluten-free products without any precise medical advice or indication, so likely causing nutritional deficiencies. Indeed, a GFD could be low in nutrients, whereas commercially available gluten-free products are generally abundant in chemical additives and preservatives, a potential cause of functional gastrointestinal symptoms.[5]

Buckwheat (*Fagopyrum esculentum*) is a promising alternative gluten-free product, which is characterized by a highly nutritive composition.[29,30] Buckwheat is an excellent source of manganese, copper, magnesium, phosphorus, vitamins and fibre.[6] This alternative cereal contains several polyphenolic antioxidant compounds with significant health-promoting actions, such as rutin, tannins and catechin. In addition, the proteins in buckwheat are representative of high-quality proteins, containing all the essential amino acids, including lysine.[31] As part of a healthy way of eating, buckwheat can significantly lower the risk of cardiovascular disease, obesity and type 2 diabetes.[32]

Although results are promising, the number of participants represents a limitation in this study. Further and larger studies are needed before drawing any firm conclusions on the effects of buckwheat products on NCGS patients. We are aware that changes in dietary and/or lifestyle habits could have affected the investigated parameters. However, before initiating the experimental trial, all patients were instructed by physicians, and by an expert dietician, to maintain their usual lifestyle habits.

In conclusion, the present study provided evidence for the positive effects of buckwheat in NCGS, thereby showing that this alternative cereal represents a dietary option for patients with NCGS. These results are certainly interesting, but further research is necessary to better investigate whether the use of alternative cereals are effective in reducing symptom severity and nutritional deficiencies in the diets of gluten sensitive patients.

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AUTHOR DISCLOSURES

The authors declare no conflicts of interest.

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