

Author's response to reviews

Title: Supplementation of the diet with the functional fiber PolyGlycoplex(R) is well tolerated by healthy subjects in a clinical trial

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Version: 2 **Date:** 10 December 2008

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REPLY TO REVIEWERS COMMENTS

The authors greatly appreciate the reviewers' time and the comments/suggestions made. Each comment was carefully considered and addressed in the article, and is also found in this cover letter, as specific answers. The tables that were generated based on the reviewers' comments could not be uploaded in table format, reason why they may appear confusing.

Respectfully,

Ioana Carabin

Comments from Reviewer #1

1. The reasons for exclusion of some subjects need to be stated specifically. The general inclusion/exclusion criteria are clear, but the reasons for researcher-led exclusions should be summarised.

A new table (Table 1) was included to describe the reasons for exclusion. The table format could not be used in this upload

Table 1. Exclusion criteria

Abnormal physical examination

History of GI disease (i.e., gastric ulcers, irritable bowel disease, history of bowel obstruction and colon cancer)

History of abdominal surgery (exception: appendectomy)

Pregnant or breast feeding women

BMI > 30 kg/m²

Metabolic disorders (i.e., diabetes, and metabolic syndrome)

Using prescription medications, H₂ blockers*, anti-acids, OTC**, dietary & herbal supplements

Involved in a weight loss program

Being treated for eating disorders

Having participated in another clinical trial in the previous month

Having received general anesthesia in the previous month

Refusing to consume the foods provided in the study

Known allergies to milk, nuts, wheat, soy, oat, and barley

Refusal to sign consent

*H₂ blockers, also known as histamine-2 receptor antagonists, are drugs that prevent or block the production of gastric acid.

**OTC – over-the-counter

2. Biochemical parameters: the authors have used an impressive array of biochemical measures and these are listed in the relevant section, but little or no detail on the methods used to assay is given. This could be stated in a table or as supplementary online information, but must be given somewhere and in some form.

The following information was included under the “Study Parameters” section: “Biochemical analyses were performed on the multi-parametric automated system Roche/HITACHI 912, Roche Diagnostics, with a bi-directional connection. All parameters were analysed using commercially available kits. No adaptation of the commercial methods was made. Methods of analysis are found in Appendix I.” The table format could not be used in this upload

APPENDIX I

Assay methods employed for biochemical, hematological and urine analysis

BIOCHEMISTRY

Conducted with ROCHE/HITACHI 912, Roche Diagnostics, Inc.

Bun/Urea UV kinetic test.

Creatinine JAFFE method with compensation. Kinetic colorimetric test.

Total protein BIURET method. Colorimetric test.

Total bilirubin JENDRASSIK GROF method. Colorimetric method.

Glucose Glucose GOD-PAP method. Enzymatic colorimetric method.
Total cholesterol Enzymatic colorimetric method.
HDL cholesterol Enzymatic colorimetric test in homogenous phase.
LDL cholesterol Enzymatic colorimetric test in homogenous phase.
Triglycerides Enzymatic colorimetric TRINDER modified final point method.
Alkaline phosphatase Enzymatic kinetic method
Alanine amino transferase. ALAT/GPT/TGP/ALT IFCC method. Enzymatic kinetic.
Aspartate amino transferase. AST/ASAT/GOT/TGO IFCC method. Enzymatic kinetic.
GGT SZASZ method (kinetic photometric method). Colorimetric method
LDH "Optimised method" according to the Germany Society of Clinical Chemistry.
CPK "Optimised method" according to the IFCC.
Sodium, potassium, chloride Indirect potentiometric analysis
Calcium Colorimetric test. Final point method with reagent control test
Uric acid Enzymatic colorimetric method
Albumin Immunoturbidimetric analysis
Magnesium Colorimetric test using a final point method for the quantitative determination of magnesium in serum or plasma
Ferritin Immunoenzymological microparticulate MEIA technique

HEMATOLOGY

Conducted with HORIBA ABX PENTRA 120 DX. Horiba Medical Diagnostics, Ltd.

The results for RBC, WBC, platelet, basophil counts, and the level of hemoglobin and hematocrit were measured.

The results for MCV, MCH, MCHC were obtained through calculations using computer software.

The differentiation of the blood elements is based on physical (light diffraction), and chemical principles (affinity for a colour) which are different for each population.

TOXICOLOGY/SEROLOGY/HORMONES

Conducted on AxSYM® ABBOTT DIAGNOSTICS

Zinc (Pasteur Cerba Laboratory) Atomic Absorption Spectrometry technique

Vitamin A (Pasteur Cerba Laboratory) HPLC technique

Vitamin B1 (Pasteur Cerba Laboratory) HPLC technique

Vitamin B6 (Pasteur Cerba Laboratory) HPLC technique

Vitamin B12 (Pasteur Cerba Laboratory) Chemiluminescence technique
Vitamin C (Pasteur Cerba Laboratory) HPLC technique
Vitamin D (1.25 OH) (Pasteur Cerba Laboratory) Radioimmunological technique
Vitamin E (Pasteur Cerba Laboratory) HPLC technique
Vitamin K (Pasteur Cerba Laboratory) HPLC technique

TSH

- Conducted on BAYER CENTAUR MACHINE - Immunoenzymological ELISA third generation technique

URINE ANALYSIS

Performed with COMBUR-Test® strips, ROCHE DIAGNOSTICS

Tested urinary pH, glucose, protein, blood & ketone bodies

The functioning of the PENTRA 120 DX, HITACHI 912 ROCHE DIAGNOSTICS and AXSYM ABBOTT conform to the norms of the ISO 9002 certification.

HPLC – high performance liquid chromatography

IFCC – International Federation of Clinical Chemistry

ELISA - enzyme-linked immunosorbent assay

HORIBA ABX Diagnostics is a division of HORIBA Ltd., Kyoto, Japan

Roche Diagnostics Combur-Test® strips, in the USA and Canada are known as Chemstrips

3. The placebo group received skim milk. Why? The justification as a placebo should be detailed.

(See below)

4. Placebo: A statement implying orosensory match would appear to back the claim that this study is double-blind, are there data to support this?

The answer to #3 and #4: The control product selected for use in the study, based on color and (texture similarity) orosensory match to the test product was a skimmed milk

powder taken as 2.5 g BID for one week and then 5 g BID for the remainder of the study.

5. The effect of the intervention on dietary intakes is not measured and is a severe limitation of the study. The conclusions about status of various nutrients needs to be linked to intake measures. These have not been made, so this limitation should be clearly stated.

The following paragraph was included at the end of the “Discussion” section. “The effects of the intervention on dietary intakes were not measured in this

clinical trial, because the focus of the study was on assessing GI tolerance. However, this intentional omission could be interpreted as a limitation of the study, which the authors plan in addressing in future clinical trials.”

6. Diet: how was the 10g/day fibre target defined.

The authors have addressed this issue in “3.5 Selection of doses for the study”. That is, the dose was selected based on literature values for similar carbohydrates.

“The tolerance of PGX was tested compared to the literature values established for other similar carbohydrates (e.g., fiber). Therefore, it was decided to evaluate the tolerance to ingestion of PGX for up to 10 g per day.”

7. Compliance - how was compliance measured, what was it scored at?

“The following formula was used to determine compliance:

$\% \text{ compliance} = \text{number of products taken} / \text{theoretical number of products to be taken} \times 100$

Compliance with the instruction provided and intake of the test and control products was assessed throughout the study. The mean average compliance at V2 and V3 (%) for the control group was 100% and for the test group was 99.6%”

8. Stool collection: SCFA levels are highly labile - the time from deposition at the study centre to analysis should be stated clearly.

The time from collection of the stool samples to analysis was generally 24 hours and never exceeded 36 hours.

9. Claims that PGX is fermentable (in the abstract) are not supported by this study which shows no alteration in faecal SCFA. This should be discussed.

The term “fermentable” was removed from the abstract. In the “Discussion” section the following information was included:

“Although the authors believe that PGX is a fermentable fiber, the stool analysis for SCFA showed no differences between test and control groups. SCFA are highly labile and it is possible that the amount of time that lapsed between sample collection to the time of analysis was too long, possibly affecting the overall results.”

Comments from Reviewer #2

Title – suggest revision in the title – “Supplementation of a functional fiber (Polyglycoplex) to the diet is well tolerated by healthy subjects”. A possible sub-title might be - “Failure to affect blood lipids, GGTP, uric acid or vitamin D or C”

Based on the reviewer’s suggestion, the title was changed to: “Supplementation of the diet with the functional fiber PolyGlycoplex is well tolerated by healthy

subjects in a clinical study.”

Abstract: The abstract is fragmented by the presence of various sub-headers.
Delete?

Sub-headings are required by the journal.

Background (p. 4; 4th paragraph) Suggest a change in sentence. “In soon to be published”..(delete “In pre-clinical testing”).

The intention of the authors was to emphasize the fact that PGX showed no toxicity in pre-clinical testing and that no patients were put at risk. The authors feel it is necessary to include a reference to that fact.

Methods (p. 6): Inclusion/exclusion criteria. This section could be condensed by omitting those “excluded”. There is somewhat of a contradiction (see page 6) about “taking prescription medications”

The authors feel that the criteria for inclusion and exclusion from the study should be fairly detailed as to respond to pre-empt questions regarding the effect of one or another action by the test subjects on the outcome of the study.

The authors agree and the contradictory sentence was deleted.

Difficulty distinguishing between “GI tolerance and “GI signs and symptoms”

These following two sentences were added to the end of this section to clarify for the reader the reason for utilizing the separate terms throughout the article.
“Signs and symptoms were recorded through out the study and their occurrences analyzed statistically. The frequency and intensity of signs and symptoms determined the threshold of GI tolerance.”

The authors need to clarify/justify why vitamin C & D were measured

Vitamin D was measured because PGX demonstrates specific physical properties including high viscosity and solubility and, there was concern for possible sequestration of fat soluble vitamins and therefore vitamin malabsorption. Consequently, vitamin A, D, E and K levels were monitored during the study. Vitamin C and B levels were followed as an assessment of the potential of PGX to result in generalized malabsorption.