MANITOBA Pulse Soybean GROWERS

P.O. Box 1760, Carman, MB
Canada R0G 0J0
T 204.745.6488
F 204.745.6213
@MbPulseGrowers
www.manitobapulse.ca

MPSG FINAL EXTENSION REPORT

PROJECT TITLE: **Mixed Beans – Characterization of Bioactive Compound Absorption and Excretion, and Relationship to Improvements in Cardiovascular Function**

PROJECT START DATE: **1 April 2014** **PROJECT END DATE:** **31 March 2016**

DATE SUBMITTED: **30 September 2016**

PART 1: PRINCIPAL RESEARCHER

PRINCIPAL

NAME:	Peter Zahradka
POSITION:	scientist
INSTITUTION:	CCARM, St. Boniface Hospital
EMAIL:	peterz@sbrc.ca
PHONE:	204-235-3507

PART 2: EXECUTIVE SUMMARY

Outline the project objectives, a summary of the activities and results, and their relevancy to pulse and soybean farmers.

Based on data obtained in a clinical study that showed consumption of 1/2 cup mixed pulses every day could improve blood flow to the legs of persons with peripheral artery disease in only 8 weeks, we hypothesized that compounds absorbed from the pulses contained in the study foods are responsible for the benefits that were obtained. Since we do not have any understanding of how fast these compounds are absorbed, nor how fast they are metabolized, we proposed an acute study in which healthy individuals would eat separately 4 types of beans (navy, red kidney, black, pinto) and to collect blood and urine samples over a 6 hour period for metabolomic analysis. This study showed that eating red kidney beans prodcuced a specific benefit, namely lowered of the tension of the blood vessel wall. Additionally, we completed a metabolomic analysis of the samples obtained during our chronic mixed bean study, where persons with peripheral artery disease ate a mixture of red kidney, navy, black and pinto beans for 8 weeks.This metabolomic analysis evealed a small number of endogenous metabolites were altered in the blood (15 of 20,805 detected) and urine (9 of 10,487 detected) of those eating the bean diet, and only 21 compounds of 1,781 detected in beans were absorbed into the blood. The ability of red kidney beans to cause vasodilation in healthy persons should help to increase the demand for these beans by persons of all ages and health status. We anticipate more information will become available as we sift the extensive data produced by these studies.

PART 3: EXPERIMENT DESCRIPTION & RESULTS

Concisely describe the experimental methods and results to date. You may include up to 3 graphs/tables/pictures in the Appendix.

Objective 1 - Acute study involving consumption of individual bean types.
1. Approval to conduct the study was obtained from Health Canada, the Research Ethics Board (University of Manitoba) and the Research Review Committee (St. Boniface Hospital)
2. The foods articles were developed through a series of taste tests to idnetify a flavouring that would not add significant number of extraneous compounds to the test, and would still make the beans palatable to the participants. All food items were flavouredidentically. The items were then prepared in sufficient volume for the entire study and frozen in individual sizes equivalent to the serving size recommended in Canada's Food Guide.
3. Healthy participants were recruited through a series of advertisements. Their medical history was taken.
4. Study visits were arranged. Participants were expected to come 1 week apart for a period of 6 weeks. A different food item was provided each week.
5. Blood samples were taken from the participants before they ate the food item, and then at 30, 60, 120, 240 and 360 min; urine samples were taken before eating the food item and then at 120 and 360 min.
6. Measurements of vascular function were taken before the study food was eaten and at 120 and 360 min.
7. The blood and urine samples were prepared for metabolmics analysis.
8. The clinical data were analyzed. The data indicate that red kidney beans reduce the pulse wave reflectance magnitude at 2 and 6 hours after consumption relative to the rice control. The black beans also reduced this value, but it did not reach statistical significance. See the Graph provided in the Appendix. These results indicate that the blood vessel wall tension was lower, which ocntrasts with theincrease typically seen after eating foods that contain saturated fat.

Objective 2 - Metabolomic analysis of samples from Mixed Bean Study
1. The blood and urine samples were thawed, extracted and analyzed by mass spectrometry. In total, 53 samples of blood and 53 samples of urine were prepared. The 53 samples represented three groups, one which ate the rice control foods, one that ate 0.3 cups of an equal mixture of red kidney, black, navy and pinto beans for the duration of the study, and one that ate 0.6 cups of the bean mixture. Each of the foods was consumed 5 times per week.
2. The data were analyzed to identify correlations between the beans eaten in the diet and the clinical outcomes.
3. The bean diet caused changes in 9 urinary metabolites and 15 plasma metabolites. These results indicate including beans in our diet can change the metabolic profile in our blood which likely explains the health benefits derived from eating more beans. However, further understanding of what these metabolites do in our bodies is necessary before it is possible to conclude how they contribute to the actions of beans.
4. Twenty-one compounds present in beans were detected in the blood of persons who ate the mixed bean diet. See Table included in the Appendix. It is expected some of these compounds may be responsible for the health benefits ascribed to beans. As well, some of these compounds may be useful to monitor bean consumption by Canadians. Additional studies will be required before a complete understanding of what these compounds do in the human body.

Objective 3 - Correlation of metabolites present in the samples obtained in Objective 1 with the clinical data collected
1. This objective has not been completed since the samples prepared for analysis have not been run on the instrument. The queue for analysis over the summer has pushed our access back until October or November.

PART 4: RELEVANCE TO FARMERS AND FUTURE RESEARCH

Describe how the project results can be captured to benefit pulse and soybean farmers (production recommendations, innovation items, marketing plans, commercialization of technology etc). Identify any future research opportunities.

The study provides evidence regarding the components in beans which are effective for improving cardiovascular function. Measuring the bioactive compounds in the bean seeds and relating their levels in blood and urine to the improvements in various study parameters will help to verify whether these compounds play a role in the beneficial actions of beans. Being an acute study, we will obtain information on the similarities and differences in bioactive compounds in the four types of beans. This in turn will enable crop breeders to select for cultivars that manufacture higher levels of these compounds.

Identifying the bioactive compounds in beans associated with improved cardiovascular function will allow for the formulation of food products with a standardized dose of bioactive compounds.

The information we have obtained will contribute to evidence for dietary recommendations for whole pulses, specifically beans, in relation to cardiovascular health. This will support marketing strategies to increase awareness that a pulse-rich diet can improve human health and in turn promote greater pulse consumption locally (Manitobans, Canadians) and internationally (export markets).

We anticipate that a future study that examines the processing fractions of red kidney beans will help determine what components of the seed can be used in the preparation of a functional food or a natural health product through concentration of the compound that is responsible for the observed health benefit.

PART 5: COMMUNICATION

List extension meetings, papers produced, conference presentations made, project materials developed.

An abstract of our results has been submitted to the 10th Canadian Puls eResearch Workshop and has been selected for an oral presentation: Red Kidney Beans and Lentils Induce Acute Vasorelaxation in Healthy Adults; J.L. Clark, A. Wilson, D. Perera, C.G. Taylor, P.Zahradka

Additional abstracts are expected based on the data we have obtained and that is described in the MSc thesis of Le Wang (defended Aug 16, 2016, Dept Human Nutritional Sciences).

A total of three manuscripts are planned for these data.

We anticipate having the results of these studies described on the Health Report, CJOB Radio, 11 am Sundays (September-June).

APPENDIX

Include up to 1 page of tables, graphs, pictures.

1,2-Dimethoxy-4-[2-(2-propenyloxy)ethenyl]-benzene
16-Oxo-palmitate
2-Dodecylbenzenesulfonic acid
3-(8,11,14-Pentadecatrienyl)phenol
4Z,7Z,10Z-octadecatrienenitrile
9-(beta-D-Ribofuranosyl)zeatin
Absinthin
cis-1,2-Diphenylcyclobutane
Dimethyl trisulfide
Hexadecyl Acetyl Glycerol
3α,7α-Dihydroxy-5β-cholestanate
5-Pentadecylresorcinol
7-beta-D-Glucopyranosyloxybutylidenephthalide
Armillaramide
Avermectin A2a monosaccharide
Palmitic acid
Erinacine D
Etamiphylline
Garcinia lactone dibutyl ester
Palmitic amide
Stearic acid