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MPSG FINAL EXTENSION REPORT

PROJECT TITLE: Effects of black and navy beans on blood vessel function and remodelling

PROJECT START DATE: 1 September 2015 **PROJECT END DATE: 1 October 2016**

DATE SUBMITTED: 30 September 2016

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.

The intent of this study was to determine which bean type (black or navy) has the greatest potency for positively modifying blood vessel function and remodeling, blood pressure and serum cholesterol levels in the spontaneously hypertensive rat (SHR) which has elevated blood pressure and stiff arteries relative to the normotensive control WKY rats. Once ethics approval was obtained and the materials for the study sourced, the diets were prepared and the animals were ordered. Once they arrived, they were randomly placed on a black bean, navy bean or control (no bean) diet, which they consumed for 8 weeks. Measurements of blood pressure, arterial stiffness and arterial morphology were the primary outcomes, with fasting serum cholesterol as a secondary objective. The study then continued for another 4 weeks - all animals were placed on the control diet; this added period was to determine how quickly the parameters returned to their normal values once the beans were no longer being consuned. Overall, the diets containing beans did not cause many changes in the animals. However, the morphology of the blood vessels was affected by the black bean diet but not the navy bean diet. Specifically, the black bean diet reduced the size of the blood vessel, and this is a positive finding given that the vessel size increases when stressed by conditions such as high blood pressure. Our expectation is that these positive results will promote local (Canadian, US) demand for Manitoba beans due to their beneficial effects on health. The intent is to overcome the low interest by Canadians to eat beans and in this way help to increase both acreage grown and the value of the crop.

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.

Part A - Effect of beans on SHR over 8 weeks.
Animals: Fifteen week old SHR (hypertensive) and WKY (normotensive) rats were obtained. After a one week acclimation period, SHR were randomly assigned to one of 3 diets (n=10/group): pulse-free control diet, black bean diet, or navy bean diet while the WKY rats (n=10) were fed the pulse-free control diet. The duration of the feeding was 8 weeks.
Diets: High quality Manitoba black and navy beans of known varieties were sourced, cooked, freeze-dried and powdered. The final semi-purified diets were based on the AIN-93G formulation and contained 30% (w/w), which does not affect feed intake or body weight. Proximate analyses was used to make adjustments in calorie, protein, carbohydrate and fibre content of the diet formulations to ensure they were isocaloric, isonitrogenous and isofibrous.
Assessments: 1) Feed intake and body weight were monitored weekly. No changes due to diet were detected.
2) Pulse wave velocity (a measure of arterial stiffness) and blood pressure were taken at baseline, week 4 and week 8. No changes due to diet were observed.
3) At termination, blood samples were taken to measure fasting lipid levels. No changes in these parameters, including total and LDL-cholesterol and triglycerides were seen.
4) At termination, a segment of mesenteric artey was extracted and used for myography. This is a procedure that can determine how elastic an artery is. No changes due to diet were obtained. Not that the technique was accurate since control SHR had significantly less elasticity than control WKY rats.
5) The aorta was removed at termination and its dimensions calculated using microscopy. Normally, the aorta will become thicker in SHR rats because it is subjected to higher pressures and so adapts to ensure the vessel does not rupture.
6) Biochemical measures of vascular function were assessed by Western blot analysis of the aorta. Two known mediators of vessel enlargement, p38MAPK and ERK1/2, were reduced by the black bean diet, however, navy beans only reduced the levels of p38MAPK and not ERK1/2. Similarly, PAK!, which promotes vascular remodelling (changes in the thickness of the blood vessel wall) was also reduced by the black bean but not the navy bean diet.
Conclusions: (i) The black bean diet can block the changes to blood vessel structure that are caused by high blood pressure. (ii) Navy beans may have some effect on blood vessels, but they are not as potent as black beans.

Part B - Effect of removal of beans for the diet (follow-up phase)
Animals and Diets: To determine the length of time that positive effects of beans continue after bean consumption is discontinued, a fourth group of SHR rats (n=10/group) was fed the black bean diet (we selected the one diet which produced some positive results) for 8 weeks (as described in Part A) and then switched these animals to the pulse-control diet for 4 weeks.
Assessments: During the initial 8 week feeding period, the same assessments were made as described in Part A except for those made after termination of the animals. Theses assessments were also done during week 12. The termination assessments were completed on week 12, which is then the 4-week follow-up phase was completed.
1) No changes were seen in body weight, feed intake, pulse wave velocity, blood pressure or arterial stiffness.
2) Myography revealed that the artery remains elastic for two weeks after the black bean diet is stopped. However, by 4 weeks, the vessel has returned to being stiff and non-elastic. This result is depicted in the Appendix figure.
3) Morphometry has not been completed yet on the washout samples.
Conclusions: The improvement in arterial function that occurs in hypertensive rats that have eaten black beans daily for 8 weeks is maintained for 2 weeks after the black beans are removed from the diet, but this protective effect is completely lost after 4 weeks without beans in the diet.

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 ability of beans to affect the function of our blood vessels in relation to their bioactive content as indicated by their colour. Knowing which bean type has an effect will provide information regarding the targets to select for further exploration. This in turn will enable crop breeders to select for cultivars that manufacture higher levels of these compounds. It will also serve as the foundation for identifying the bioactive copounds responsible for the positive effects we have seen. By 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.

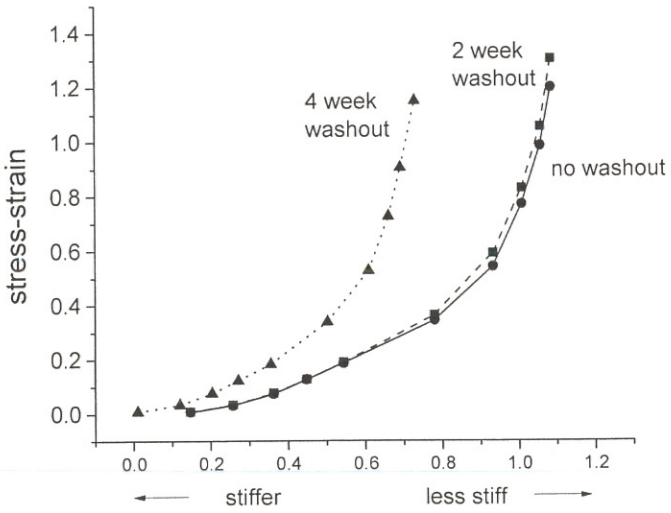
A total of three manuscripts are planned for these data. Two abstracts of our results have been submitted:
1. Loader T, Clark J, Taylor C, Zahradka P. Black beans reduce hypertension-related vascular remodeling: A dietary intervention study in spontaneously hypertensive rats. submitted to the 10th Canadian Pulse Research Workshop, Winnipeg, Oct 26-28, 2016
2. Loader T, Clark J, Taylor C, Zahradka P. Vascular remodeling due to hypertension can be reduced by eating black beans: A dietary intervention study in spontaneously hypertensive rats. submitted to the 10th American Heart Association meeting, New Orleans, Nov 12-16, 2016

Additional abstracts are expected based on the data we have obtained and they will form part of the thesis of both Tara Loader (MSc) and Jaime Clark (PhD).

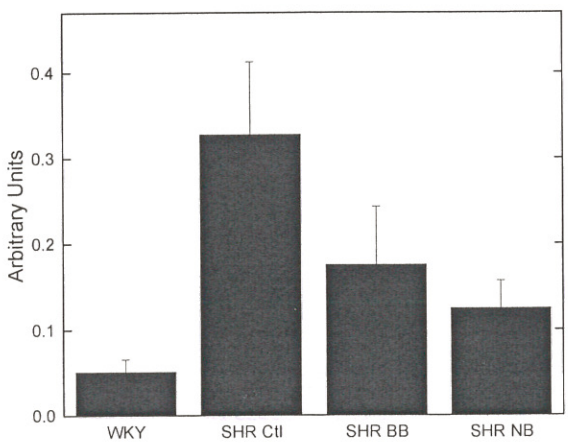
We also anticipate having the results of these studies described on the Health Report, CJOB Radio, 11 am Sundays (September-June).

APPENDIX

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



Stress-strain graph derived from myography of mesenteric artery of hypertensive SHR rats fed a diet consisting of 30% black beans every day for 8 weeks. Subsequently, the arterial performance was measured in i) a group of rats immediately at the end of the 8 week period (no washout), ii) a group placed on a bean-free diet for 2 weeks (2 week washout) and iii) a group placed on a bean-free diet for 4 weeks (4 week washout). The data indicate the beneficial effects of black beans continue for at least 2 weeks after consumption stops, but that these benefits are lost after 4 weeks.



Active p38MAPK in aorta of hypertensive SHR animals fed control diet (Ctl), black bean (BB) or navy bean (NB) for 8 weeks. Control, normotensive WKY rats received the control diet. Higher levels of p38MAPK are associated with greater enlargement of blood vessels.