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MPSG FINAL EXTENSION REPORT

PROJECT TITLE: Cardiovascular Health Benefits of Soybean Crops

PROJECT START DATE: 30 May 2016

PROJECT END DATE: 31 March 2018

DATE SUBMITTED: 31 March 2018

PART 1: PRINCIPAL RESEARCHER

PRINCIPAL

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PART 2: EXECUTIVE SUMMARY

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

We have completed a series of experiments using the spontaneously hypertensive rat (SHR) that showed consumption of whole pulses (30% w/w) caused a significant reduction in blood pressure, altered blood vessel morphology and decreased serum cholesterol levels. Although prior human studies had used isoflavones isolated from soybeans, and these studies showed arterial stiffness was reduced, further examination to determine whether whole soybeans might be similarly effective, as we have seen with whole pulses, have not been done.

Consequently, our objective was to examine the effects of dietary soybean consumption on blood pressure, arterial stiffness, serum cholesterol and antioxidant/inflammatory status in the spontaneously hypertensive rat model.

Based on the results of this study, it is possible to conclude that the soybean diet can significantly reduce the levels of the main serum lipids without altering body weight or serum glucose. Markers of inflammation or antioxidant status were also unchanged by the soybean diet. With respect to the functioning of the blood vessels, the diet did not affect blood pressure or blood flow rates, however, there was a trend to lower pulsatility index, suggesting the diet enhanced the elasticity of the blood vessels, even in the absence of a change in vessel morphology. Overall, the study has shown that consuming soybeans has both metabolic and vascular benefits. The information that eating whole soybeans can improve overall cardiovascular health should benefit farmers marketing their products in North America, thus leading to increased demand as well as indicating that production of novel foods containing soybeans would be favourable.

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.

METHODS

- Soybeans (AC Springfield) grown in Manitoba in 2017, were cooked by boiling, freeze-dried, and milled (powdered) for incorporation into semi-purified rodent diet. The soybean diet was formulated to contain 30% w/w cooked and freeze-dried soybeans
- Samples of whole uncooked soybeans, and the cooked, freeze-dried, milled soybeans were sent for proximate analysis, which was needed to formulate the diet (ie. isocaloric and isonitrogenous).
- The hypertensive (SHR) and normotensive (Wistar) rats (Charles River Laboratories) were randomized to the soybean diet (n=8) or control diet (n=8) while the Wistar rats received the control diet (n=8) for 8 weeks.
- Body weights were obtained weekly throughout the study.
- Blood pressure and arterial stiffness was assessed non-invasively at baseline and 8 weeks.
- Rats were fasted 12 hours before collecting of blood and aorta samples. The mid-section of the aorta was used to prepare frozen sections using OCT as the embedding media, which were stained using an Elastin Stain kit (Sigma Aldrich) to differentiate between elastin (black), muscle (yellow) and collagen (red). Medial thickness was measured.
- Serum was analyzed using a Cobas C111 auto-analyzer (Roche Diagnostics GmbH) for lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides) and glucose.
- Antioxidant and inflammatory status were assessed using serum and commercial kits for OxiSelect In Vitro ROS/RNS Assay kit (Cell Biolabs), OxiSelect Total Antioxidant Capacity (TAC)(Cell Biolabs) and soluble tumor necrosis factor soluble receptor II Cusabio).
- For statistical analysis, time course data and end-point data were analyzed by repeated measures ANOVA and one-way ANOVA, respectively, followed by post-hoc means testing with Duncan's multiple range test. Data were log-transformed if not normal and homogeneous. Outliers ($\pm 3 \times$ standard deviation) were removed before analysis. $P < 0.05$ was accepted as significant.

RESULTS

- The soybean diet did not affect body weight gain over the 8 weeks of the study.
- No differences were observed in the primary determinants of arterial stiffness (peak and minimum flow velocities) between groups. There was a trend towards lower pulsatility index (PI) in the soybean fed group, indicating a decline in shear stress on the arterial wall and less arterial stiffness.
- The blood pressure of the SHR group was significantly higher than the normotensive Wistar control group; however, there were no differences in blood pressure between the soybean fed and the pulse-free SHR control groups.
- The soybean fed group had lower fasting serum levels of all lipids measured (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides) relative to the control SHR group. Interestingly, the SHR had lower levels of all lipids relative to the normotensive control Wistar group, suggesting there are metabolic differences between these animals in addition to the blood pressure.
- There were no differences in fasting serum glucose levels among groups.
- No differences were observed in reactive oxygen species (ROS) levels among groups. Total serum antioxidant activity was equivalent for all groups; no changes were observed as a result of the intervention.
- No differences were observed in soluble tumor necrosis factor receptor II levels between groups suggesting Inflammation status was the same for all groups.
- There were no differences between the SHR control and SHR soybean-fed groups for aorta media thickness.



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.

This is the first and only study to date to examine the potential effect of whole soybeans on cardiovascular health. A significant take-home message is the fact that soybeans were able to reduce serum lipid levels, both cholesterol and triglycerides, thus providing a strong indication that eating soybeans is beneficial. Additionally, our study has unique data showing soybean consumption can positively influence the properties of blood vessels. Specifically, we observed greater elasticity in the blood vessels of rats fed the soybean diet. These data imply that soybeans provide additional health benefits beyond those expected with lipid lowering. This sets the stage for further studies in animal models and humans to examine each aspect in greater detail, particularly in relation to blood vessel elasticity.

Since soybeans are not typically included in the North American and European diets, there is a market for developing food products beyond tofu and edamame. The development of food products also needs to consider the processing methods that could be applied to soybeans to provide health benefits that are equivalent to or enhanced compared to whole soybeans.

It is noteworthy that the positive results achieved in the present study were obtained using a soybean variety developed for Canadian farmers and grown in Manitoba. We do not know whether the benefits can be generalized to all soybean varieties or are specific to AC Springfield. However, we would like to believe that these beneficial properties are a product of the specific variety and the unique environmental conditions found in Manitoba which would further boost demand for product from Manitoba soybean growers. Further research would be needed to establish the importance of the genotype and environment interaction.

PART 5: COMMUNICATION

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

Since the project was completed just prior to the deadline, there has been no opportunity to communicate the study results via a conference or publication at this time. We have published a review paper in a top class nutrition journal (Nutrients) which describes the benefits associated with soybean consumption on diabetes and associated complications. We anticipate publishing at least one scientific article in the next year.



APPENDIX

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

