ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 56, N.3, 121-127, 2024

Kenta Horimukai, Misako Kinoshita, Noriko Takahata

Predicting the outcome of the buckwheat oral challenge test: a first evaluation assuming a single serving of boiled buckwheat noodles

Department of Pediatrics, Jikei University Katsushika Medical Center, Tokyo, Japan

Key words

Child; buckwheat hypersensitivity; oral challenge test.

Corresponding author
Kenta Horimukai
Department of Pediatrics
Jikei University Katsushika Medical Center
6-41-2 Aoto, Katsushika-ku
Tokyo 125-8506, Japan
ORCID: 0000-0003-3296-2207
E-mail: khorimukai@jikei.ac.jp

Doi
10.23822/EurAnnACI.1764-1489.315

Impact statement

Buckwheat-specific IgE levels poorly predict food challenge outcomes despite increased serving size; prior buckwheat-induced symptoms strongly correlate with positive challenges, emphasizing patient history's importance.

Summary

Background. *Global increase in buckwheat consumption has led to a surge in buckwheat allergy reports. However, studies scrutinizing the predictive accuracy of buckwheat-specific immunoglobulin E (IgE) antibody levels in correlation with symptom manifestation remain limited. A critical concern is the discrepancy between the total buckwheat amount featured in prior studies and the quantity consumed per occasion. We aimed to determine open Oral Food Challenge (OFC) positivity rates with buckwheat, using a single serving of boiled buckwheat noodles, and assess the predictability of positive responses using buckwheat-specific IgE levels.* ***Methods.*** *Patients aged 20 years or younger, suspected of buckwheat allergy, were subjected to an OFC involving consumption of 100 g (4,800 mg of protein) of boiled buckwheat noodles for those under six years, and 200 g (9,600 mg of protein) for those six years or older. The predictive accuracy of the OFC, corresponding with buckwheat-specific IgE antibody levels, was evaluated using Receiver Operating Characteristic (ROC) analysis.* ***Results.*** *Our study involved 80 patients who undertook a buckwheat OFC. Among these, 14 (17.5%) tested positive for a buckwheat allergy, with 3 (3.8%) developing anaphylaxis. The comparative analysis of buckwheat-specific IgE antibody levels did not offer a reliable predictive measure for OFC outcomes. However, a past history of symptom manifestation following buckwheat consumption was significantly correlated with a positive OFC.* ***Conclusions.*** *Forecasting OFC outcomes based on buckwheat-specific IgE antibody levels poses a challenge, even when taking into account the total quantity of buckwheat that can be consumed in a single occasion.*

Introduction

Buckwheat is a widely cultivated and consumed crop that is used as a primary ingredient in various traditional dishes worldwide. It has gained popularity in the European market due to its nutritional value and suitability for gluten-free products (1, 2). In Italy, however, buckwheat allergies account for approximately 7% of all identified food allergies (3). This finding is supported by a separate clinical study that reported buckwheat allergies in approximately 3.6% of patients evaluated at 18 allergy clinics across Italy (4).

Therefore, accurate diagnosis of buckwheat allergy has become increasingly crucial. However, the largest study conducted to date, involving 419 individuals with suspected buckwheat allergy, demonstrated a low predictive accuracy for symptom onset during a buckwheat oral food challenge (OFC) when using buckwheat-specific IgE antibody levels (5). Additionally, previous studies used a single serving size of boiled buckwheat noodles that was relatively low, ranging from 64 to 80 g (equivalent to 3,072 to 3,840 mg of buckwheat protein). This suggests the need for a study to investigate the rate of symptomatic positivity with a higher total serving size (5-8). In this research,

we conducted a buckwheat OFC with a serving size of 100 grams (equivalent to 4,800 mg of protein) for subjects under 6 years of age and 200 grams (equivalent to 9,600 mg of protein) for those aged 6 and above, aiming to investigate the positive predictive accuracy of buckwheat-specific IgE antibody levels. This study represents the first cross-sectional case series where the total serving size of buckwheat was set higher than in previous studies.

Materials and methods

Study design, ethics statement and informed consent

In this cross-sectional study, we administered open OFC to patients under 20 years of age suspected of buckwheat allergy. The amount of boiled buckwheat noodles typically consumed in Japan (100 g for children under six years of age, and 200 g for those over six) was employed for the OFC. We retrospectively evaluated the accuracy of predicting symptom development during OFC based on buckwheat-specific IgE antibody levels, using data obtained from electronic medical records.

We ensured the stringent observance of ethical standards in the course of the study. Parents or guardians were thoroughly briefed, both verbally and in writing, about potential risks associated with OFC. Written informed consent was acquired from the parents or legal guardians of all subjects. The study protocol received approval from the Ethics Review Committee of Jikei University Hospital (approval number 35-027 [11648])). The study was conducted in adherence to the ethical guidelines for life science and medical research involving human subjects as stipulated by Japanese standards. Moreover, we ensured full compliance with the principles enshrined in the Declaration of Helsinki throughout the study.

Objectives

The study, conducted at the Department of Pediatrics, The Jikei University Katsushika Medical Center in Tokyo, from January 1, 2012, to April 30, 2023, aimed to assess OFC in patients under 20 years of age suspected of buckwheat allergy. The inclusion criteria were patients with a history of buckwheat-related symptoms or a positive buckwheat-specific IgE antibody level, and no prior consumption of buckwheat. Patients who underwent an oral boiled buckwheat noodles challenge of 100 g for those under six years of age, and 200 g for those over six, were considered for the total buckwheat load. Those scheduled for a lower total load were excluded.

Total immunoglobulin E (IgE) levels and serum-specific IgE antibody titers

We utilized the ImmunoCAP assay system (Thermo Fisher Scientific, Uppsala, Sweden) to measure total immunoglobulin E (IgE) levels and serum-specific IgE antibody titers. For this study, we used buckwheat-specific IgE antibody measurements taken within one year, both preceding and following the OFC. Specific IgE antibody titers below 0.1 UA/mL were recorded as 0.09 UA/mL, and those above 100 UA/mL as 101 UA/mL. Notably, in January 2013, the lower limit of accuracy for the commercial ImmunoCAP assay system in Japan was revised from 0.35 UA/mL to 0.1 UA/mL. Consequently, for consistency, buckwheat-specific IgE antibody levels less than 0.35 UA/mL measured before January 2013 were adjusted to 0.09 UA/mL in our study.

OFC

The boiled buckwheat noodles OFC was conducted by administering buckwheat in specific fractions. For a total load of 100 g of boiled buckwheat noodles (buckwheat protein equivalent 4,800 mg), subjects consumed buckwheat in three divided servings of 10 g, 30 g, and 60 g at 30-minute intervals. For a total load of 200 g of boiled buckwheat noodles (buckwheat protein equivalent 9,600 mg), subjects ingested buckwheat in three divided servings of 25 g, 75 g, and 100 g. All patients underwent the oral challenge as inpatients and were monitored until the day following the test. Symptoms observed during the oral challenge were evaluated using a grading system based on the Sampson classification (9). All OFCs were performed in an inpatient setting and monitored through the next day.

Symptoms observed during the OFC were categorized into distinct groups: cutaneous symptoms (urticaria, erythema, pruritus), respiratory symptoms (cough, wheezing, hoarseness, dyspnea), gastrointestinal symptoms (vomiting, diarrhea, prolonged abdominal pain), cardiovascular symptoms (hypotension), and neurological symptoms (confusion).

If a positive result was determined by the OFC physician, patients under seven years of age were treated with antihistamines like fexofenadine or epinastine, while patients over seven were administered loratadine. The variation in types of antihistamines administered based on age was due to changes in, or limited availability of, the drugs employed at our institution. In addition, based on the specific pharmacotherapy available at the hospital, inhaled beta-adrenergic agonists (such as salbutamol), corticosteroids (prednisolone), and intramuscular epinephrine into the lateral thigh muscle were administered as needed. The final determination of whether the OFC results were positive, negative, pending, or indicative of anaphylaxis was made by the physician. “Pending” referred to cases where there were no clear objective clinical symptoms, making it difficult to render a positive determination. The details of the OFC were retrospectively confirmed from the medical records.

Endpoints

The primary endpoints of the study were as follows: the proportion of patients who tested positive for OFC with a total boiled

buckwheat noodles load of 100 g (< 6 years) and 200 g (≥ 6 years), sensitivity and specificity based on buckwheat-specific antibody levels, and the area under the curve (AUC) for the receiver operating characteristic (ROC) analysis.

Statistical analysis

Continuous variables were summarized using medians and interquartile ranges (IQRs), whereas nominal variables were represented as numbers and percentages. For the comparison of nominal variables, we applied Fisher's exact test, and for continuous variables, the Mann-Whitney U test was employed. We used the ordinary logarithm to transform Total IgE values and specific IgE antibody titers logarithmically. We considered a P-value less than 0.05 as indicative of statistical significance. To assess the predictive performance of buckwheat-specific IgE antibody titers, we generated ROC curves and calculated AUC. We determined specificity and sensitivity based on the point on the ROC curve nearest to the upper-left corner. We conducted statistical analyses using IBM SPSS Statistics for Windows, Version 17.0 (IBM Corp., Armonk, New York, United States). Additionally, we employed the graphical interface for R, EZR version 1.60 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is affiliated with The R Foundation, to facilitate data analysis.

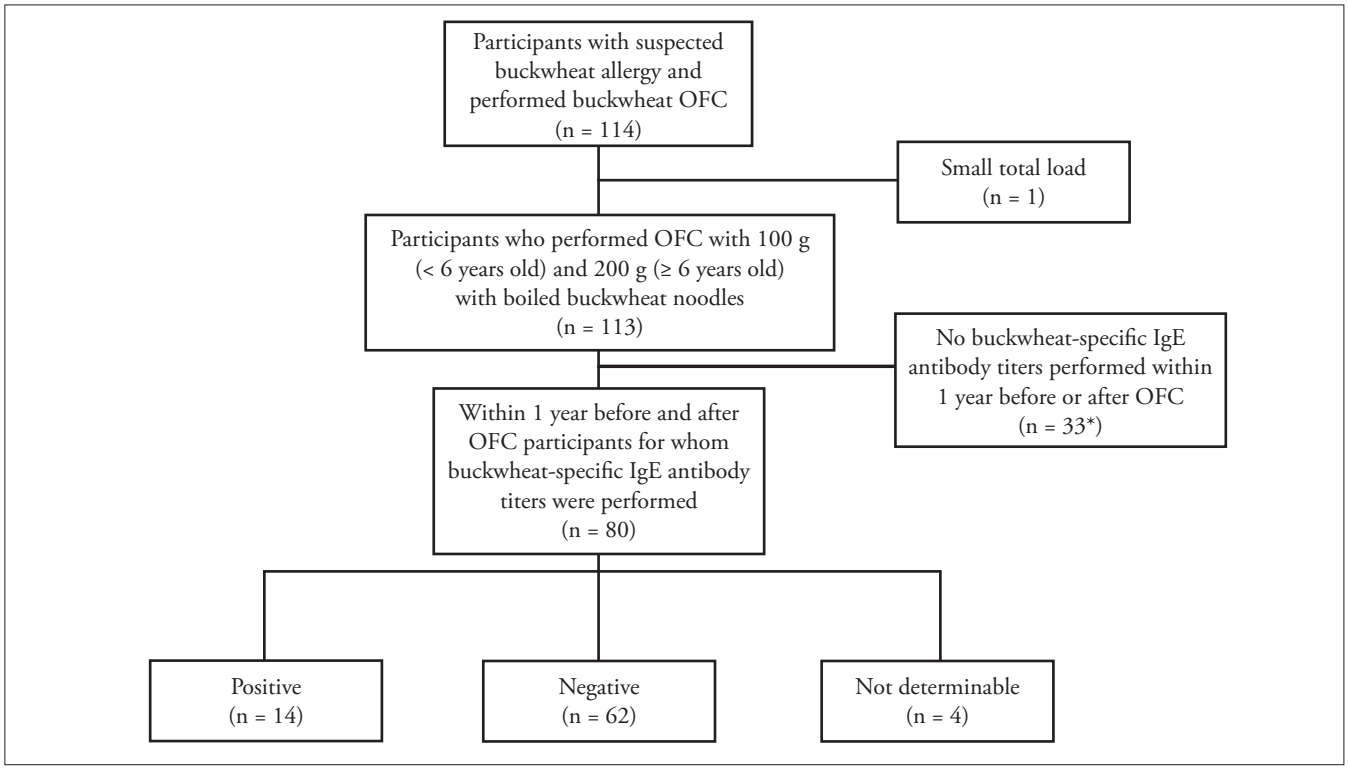
Results

Patient characteristics

Throughout the study period, 114 patients were set for a boiled buckwheat noodle OFC. Among these, 113 patients proceeded with the OFC, adhering to the total load designated in this study: 100 g for patients below the age of 6 and 200 g for those above 6 years old. Out of these patients, 80 had buckwheat-specific Immunoglobulin E (IgE) antibody titers measured within one year preceding or following the OFC (**figure 1**).

Therefore, 80 patients (56 males, constituting 70.0%) fulfilled the inclusion criteria for this study. The median age at the time of the OFC was 73 months (ranging between 23 to 203 months). At the time of the OFC, 11 (13.8%) had previous exposure to buckwheat consumption, 8 (10.0%) reported a history of symptoms triggered by buckwheat consumption, and 4 (5.0%) had experienced anaphylaxis following buckwheat exposure (**table I**).

Figure 1 *- Flowchart of this study*



*OFC: oral food challenge; *these subjects underwent buckwheat OFC and IgE antibody evaluations, but buckwheat-specific IgE wasn't assessed within a year of the OFC, largely because parents were wary of prior positive IgE findings and past buckwheat symptoms.*

Table I *- Patient characteristics (n = 80).*

Characteristics	Total
Sex	
Male	56 (70.0)
Female	24 (30.0)
Age (months)	73 [44.5-106.25]
History of symptom induction by buckwheat	8 (10.0)
History of anaphylaxis due to buckwheat	4 (5.0)
Current bronchial asthma	26 (32.5)
Buckwheat-specific IgE (UA/mL)	1.62 [0.66-3.98]
Total IgE (IU/mL)	829 [263-1,565]

Data are presented as n (%) or median [interquartile range]; IgE: immunoglobulin E.

OFC result

Of the participants, 14 subjects (17.5%) tested positive during the OFC, while 62 (77.5%) tested negative. A total of 4 (5.0%) showed minor symptoms, such as localized oral cavity itching, and were subsequently classified as inconclusive OFC results. 6 subjects (7.5%) showed symptoms before the maximum OFC dose: 100 g for children under 6 and 200 g for those 6 and older. Of these, 2 were positive at 25 g and 4 at 75 g.

Anaphylaxis was noted in three patients (3.8%), with one patient (1.3%) requiring an intramuscular adrenaline injection. In two cases, symptoms began to alleviate while the administration of adrenaline was under consideration, leading to the physician's decision against its use. There were no recorded cases of severity Grade 4 or higher.

Comparative analysis of OFC outcomes in positive and negative groups

OFC results were compared between 14 cases in the symptom-positive group and 62 cases in the negative group, excluding 4 cases with minor and difficult-to-identify symptoms (**table II**). In this study, the group with a history of symptom induction from previous buckwheat consumption had a significantly higher positive rate in the buckwheat OFC compared to those without such a history (28.6% *vs* 6.5%; p = 0.016).

A Receiver Operating Characteristic (ROC) analysis of the buckwheat-specific IgE antibody titer, used for predicting OFC positivity, yielded an Area Under the Curve (AUC) of 0.543, which was deemed non-significant. A similar study was carried out utilizing the ratio of buckwheat-specific IgE antibody titer to total IgE titer. However, the AUC was 0.486, which also demonstrated a lack of significance (**figure 2**).

Association between past symptom induction during buckwheat consumption and outcomes of OFC

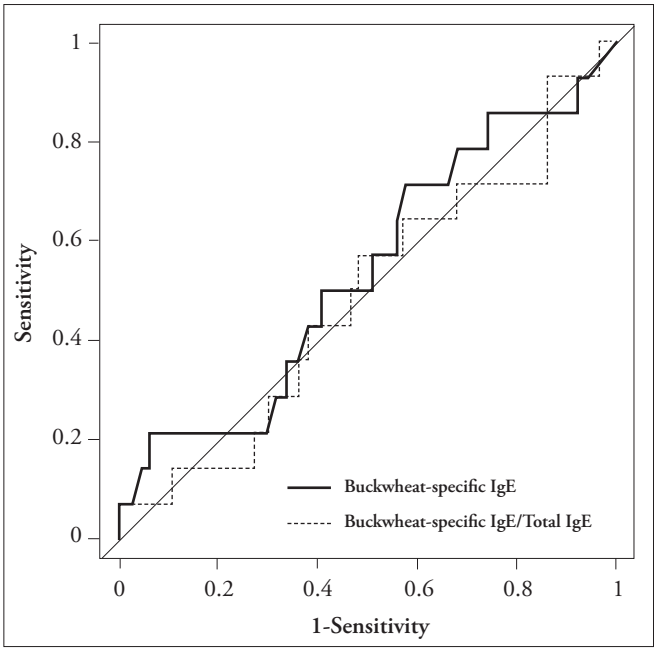
The OFC positivity rate for buckwheat was evaluated in eight individuals with a history of symptom onset following buckwheat consumption. Of these, 4 out of 8 patients who had previously experienced symptoms following buckwheat ingestion (50.0%), and 10 out of 72 subjects (13.9%) without such a history, tested positive for the buckwheat OFC. This difference was statistically significant (p = 0.028). Even when four subjects with inconclusive buckwheat OFC outcomes were excluded from the analysis, the significant difference persisted (p = 0.034).

Table II *- Comparison of patient characteristics and laboratory findings in positive versus negative buckwheat OFC (100 and 200 g of boiled buckwheat noodles in children < 6 and ≥ 6 years, respectively) results.*

Result of OFC	Positive (n = 14)	Negative (n = 62)	P-value
Sex			0.568
Male	11 (78.6)	44 (71.0)	
Female	3 (21.4)	18 (29.0)	
Age (months)	84.5 [51.75-137.50]	70.5 [43.0-102.25]	0.269
History of symptom induction by buckwheat	4 (28.6)	4 (6.5)	**0.016**
History of anaphylaxis due to buckwheat	2 (14.3)	2 (3.2)	0.096
Current bronchial asthma	3 (21.4)	22 (35.5)	0.315
Buckwheat-specific IgE (UA/mL)	1.81 [0.72-4.6275]	1.47 [0.620-3.927]	0.862
Total IgE (IU/mL)	650.5 [336.5-2,379.5]	862 [249-1,541.75]	0.615

Data are presented as n (%) or median [interquartile 25^{th}-75^{th} percentiles (range)]; OFC: oral food challenge; IgE: immunoglobulin E; $p < 0.05$ was considered statistically significant.

Figure 2 - *Receiver operating characteristic (ROC) curves for diagnostic accuracy of buckwheat allergy based on buckwheat-specific immunoglobulin E (IgE) antibody levels and the ratio of buckwheat-specific IgE/total IgE values.*



Comparison of groups with symptoms at below-planned vs planned total loads

Of 6 subjects positive for OFC at ≤ 75 g and 8 subjects at ≥ 100 g, the buckwheat-specific IgE antibody titers were 3.645 ± 5.085 UA/mL and 5.656 ± 9.557 UA/mL, respectively, with no significant difference (p = 0.622).

Discussion and conclusions

To our knowledge, this is the first investigation utilizing a typical single-serving amount of boiled buckwheat noodles for an OFC and evaluating the predictive accuracy of OFC outcomes based on buckwheat-specific IgE antibody titers. Buckwheat, a globally cultivated and consumed food, is a staple ingredient in various traditional cuisines worldwide. Its popularity extends beyond Asia, and it has seen growing acceptance in the European market due to its nutritional value and suitability for gluten-free products (1, 2, 10).

Hence, it is anticipated that both the frequency of buckwheat consumption and the quantity ingested per meal will increase. Previous studies on buckwheat OFCs have generally found that the total amount of boiled buckwheat noodles ranges from 64 to 80 g (equivalent to 3,072 to 3,840 mg of buckwheat protein – about 30 g to 40 g of buckwheat flour) (5-8). However, numerous cooking recipe websites from various regions commonly showcase buckwheat-based dishes, in which the total buckwheat content often surpasses the amounts used in previous research studies (11-14). In other words, a gap has developed between the total buckwheat load from previous studies and the total amount of buckwheat actually consumed at one time.

One obstacle to the widespread use of buckwheat OFC is the problem of total load. Clinical trials involving chicken eggs, milk and wheat suggest that as the cumulative intake during an OFC increases, so does the risk of symptom manifestation (15). In other words, even if a moderate dose (64-80 g of boiled buckwheat noodles) of OFC is negative, a single intake of an even higher dose (100-200 g of boiled buckwheat noodles) may cause concern for both patient and physician. As a result, there is a need for an OFC that corresponds with the quantity of buckwheat typically consumed in a single serving.

This study was conducted using a single serving of boiled buckwheat noodles. We administered an OFC with a total buckwheat intake of 100 g (equivalent to 4,800 mg of protein – about 50 g of buckwheat flour) for children under six years of age, and 200 g (equivalent to 9,600 mg of protein – about 100 g of buckwheat flour) for those older, resulting in a symptom induction rate of 17.5%. This outcome appears reasonable when compared to the largest cross-sectional study performed on 419 individuals, which reported a positive rate of 10.5% (5).

This study aligns with the results of previous studies in several ways. For example, it remains difficult to predict OFC positivity from buckwheat-specific IgE antibody titers, and a history of past symptom induction predicts OFC outcome. Previous studies have shown that patients whose symptoms were induced by ingestion of the target allergen in the past have a higher rate of symptom induction during OFC (16).

Our study diverges from previous findings in several respects. The incidence of anaphylaxis in our investigation was lower than in the previous study by Yanagida *et al.* (5). For instance, in Yanagida *et al.*'s study (5), 54.5% of patients who tested positive in the OFC developed anaphylaxis, whereas in our study, anaphylaxis occurred in only 21.4% of patients. The decision to administer OFC was made by an outpatient physician, which may have excluded patients with a history of anaphylaxis. Despite this, the incidence of anaphylaxis from buckwheat OFC, where buckwheat-specific IgE antibody titers is still estimated to be higher than in previous studies with various foods. For instance, a review of 6,377 open OFC cases conducted at 5 U.S. facilities estimated anaphylaxis to be 2% of the 14% positive rate for OFC (17). This study underscores the difficulty of predicting OFC symptom development based on buckwheat-specific IgE antibody titers in real-world scenarios. Moving forward, strategies to improve the accuracy of positive predictions for buckwheat OFC are needed. Kajita *et al.*, for example, reported an improvement in the accuracy of OFC

outcome prediction by considering the ratio of buckwheat-specific IgE antibody titer to the total IgE titer (8). However, our study did not replicate the effects reported by Kajita *et al*. They found that 27.0% of patients tested positive for OFC with approximately 80 g of boiled buckwheat noodles, which may be influenced by a higher rate of OFC positivity than in previous studies and our study.

This study has few limitations. First, the group in which the OFC was conducted was a cross-sectional study with a retrospective design, where the total load was predetermined by the outpatient physician. However, the study also encompassed cases with a history of anaphylaxis, and it was inferred that these groups were still at higher risk of inducing allergic symptoms. Thus, while bias may exist, its effect is minor and remains important as a result of OFC conducted in the real world.

Second, handling specific IgE antibody titers posed a challenge: as the accuracy of the lower limit for testing food-specific IgE antibody titers improved, the previous test group of less than 0.35 UA/mL was considered as 0.09 UA/mL. However, coincidentally, cases with buckwheat-specific IgE antibody titers less than 0.35 U_A/mL were not included in this study, meaning the results were not influenced by this factor.

Lastly, the number of cases was limited. However, this study is still considered valuable as it is the first to examine the total load set for the intake of one serving of buckwheat over a period of more than 10 years.

Given these circumstances, additional research on buckwheat components is necessary, as predicting buckwheat OFC results from buckwheat-specific IgE antibody titers remains challenging (18). For instance, the IgE antibody titers for Fag e3, Fag e 2, and Fag e 5 have been reported to significantly improve the accuracy of positive prediction of buckwheat allergy (7, 19).

In our study, these buckwheat component tests were not measured because it was not practical to use them routinely, but we believe they will become even more crucial in the future. Further research is desirable to establish a method for buckwheat OFC and a test with high predictive accuracy for symptom induction prior to OFC.

Fundings

None.

Contributions

KH: conceptualization, study design, sample collection, data analysis, writing – original draft. MK: data collection, writing – original draft. NT: study design, writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgements

We extend our sincere gratitude to our colleagues for conducting OFC safely and professionally.

References

1. Zhou, M, Tang Y, Deng X, Ruan C, Kreft I, Tang Y, et al. Overview of buckwheat resources in the world. Buckwheat germplasm in the world. 2018;1-7. doi: 10.1016/B978-0-12-811006-5.00001-X.
2. Sofi SA, Ahmed N, Farooq A, Rafiq S, Zargar SM, Kamran F, et al. Nutritional and bioactive characteristics of buckwheat, and its potential for developing gluten-free products: An updated overview. Food Sci Nutr. 2022;11(5):2256-76. doi: 10.1002/fsn3.3166.
3. Heffler E, Nebiolo F, Asero R, Guida G, Badiu I, Pizzimenti S, et al. Clinical manifestations, co-sensitizations, and immunoblotting profiles of buckwheat-allergic patients. Allergy. 2011;66(2):264-70. doi: 10.1111/j.1398-9995.2010.02469.x.
4. Badiu I, Olivieri E, Montagni M, Guida G, Mietta S, Pizzimenti S, et al. Italian study on buckwheat allergy: prevalence and clinical features of buckwheat-sensitized patients in Italy. Int J Immunopathol Pharmacol. 2013;26(3):801-6. doi: 10.1177/039463201302600328.
5. Yanagida N, Sato S, Takahashi K, Nagakura KI, Ogura K, Asaumi T, et al. Reactions of Buckwheat-Hypersensitive Patients during Oral Food Challenge Are Rare, but Often Anaphylactic. Int Arch Allergy Immunol. 2017;172(2):116-22. doi: 10.1159/000456008.
6. Maruyama N, Sato S, Yanagida N, Cabanos C, Ito K, Borres MP, et al. Clinical utility of recombinant allergen components in diagnosing buckwheat allergy. J Allergy Clin Immunol Pract. 2016;4(2):322-3.e3. doi: 10.1016/j.jaip.
7. Yanagida N, Sato S, Maruyama N, Takahashi K, Nagakura KI, Ogura K, et al. Specific IgE for Fag e 3 Predicts Oral Buckwheat Food Challenge Test Results and Anaphylaxis: A Pilot Study. Int Arch Allergy Immunol. 2018;176(1):8-14. doi: 10.1159/000487135.
8. Kajita N, Yoshida K, Morikawa E, Hirao K, Yokoyama S, Narita M. Predictor of buckwheat allergy in children based on challenge test results: a retrospective observational study in Japan. Eur Ann Allergy Clin Immunol. 2022;54(4):183-8. doi: 10.23822/EurAnnACI.1764-1489.224.
9. Sampson HA. Anaphylaxis and emergency treatment. Pediatrics. 2003;111(6 Pt 3):1601-8.
10. Ohsawa R, Matsui K, Yasui Y. Current research and future prospects in common buckwheat (Fagopyrum esculentum). Breed Sci. 2020;70(1):1. doi: 10.1270/jsbbs.70.1.
11. Giallo Zafferano: Torta di grano saraceno. Available at: https://ricette.giallozafferano.it/Torta-di-grano-saraceno.html. [Italian]. Last access date: 06/06/2023.
12. Giallo Zafferano: Crostata di grano saraceno. Available at: https://ricette.giallozafferano.it/Crostata-di-grano-saraceno.html. [Italian]. Last access date: 06/06/2023.
13. Kwestia Amaku. Makaron Gryczany Z Grillowanym Łososiem. Available at: https://www.kwestiasmaku.com/przepis/makaron-gryczany-z-grillowanym-lososiem. [Polish]. Last access date: 06/06/2023.
14. Marmiton. Crêpes au Sarrasin farcies à l'oeuf, fromage et jambon. Available at: https://www.marmiton.org/recettes/recette_crepes-au-sarrasin-farcies-a-l-oeuf-fromage-et-jambon_71106.aspx. [French]. Last access date: 06/06/2023.

15. Yanagida N, Minoura T, Kitaoka S, Ebisawa M. A three-level stepwise oral food challenge for egg, milk, and wheat allergy. J Allergy Clin Immunol Pract. 2018;6(2):658-60.e10. doi: 10.1016/j.jaip.2017.06.029.
16. Miceli Sopo S, Gurnari G, Monaco S, Romano A, Liotti L, Cuomo B, et al. Predictive value of the number of adverse reaction episodes for the IgE-mediated food allergy diagnosis. Allergol Immunopathol (Madr). 2019;47(3):277-81. doi: 10.1016/j.aller.2018.10.006.
17. Akuete K, Guffey D, Israelsen RB, Broyles JM, Higgins LJ, Green TD, et al. Multicenter prevalence of anaphylaxis in clinic-based oral food challenges. Ann Allergy Asthma Immunol. 2017;119(4):339-48.e1. doi: 10.1016/j.anai.2017.07.028.
18. Satoh R, Jensen-Jarolim E, Teshima R. Understanding buckwheat allergies for the management of allergic reactions in humans and animals. Breed Sci. 2020;70(1):85-92. doi: 10.1270/jsbbs.19051.
19. Geiselhart S, Nagl C, Dubiela P, Pedersen AC, Bublin M, Radauer C, et al. Concomitant sensitization to legumin, Fag e 2 and Fag e 5 predicts buckwheat allergy. Clin Exp Allergy. 2018;48(2):217-24. doi: 10.1111/cea.13068.