Bean/Bran Enriching Nutritional Eating For Intestinal health & Cancer Including Activitiy for Longevity (BENEFICIAL)

Bridget A Baxter[1], Melanie N Beale[3], Hillary V Smith[2], Hannah Haberecht[1], Sangeeta Rao[4], Sarah Hibbs-Shipp[5] Heather J Leach[3] & Elizabeth P Ryan[1]

1. Department of Environmental and Radiological Health Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523
2. Colorado School of Public Health, Colorado State University, Fort Collins, Co 80523
3. Department of Health and Exercise Science, College of Health & Human Sciences, Colorado State Uniersity, Fort Collins, CO 80523
4. Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523
5. Colorado State University Ventures, Fot Collins, CO 80523

INTRODUCTION

Healthy diet and physical activity substantially reduce the risk of colon cancer and other associated chronic diseases. Navy beans and rice bran fed to mice showed inhibition of colon carcinogenesis and were able to modulate the gut microbiome communities in colorecetal cancer survivors (NCT01929122). Beans/Bran Enriching Nutritional Eating For Intestinal health & Cancer Including Activity for Longevity (BENEFICIAL) is a new human clinical research study designed to idenify whether navy bean and rice bran combined with regular physical activity, can lower the risk of colorectal cancer. National recommendatins for colorectal cancer survivors are regular physical activity (150 min/week) and a diet rich in fiber (30g/day). In 2018, a pilot 3-month intervention trial was completed with 23 particpants that had one or more polyps removed within the last 3 years. We hypothesized that daily consumption of navy beans and rice bran would improve Healthy Eating Index (HEI) compared to a Fibersol®-2 control supplement, and elicit distinct changes to metabolism.

MATERIALS & METHODS

1) Recruitment: Healthy adults who had one or more adenomatous polyps removed within the last 3 years and no cancer diagnosis.

2) Diet Intervention: Participants consumed 1-3 study food items daily for 12 weeks. Recipes and 3-day food logs were analyzed using NutritionistPro™ diet analysis software. Participants completed 3-day food logs at week 0, week 6 and week 12 during the study, which included recording all foods and drinks consumed on two weekdays and one weekend day. We used ASA24 output to generate a HEI, according to the 2015 Dietary Guidelines for Americans. Rice bran and navy beans were computational additions to ASA24 output for HEI.

3) Physical Activity Guidelines: Participants met with a health and exercise science trained graduate student who provided a one-hour physical activity education session specific to colon cancer prevention.

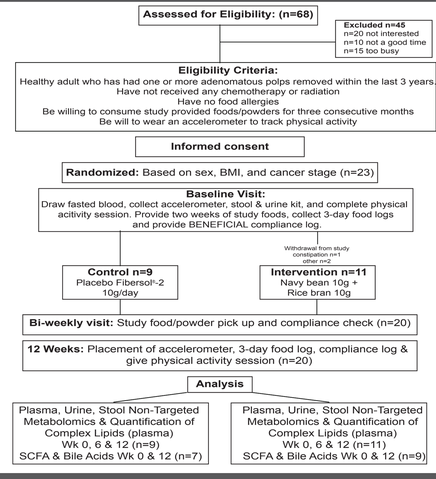
4) Physical Activity activPal™: Moderate to vigours physical activity, light and sedetary minutes was measured for 7-days, 24 hours using an activePal™ accelerometer at week 0 and 12.

5) Plasma, Urine and Stool Metabolomes: Sample preparation was conducted using 80% methanol extract ion. Metabolomes analyzed by Metabolon, Inc. using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS/MS). Proteomics and Metabolomics Facility at Colorado State University, quantified stool bile acids and short chain fatty acids using gas chromatography mass spectrometry (GC/MS).

6) Statistical analysis: A repeated measures 2-way ANOVA was applied to analysis of Non-targeted metabolomics and complex lipids. Linear mixed models were applied for SCFA and bile acids (SAS). A p-value of 0.05 was used to evaluate statistical significance.

Clinical Trial Approval #17-7464H

Figure 1. Study Design



Study Participants' Baseline Characteristics & Intervention

Characteristic (n=20)	Control (n=9)	Intervention (n=11)
Age (years)	58.9 ± 7.8	59.18 ± 9.3
Sex		
Males (%)	4 (45%)	6 (55%)
Females (%)	5 (55%)	5 (45%)
BMI (kg/m^2)	29.5 ± 2.1	25.9 ± 3.5
Normal weight (20-25%)	0	5
Overweight (25-30%)	5	4
Obese (30% +)	4	2
Colon polyps stage		
Stage 0 < 6mm	7	9
Stage 1 > 6mm	2	2

A one-hour pysical activity education session specific to colon cancer prevention is provided to particpants.

Physical Activity Guidelines:
150 minutes of moderate intensity activity per week
OR
75 minutes of vigorous intensity activity per week
Plus
Two days of resistance training for at least 30 minutes

activePAL™:
The activePAL™ was used to collect participants physical activity for one week at week 0 & week 12. Worn on the thigh, to quantify free living sedetary, upright and and ambulatory activity, and provides the evidence to link sedentary behaviors to chronic disease

	Calories (Kcal)		Fiber (g)	
	Control	Intervention	Control	Intervention
Berry smoothie	161	184	6.5	10.5
Baked pasta marinara	242	378	4.1	13.4
Tomato basil soup	166	159	2.2	6.9
Homemade chili	243	331	7.7	12
Caraway crackers	337	335	6.3	11
Cheesy biscuit	171	220	0.4	5.1
Cranberry/apple granola	356	334	7.7	10.4
Energy date bites	165	265	3.5	10.3
Hummus	116	144	10.8	13.8
Butternut squash soup	267	273	5.5	8.8
Southwest chicken bake	341	331	3.3	7.7
Pizza	270	240	4.1	13.5
Chipotle meatball	216	442	0.6	5.5
Banana nut muffin	251	253	3	6.7
Waffle	267	172	0.4	5.2
Study powders	11	63	8.8	5.6

Two foods and one study powder packet were consumed by all participants. Intervention received 10g rice bran/10g navy bean per meal/snack. Control study foods were macronutrient matched.
Study powder packet contains 10g Fibersol®-2 (soluble corn fiber) or 10g rice bran/10g navy bean powder. Study powder packets were self-incorporated by participants into daily diet.

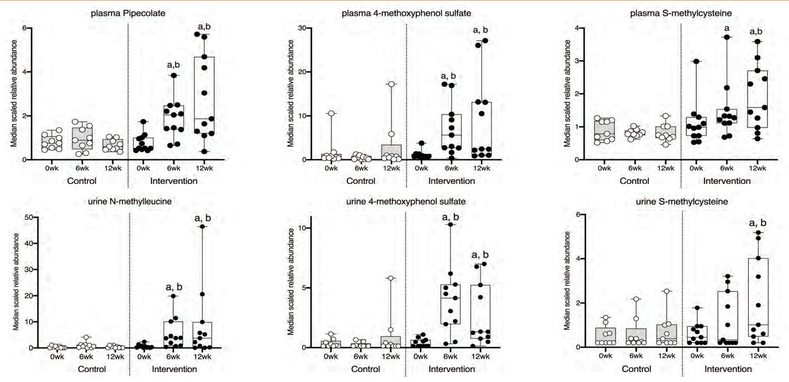
RESULTS: Non-Targeted Metabolomics, Quantification of Complex Lipids (Plasma) & Stool Bile Acids

Table 1. Navy bean/rice bran consumption for 6 weeks & 12 weeks modulated plasma & urine profiles, while minimal changes occurred over time in the control group.

Plasma Fold Change									
Super Pathway	Sub Pathway	Biochemical Name	Control			Intervention			
			Wk6/Wk0	p-value	Wk12/Wk0	p-value	Wk6/Wk0	p-value	Wk12/Wk0	p-value
Amino Acids	Lysine	pipecolate	1.17	0.848	0.83	0.467	**2.46**	0.0004	**3.62**	0.324
	Tyrosine Metabolism	4-methoxyphenol sulfate	**0.27**	0.079	1.67	0.958	**6.42**	0.0008	**8.34**	0.997
	Methionine, Cysteine, SAM and Taurine Metabolism	methionine sulfone	1.04	0.910	0.98	0.616	**1.16**	0.002	**1.20**	0.912
		S-methylcysteine	0.92	0.823	0.92	0.694	**1.32**	0.097	**1.70**	0.195
		S-methylcysteine sulfoxide	0.72	0.301	0.85	0.503	1.17	0.271	**1.72**	0.235
Phytochemicals	Benzoate Metabolism	3-methoxycatechol sulfate (2)	1.19	0.886	1.48	0.579	**2.77**	0.014	**3.31**	0.822
Urine Fold Change									
Super Pathway	Sub Pathway	Biochemical Name	Control			Intervention			
			Wk6/Wk0	p-value	Wk12/Wk0	p-value	Wk6/Wk0	p-value	Wk12/Wk0	p-value
Amino Acids	Tyrosine Metabolism	phenol sulfate	0.83	0.67713	1.61	0.336	1.5	0.113	1.08	0.474
		4-methoxyphenol sulfate	0.55	0.36019	1.47	0.471	**7.72**	0.000	**5.8**	0.0005
	Leucine, Isoleucine and Valine Metabolism	N-methyleucine	2.78	0.13223	0.85	0.981	**8.95**	0.000	**11.87**	0.0005
	Methionine, Cysteine, SAM and Taurine Metabolism	S-methylcysteine	1.05	0.88568	0.89	0.903	1.44	0.336	**2.92**	0.002
		S-methylcysteine sulfoxide	1.01	0.793	0.99	0.614	1.1	0.504	**2.02**	0.017
	Urea cycle	N2,N5-diacetylornithine	1.09	0.45533	0.96	0.977	**1.65**	0.001	**1.55**	0.002
Nucleotide	Purine Metabolism	urate	1.12	0.50543	0.99	0.888	**1.25**	0.009	**1.34**	0.002
Cofactors & Vitamins	Tocopherol	gamma-CEHC	0.62	0.69342	0.66	0.466	**2.36**	0.013	**2.23**	0.047
		gamma-CEHC glucuronide*	**0.55**	0.01979	0.73	0.241	**1.51**	0.019	1.36	0.171
	Pterin Metabolism	pterin	1.02	0.82938	0.89	0.697	**1.3**	0.037	**1.75**	0.0004
Phytochemicals	Benzoate Metabolism	3-methoxycatechol sulfate (2)	2.26	0.22723	1.1	0.862	**2.62**	0.009	**1.96**	0.052

Values presented are fold-change of the mean relative abundance within control and intervention groups at 6 and 12 weeks compared to their baseline ($p \leq 0.05$) . Statistically significant metabolites are **bold.**

Figure 2. Amino acid metabolites were impacted after 6 and 12 weeks of navy bean/rice bran intake in plasma and urine metabolomes.



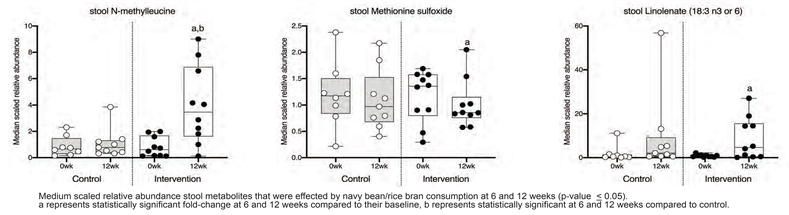
Medium scaled relative abundance plasma A. and urine B. metabolites that were effected by navy bean/rice bran consumption at 6 and 12 weeks (p-value ≤ 0.05).
a represents statistically significant fold-change at 6 and 12 weeks compared to their baseline, b represents statistically significant at 6 or 12 weeks compared to control.

Table 2. Navy bean/rice bran consumption for 12 weeks modulated stool profiles, while minimal changes occurred over time in the control group.

Stool Fold Change						
Super Pathway	Sub Pathway	Biochemical Name	Control		Intervention	
			Wk12/Wk0	p-value	Wk12/Wk0	p-value
Amino Acids	Leucine, Isoleucine and Valine Metabolism	N-methylleucine	1.25	0.919	**4.88**	0.0005
	Methionine, Cysteine, SAM and Taurine Metabolism	methionine sulfone	**0.82**	0.073	**0.66**	0.047
Lipids	Long Chain Polyunsaturated Fatty Acid (n3 and n6)	linolenate [alpha or gamma; (18:3n3 or 6)]	4.93	0.085	**10.14**	0.045
	Fatty Acid, Dicarboxylate	dodecanedioate (C12-DC)	0.98	0.906	**1.83**	0.048
		hexadecanedioate (C16-DC)	1.49	0.119	**1.83**	0.008
	Phospholipid Metabolism	trimethylamine N-oxide	0.65	0.528	**0.37**	0.047
Nucleotide	Purine and Pyrimidine Metabolism	methylphosphate	1.68	0.435	**2.21**	0.045
Cofactors & Vitamins	Nicotinate and Nicotinamide Metabolism	nicotinamide	**0.65**	0.048	**2.44**	0.026

Values presented are fold-change of the mean relative abundance within control and intervention groups at 12 weeks compared to their baseline (p-value≤ 0.05). Statistically significant metabolites are **bold.**

Figure 3. Amino acid and lipid metabolites were impacted after 12 weeks of navy bean/rice bran intake in stool metabolomes.



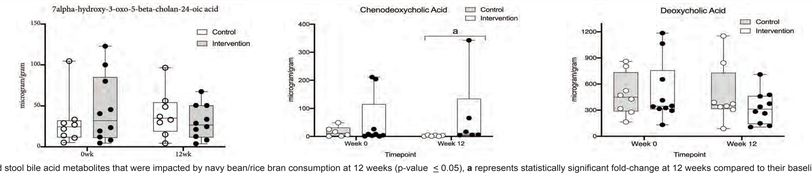
Medium scaled relative abundance stool metabolites that were effected by navy bean/rice bran consumption at 6 and 12 weeks (p-value ≤ 0.05).
a represents statistically significant fold-change at 6 and 12 weeks compared to their baseline, b represents statistically significant at 6 and 12 weeks compared to control.

Table 3. Navy bean/rice bran consumption for 12 weeks modulated stool bile acid profiles.

Bile Acids	Control		Intervention		Control Intervention	
	Week 0 (n=8)	Week 12 (n=8)	Week 0 (n=9)	Week 12 (n=9)	p-value Week 0	p-value Week 12
Deoxycholic Acid	481 ± 245.1 (450)	470.2 ± 322.3 (325.5)	404.2 ± 364.2 (325.5)	328.13 ± 364.2 (325.5)	0.25	0.31
Chenodeoxycholic Acid	16.5 ± 15.8 (16.4)	5.4 ± 6.5 (3.4)	42.8 ± 73.8 (16.5)	50.7 ± 104.1 (16.5)	0.91	**0.005**
3_Oxocholic Acid	1.6 ± 1.5 (1.1)	1.1 ± 0.85 (1.1)	4.3 ± 3.9 (3.4)	17.3 ± 14.6 (25.5)	0.32	**0.022**
Glycocholic Acid	4.3 ± 8.4 (1.8)	1.2 ± 0.81 (0.97)	2.9 ± 3 (1.7)	3.5 ± 5 (1.29)	0.7	0.38
Taurodeoxycholic Acid	0.86 ± 0.6 (1.24)	1.5 ± 1.5 (1.21)	5.0 ± 13.1 (1.21)	0.94 ± 0.73 (0.72)	0.63	0.68
7alpa-Hydroxy-3-oxo-5beta-cholan-24-oic acid	30.73 ± 31.38 (24.4)	40.3 ± 26.4 (37)	3138.63±11184.6 (19.37)	30.2 ± 2064 (26.7)	0.58	0.45

Values presented are mean ± standard deviation (median). P-values represent control compared to intervention groups at 0 and 12 weeks (p≤ 0.05). SAS was used for all statistical analyses.
Statistically significant metabolites are **bold.**

Figure 4. Stool bile acid metabolites were impacted after 12 weeks of navy bean/rice bran.



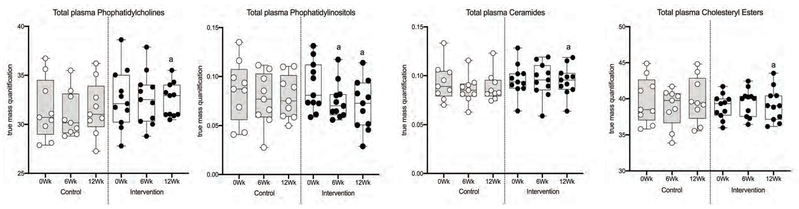
Quantified stool bile acid metabolites that were impacted by navy bean/rice bran consumption at 12 weeks (p-value ≤ 0.05), **a** represents statistically significant fold-change at 12 weeks compared to their baseline.

Table 3. Fold changes in complex lipid from plasma after 6 and 12 weeks of navy bean/rice bran consumption.

Sub Pathway	Biochemical Name	Control				Intervention			
		Wk6/Wk0	p-value	Wk12/Wk0	p-value	Wk6/Wk0	p-value	Wk12/Wk0	p-value
Phosphatidylcholines	Total PC	1.06	0.209	0.98	0.817	0.95	0.195	**0.92**	0.032
Phosphatidylinositols	Total PI	0.92	0.677	0.92	0.616	**0.80**	0.029	**0.72**	0.001
Ceramides	Total CER	1.01	0.806	0.95	0.195	0.94	0.183	**0.90**	0.036
Cholesteryl Esters	Total CE	1.06	0.299	0.99	0.812	0.96	0.336	**0.92**	0.043

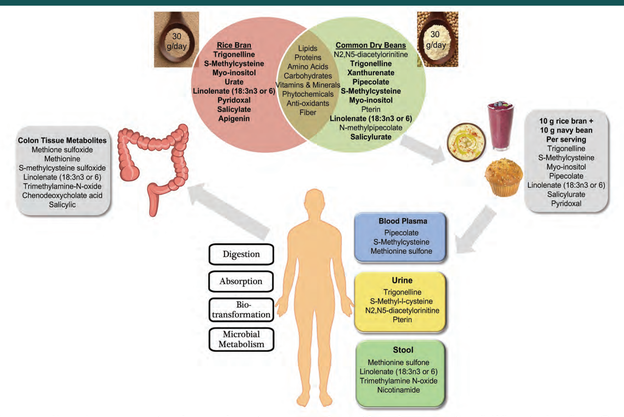
Values presented are fold-change of the mean relative abundance within control and intervention groups at 6 and 12 weeks compared to their baseline ($p \leq 0.05$) . Statistically significant metabolites are **bold.**

Figure 5. Quantification complex lipids after 6 and 12 weeks of navy bean/rice bran intake in plasma.



True mass quantification of complex lipids in plasma that were impacted by navy bean/rice bran consumption at 6 and 12 weeks (p-value ≤ 0.05).
a represents statistically significant fold-change at 6 and 12 weeks compared to their baseline.

CONCLUSION & FUTURE DIRECTIONS



Next we will enroll a larger study cohort, examine the impact of lifestyle for 6 months (increased fiber and physical activity) on right and left colon tissue metabolites and develop biomarkers for colon cancer risk reduction.

RESULTS

- We demonstrated pilot feasability of modulating metabolism following dietary fiber intake and controlling for physical activity.
- Complex lipids in blood were impacted after 6 and 12 weeks of navy bean/rice bran intake.
- Navy bean/rice bran intervention group decreased total plasma cholesteryl ester (CE) over time when compared to no change in the control group.
- The reduction in total phosphatidylcholines (PC), phosphatidylinositols (PI) and ceramides (CER) following the navy bean/rice bran intervention merit additional attention as biomarkers for reducing colorectal cancer risk.
- The increase in pipecolate and S-methylcysteine in plasma metabolomes merit addtiontional attention as biomarkers of intake for navy bean and rice bran.

REFERENCE

Metabolite Profile Comparisons Between Ascending and Descending Colon Tissue in Healthy Adults. Bridget A. Baxter, Kristopher D. Parker, Michael J. Nosler, Sangeeta Rao, Rebecca Craig, Catherine Seiler and Elizabeth P. Ryan. Department of Radiological Sciences, Colorado State University, Fort Collins, CO 80521, USA *World Journal of Gastroenterology* 2020 Jan 21; 26(3): 335-352.

ACKNOWLEDGEMENTS

This work was funded by University of Colorado Cancer Cente (P30CA046934), Colorado School of Public Health provided a Graduate Research Associate student funds. Human Performance Clinical Research Lab at CSU provided space to conduct the study. We would like to thank the research staff at University Colorado Health Cancer Center for assisting in recruitment. Lastly a big thank to our BENEFICIAL participants.