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Tolerance and Efficacy of Glutalytic™: A Randomized, Double-Blind, Placebo-Controlled Study

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Abstract

Background: Non-celiac gluten sensitivity (NCGS) symptoms can overlap the gastrointestinal symptoms of Celiac disease (CD) and wheat allergies without triggering allergy mechanisms or observable damage to the small intestine. Diagnosis is limited to a gluten challenge while a gluten-free diet is currently the treatment most often recommended. Oral protease supplementation may help alleviate symptoms of NCGS.

Methods: Thirty-seven adults age 19 to 64 years, with or without self-reported gluten intolerance and/or sensitivity or undefined gastrointestinal problems were recruited into a double-blind, randomized, placebo-controlled study. On the other hand, subjects with a diagnosis of Celiac disease were excluded from the study. Participants were randomized into Glutalytic™ enzyme product and placebo groups. All were instructed to consume 3 capsules daily with a meal for 30 days. The tolerance and efficacy of Glutalytic™ was assessed using C-reactive protein (CRP) tests, gliadin antibody panels, and Gastrointestinal Symptom Index Questionnaires collected at the beginning, middle, and conclusion of the study.

Results: As an oral enzyme supplement, Glutalytic™ consumption lowered the overall mean in deamidated gliadin IgA antibodies during the study period (P=0.024) based on a 95% confidence interval. The Glutalytic™ group experienced a significant reduction in gastrointestinal reflux from 1.64 to 1.14 (P=0.038) between baseline and midpoint. Additionally, Glutalytic™ consumption reduced food cravings (P=0.04).

Conclusion: Consumption of Glutalytic™ reduced deamidated gliadin IgA antibodies over time. During the first 14 days of consumption, gastrointestinal reflux and food cravings were significantly reduced.

Keywords: Non-celiac gluten sensitivity; Gluten intolerance; Gluten sensitivity; Gastrointestinal symptoms; Food cravings; Reflux; Deamidated gliadin; IgA; Clinical trial; Glutalytic

Abbreviations: NCGS: Non-celiac Gluten Sensitivity; CD: Celiac Disease; WA: Wheat Allergy; CMP: Comprehensive Metabolic Panel; CRP: C-reactive Protein; IgA: Immunoglobulin A; IgG: Immunoglobulin G.

Introduction

Non-celiac gluten sensitivity (NCGS) is a condition characterized by intestinal and extra-intestinal symptoms attributed to the ingestion of gluten in individuals who do not test positive for celiac disease (CD) or wheat allergy (WA) [1-3]. The prevalence of NCGS in the general population is still unclear as there are no validated diagnostic tests specific to this condition. The symptoms of NCGS overlap signs of CD and WA [1,3] while some symptoms and diagnostic criteria of irritable bowel syndrome (IBS) [1] further obscure the development of diagnostic testing. Current diagnostic tests for CD and WA do not apply to NCGS as it presents without autoantibodies, increased gut permeability or villous atrophy [4]. Although specific biomarkers for NCGS remain lacking; recent research shows that the immune activation of NCGS is caused by a distinctly different intestinal mucosal response [4] and that NCGS presents a different pro-inflammatory immune response [5]. Therefore, individuals who've experienced the immune response may search for ways to eliminate NCGS. Although some may have attempted to completely eradicate gluten from daily diets, cross-contamination of foods and compliance may prove a difficult task. A strong alternative, that compensates for the inevitable consumption of gluten, is oral supplementation with proteases.

Proteases are enzymes that catalyze hydrolysis of the peptide bonds that link amino acids together [6]. Proteases aid in the breakdown of substances including gluten and offer affected individuals an alternative approach to their dietary needs without extreme dietary restrictions. Lahdeaho et al. [6] developed a study to test the possibility of Glutenase ALV003 reducing gluten-induced small intestinal injury in patients with celiac disease. The study revealed that the use of "orally administered gluten-specific proteases was able to reduce gluten-induced mucosal injury in CD patients" [6].

The present study assessed the tolerance and efficacy of Glutalytic™, an enzyme supplement, in a double-blind, randomized, placebo-controlled fashion with daily intake of three capsules of Glutalytic™ or placebo with a meal for 30 days. Tolerance and efficacy were evaluated through a comprehensive metabolic panel, C-reactive protein levels (CRP-an inflammatory marker), a gliadin antibody profile (IgG, IgA, EIA), and a series of gastrointestinal symptom index questionnaires.

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Methods

Subjects

The purpose of this study was to compare the effects of Glutalytic™, a dietary supplement, versus placebo, on symptoms characteristic of gluten sensitivity [1,3]. This study was designed for healthy adults 18 years of age or older with or without self-reported gluten intolerance and/or sensitivity or undefined gastrointestinal problems. All participants were designated as low risk. Subjects with a known diagnosis of CD were excluded from the study. Participation was voluntary and subjects were able terminate their participation in the study at any time without penalty.

All participants were recruited through print advertising in the greater Atlanta area, and in La Crosse Wisconsin. Of an initial 53 participants who enrolled and completed screening, 37 completed the study. The participants included males and females ages 19 to 64 years (mean of 33.2 ± 12.2 years) (Table 1). Gluten knowledge was assessed at baseline and reported in this study (Table 1). Randomization of pill assignments, placebo and product groups were performed using the SPSS randomizer function.

Participants reviewed, signed, and completed the initial documents packet which consisted of the following forms: informed consent, authorization to use or disclose health information, consent to draw blood, health questionnaire, and a W9. A diagnostic baseline was achieved through the completion of a self-reported initial gastrointestinal symptom index questionnaire and an initial 12-hour fasting blood sample, which was taken and tested by a third party. After individual baselines were established, capsules were randomly assigned and distributed in person or by mail. If capsules were sent by mail, subjects verified the capsule type received (i.e. Product A or B).

Capsule consumption

Participants were instructed to consume the assigned capsule three times daily with a meal for 30 days. If a dose was missed, participants were instructed to report it to the researcher immediately. Recurring incidences of missed doses were taken into consideration for possible exclusion from the study. Participants were also instructed to maintain a dietary intake record throughout the duration of the study. The intermediate gastrointestinal index symptom questionnaire was completed 14 days after the first capsule was taken. If adverse reactions beyond any gluten intolerance and/or sensitivity symptoms common to the participant were experienced, instructions were given to report all questions, concerns, and symptoms to the researchers immediately and a medical professional would be consulted if necessary.

	Enzyme	Control	Total
Participants enrolled	4 (38.89)	22 (61.11)	36
Age in years*	30.43 ± 11.81 (19-52)	35.09 ± 12.49 (19-64)	33.28 ± 12.28 (19-64)
Gender *n* (%)			
Male	4 (11.11)	3 (8.33)	7 (19.4)
Female	10 (27.78)	19 (52.78)	29 (80.6)
Gluten-free diet compliance			
Excellent	0	0	--
Good	0	0	--
Fair	4 (11.11)	5 (13.89)	9 (25)
Very Poor	2 (5.56)	5 (13.89)	7 (19.44)
Does not follow GFD	8 (22.22)	12 (33.33)	20 (55.56)
	14 (38.89)	**22 (61.11)**	**36**

Data reported as n (%)
*Reported as mean, SD and (range)

Table 1: Participant characteristics and baseline assessment of gluten-free diet compliance.

Gastrointestinal symptoms questionnaire

Upon completion of capsule consumption, participants submitted dietary intake records and completed the final fasting blood sample taken and tested by the same third party that performed the initial sampling and testing. Gastrointestinal Symptom Index Questionnaires were modified from the Celiac Symptom Index Questionnaire [7]. Additional questionnaires were completed by each participant prior to or on day 15 and on the last day (day 30) of capsule consumption.

Biomarker collection

A comprehensive metabolic panel (CMP) was performed in order to ensure that Glutalytic™ was tolerated by the experimental population. Glucose was used to measure blood sugar levels, as it is the most direct way of determining a patient's likelihood of developing diabetes as a chronic disease. Biomarkers such as BUN, creatinine, and BUN/creatinine ratio were used to determine kidney function. Liver biomarkers (protein, albumin, globulin, albumin/globulin ratio, bilirubin, alkaline phosphatase, AST, and ALT) were used to determine the state of nutrition of each participant. Fluids and electrolytes were indicators of nerve and muscle activity as well as electrolyte balance. C-reactive protein (CRP) was followed as an inflammatory marker commonly elevated due to burns, trauma, infections, inflammation, arthritis, and certain cancers. Finally, a gliadin antibody panel was used to detect anti-gliadin antibodies. The gliadin antibody panel aids in the diagnosis of celiac disease, wheat allergy, and non-celiac gluten related disorders.

Statistical analysis

A Box's M test was used to analyze the homogeneity of covariance matrices and assumptions about the gathered data. Within-subjects contrast was used to calculate the significance of the error between the time points. In the case where the data assumptions above were supported, a general linear model (GLM), more specifically a MANOVA, was used to determine the products effect on time by test group. Significance was determined where $\alpha < 0.05$ for Wilk's Lambda, based on the industry standard as a supplement and not a medication intended to cure or treat disease. However, for additional analysis on C-reactive protein and Gliadin antibody panels, significance was also assessed where $\alpha < 0.01$ for Wilk's Lambda. The analysis was performed on SPSS Version 22.0 (IBM Corp., Armonk, NY).

Data transfer

For the purpose of this study, the first round of participants' data was manually transcribed and revised by senior staff. Subsequent volunteers were electronically added to the study via direct import from SurveyMonkey.com in a .csv file. Upon importation, data was pooled into a master data file for analysis.

Results

Blood analysis

Levels of the inflammatory marker CRP vary from day-to-day and by time of day in which the sample is provided. The results of the CMP are shown in Table 2, and the results for the Gliadin IgG/IgA antibody profile, and CRP analysis are shown in Tables

CMP	Reference Interval	Baseline ENZ	Baseline CON	Final ENZ	Final CON
Glucose, Serum (mg/dL)	65 - 99	90.60 ± 9.91	89.80 ± 6.78	89.60 ± 5.94	84.50 ± 7.15
BUN (mg/dL)	6 - 20	13.60 ± 4.04	14.00 ± 5.08	13.40 ± 6.31	14.50 ± 5.60
Creatinine, Serum (mg/dL)	0.57 - 1.00	0.92 ± 0.14	0.96 ± 0.26	0.90 ± 0.19	0.97 ± 0.30
eGFR (Non-African - American) (mL/min/1.73)	>59	97.8 ± 19.23	87.70 ± 21.46	100.80 ± 19.43	86.10 ± 22.76
eGFR (African American) (mL/min/1.73)	>59	113.00 ± 22.33	101.00 ± 24.65	116.20 ± 22.30	89.40 ± 37.67
BUN/Creatinine Ratio	8 - 20	14.60 ± 2.61	13.44 ± 3.29	14.60 ± 4.16	13.67 ± 4.42
Sodium, Serum (mmol/L)	134 - 144	139.80 ± 1.30	141.00 ± 2.11	139.80 ± 0.84	140.00 ± 2.67
Potassium, Serum (mmol/L)	3.5 - 5.2	4.04 ± 0.34	4.35 ± 0.47	4.06 ± 0.21	4.25 ± 0.30
Chloride, Serum (mmol/L)	97 - 108	101.20 ± 3.19	101.70 ± 2.11	101.20 ± 0.84	100.30 ± 2.31
Carbon Dioxide, Total (mmol/L)	18 - 29	23.80 ± 2.17	24.10 ± 1.79	23.00 ± 1.41	24.00 ± 1.63
Calcium, Serum (mg/dL)	8.7 - 10.2	9.14 ± 0.26	9.52 ± 0.40	9.18 ± 0.28	9.44 ± 0.28
Protein, Total, Serum (g/dL)	6.0 - 8.5	6.84 ± 0.36	6.75 ± 0.31	6.78 ± 0.36	6.81 ± 0.41
Albumin, Serum (g/dL)	3.5 - 5.5	4.36 ± 0.18	4.48 ± 0.30	4.34 ± 0.15	4.48 ± 0.15
Globulin, Total (g/dL)	1.5 - 4.5	2.48 ± 0.40	2.27 ± 0.24	2.44 ± 0.36	2.33 ± 0.29
A/G Ratio	1.1 - 2.5	1.78 ± 0.29	2.00 ± 0.32	1.78 ± 0.25	1.94 ± 0.30
Bilirubin, Total (mg/dL)	0.0 - 1.2	0.60 ± 0.35	0.48 ± 0.25	0.58 ± 0.30	0.50 ± 0.25
Alkaline Phosphatase, S (IU/L)	39 - 117	53.60 ± 18.19	68.80 ± 20.29	55.40 ± 17.54	59.10 ± 24.76
AST (SGOT) (IU/L)	0 - 40	18.80 ± 2.78	18.10 ± 4.90	20.40 ± 5.68	18.20 ± 3.58
ALT (SGPT) (IU/L)	0 - 32	15.20 ± 5.54	14.20 ± 3.04	19.20 ± 8.44	15.60 ± 4.81

Values are expressed as mean ± standard deviation of the mean.
†=individual results with an absolute z-score of above 3.29 is not reflected above and is subject to change with more participants finishing

Table 2: CMP: range reference and mean levels sampled at baseline and study conclusion.

Gliadin IgG/IgA	Reference interval	Baseline ENZ	Baseline CON	Final ENZ	Final CON
Deamidated Gliadin Abs, IgA		3.20 ± 1.30	5.00 ± 1.63	3.00 ± 1.22	5.30 ± 2.26
Negative	0 - 19				
Weak Positive	20 - 30				
Moderate to Strong Positive	> 30				
Deamidated Gliadin Abs, IgG		2.60 ± 1.34	3.00 ± 1.33	3.00 ± 1.00	2.90 ± 1.10
Negative	0 - 19				
Weak Positive	20 - 30				
Moderate to Strong Positive	> 30				

Table 3: Gliadin IgG/IgA antibody profiles sampled at baseline and study conclusion.

C-Reactive Protein, Quantitative (mg/L)	Reference Interval	Baseline ENZ	Baseline CON	Final ENZ	Final CON
	0.0 – 4.9	1.12 ± 1.29	5.27 ± 12.06	0.74 ± 0.59	1.84 ± 1.19

Table 4: CRP: Range reference and mean levels sampled at baseline and study conclusion.

3 and 4 respectively. With variability noted, the initial CRP levels were statistically indistinguishable between groups, as well as the difference between the final time points of the two CRP levels using an independent T-Test at a confidence level of 95% ($\alpha \leq 0.05$) as well as 99% ($\alpha \leq 0.01$). The deamidated gliadin IgA antibody, is rated high in both sensitivity and specificity, thus resulting in a high positive predictive value. Significant differences between the test groups over time was demonstrated with an independent T-test (P=0.024 $\alpha \leq 0.05$) (Figure 1). Furthermore, when using an independent T-Test at ($\alpha \leq 0.01$), there was a near significant difference between the mean IgA of Enzyme group vs placebo group at the study conclusion (P=0.018) but a difference in average IgA was also evident at baseline (P=0.021) however, slightly less significant. The change in time remained as shown in Figure 2 for $\alpha \leq 0.01$. As anticipated, all other biomarkers had insignificant changes as a result of both time and test group.

Gastrointestinal symptom index questionnaire

The most significant change relates to the amount of gastrointestinal reflux experienced by participants between the baseline and midpoint of the study. The Glutalytic group experienced a significant drop in mean GI symptom scores from 1.64 to 1.14 (P=0.038), while the placebo group's score only experienced a slight reduction from 1.36 to 1.27. Additionally, there was a significant effect of Glutalytic on cravings (P=0.04). There was a nearly significant effect on bloating with subjects consuming Glutalytic (P=0.065). All tests were analyzed using an independent T-Test ($\alpha \leq 0.05$) (Figure 3).

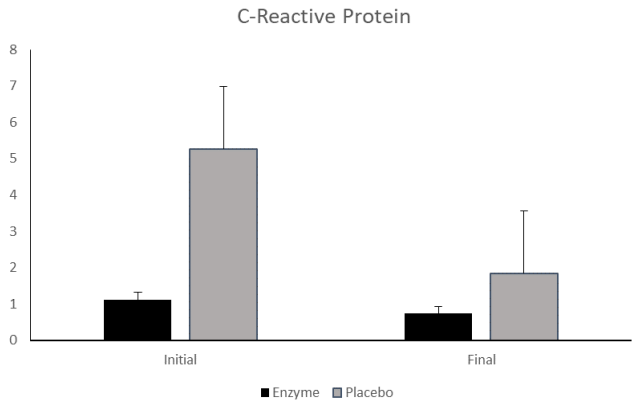


Figure 1: C-reactive protein averages for the enzyme group and placebo group from the baseline sample measures and the final sample collection measures.

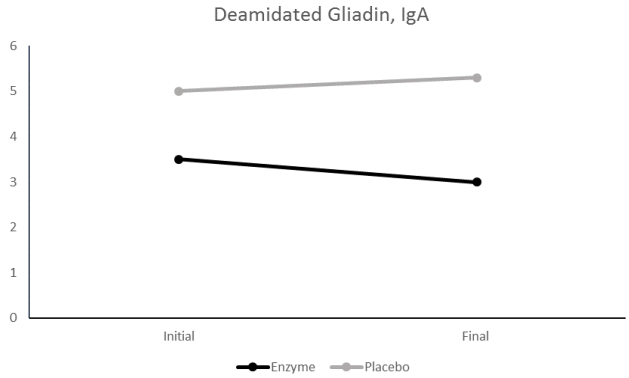


Figure 2: Deamidated gliadin, IgA average change from baseline to final sample measures.

Discussion

Dependable diagnostic tests for NCGS are unavailable and NCGS specific biomarkers remain insufficient for reliable diagnosis. Diagnosis therefore requires first excluding CD and WA, followed by a gluten-free-diet regimen, and finally a gluten challenge to verify gluten dependent symptoms. Many individuals self-diagnose NCGS without seeking medical consultation and without following strict regimen to determine gluten symptom dependence [1,5]. The development of diagnostic tests and treatments are made even more difficult by the genetic variability in gluten grain proteins and in the human mucosal immune systems [2,8]. Gluten grain species variability is owed in part to genotype determined protein structure and changes in technological processes involved in food production [8]. Additionally, research on mucosal immune gene expression shows that CD and NCGS pathogenesis are mediated differently [8]. Pathogenesis in CD is mediated dually by innate and adaptive immune systems, while NCGS is primarily an innate immune response [8].

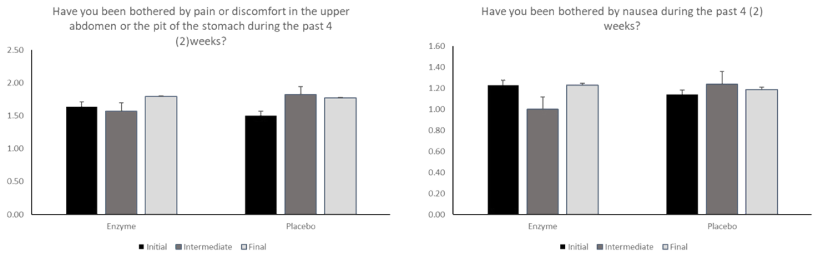
Many individuals on the gluten sensitivity spectrum are faced with the choice between a GFD and a strict dietary regimen which often lead to increased food costs or losses in personal health and quality of life. Proteolytic enzymes, via oral supplements, may offer a cost-effective alternative to reduce or alleviate symptoms in gluten sensitive individuals. A randomized, double-blind, placebo-controlled clinical trial in 2014 demonstrated the effectiveness of Glutenase ALV003 in reducing gluten-induced mucosal injury [6]. The *Aspergillus niger*-derived prolyl endoprotease (AN-PEP) in a double-blind, placebo-controlled study from 2015 was shown to degrade almost all ingested gluten in the stomach within a one hour period. Bacterial and fungal proteases have also been shown to reduce gluten concentrations and gluten-related inflammation [9,10]. While the results from these studies are promising, more investigations are warranted to adequately define NCGS and identify reliable biomarkers for testing and diagnosis.

Conclusion

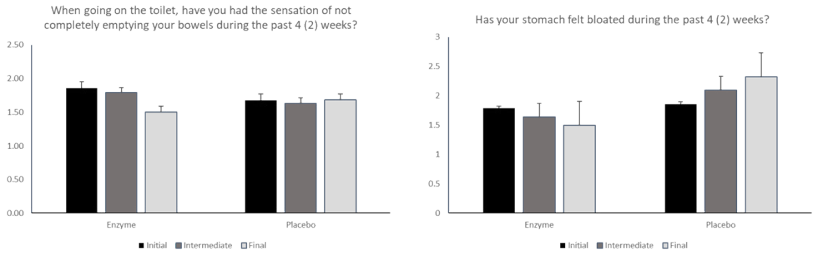
In the present study, Glutalytic™ consumption was associated with a decrease in deamidated gliadin IgA antibody over time from baseline to conclusion and with significant differences between the Glutalytic™ group and placebo group at study conclusion. Consumption of Glutalytic™ also reduced reports of gastrointestinal reflux and food cravings from the baseline to midpoint of the study as well as the attenuation of bloating symptoms.

Limitations

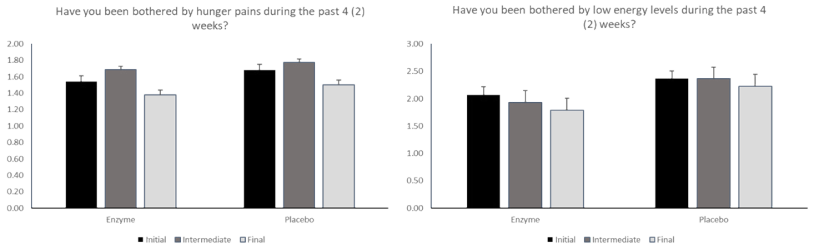
Limitations in this study involve the complicated nature of dependable diagnostics and specific biomarkers for reliable diagnosis. NCGS is often self-diagnosed with no medical consultation and do not follow a strict regimen regarding their gluten sensitivity. Diagnostic tests are also difficult because of the genetic variability in gluten grain


Have you been bothered by pain or discomfort in the upper abdomen or the pit of the stomach during the past 4 (2)weeks?
Enzyme
Placebo
Initial
Intermediate
Final

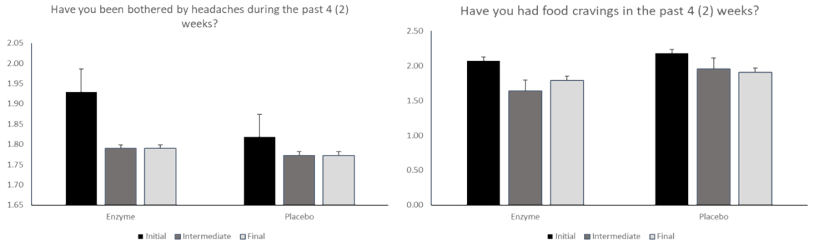
Have you been bothered by nausea during the past 4 (2) weeks?
Enzyme
Placebo
Initial
Intermediate
Final


When going on the toilet, have you had the sensation of not completely emptying your bowels during the past 4 (2) weeks?
Enzyme
Placebo
Initial
Intermediate
Final

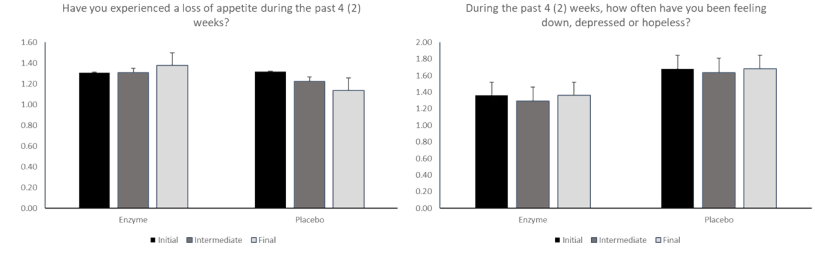
Has your stomach felt bloated during the past 4 (2) weeks?
Enzyme
Placebo
Initial
Intermediate
Final


Have you been bothered by hunger pains during the past 4 (2) weeks?
Enzyme
Placebo
Initial
Intermediate
Final

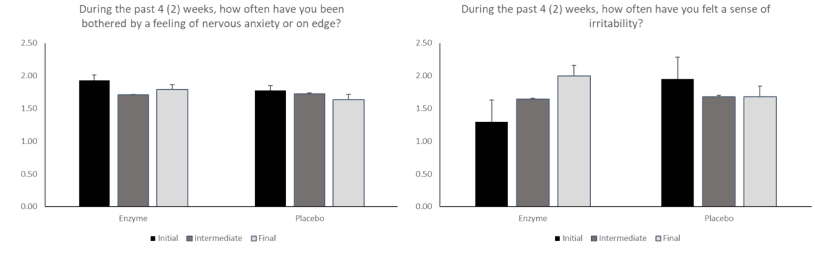
Have you been bothered by low energy levels during the past 4 (2) weeks?
Enzyme
Placebo
Initial
Intermediate
Final


Have you been bothered by headaches during the past 4 (2) weeks?
Enzyme
Placebo
Initial
Intermediate
Final

Have you had food cravings in the past 4 (2) weeks?
Enzyme
Placebo
Initial
Intermediate
Final


Have you experienced a loss of appetite during the past 4 (2) weeks?
Enzyme
Placebo
Initial
Intermediate
Final

During the past 4 (2) weeks, how often have you been feeling down, depressed or hopeless?
Enzyme
Placebo
Initial
Intermediate
Final


During the past 4 (2) weeks, how often have you been bothered by a feeling of nervous anxiety or on edge?
Enzyme
Placebo
Initial
Intermediate
Final

During the past 4 (2) weeks, how often have you felt a sense of irritability?
Enzyme
Placebo
Initial
Intermediate
Final


During the past 4 (2) weeks, how frequently have you had concentrating on things, such as reading the newspaper or watching TV?

During the past 4 (2) weeks, how frequently have you been bothered with skin problems, such as acne, rashes, or itchiness?

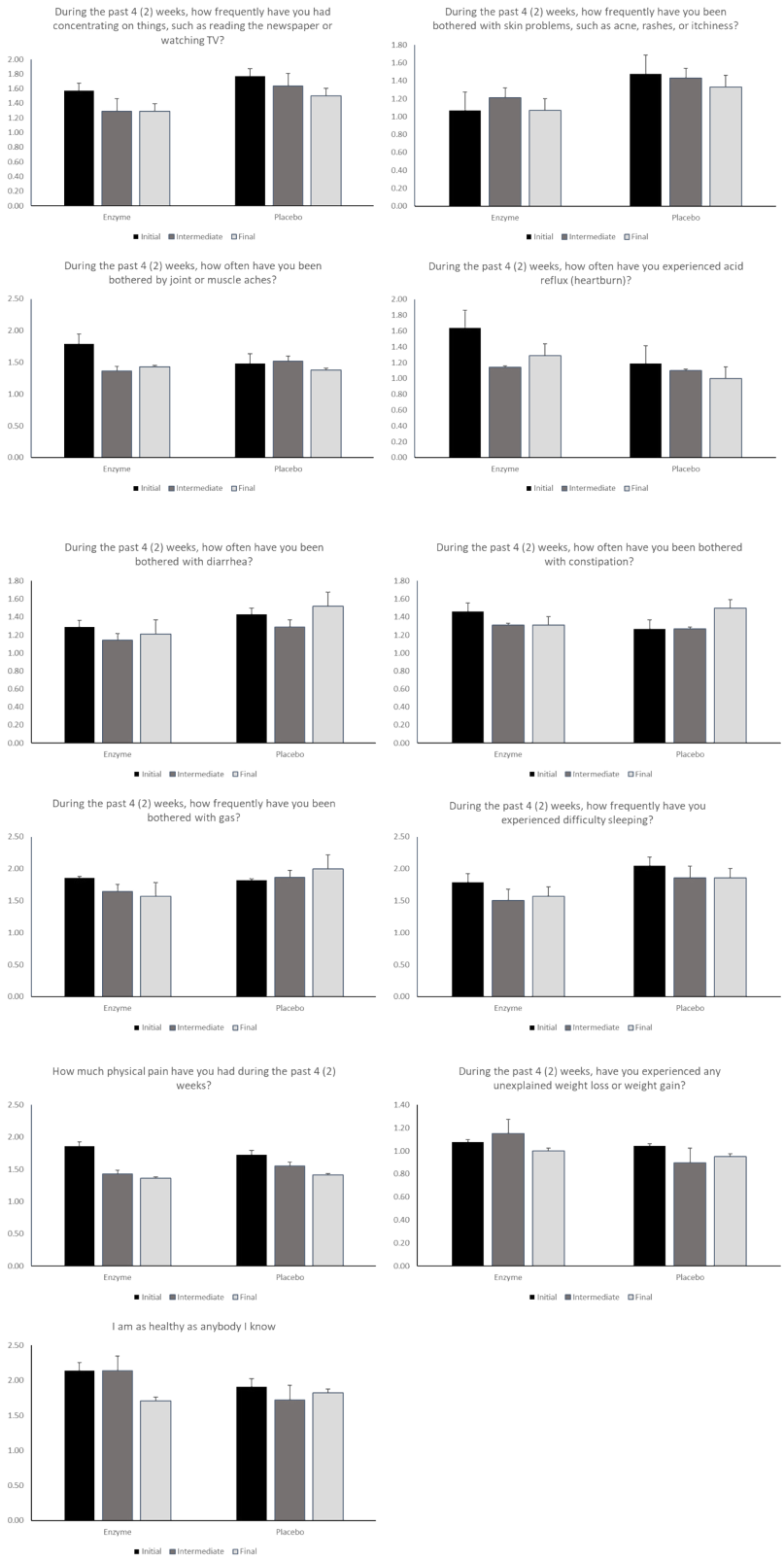


Figure 3: Gastrointestinal symptom questionnaire mean scores from the baseline, midpoint and final questionnaires for the enzyme and control groups.

proteins and the human mucosal immune system. Furthermore, careful interpretation of confidence intervals must be considered due to the small sample size of the study.

Future Studies

Recommendations for future studies involve recruiting a larger group of participants in order to gain additional statistical significance and power analysis. A larger subject pool would give more insight as to the physiological reaction to NCGS. Future studies are also warranted to assess and substantiate the clinical efficacy of trials supplementing proteases for gluten sensitivity.

Authors' Contributions

JD conceived the study, collaborated in its design and also participated in subject recruitment and drafted the manuscript. JS carried out the statistical analysis. AC helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgement

We would like to acknowledge WalkinLabs for collecting and analyzing all blood draws.

Disclosure Statement

In accordance with Taylor & Francis policy and my ethical obligation as a researcher, I am reporting that I have received funding from Deerland Enzymes and Deerland Probiotics a company that may be affected by the research reported in the enclosed paper. I have disclosed those interests fully to Taylor & Francis, and I have in place an approved plan for managing any potential conflicts arising from my employment.

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